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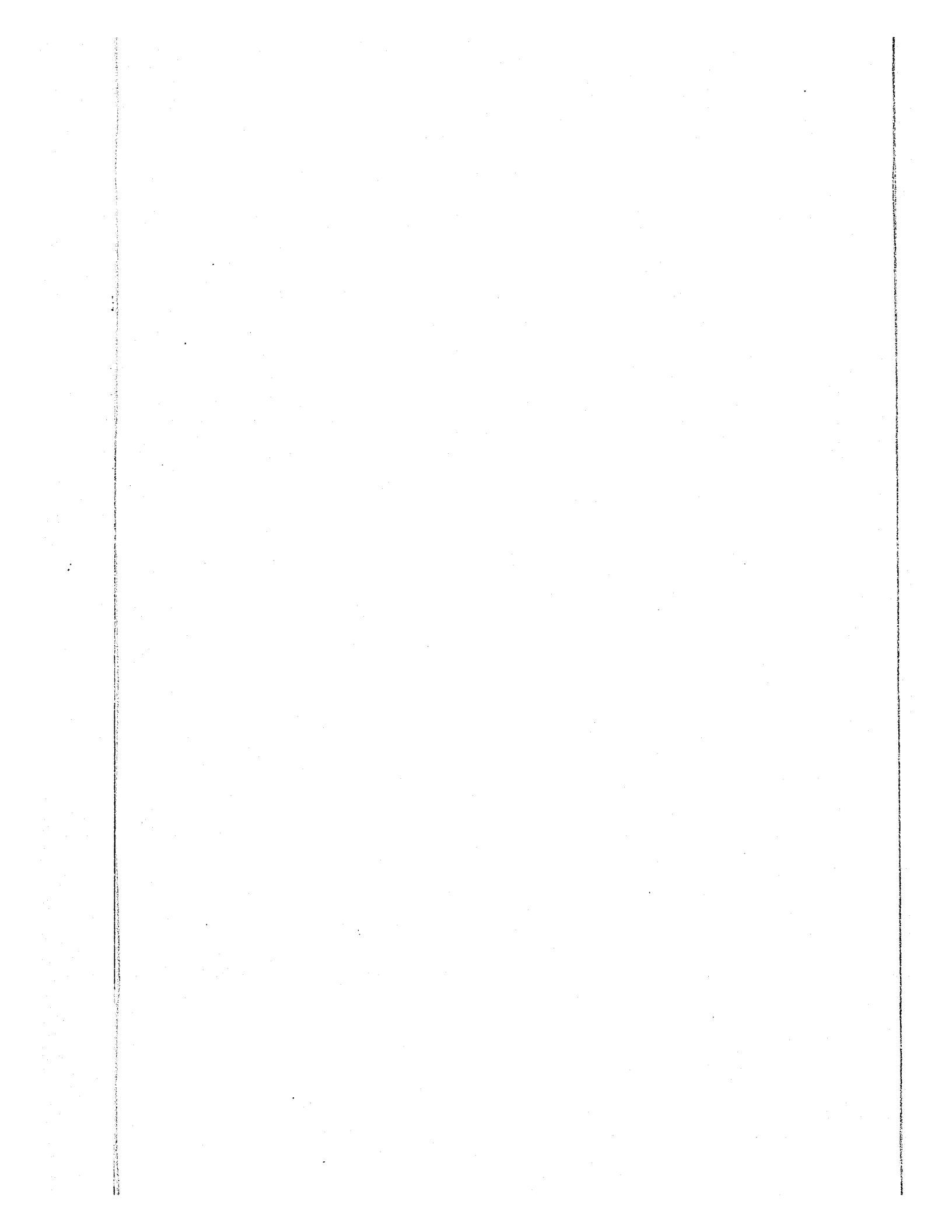
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STUDY OF THE BLOOD-BRAIN BARRIER
WITH EXPERIMENTAL SUBARACHNOID HEMORRHAGE

BY

ERICO R. CARDOSO, M.D.

THESIS

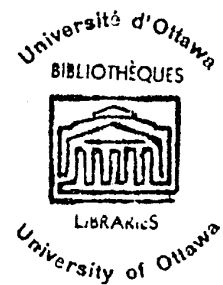
Submitted to the School of Graduate Studies in partial fulfilment
of the requirements for the Degree of Master of Science.

Department of Anatomy

Faculty of Medicine

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NOVEMBER, 1977



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ABBREVIATIONS

BBB	BLOOD-BRAIN BARRIER
BP	ARTERIAL BLOOD PRESSURE
CBF	CEREBRAL BLOOD FLOW
CBV	CEREBRAL BLOOD VOLUME
CNS	CENTRAL NERVOUS SYSTEM
CPP	CEREBRAL PERFUSION PRESSURE ($CPP = BP - ICP$)
CSF	CEREBROSPINAL FLUID
CVR	CEREBROVASCULAR RESISTANCE ($CVR = CPP/CBF$)
EB	EVANS BLUE
ECS	EXTRACELLULAR SPACE
EM	ELECTRON MICROSCOPIC
HRP	HORSERADISH PEROXIDASE
ICP	INTRACRANIAL PRESSURE
MP	MICROPEROXIDASE
SAH	SUBARACHNOID HEMORRHAGE
5-HT	5-HYDROXYTRYPTAMINE, SEROTONIN

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ACKNOWLEDGEMENTS

ABBREVIATIONS

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I. INTRODUCTION

A. HISTORICAL CONCEPT OF THE BLOOD-BRAIN BARRIER

The first investigation of the blood-brain barrier (BBB) was carried out by Ehrlich in 1885, who observed the difference in appearance of the central nervous tissue when acidic and basic dyes were injected subcutaneously in experimental animals. The acidic dye, coerulein-S, failed to stain the brain. He interpreted the results as a failure of the nervous tissue to impregnate with the dye. No mention was made of a possible barrier mechanism, which would prevent the substance contacting the brain parenchyma, (reviewed by Friedmann, 1942; Davson, 1976).

The concept of a blood-brain barrier (Blut-Gehirn-Schanke) was clearly established in 1913 after Goldmann's "first and second experiments" (reviewed by Bakay, 1956). In the first experiment (1909), he injected the acidic dye trypan blue intravenously and observed its distribution through all body tissues, except the central nervous system (CNS), which remained "snow-white", although the choroid plexus was stained. When the dye was injected into the cerebrospinal fluid (CSF) in the second experiment (1913), heavy staining of the whole brain and meninges was seen, with granular accumulations of the dye in all types of cells. Furthermore, no sign of neurotoxicity was noted during the first experiment. This is in striking contrast to the neurological symptoms, such as seizures, paralysis and death, displayed by animals during the second experiment. Goldmann was the first investigator to use trypan blue for cerebral vascular permeability studies (reviewed by Davson, 1967).

Simultaneous investigations by others on the comparison of the toxic effects of certain substances, when injected into the CSF and blood, also contributed to the establishment of a blood-brain barrier concept. Intravenous injection failed to induce the toxicity observed after administration into the CSF, even when higher doses were used. This was observed, for instance, with bile salts by Biedl and Kraus, with potassium ferrocyanide by Lewandowsky, and with diphtheria toxin by Roux and Borrel (reviewed by Friedemann, 1942 and Bakay, 1956).

Subsequently it was demonstrated by several investigators (Rawson, 1943; LeVeen and Fishman, 1947; Allen and Ovahovats, 1950; Steinwall and Klatzo, 1966; Freedman and Johnson, 1969; Clasen et al., 1970), that the acidic dyes, such as trypan blue and its isomer Evans blue, bind to the plasma proteins (mostly to albumin) immediately after intravenous injection. Albumin contains up to 70 binding sites for these compounds (LeVeen and Fishman, 1947). It has been shown that at the dose of 100 mg/kg body weight, all the Evans blue is bound to plasma proteins (Rawson, 1943; Johansson et al., 1974). The dye-protein complex has a molecular weight of approximately 68,500 (in Rapoport, 1976a) and a diameter of 76\AA (Scheraga, 1961). Therefore, the previous results concerning the blood-brain barrier must be re-interpreted in terms of a barrier to large molecules. Tschirgi (1950) repeated Goldmann's "second experiment", injecting trypan blue mixed with serum into the subarachnoid space. There was a failure of brain staining.

Further studies on the blood-brain barrier phenomenon can be divided into two types: 1) attempts to define the barrier in terms of

the anatomic site. These studies involve permeability properties of a non-specific type. Protein tracers have been very useful tools for such an approach, providing practical and accurate qualitative microscopic traces. These morphological investigations have been extended to the study of neuropathologic and neurophysiologic insults which disrupt the barrier. From the results of altered permeability under various abnormal situations, conclusions can be drawn regarding the structure and functions of the BBB. 2) Physiologic studies concerned with the concentration of substances in the brain. These investigations deal with the barrier properties to specific substances and the active transport mechanisms involved in the "carrying" process across the BBB. The barrier is defined in terms of the compound being tested. These studies include substances which inhibit or facilitate specific BBB transport. This second line of investigation emphasizes the dynamic concept of the BBB, which holds not only barrier properties, but also facilitates the entry of essential nutrients to the nervous tissue. The present investigation deals with a pathological aspect and its possible implications with the BBB to proteins.

B. HISTORICAL EVOLUTION OF THE MORPHOLOGICAL LOCALIZATION OF THE BBB

Historically, three main theories have been developed concerning the anatomical substrate for the barrier to proteins. These are based on: 1) the perivascular glial end-foot processes, 2) the small volume of the cerebral extracellular space (ECS), and 3) special features of the cerebral vascular endothelium. Other morphological features of the brain capillaries have been hypothesized as responsible for the barrier effect, but their role has not been universally accepted. They include: a) capillary pericytes (Kristensson and Ollson, 1973; van Deurs, 1976), b) a continuous basement membrane (Bennett et al., 1959), c) the paucity of contractile protein in the cerebrovascular endothelial cells (Becker and Shustak, 1972), d) the presence of ground substance, (Hess, 1955), e) the absence of connective tissue (King, 1938), f) the higher density of mitochondria in the cerebrovascular endothelial cells when compared to other tissues (Oldendorf and Brown, 1975), and g) the absence of phosphatase activity in the pinocytotic vesicles of cerebral endothelial cells (Torack and Barnett, 1969).

1. Perivascular end-feet theory

The perivascular glial end-feet concept was formulated in 1928 by Schaltenbrand and Bailey and further developed by Hauptmann and Garter, Hoff (reviewed by Bakay, 1956) and Tschirgi (1950; 1952). The development of this theory was based on the assumption that, due to the anatomical similarity and continuity of the superficial pia-glial

membrane with the perivascular glial sheath, both membranes should possess the same permeability properties. A strong argument against the theory was provided by Goldmann's and Spatz's results (reviewed by Tschirgi, 1952), who demonstrated that saline solutions of trypan blue injected in the CSF led to brain staining. Therefore, the superficial pia-glial membrane was considered not to be a barrier to the dye, and the same should apply to the perivascular glial sheath. Since Tschirgi (1950) demonstrated that solutions of trypan blue in plasma did not stain the brain after application onto the cortex surface, the evidence favoured the perivascular glial sheath theory. Again, if the protein bound dye did not permeate the superficial pia-glial lining, it should not go through the perivascular glial sheath. Tschirgi (1952) reinforced his theory with the argument based on the post-mortem diffusion of dye into the artery wall. Therefore, the structures responsible for barrier effect should be outside the vessel wall. It was demonstrated later (Westergaard and Brightman, 1973) that the size of arteries Tschirgi was working with are devoid of BBB and his results could not be extrapolated to arterioles and capillaries.

Further elucidation of the morphology of the perivascular glial sheath was prevented by difficulties of nervous tissue histologic staining and the low resolution of the light microscope. More accurate information was only provided after the advent of the electron microscopy. In 1957, Maynard et al. demonstrated that the

astrocyte end-foot processes do not form a continuous sheath around the capillaries of rat cortex. The authors calculated that these processes cover just about 85% of the vessel surface. It was shown later that species differences exist. Brightman et al., (1970b) reported that in sharks, whose brain capillaries are porous, the fused end-foot processes represent the main barrier structure. However, this is not applicable to mammals, as was convincingly demonstrated by Brightman et al., (1969; 1970b) and Feder et al., (1969). These investigators used horseradish peroxidase (HRP) and microperoxidase (MP) and injected them into the blood stream and into the cerebral ventricles. When injected intravenously, both tracers were confined to the lumen of the brain vessels, not penetrating through the cerebral endothelial lining. When injected into the ventricles, both tracers crossed the ependymal lining, migrated through the extracellular space and were visualized between the perivascular glial end-foot processes, going as far as the contraluminal surface of the endothelial cells. It was, therefore, demonstrated that the interspace between perivascular glial membranes was permeable to molecules as large as 43,000 molecular weight (HRP) and that the vascular endothelium prevented the diffusion of the molecules as small as 2,000 molecular weight (MP) in both directions. Recently Pappenheimer (1970), reformulated the astrocyte end-foot theory, proposing a model for the blood-brain barrier based on the combined action of the capillary endothelial cells and the astrocytic processes. He

proposes that the endothelium is unable to carry out all the metabolic functions of selective transport and facilitated diffusion attributed to it. Instead, he advocates that unspecific passive permeability mechanisms occur at the endothelial level, blocking larger molecules and allowing restricted diffusion of small particles. Enzymatic activities would take place in the underlying astrocytic membrane. Torack and Barnett (1964) demonstrated the unique localization in the basement membrane and glial processes of brain capillaries of nucleoside phosphatase activity; this enzyme is normally located in the pinocytotic vesicles of capillaries without barrier characteristics. This evidence seems to support Pappenheimer's theory.

2. Small ECS Theory

Simultaneously with the initial EM studies indicating virtual absence of brain ECS (Schultz et al., 1957; Farquhar and Hartmann, 1957), Maynard et al., (1957) postulated that the movement of particles from the vessel lumen to the brain parenchyma was prevented by the small extracellular space of the nervous system limiting large molecule migration. The figures found for the ECS volume calculated from morphological measurements were demonstrated to be inaccurate according to physiological methods (Davson and Bradbury, 1965; Levin et al., 1970), probably due to fixation artifacts (Van Harreveld, 1965).

The small ECS theory was defended by other authors such as Edstrom (1958) and Dobbing (1963). A supporting argument for this

theory was the ubiquitous presence of connective tissue surrounding the capillaries in areas where the BBB was absent (Dempsey and Wislocki, 1955; Van Breeman and Clemente, 1955; Wislocki and Leduc, 1952; Brown, 1961). Absence of BBB has been reported in the choroid plexus (Goldmann, 1909; Tennyson and Pappas, 1961; Becker et al., 1967), neuro-hypophysis (Palay, 1957), area postrema (Wislocki and Putnam, 1920), tuber cinereum (Klatzo et al., 1962), intercolumnar tubercle (Putnam, 1922; Wislocki and Leduc, 1952) and pineal body (Wislocki and Leduc, 1952; Wolfe, 1965; Moller et al., 1976).

The concept that a small ECS could explain the BBB was strongly rejected because of available evidence (Davson and Spaziani, 1959). These authors found that substances such as sucrose, p-aminohippurate and I¹³¹ penetrate the muscle but not the brain, in vivo. In contrast, they penetrate slices of both tissues in vitro at similar rates. Therefore, the in vivo difference should not be due to the lack of brain extracellular space (Davson and Spaziani, 1959). In addition, Bodenheimer and Brightman (1968) demonstrated that, in certain amphibians possessing large cerebral perivascular space (0.5 μ m), a BBB was still present, similar to that of the mammalian brain. Furthermore, the ECS of the mammalian brain was demonstrated, in later studies, to allow free migration of intraventricularly injected large molecules, such as ferritin (Brightman, 1965) and protein (HRP) (Brightman and Reese, 1969; Brightman et al., 1970b). Therefore, there is evidence that the small ECS does not constitute a morphological substrate for the BBB.

3. The Cerebrovascular Endothelium Theory

Studies initiated by Riser in 1929, and elaborated in the 1930's by Spatz, Friedman and Elkeles, and Broman (reviewed by Broman and Lindberg-Broman, 1945) supplied strong evidence for the structural localization of the blood-brain barrier within the vessel wall. It was then well established that trypan blue (or other dyes of the same kind) does not cross the cerebral vessel wall under normal conditions. These dyes became an important tool for tracing cerebral vascular lesions. The first pioneer work in this line was carried out by Macklin and Macklin, 1920, who studied vascular lesions following mechanical trauma to the CNS (reviewed by Broman and Lindberg-Broman, 1945).

The advent of the electron microscope and the improvement of brain fixation techniques, by in situ perfusion, disclosed new details of the structural features of the BBB. A vascular endothelial barrier was identified using intravenously injected electron opaque tracers such as thorotrast, (Donahue, 1964; Lampert and Carpenter, 1965) and colloidal iron oxide (Clawson et al., 1966). However, this barrier was not unique for brain capillaries, since it was also seen in many other organs (Jennings, 1962). Using HRP as a tracer, Reese and Karnovsky (1967) convincingly demonstrated a specific barrier to that protein in the brain capillary endothelium. The anatomical substrate of this barrier was seen to consist of two main features: intercellular junctions of the type zonula occludens (tight junctions) and paucity of pinocytotic vesicles. The presence of vascular endothelial cells

attached by tight junctions is exclusive to a few areas in the body, such as the brain (Muir and Peters, 1962; Brightman and Reese, 1969), endoneurium (Olsson, 1968), retina (Shakib and Cunha-Vaz, 1966; Karnovsky, 1967), and inner ear (Wade and Karnovsky, 1974). The tight junctions are absent in areas of the brain devoid of BBB (Hashimoto and Hama, 1968). The tight junctions completely surround the cell circumference constituting a continuous belt of closely apposed membranes. The two neighbouring membranes are linked by fibrillar strands (Wade and Karnovsky, 1974; Sandri et al., 1977). It has been suggested that the permeability properties are dependent on the special alignment of these fibrils. Further studies by Brightman, et al., (1969; 1970a) and Feder et al., (1969), demonstrated that the only continuous cellular layer between the circulation and the ECS of the brain is represented by the vascular endothelium.

Pinocytotic vesicles have been suggested to play a role in the trans-cellular transport of larger molecules across the endothelial cells (Palade, 1953). The small number of these vesicles in the cerebral vascular endothelium, when compared with other organs (Karnovsky, 1965a), associated with the lack of evidence of vesicle discharge in the contraluminal endothelial border under normal conditions, suggest that these structures may represent a BBB component (Reese and Karnovsky, 1967). This theory is reinforced by the absence of phosphatase activity in these vesicles, as opposed to pinocytotic vesicles elsewhere in the body (Torack and Barnett, 1964). A con-

vincing demonstration that the paucity of pinocytotic vesicles constitutes a morphological manifestation of the BBB phenomenon would require the visualization of tracers in the basement membrane and/or neurophil under circumstances which would selectively cause increased vesicular transport. The majority of agents that cause injuries to the cerebrovascular endothelium, act concomitantly by intensifying pinocytotic transport and opening tight junctions. Therefore, the contribution played by pinocytosis is difficult to evaluate. Furthermore, the increase in number of pinocytotic vesicles seems to be a very sensitive event, having been described even as a consequence of HRP injection itself (Cotran and Karnovsky, 1967) or intracarotid normal saline perfusion (Waldron et al., 1974). No appreciable amount of HRP had been observed in the basement membrane as a consequence of the selective increase of pinocytosis until the description by Westergaard et al., (1975, 1976) of increased cerebrovascular permeability due solely to increased pinocytosis. Their findings suggest that increased vesicular transport plays a role in abnormally increased cerebrovascular permeability to protein.

Another example that morphological alteration of structures displaying BBB functions can corroborate the role played by those structures under normal conditions is provided by changes of the tight junctions under certain circumstances. It has been demonstrated that widening of the tight junctions under certain circumstances is accompanied by extravascular leakage of proteins. This occurs, for

instance, after osmotic endothelial cell shrinkage due to intravascular hyperosmolarity (Brightman et al., 1973) or following intense increase of intraluminal pressure, presumably causing endothelial cell over-stretch (Johansson et al., 1974).

C. SOME PATHOLOGICAL ALTERATIONS OF THE BBB

Studies of the effect of damaging agents on the BBB permeability were initiated by Macklin and Macklin, and were followed by many other clinical and experimental studies (see reviews by Broman and Lindberg-Broman, 1945; Brightman et al., 1970a; Katzman and Pappius, 1973; Rapoport, 1976a). Conditions where BBB damage has been observed include: inflammation (Lampert and Carpenter, 1965; Som et al., 1972), arterial hypertension (Byrom, 1954; MacKenzie et al., 1976; Hazama et al., 1976), seizure (Lorenzo et al., 1972; 1975), severe increase of intracranial pressure (Petersén and Zwetnow, 1967) ischemia (Hossmann and Olsson, 1971a; Ito et al., 1976), trauma (Vise et al., 1975; Beggs and Waggener, 1976), stab wound (Hirano et al., 1969), cerebral tumours (Bakay, 1968), and intense hypo and hypercapnia (Cutler and Barlow, 1966; Roth et al., 1966). In addition, damage to the BBB has been induced by thermal (Klatzo et al., 1958) and chemical (Broman and Lindberg-Broman, 1945) injuries, and by radiation (Miquel and Haymaker, 1965). In this work, attention will be focused on two BBB damaging agents: acute arterial hypertension and intracarotid perfusion of toxic agents.

1. BBB and Arterial Hypertension

Cerebral changes following a rise in arterial blood pressure were initially noted from clinical observations. They were first

attributed by Allbutt (reviewed by Byrom, 1954) to the uremia associated with hypertension, but subsequently have been elucidated as specific hypertensive symptoms (Volhard and Fahr, reviewed by Byrom, 1954). The whole clinical and pathological picture was named "hypertensive encephalopathy" by Oppenheimer and Fishberg (1928). Byrom (1954) was the first author to demonstrate experimentally increased cerebrovascular permeability in chronically hypertensive rats, using Goldblatt's renal hypertension technique. Byrom observed trypan blue extravasation in the grey matter, associated with focal ischemic lesions and increased water content in the discoloured areas. He also described diffuse spasm of small pial arteries, and segmental arteriolar spasm of larger arteries upon direct observation of cerebral blood vessels. A similar phenomenon was seen in mesenteric vessels. These results were confirmed by studies on monkey cortical vessels by Rodda and Denny-Brown (1966). The above data suggest that vascular leakage was due to vasoconstriction. However, Giese (1961; 1963; 1964) and Goldby and Beilin (1972) observed that increased endothelial permeability to colloidal carbon in acute hypertension was related only to the dilated segments of the mesenteric arteries. Segments showing severe constriction were never affected. The altered permeability was attributed to a hydrostatic increase of the luminal pressure leading to higher tangential wall tension in segments with larger diameter. These studies also suggested that the passage of carbon particles into the arterial media might be due to disruption of the intercellular endothelial junctions. Byrom (1969) changed his views regarding the role played by

alternating vasoconstriction and vasodilatation of cerebral pial vessels and discussed the possible importance of vasodilatation, referring to Giese's work (1964).

Meanwhile, clinical and experimental studies suggested that the increase in brain water content due to increased vascular permeability following arterial hypertension was directly related to CNS manifestations (Oppenheimer and Fishberg, 1928; Alajouanine, 1947; Thurel, 1953).

Two experimental models have been used for studies of the effect of increased blood pressure (BP) on cerebrovascular permeability: hypertensive encephalopathy and acute hypertension. The former could be achieved by the use of renal artery clipping techniques (Byrom, 1954; Giacomelli, 1970), adrenal regeneration (Robertson et al., 1970), or selected strains of spontaneously hypertensive rats (Okamoto and Aoki, 1963; Hazama et al., 1976). Pharmacological hypertension (Johansson, 1974a; 1974b; Dinsdale et al., 1974) and intra-arterial injection under high pressure (Haggendal and Johansson, 1972b; Rapoport, 1975) are used for an acute rise of blood pressure.

At the present time, two pathophysiological theories have been proposed to explain the increased cerebrovascular permeability due to arterial hypertension. These are the "vasospasm theory" and the "vasodilation theory". Fig. 1 illustrates the changes in the pial arteries during acute hypertension as viewed by both theories. A controversy about whether hypertension leads to either vasospasm or

14-A

FIGURE 1

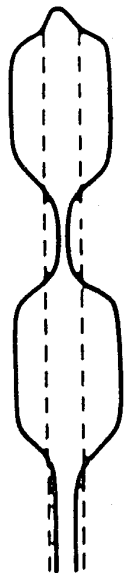
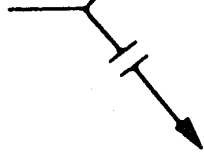
FIGURE 1: Diagram of the changes observed in the pial arteries during acute hypertensive episode. The left side represents the "vasospasm theory" and the right side the "vasodilatation theory".

(from Mackenzie et al., 1976).

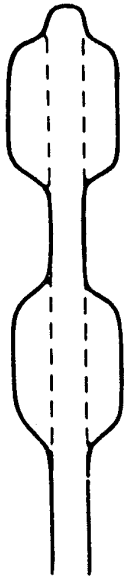
Pial arteriolar caliber
during Normotension



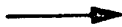
Pial arteriolar caliber
during moderate Hypertension



Sausage string during extreme
Hypertension: Vasospasm theory



Sausage string during extreme
Hypertension: Forced vasodilatation



Generalized vasodilatation with
sustained Hypertension

FIG. 1

vasodilation exists for over a century. In this regard, the "vasospasm" and "vasodilation" theories for increased cerebrovascular permeability could be considered extrapolations of these arguments. According to Byrom (1969), as early as 1859, Kussmaul and Tenner postulated that tissue dysfunction caused by hypertension was due to excessive arteriolar constriction. Around 1871, Traube was against such a concept, suggesting excessive arteriolar dilatation to be the main event. The first theory postulates that the BBB breakdown is due to localized small ischemic areas caused by spasm of hyperactive terminal arterioles in response to the hypertensive insult (Meyer et al., 1960; Kung et al., 1968; Robertson et al., 1970; Finnerty, 1972; Dinsdale et al., 1974). This concept is based on the observation of constriction of cerebral blood vessels associated with ischemic changes and increased permeability to proteins (Byrom, 1954; Rodda and Denny-Brown, 1966). This theory has been re-structured recently by Dinsdale et al., (1971/72; 1974), who observed that the BBB leakage was located basically in the arterial boundary zones, where they found decreased cerebral blood flow during acute hypertension (Dinsdale et al., 1974). They postulated that, in spite of a global increase of cerebral blood flow during the hypertensive episode, focal areas in the boundary zones displayed decreased flow due to spasm of the vessels with a high degree of reactivity. The areas of increased permeability to proteins were coincident with the ischemic changes.

The second theory advocates that the opening of the BBB caused by hypertension is due to a hydrostatic increase of the intraluminal pressure in dilated segments of cerebral arterioles and capillaries (Johansson et al., 1974b; MacKenzie et al., 1976; Rapoport, 1976a). The tangential tension on the vessel wall is directly proportional to the luminal pressure and to the vessel diameter (Poiseuille's law). The enhanced wall tension would cause widening of the tight junctions, providing a route for plasma extravasation (Fig. 2).

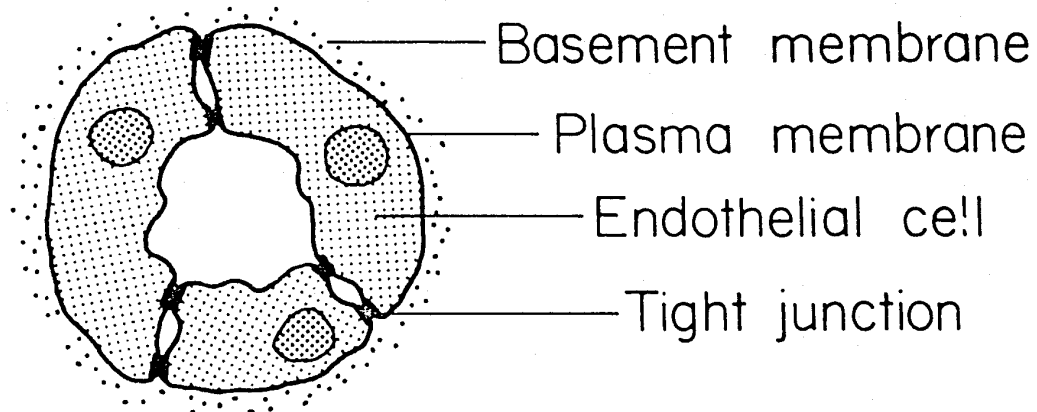
The explanation for BBB leakage according to this theory, is based on a "breakthrough" of the upper limit of the cerebrovascular autoregulation (Johansson et al., 1974a,b). It is known that the cerebral blood vessels are provided with autoregulatory adjustments which maintain a constant cerebral blood flow (CBF) in spite of wide variations in blood pressure (Lassen, 1974). This is due to contraction and relaxation of small resistive pre-capillary vessels to keep a steady capillary blood flow. With further BP rise, the small cerebral arteries start to display alternate zones of constriction and dilatation ("sausage-string phenomenon"). If BP levels are maintained or further increased, these arteries develop a forced dilatation. The autoregulation is then lost and the increased luminal pressure is transmitted upstream into the capillaries and venules (MacKenzie, 1976; Haggendal and Johansson, 1972a). From this point

16-A

FIGURE 2

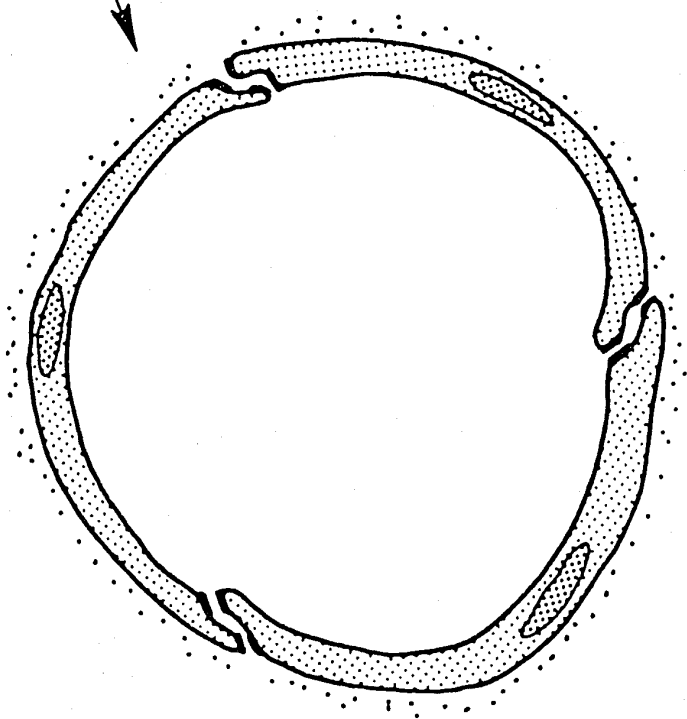
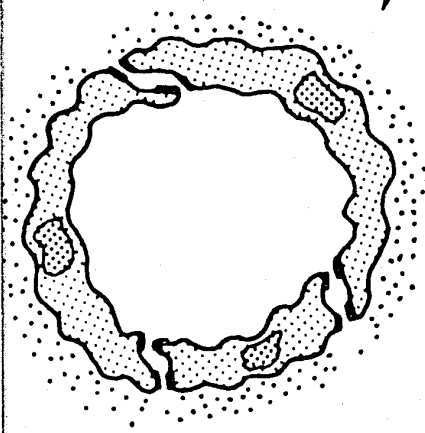
FIGURE 2: Pathophysiological model for the tight junction opening caused by a: cell shrinkage in a hypertonic environment and b: endothelial cell stretching caused by capillary dilatation following increased intraluminal pressure.
(from Rapoport, 1976a)

NORMAL CAPILLARY



OSMOTIC SHRINKAGE

DILATION



A

B

FIG. 2

on, the CBF varies linearly with blood pressure changes. The critical point is said to be around 135 mm Hg for humans (Lassen and Agnoli, 1973; Strandgaard et al., 1973) and 240 mm Hg for cats (MacKenzie et al., 1976). It has been demonstrated that BBB breakdown only occurs when the BP is raised beyond these limits (MacKenzie et al., 1976). Therefore, the BBB leakage is associated with loss of autoregulatory adjustments (Pannier and Leusen, 1975).

Another convincing piece of evidence that BBB opening depends on increased transmitted pressure into the capillary bed is provided by the damage caused by hypertension induced by a short lasting wave of increased pressure. This is achieved by intra-arterial injection of normal saline or blood into the carotid artery (Haggendal and Johansson, 1972b; Rapoport, 1975). The autoregulatory adjustments to increased BP take about 30 to 120 seconds to occur (Rapela and Green, 1974). If a quick pulse of high pressure takes place before this period of time, the capillaries will be overloaded before autoregulation happens. A 10-second pulse pressure of about 200 mm Hg into the internal carotid artery of rats is enough to cause BBB breakdown (Rapoport, 1975).

The vasodilatation theory is also supported by the following indirect evidences: a) increased vascular permeability associated only with dilated arterial zones in other areas of the organism

(Giese, 1964; Goldby and Beilin, 1972), b) accentuated hypertensive BBB leakage in previously dilated cerebral blood vessels by the use of papaverine or by hypercapnia (Johansson, 1974a), c) absence of BBB breakdown following "stepwise" increase of blood pressure to the same levels which cause lesions when BP is quickly elevated (Haggendal and Johansson, 1972a) (the stepwise increase would allow time for autoregulation to take place), d) local cerebral blood flow increase associated with areas displaying BBB leakage (Johansson, 1974b), e) enhanced cerebral venous pressure during a hypertensive episode (Haggendal and Johansson, 1972a; 1972b).

Structurally, it had been suggested that the blood-borne tracers cross the cerebral vascular endothelium during hypertension through two main routes: widening of the tight junction and increased vesicular transport (Olsson and Ossmann, 1970a; Hansson et al., 1975). The junction opening is a graded and not an all-or-none phenomenon, as postulated by Giacomelli et al., (1970). They found that the endothelial intercellular cleft under hypertensive encephalopathy was permeable to particles as large as HRP molecules (50-60Å diameter) but not to colloidal carbon ones (about 250Å diameter).

Acute hypertensive insult has been used as an experimental tool to damage the BBB previously exposed to a potentially harmful agent. An association of effects is proposed to occur, leading either to an increased or to a decreased vulnerability of the cerebral blood vessel, with consequent BBB breakdown. Thus, a semi-quantitative

analysis of the extension of the lesions can be done, by comparing the effect of hypertension alone and that of a harmful agent followed by hypertension. On this basis, arterial hypertension has been associated, for instance, with brain irradiation (Blomstrand et al., 1975a) and ischemia (Spatz et al., 1976). The cerebral vessels seem to be protected against BBB leakage caused by acute hypertension during the immediate period following release of total ischemia (Olsson and Hossman, 1970; Hossman and Olsson, 1971), after total adrenalectomy (Dinsdale et al., 1976), and after dexamethasone treatment (Blomstrand et al., 1975b). In contrast, more prolonged observation after the release of total cerebral ischemia (Spatz et al., 1976) and after radiation (Blomstrand et al., 1975a) reveals potentiation of the BBB damage caused by acute arterial hypertension.

2. BBB Damage Caused by Intracarotid Perfusion of Toxic Substances

The discovery of increased cerebrovascular permeability caused by a chemical agent was made by Skoog in 1937, working on guinea-pigs with a sheep-hemolytic rabbit serum (reviewed by Broman and Lindberg-Broman, 1945). Broman and Lindberg-Broman (1945) were the first investigators to perform a thorough study of diffuse BBB damage caused by several unrelated chemical agents. They established the basis for the technique of carotid perfusion and pial application of substances, which became extensively used in further studies by other authors. The common carotid artery perfusion technique was used later

to study the effect of radiologic contrast agents on cerebral blood vessels (Broman and Olsson, 1956), in resemblance with a standard human angiogram. The method was modified and improved by Steinwall (1958), who described a method of perfusion of test substances into the internal carotid artery of the rabbit. Perfusion was performed after proximal ligation of the external carotid artery. Concomitant visualization of the pial vessel being filled with the perfusing solution was achieved through a craniectomy.

After extensive work on chemical BBB damage, Steinwall (1961; 1964) classified the changes in the cerebrovascular permeability in two main groups: 1) non-specific BBB leakage, due to generalized alterations of the BBB properties, caused, for instance, by higher concentrations of mercuric chloride, (HgCl_2) (Olsson and Hossman, 1970a), or endotoxins (Clawson et al., 1966), and 2) selective changes in BBB permeability to specific substances, for example, inhibition of glucose and amino acid transfer, caused by lower concentrations of HgCl_2 (Steinwall and Klatzo, 1966; Steinwall, 1968).

Mercuric chloride is known to be a potent toxic substance. It has been demonstrated to have a high affinity for amino acids, proteins, (both free and membrane-bound) purines and pyrimidines (reviewed by Vallee and Ulmer, 1972). Following intravenous injection, 99% of the plasma HgCl_2 is bound to proteins (Steinwall and Olsson, 1969). It is believed that HgCl_2 exerts its toxic effects on cell

membranes by binding to their proteins. It possesses high affinity for the sulfhydryl (-SH) groups of cysteine (Brown et al., 1967). This reaction probably accounts for the blockage of enzyme-mediated active transport mechanisms (Vallee and Ulmer, 1972; Clarkson, 1972). The inhibition by mercuric compounds of several enzymes such as glucose-6-phosphatase, alkaline phosphatase, succinic dehydrogenase, adenosine triphosphatase has been reported (Chang et al., 1973). Part of the cellular toxicity of mercuric compounds is probably due to organelle damage. Various patterns of mitochondrial injury have been described, associated with decreased mitochondrial enzymatic function (Desnoyers and Chang, 1973; Ware et al., 1973). It has also been shown that mercuric compounds have a high affinity for lysosomes of liver cells (Vallee and Ulmer, 1972). Inhibition of enzyme activities and ultrastructural changes such as the above should be involved with the damage to the BBB by mercuric chloride.

Intraperitoneal, intravenous or intracarotid injection of mercury compounds causes BBB breakdown (Steinwall and Klatzo, 1966; Steinwall, 1968; Chang and Hartmann, 1972; Ware et al., 1974). The tight junctions between cerebrovascular endothelial cells are dynamic structures which depend on the functional integrity of the cell membranes (Hirano et al., 1969). They consist of a network of fibrillar strands with specific spatial orientation which seems to determine their effectiveness as a barrier (Wade and Karnovsky, 1974). Such fibrillar organization can be deformed by osmotic or hydrostatic

stresses (Rapoport, 1976a). The tight junctions then become temporarily "leaky", till a fibrillar rearrangement takes place. Such active re-organization is evidence for a dynamic status of the tight junctions.

It has been demonstrated that HRP leakage through the cerebrovascular endothelium occurs following HgCl_2 intoxication because of the widening of the tight junctions and increased vesicular transport (Ware et al., 1974). The BBB damage following HgCl_2 intoxication is considered permanent (Chang and Hartmann, 1972). These ultrastructural studies regarding morphological alterations of BBB induced by mercury were performed on animals subject to intravenous administration of mercuric compounds (Ware et al., 1974). However, it has been stressed that the pattern of the distribution of Evans blue fluorescence in the BBB structures and cerebral tissue is similar, whether HgCl_2 is injected intravenously or via carotid perfusion (Steinwall and Olsson, 1969). Therefore, it is likely that intra-carotid perfused animals will display similar structural changes to those observed by fluorescence microscopy for animals that had had intravenous administration of the mercuric compounds.

It is worth noting that the non-specific BBB damaging agents such as toxic substances, hypertension, hypercapnia, cause a diffuse BBB damage. The pattern of the tracer extravasation is discontinuous and the distribution of vessel damage is scattered and patchy. It is also a general observation that the intensity of the damage varies

from one animal to another (Steinwall, 1968; Olsson and Hossmann, 1970a).

In this work, mercuric chloride was employed as a chemical damaging agent of the BBB.

3. BBB Breakdown with Meningitis

Acute inflammatory reactions produce increased permeability of systemic blood vessels, as demonstrated by the vascular labelling with colloidal carbon (Majno et al., 1961). Serotonin (5-hydroxytryptamine, 5-HT) and histamine are thought to play an important role in causing endothelial cell contraction which increases the space between endothelial cells and leads to vascular leakage (Majno et al., 1961). In contradistinction, the cerebral endothelial cells lack contractile properties because they are devoid of contractile proteins (Becker and Shustak, 1972). Therefore, enhanced cerebral vascular permeability during inflammation seems to be due to other mechanism(s). The migration of white blood cells across the cerebral vessels which occurs with meningitis is accompanied by an enhanced vascular permeability to proteins (Som et al., 1972). The increase of protein in the CSF is secondary to this alteration (Prockop and Fishman, 1968). Proteins and leukocytes are thought to cross the vascular endothelium most likely through the widened tight junctions (Nelson et al., 1962; Shabo and Maxwell, 1971).

4. BBB Breakdown Induced by Serotonin

Serotonin has been demonstrated to cause increased permeability of vascular endothelium to plasma proteins and induce edema formation when injected subcutaneously (Benditt and Rowley, 1956). Serotonin has been demonstrated to increase cerebrovascular permeability to plasma proteins following intra-arterial (Swank and Hissen, 1964) or intracerebral (Bulle, 1957) injection. The cerebrovascular endothelium has been shown to lack considerable amounts of contractile protein (Becker and Shustak, 1972). Therefore, the increased permeability to proteins induced by serotonin could be caused by a mechanism other than endothelial cell contraction and intracellular cleft widening. This was demonstrated by Westergaard (1975), who injected serotonin solutions of different concentrations in the cerebral ventricle of mice. He found that vascular endothelial tight junctions were impermeable to HRP and that the serotonin-induced leakage of the protein was due to increased vesicular transport across the endothelial cells.

5. BBB Breakdown Caused By Loss of Cerebrovascular Auto-regulation

The cerebral vasculature is supplied with regulatory mechanisms, which adjusts the cerebral blood flow (CBF) according to hemodynamic variations and metabolic needs. Furthermore, under normal conditions the cerebral blood vessels respond promptly to changes of the arterial pCO_2 (reviewed by Betz, 1972, and Langfitt, 1974). Both

properties have been reported to be abolished after some pathological conditions. Cerebral trauma (Reivich et al., 1969), intense rise of intracranial pressure (Langfitt et al., 1965; Kjallquist et al., 1969; Haggendal et al., 1970a; 1970b; Zwetnow, 1970), seizure (Plum et al., 1968; Howse et al., 1974), intense hypercapnia (Cutler and Barlow, 1966), and acute arterial hypertension (Johansson et al., 1974b; Pannier, 1975) cause a long lasting dilation of the small pre-capillary vessels ("reactive hyperemia or vasomotor paralysis") which become unresponsive to PaCO₂ variations, metabolic needs and BP changes. Once the pre-capillary resistance is abolished, the capillary lumen is dilated and the endothelium becomes vulnerable to damage caused by enhanced intraluminal pressure (Rapoport, 1976a). The increased pressure is then transmitted to the cerebral venous bed (Haggendal and Johansson, 1972a). BBB breakdown has been demonstrated after all these circumstances (Cutler and Barlow, 1966; Petersén, 1967; Lorenzo et al., 1972; 1975; Johansson et al., 1974; Lassen, 1974; Rapoport, 1976b). Rapoport (1976a) has proposed a common pathophysiological model to explain the BBB breakdown after such injuries. It is based on the temporary widening of the tight junction due to the over-stretch of the endothelial cells caused by the hydrostatic increase of intraluminal pressure (Fig. 2). Following the BBB opening, the rate of entry of plasma constituents into the brain ECS will depend on the intravascular pressure levels. It has been demonstrated that the volume of abnormal

transfer through the endothelium is proportional to the BP values (Klatzo, 1962; 1967; Katzman and Pappius, 1963; Reulen et al., 1977).

6. BBB Breakdown and Brain Edema

According to Klatzo (1967) brain edema can be classified into two types: cytotoxic and vasogenic. Metabolic cellular dysfunction is the original event in the former case leading to intracytoplasmic accumulation of water by neurons and glia. On the other hand, vasogenic edema is primarily due to increased cerebrovascular permeability which causes the escape into the cerebral ECS of plasma constituents commonly retained in the intravascular space by the intact BBB. Therefore, BBB breakdown is a sine qua non condition for the definition of vasogenic brain edema. However, the correlation between BBB breakdown on one side, and edema formation and appearance of clinical manifestations on the other side, has not been well defined. It was initially thought that BBB breakdown necessarily leads to brain edema. Furthermore, on the basis of clinical and experimental grounds, it was common belief that BBB breakdown accounts for neurological symptoms. (Alajouanine, 1946/1947; Thurel, 1953; Rapoport and Levitan, 1974; reviewed by Feindel, 1976). It has been demonstrated that brain edema occurs only in areas where BBB breakdown takes place following a patchy BBB damage caused by acute hypertension (Nag et al., 1976). However, Rapoport (1976a) drew attention to the complexity of the subject, stressing the fact that BBB breakdown constitutes just

one variable out of a group involved with brain edema formation. Rapoport et al., (1976b) have demonstrated that weak to moderate BBB breakdown can occur without developing brain edema. Therefore, it seems that neurological manifestations are dependent upon the intensity and duration of the BBB breakdown.

D. SUBARACHNOID HEMORRHAGE

Subarachnoid hemorrhage (SAH) is a clinical syndrome caused by numerous etiological entities (Heidrich, 1972). The most common cause is by far the abrupt rupture of an aneurysmal malformation of a vessel of the circle of Willis (Richardson and Hyland, 1941; Walton, 1956). A series of events which follow the initial bleeding includes systemic and CNS changes (Richardson and Hyland, 1941). The severity of the clinical course following SAH varies within a wide range, going from mild symptoms to deep coma and death (Hyland, 1950). The manifestations related to the CNS depend on the presence of the blood itself in the subarachnoid compartment (Smith, 1963; Fein, 1975), and on the occurrence of complicating factors, such as spasm of the major vessels, (reviewed by Heros et al., 1976), cerebral hematoma (Richardson and Hyland, 1941), hydrocephalus (Galera and Greitz, 1970; Yasargil et al. 1973; Vapalahti et al., 1976) and increased intracranial pressure (Nornes, 1973; Hayashi et al., 1977). An intriguing event in the clinical course of some patients who suffered subarachnoid bleeding is a progressive clinical deterioration after days of apparent recovery (Walton, 1956). The interpretation of the phenomenon is controversial. Some authors attribute the change to ischemia, secondary to spasm of vessels of the circle of Willis (Fletcher et al., 1959; Allcock and Drake, 1965; reviewed by Millikan, 1975). Their conclusions are based on pathological ischemic changes

observed in those cases (Connolly, 1962; Crompton, 1964a; 1964b; 1973), but this correlation has been questioned more recently, based on lack of correlation between vasospasm on one hand, and clinical deterioration and decrease of CBF on the other hand (James, 1968; Hashi et al., 1972; Petruk et al., 1972; Martins et al., 1975; Millikan, 1975; Hayashi et al., 1977). It has been commonly said that arterial spasm plays only a limited role in the pathogenesis of the encephalopathy which follows SAH (Smith, 1963; Martins et al., 1975). More recently, other pathophysiological events with possible deleterious effects have been suggested. For instance, there is some evidence pointing to cerebral metabolic changes (Hashi et al., 1972a; Fein, 1975; 1976), altered vascular reactivity (Hammer and Kuhner, 1976; Sakurai et al., 1976), concomitant intracranial hypertension (Hayashi et al., 1977), and microcirculatory disturbances (Asano and Sano, 1977) as factors potentially responsible for the clinical deterioration which can follow SAH.

Hypertension is a common occurrence during the course of SAH (Dekaban and McEachern, 1952; James, 1968; 1972; Heidrich, 1972; Ransohoff et al., 1972; Adams et al., 1976). There are suggestions that patients presenting hypertensive episodes after SAH have a troublesome clinical course (Jacobson, 1954; Walton, 1956; Adams, 1976; Hamer and Kuhner, 1976; Nibelink et al., 1977). Attempts to explain this association are based on three possibilities: a) the

hypertension is a secondary symptom due to damage and disruption of vegetative control centres (Walton, 1956; Stornelli and French, 1964; Heidrich, 1972), reflecting a more severely injured brain; b) the arterial hypertension represents a compensating adjustment secondary to increased intracranial pressure (Nornes, 1973); c) the hypertension, due to whatever pathophysiological mechanisms, will cause further damage to the brain tissue, accounting for the clinical deterioration. In other words, arterial hypertension would be causing further damage to the brain tissue.

Several events are known to occur after SAH. Attention will be directed to: 1) blood-induced meningeal irritation, 2) considerations of the possible role played by serotonin after SAH, 3) post-SAH brain edema, and 4) vascular dysfunction which follows SAH.

1. Blood-Induced Meningitis

The clinical signs of meningeal irritation which follow SAH have been demonstrated to be due to an inflammatory meningeal reaction starting within the first few hours after SAH (Hammes, 1944). The liberation of hemoglobin degradation products has been suggested to be responsible for the meningeal irritating effects of blood. Jackson (1949) demonstrated meningeal cellular reaction following intracisternal injection of bilirubin and bilirubin-protein complex. Pathological examination of brains from patients who died at different times after SAH demonstrated the migration of white blood cells from

subpial vessels into the subarachnoid space. This process was seen to start within the first hours after SAH (Hammes, 1944; Debakan and McEachern, 1952). The increased number of cells in the cerebrospinal fluid (CSF) and the subarachnoid adhesions and fibrosis which sometimes occurs after SAH are the result of this inflammatory reaction (Bagley, 1928).

2. Serotonin Related Aspects

Serotonin is known to be released by platelets during the clotting process (Zucher, 1947; Boumeaux and Lecompte, 1954; Zucher and Borrelli, 1955). The platelets are the blood fraction with the highest serotonin concentration (Zervas et al., 1973). Serotonin is thought to be released during blood clotting after SAH. Hashi et al., (1972) found enhanced 5-HT levels in the cerebral venous blood following SAH. Serotonin, as well as other blood vasoactive substances, has been suggested to play an important pathophysiological role in the genesis of vascular dysfunction following SAH (Raynor et al., 1961; Smith, 1963; Zervas et al., 1973; reviewed by Heros et al., 1976). Intracisternally injected serotonin, at concentrations equivalent to that thought to be present in CSF of patients after SAH (Contractor, 1964; Allen, 1974) produces changes in vessel calibre and CBF changes (Raynor et al., 1961; Hashi et al., 1972a). These changes resemble those seen following subarachnoid injection of blood. Serotonin, as well as platelet concentrates, were found to promote cerebral blood

vessels constriction very effectively (Zervas et al., 1973). Blood with markedly decreased 5-HT levels loses its ability to cause vasospasm (Zervas et al., 1974). On the other hand, it has been suggested that serotonin also accounts for vascular changes occurring during other cerebral pathological processes, such as ischemia and trauma (Mrsulja et al., 1976; Osterholm and Meyer, 1969).

In summary, the above data seem to indicate that the increased concentration of serotonin in the CSF and in the brain tissue after SAH could be, at least partially responsible for the vascular dysfunction which follows SAH.

3. Vascular Dysfunction Post-SAH

Under normal circumstances, two well defined modes of cerebrovascular autoregulatory adjustments take place: 1) the CBF is adjusted according to the cerebral metabolic needs (reviewed by Betz, 1972; Langfitt, 1974) and 2) the CBF is kept constant during wide variations of the systemic blood pressure (Lassen, 1974). Both processes are achieved by variations in the diameter of the pre-capillary resistance vessels. For instance, during hypertension the vessels constrict, and vice-versa. However, uncoupling of the blood flow - O_2 consumption autoregulatory mechanism (Fein, 1976), and impairment of the control during BP changes (Hashi et al., 1972b) have been described to occur after SAH.

It is generally accepted that the presence of blood in the subarachnoid space leads to a global disturbance of the cerebral

vascular reactivity (du Boulay et al., 1972; Heilbrun et al., 1972). The best defined changes are spasm of the major conductive arteries (reviewed by Heros et al., 1976) and disturbed reactivity of the pre-capillary resistive vessels (Yamaguchi and Waltz, 1971; Heilbrun et al., 1972; Hashi et al., 1972b; Petruk et al., 1972; Fein, 1976). These alterations take place within the first few minutes following SAH induction. The factors involved in the pathogenesis of these phenomena are subject to controversy. Some variables have been suggested as possibly being involved in the pathophysiology of post-SAH vascular dysfunction: enhanced activity of the autonomic vasa nervorum induced by blood substances (Rosenblum and Guilianti, 1973), liberation of blood vasoactive substances (Zervas et al., 1973) released during the clotting process (Kapp et al., 1968), and metabolic changes interfering with the perivascular environment (Hashi et al., 1972a). Vasospasm of major arteries has been demonstrated in various species during the immediate period after experimental SAH (reviewed by Heros et al., 1976). Some authors propose that it is associated with decreased CBF (Ferguson et al., 1972; Petruk et al., 1974), whereas others claim that it is related to an increased CBF (Hashi et al., 1972a). In addition, a poor correlation between the two phenomena has been reported by other authors (Simeone et al., 1972; Bergval et al., 1973). Disregarding the CBF variation, some investigators have reported that the cerebral

vascular resistance ($CVR = CPP/CBF$) remained constant (Martins et al., 1975; Fein, 1976), whereas others observed a decrease of this measurement (Hashi et al., 1972a). The consistency or decrease of the CVR in association with spasm of major arteries constitute an interesting finding. In order to explain these results, simultaneous dilatation of pre-capillary resistant vessels has been proposed based on indirect evidence, since the presumably dilated vessels are beyond angiographic resolution or pial inspection (Hashi et al., 1972b; Simeone et al., 1972; Grubb et al., 1977). Furthermore, this hypothesis is reinforced by a report of increased cerebral blood volume, simultaneous with large artery spasm following SAH in humans (Grubb et al., 1977). Sengupta et al., (1971/72) reported similar findings following carotid artery ligation in baboons. Vasodilatation of the microcirculation was presumed to occur in the ipsilateral hemisphere. It was suggested that a small vessel dilatation was part of a compensatory mechanism to decrease the CVR and facilitate the CBF maintenance. Moreover, presumably dilated vessels show anomalous reactivity to arterial pCO_2 changes following both carotid ligation and SAH (Sengupta et al., 1971/72; Hashi et al., 1972b). The reactivity is globally diminished but the response is more intense to the constrictor stimulus (decreased pCO_2) than to the dilating one (increased pCO_2). This is opposite to the normal response (Petruk et al., 1974). This abnormal response pattern is in agreement with the

hypothesis of previously dilated resistance vessels, which would be expected to constrict more easily than to dilate. In summary, it seems to be widely accepted that SAH induces a vascular dysfunction leading to spasm of major cerebral vessels and dilatation of small pre-capillary ones.

4. Brain Edema and SAH

It is well acknowledged that brain edema can occur after SAH (Smith, 1963). It has been suggested that a correlation exists between edema and ischemic changes, possibly due to spasm of major vessels (Robertson, 1949; Stornelli and French, 1964).

Autopsy reports of patients who died from subarachnoid hemorrhage usually indicate ischemic degeneration and changes compatible with brain edema (Feigin, 1955; Smith, 1963; Crompton, 1973). To our knowledge, the occurrence of brain edema after subarachnoid hemorrhage has not yet been experimentally investigated in a critical manner.

After SAH, blood substances or their degradation products could theoretically cause brain edema by primary action on the cerebral cell metabolism and/or cerebral vascular permeability. Another possibility is that brain edema would occur only after secondary ischemic changes which could take place later in the clinical course of a SAH. Brain metabolic inhibition after SAH has been suggested by experimental (Fein, 1975; 1976) and clinical (EEG) (Parkes and James, 1971) findings. It is known that cellular metabolic inhibition interferes with trans-membrane ionic transport leading to

intracellular accumulation of ions and water (Leaf, 1973). If metabolic disturbance after SAH is intense enough to cause those ionic transfer changes, this could be the possible mechanism for brain edema following SAH. On the other hand, a BBB breakdown to protein is the basic condition for the definition of vasogenic edema (Klatzo, 1967), in spite of the fact that BBB breakdown does not always lead to brain edema (Rapoport et al., 1976a).

E. PROBLEM FORMULATION

The integrity of the BBB is essential for the maintenance of a constant physiochemical environment of the nervous tissue. Following BBB breakdown the brain parenchyma is invaded by plasma constituents; some of these substances are originally absent in the tissue, while others reach concentrations different from their normal level. BBB breakdown has been held responsible for clinical deterioration, edema formation and brain dysfunction (reviewed by Brightman et al., 1970a; Rapoport, 1976a). It occurs under several pathological conditions, such as brain tumour, ischemia, infarct, acute arterial hypertension, seizure, trauma, and intense intracranial hypertension. The pathophysiology of BBB breakdown is still not well defined in many of these circumstances. Common pathophysiological models of BBB damage have been proposed to explain the BBB breakdown associated with the above entities (see Introduction).

The state of the BBB following SAH has not been investigated yet. SAH is a clinical entity still poorly understood. For many years, research has been directed to spasm of major cerebral blood vessels and the consequent ischemia of the subserved territory of blood supply. The encephalopathy which can occur during the clinical course of a SAH is also poorly understood. The previously accepted correlation between clinical manifestations and the presence of spasm in major vessels has been questioned as a result of CBF measurements. Investigators have recently considered other possible pathophysiological mechanisms to explain

the symptoms seen after SAH. Metabolic disturbances, no-reflow phenomenon, and increased intracranial pressure are some of the events that have been suggested as being involved with the mechanism(s) responsible for the post-SAH encephalopathy. In the present work, the BBB breakdown is considered as a pathophysiological factor which could account at least partially for that encephalopathy. The problem that is posed is: "what is the state of the BBB after SAH?" The possibility of a relationship between SAH and BBB dysfunction arises from the consideration of several pathophysiological phenomena observed in the post-SAH syndrome. These are: post-SAH meningitis, subarachnoid release of serotonin by blood, and altered vascular reactivity following SAH. All these changes could lead to BBB breakdown.

The nature of the post-SAH brain edema has not been investigated yet. Its etiology could be related to possible alterations of permeability properties of the cerebral vasculature after SAH. Post-SAH brain edema could be classified according to the "leakiness" of the BBB. If the BBB is found to be "leaky" to proteins, this edema could be classified as vasogenic. If it is not found to be "leaky", no further classification could be attempted, because it has been demonstrated that brain edema can be caused by increased cerebrovascular permeability to plasma constituents other than proteins (O'Brien et al., 1974).

Spasm of major vessels and dilation of pre-capillary small vessels are believed to be manifestations of a global vascular dysfunction that follows SAH. Impairment of the autoregulatory mechanisms is another aspect of this altered vascular reactivity which takes place after SAH.

BBB breakdown has been demonstrated under several circumstances associated with loss of autoregulation, such as trauma, intense hypercapnia, seizures, acute intracranial hypertension. The impaired autoregulation which follows SAH is not as intense as that seen under the above-mentioned circumstances. A second question arises: "is the post-SAH disturbed autoregulation enough to cause BBB breakdown"? The present hypothesis to explain BBB breakdown under conditions associated with loss of autoregulation is based on the transmission of arterial blood pressure to the capillary bed. This concept is reinforced by the evidence of potentiation of the BBB breakdown and subsequent CNS infiltration with plasma constituents as a consequence of arterial hypertension. Therefore, the association of acute hypertension with SAH could potentiate a possible BBB breakdown. It could also initiate it in case the cerebrovascular autoregulatory impairment had not been enough to damage the BBB by itself. On the other hand, SAH followed by arterial hypertension is a common clinical occurrence. It has been suggested that hypertension, when following SAH, causes deleterious effects manifested by a troublesome clinical course. A BBB breakdown with enhanced extravasation of plasma constituents into the brain tissue caused by arterial hypertension, could be the mechanism behind the phenomenon.

The increased intraluminal pressure induced by acute arterial hypertension on the cerebral circulation could be affected by vasospasm of major vessels caused by SAH. The results of this association are difficult to predict. One can only speculate about the possible blood

pressure changes in the segment distal to the constricted arterial area. An approach to this problem would be the use of a BBB damaging agent that would not act through hemodynamic changes. This could be accomplished by intravascular perfusion of a chemical agent.

In the present work the permeability properties of the BBB to proteins were investigated during acute stages following SAH. The SAH was simulated experimentally by the injection of fresh arterial blood into the cisterna magna. Two other experiments were performed: 1) hypertension was induced after SAH, and 2) a BBB chemical damaging agent, mercuric chloride was applied after SAH. The BBB permeability properties were monitored by the use of the fluorescent tracer, Evans blue, which is known to form complexes with the plasma proteins.

II. MATERIALS AND METHODS

Two different types of agents were used to damage the integrity of the BBB. In the first group of experiments, referred to as Experiment A, acute hypertension was the damaging agent. There were three sub-groups: I: the control study of hypertensive damage of the BBB, II: the study of the BBB to proteins during the acute stage following SAH, and III: the study of BBB to proteins when acute arterial hypertension was induced during the acute stage following SAH.

The second group of experiments, referred to as Experiment B, was designed to study the permeability of the BBB to proteins under conditions of intracarotid perfusion with a vascular damaging agent, HgCl_2 , during acute stages following SAH.

A. EXPERIMENT A

Cats, unselected with respect to sex, weighing between 2.2 and 6.5 kg were anesthetized with 30 mg/kg body weight of intravenous sodium pentobarbital.* They were maintained under light anesthesia with further small doses of pentobarbital as required. The animals were placed in a supine position on the operating table and the right femoral artery and vein were cannulated. The arterial catheter (Portex** cannula set, I/D 1.19 mm) was connected to a P23Db Statham pressure transducer*** for continuous blood pressure monitoring. A tracheostomy was performed. The

* Nembutal - Abbott Laboratories Ltd., Montreal, Quebec, Canada

** Portex Ltd., Hythe, Kent, England

*** Statham - Hato Rey, Puerto Rico, U.S.A.

animals were paralyzed with 10 mg/kg body weight per hour of gallamine* and artificially ventilated with an AC Palmer** animal ventilator (respiratory rate - 26 per minute and stroke volume 14 to 25 ml, according to blood gas values). Room air, supplemented by 100% oxygen was used to supply the ventilator. The O₂ flow was controlled by the arterial pO₂. The arterial blood gases and pH were monitored throughout the experiment using a model 213 Instrumentation Laboratory*** gas analyzer. The measurements were taken at least four times during each experiment.

The right femoral artery and vein catheters were advanced as far as the aorta and inferior vena cava respectively. The arterial line was used for blood pressure monitoring and gas sampling. The venous line was used for the injection of substances. Both catheters were kept patent by occasional flushing with heparinized saline (500 units of Heparin**** per 100 ml of physiological saline). The animals were turned to the prone position and electrocardiogram and rectal temperature monitoring were started. The body temperature was kept around 38°C with the use of an electric heating pad. The temperature was monitored with a rectal probe connected to a Yellow Springs recorder*****

- * Flaxedil - Poulenc Ltd., Montreal, Quebec, Canada
- ** C.F. Palmer Ltd., London, England
- *** Instrumentation Laboratory Inc., Lexington, Mass., U.S.A.
- **** Sodium Heparin, Abbott Laboratories Ltd., Montreal, Quebec, Canada
- ***** Tele-thermometer, Yellow Springs, Ohio, U.S.A.

The head was placed in a stereotaxic head holder and the animals which were to undergo a subarachnoid hemorrhage had dissection of the posterior aspect of the neck. The atlanto-occipital ligament was exposed and the cisterna magna punctured using a 1½"/38 mm Cathlon IV* catheter. This catheter was connected to a P23Db Statham pressure transducer for intracranial pressure recording. Arterial blood pressure and intracranial pressure were continuously monitored using an Electronics for Medicine DR-8** polygraph, connected in series to the pressure transducers and to a model 7 Grass polygraph*** for intermittent recording (Figure 13). Electrocardiograms were taken at the same time with the polygraph system as described above.

After blood gases and vital signs were stabilized, 4 ml of non-heparinized autogenous arterial blood was manually injected into the cisterna magna over a four minute period. The head was kept at 45⁰ flexion, which facilitated an even distribution of the blood over the whole brain surface. The CSF pressure was monitored during the injection, through the same catheter used for injection, by means of a 4-way stopcock, and not allowed to rise beyond 40 mm Hg below the arterial diastolic pressure. The animal was slowly injected with 100 mg/kg body weight of Evans blue**** diluted to 2% with Elliott's B solution***** (Elliott and Jasper, 1949) 30 minutes after the sub-

- * Cathlon IV catheter - Arbrook Ltd., Peterborough, Ontario, Canada
- ** Electronics for Medicine Inc., White Plains, New York, U.S.A.
- *** Grass Instrument Co., Quincy, Mass., U.S.A.
- **** Evans Blue - Fisher Scientific Company, Fair Lawn, New Jersey, U.S.A.
- ***** Elliott's B Solution - Baxter Laboratories of Canada Limited,
Malton, Ontario, Canada

arachnoid blood injection. This was followed by injection in some animals of 100 mg/kg body weight of a 30% solution of horseradish peroxidase Type II*, diluted with saline just prior to use. Both tracers were injected intravenously followed immediately by an intravenous injection of 0.1 - 0.4 mg/kg body weight of metaraminol**, also diluted in saline, over a three minute period. The injection was manually controlled according to the blood pressure level. Most of the time further supplemental doses of metaraminol were required to maintain high blood pressure levels.

The main steps of each experimental group in Experiment A are represented in Figures 3, 6 and 8.

The animals were sacrificed by exsanguination through a right atrial incision, 15 minutes after the hypertension was induced. In situ perfusion-fixation was immediately started by means of a 12 gauge blunt needle placed in the ascending aorta through an incision in the left ventricle. Intravascular blood wash-out was carried out for two minutes with saline followed by infusion of 500 ml of 1/3 strength Karnovsky's fixative, and then 600 ml of full strength Karnovsky's fixative (5% glutaraldehyde, 4% paraformaldehyde) (Karnovsky, 1965b), in 0.1 M cacodylate buffer, pH 7.2-7.4, according to well established techniques (Reese and Karnovsky, 1967; Hossmann and Olsson, 1971a; Hansson et al., 1975). The perfusion pressure was kept constant at

* HRP, Type II - Sigma Chemicals Corp., Illinois, U.S.A.

** Metaraminol Bitartrate - Aramine, Merck, Sharp & Dohme, Kirkland, Quebec, Canada

the level of the animal's initial systolic blood pressure. Half of the animals in this group were perfused with 1200 ml of 10% phosphate buffered formalin, instead of Karnovsky's fixative.

The brains were immediately removed, examined and photographed. The occurrence of lesions was checked visually and subsequently under the operating microscope. The brains were kept immersed in the same fixative used for perfusion.

The fluorescence microscopy results described in the present work belong to the group of animals treated with formalin. The specimens treated with HRP and perfused with Karnovsky's fixative were examined by electron microscopy. The results obtained will be reported elsewhere. Sections were made after 24 hours of immersion. Coronal sections, 2 mm thick, starting at the sigmoid sulcus (approximately anterior coordinate 27; Snider and Niemer, 1961) were continued posteriorly including forebrain, cerebellum and brainstem. Stained and unstained areas of the control group were selected for fluorescence microscopy. Frozen sections (10.25 μ m) were obtained with a cryostat and/or a freezing microtome and mounted in 50% glycerin-water. The sections were examined under a Zeiss Universal Photomicroscope equipped with an HBO-200 UV lamp. A combination employing BG12 as an exciter and 53 and 44 as barrier filters was used. GAF, ASA 500, Ansco-chrome film was used for photomicrographs.

B. EXPERIMENT B

Successful experiments were carried out on 15 cats of either sex, weighing between 2.4 and 4.9 kg. The techniques used for anesthesia, artificial ventilation, vessel cannulations, temperature control, and monitoring of arterial blood pressure and blood gases were the same as described for Experiment A. Arterial blood pressure and intracranial pressure recordings also followed the previous description.

After the initial surgical preparation (including tracheostomy and vessel cannulation), the animals were turned on the ventral surface and the head was placed in a stereotaxic head-holder. A 1 cm diameter cranial window was made over the left parietal lobe, keeping the dura mater intact, in order to check that the injection of mercuric chloride filled the pial vessels. This was seen through the transparent dura mater. Special care was taken during the bone opening to avoid damage to the BBB caused by overheating by the electrical drill. Four brass screws were symmetrically placed over the frontal and parietal lobes, about 6 mm from the midline. The anterior screws were inserted just behind the coronal suture on each side and the posterior ones about 2.5 cm from that suture. The screw tips were prevented from getting in contact with the dural surface to avoid mechanical BBB damage. The screws were connected to a model 7 Grass polygraph and the electroencephalogram was taken by means of bipolar differential recording from the frontal and parietal electrodes on each side. The animals

which would be submitted to SAH had the posterior aspect of the neck dissected, the cisterna magna cannulated, and the ICP recorded as previously described. Once vital signs and blood gases were stable, a subarachnoid injection of 4 ml of autogenous blood was given, following the technique described in Experiment A. The animals belonging to the control group did not have the neck dissected, nor was the subarachnoid blood injection given.

Cats from both control and test groups were injected intravenously with 100 mg/kg body weight of a 2% solution of Evans blue in Elliott's B solution* (Elliott and Jasper, 1949). In the test group, this injection was given immediately after the subarachnoid blood injection.

Around 22 minutes after the Evans blue injection, the animals from both groups were turned on their right side and the left common carotid artery was cannulated using an Abbott Venocath-14 catheter.** The vessel was ligated proximal to the cannula insertion. The catheter was not advanced any further than 1 cm distally, to avoid occlusion of branches of the common carotid artery with the cannula tip. Special care was taken to prevent flexion and rotation of the animal's neck. It has been shown that if these movements occur during injection, they will interfere with the flow of substances injected into the brain (Broman and Olsson, 1956). The intracarotid perfusion of mercuric chloride*** was initiated in animals of the test group 30 minutes after the start of the subarachnoid blood injection. Correspondent timing

* Baxter Laboratories, Malton, Ontario, Canada

** Abbott Laboratories, Chicago, Ill., U.S.A.

*** Mercuric chloride, Fisher Scientific Comp., Fair Lawn, New Jersey, U.S.A.

was observed in the control group (26 minutes after Evans blue injection). The solution was injected under constant pressure, about 10 mm Hg above the systolic blood pressure. The injection time was 45-60 seconds. The amount injected varied from 60-100 ml. A solution of 6×10^{-5} M mercuric chloride was freshly prepared with normal saline, and warmed to body temperature. In a few instances, the pH was checked and verified to be in the physiological range. The pial vessel filling with the injected material was checked by an assistant by observation through the craniectomy. The injection pressure was monitored by means of a P23Db Statham pressure transducer connected in parallel with the injecting catheter (Fig. 13).

The main experimental events undergone by animals in Groups A and B are represented in Figures 14 and 17, respectively. Figure 13 shows a diagram of the experimental set-up used in Experiment B.

The animals were exsanguinated through a right atrial incision 30 minutes after the HgCl_2 injection. In situ perfusion fixation was begun immediately after, with 10% phosphate buffered formalin. After a quick intravenous flush with normal saline, the perfusion was performed through the ascending aorta and through the cannula left in the common carotid artery. The perfusion pressure was kept constant at the level of initial systolic arterial blood pressure. The total volume of formalin perfusate amounted to 1200 ml.

Brain removal, inspection, sectioning, preparation and examination of sections under the fluorescence microscope were performed

as previously described for Experiment A. All the animals in Experiment B had their brains microscopically examined. The EEG tracings were analysed by a person who was unaware of the groups to which the animals belonged.

III. RESULTS

A. EXPERIMENT A

These results refer to 20 cats which were successfully investigated for the effects of SAH alone and the association of SAH and acute pharmacological hypertension (Table I). The results include only the animals under stable physiological conditions throughout the whole experimental procedure. Special attention was given to keep identical standard conditions regarding operating procedure, monitoring techniques and timing for all the animals. The mean values and standard errors of arterial blood samples for all animals included in Experiment A were:

pH 7.293 ± 0.004

PaCO₂ 31.758 ± 0.400

PaO₂ 116.963 ± 1.469

These are the normal values of blood gases and pH for cats (Ngai, 1957; Bygdman, 1963; Fink and Schoolman, 1963; MacKenzie et al., 1976). Ten animals were excluded from the analysis of the results because of experimental failure. In most cases, this was due to hypersensitivity to large doses (100 mg/kg) of intravenous HRP. This has been previously described, (Cotran and Karnovsky, 1967) and interpreted as a histamine mediated reaction. In the present experiments, that reaction was manifested by acidemia, hypercapnia and hypoxia. In addition, metaraminol failed to induce a hypertensive response. It was common to find intra-pulmonary infiltrates of blue coloured fluid in these

animals. Other animals were also excluded because a) the metaraminol failed to induce a satisfactory increase of blood pressure and b) because of pulmonary edema, probably secondary to arterial hypertension.

The 20 animals included in the results were divided into three groups: - one control and two test groups. The control group (referred to as Group I in Table I and Fig. 3) was formed by animals which underwent only acute hypertension after intravenous injection of the tracer(s). The first test group (Group II in Table I and Fig. 6) was designed to study the effect caused by SAH on the BBB. The animals in this group underwent only cisternal injection of blood and intravenous injection of tracer(s). The other test group (Group III in Table I and Fig. 8) included animals which underwent SAH, intravenous injection of tracers, and acute hypertension, in this order.

1. Group I

Included in Group I are animals that displayed an abrupt increase of systolic arterial blood pressure of more than 95 mm Hg from the initial level. It has been demonstrated that an acute hypertensive insult is not effective in damaging the blood brain barrier if it does not fulfill these requirements (Johansson et al., 1970; Haggendal and Johansson, 1972a; Auer, 1977). The mean and standard error for the values of blood pressure rise, induced by metaraminol were 74.4 ± 1.9 mm Hg for the mean arterial blood pressure and 101.5 ± 3.5 mm Hg for the systolic arterial blood pressure (Fig. 10). The

50-A

TABLE I

TABLE I: Results of Experiment A

EXPERIMENTAL GROUPS	NUMBER OF ANIMALS	ANIMALS WITH BBB TOTAL	BBB BREAKDOWN PERCENT
I ACUTE ARTERIAL HYPERTENSION	8	7	87.5
II SAH	5	0	0
III SAH AND ARTERIAL HYPERTENSION	7	0	0

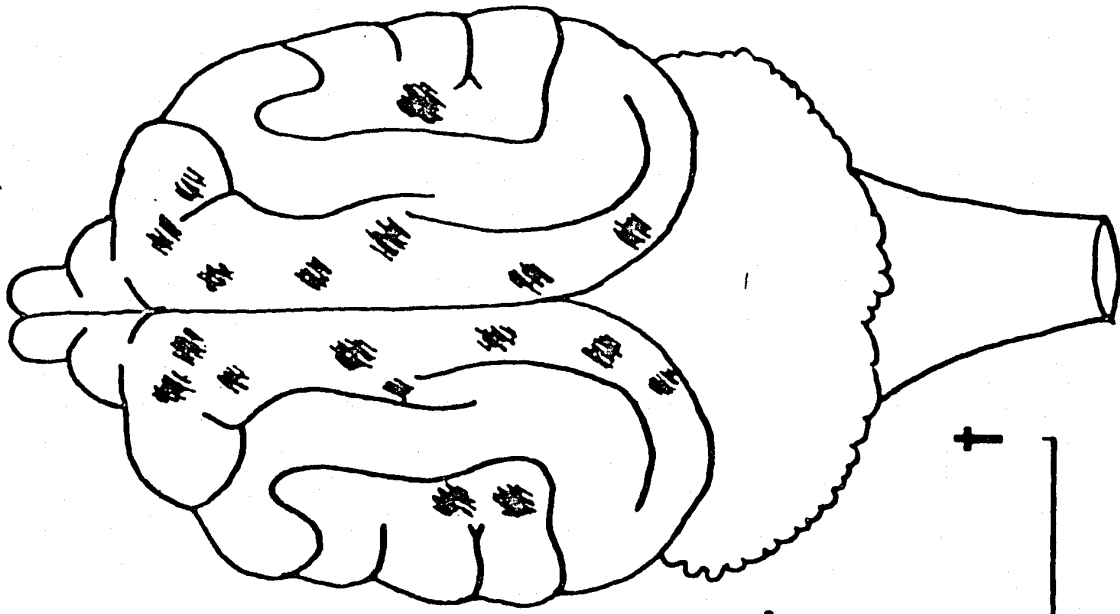
TABLE I

50-C

FIGURE 3

FIGURE 3: Diagrammatic representation of the main steps and results
for animals in Group I of Experiment A.

50-D



GROUP I

HYPERT.

t



15 min

FIG. 3

average and standard error for the length of time required for the blood pressure to reach its maximum value was 8.33 ± 0.64 seconds. Fig. 4 represents a typical recording showing the effect of metaraminol on the arterial blood pressure. There was an abrupt increase of blood pressure with widening of the pulse pressure. It led frequently to the appearance of cardiac arrhythmias within the first minutes following the injection. The arrhythmias were transitory and did not seem to interfere with the blood pressure values. The length of time during which blood pressure stayed elevated showed a wide individual variation. Some of the animals responded to subsequent doses of metaraminol during the slow return of blood pressure to previous levels. These findings were in agreement with previously published results (Johansson et al., 1970). It has been demonstrated that the effectiveness of acute hypertensive insult in inducing BBB breakdown is directly proportional to the intensity of the blood pressure rise and inversely proportional to the length of time it takes to elevate. The length of time during which the blood pressure remains elevated does not influence the final results (Haggendal and Johansson, 1972b; Rapoport, 1975a; Auer, 1977).

Under macroscopic examination (gross inspection and visualization under the operating microscope), the Group I animals showed abnormal areas of discoloration, mostly localized in the grey matter of the cerebral hemispheres. They occasionally occurred in the thalamus and cerebellum. No lesions were seen in the brainstem in the present series. They displayed a scattered distribution, preferentially in

51-A

FIGURE 4

FIGURE 4: Typical curve of arterial blood pressure induced by intravenous injection of metaraminol. There was an abrupt increase in systolic and diastolic pressures with widening of the pulse pressure. It commonly led also to the appearance of ventricular arrhythmias (represented by the irregularity of pulse rhythm in the tracer) which basically did not affect the blood pressure values.

51-B

TIME: sec

BP
mm Hg
300
200
100
0

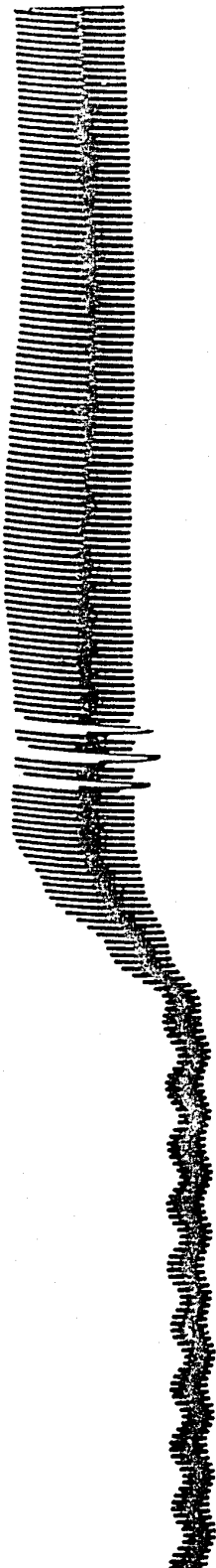


FIG. 4

the antero-lateral and parasigmoid gyri (named according to Snider and Niemer, 1961) with a tendency to symmetrical distribution. In spite of the usual faint blue discoloration, their limits were well defined (Fig. 5a and 5b). They varied in size from pinpoint to diffuse areas, measuring up to 3 mm in diameter. A conglomeration of confluent pinpoint extravasations were frequently seen. In a few instances, the blue extravasation extended through the entire depth of the grey substance and reached the underlying white matter. Blue discoloration was also seen in areas devoid of BBB such as the median eminence, pineal gland, neurohypophysis and area postrema.

The areas with abnormally extravasated dye-protein complex were identified under the fluorescence microscope by means of an evident red fluorescence, mostly localized diffusely in the neurophil and in the cell bodies (Fig. 5d). In a few instances the fluorescent material was confined to the vessel wall. There was a striking contrast between the abnormal findings of a red fluorescence against the usual dark greenish autofluorescence displayed by the nervous tissue when treated under the present methods of fixation. In normal areas, without EB exudation the brain vessels were identified as "negative" areas giving a similarity with a "honey-comb" (Fig. 5e and 5f). Dots displaying strong yellow fluorescence were usually seen in relation with vessels. They represent artifacts of lipofuscin pigment. The intravascular tracer had been washed out by the perfusion-fixation solution. The outermost cortical

52-A

FIGURE 5

Fig. 5: Photographic documentation of macro and microscopic specimens from Group I.

Fig. 5a: Gross aspect of the superior surface of a cat's brain, showing areas of blue discoloration (arrows).

Fig. 5b: The same brain shown above is seen under the operating microscope.

52-B

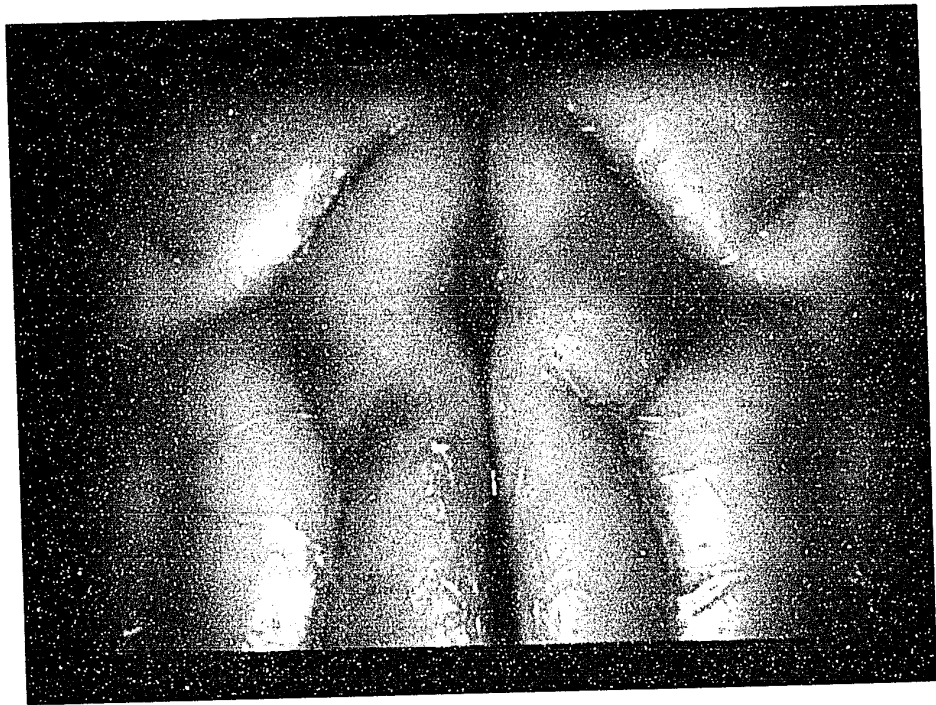
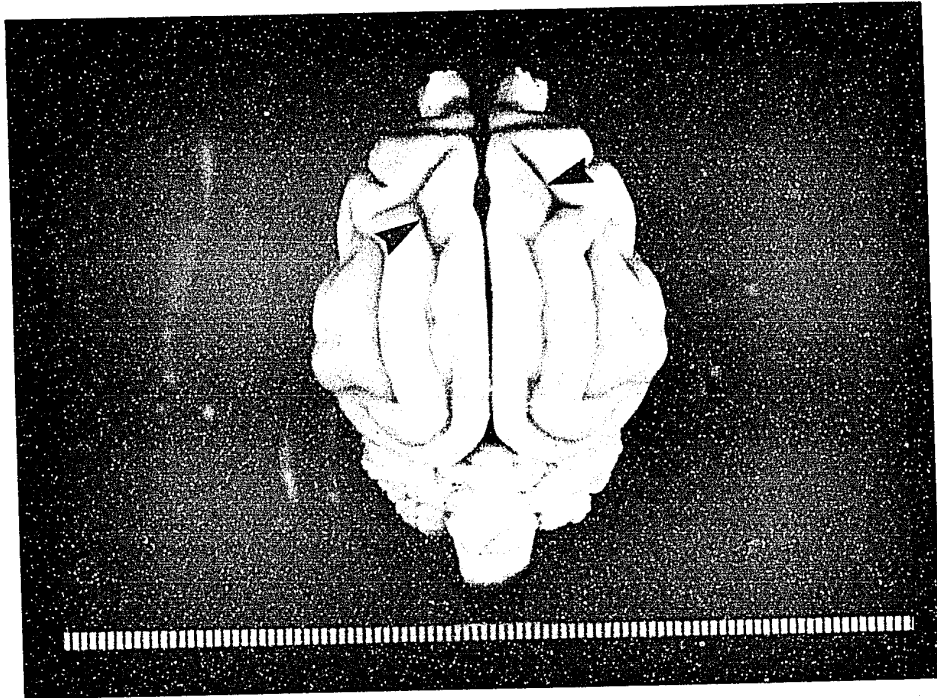


FIGURE 5c: Coronal section demonstrating faint blue discoloration at the apex of both gyri (arrows).

FIGURE 5d: Fluorescence photomicrograph from an area with BBB breakdown. The neurons are seen traced by the Evans blue-protein complex, which displays a red fluorescence. The tracer is also present in the neuropil and cell processes. The strong yellow fluorescence is due to an artifact due to lipofuscin pigment.
(bar = 100 μ m, 20 μ m thick section).

52-D

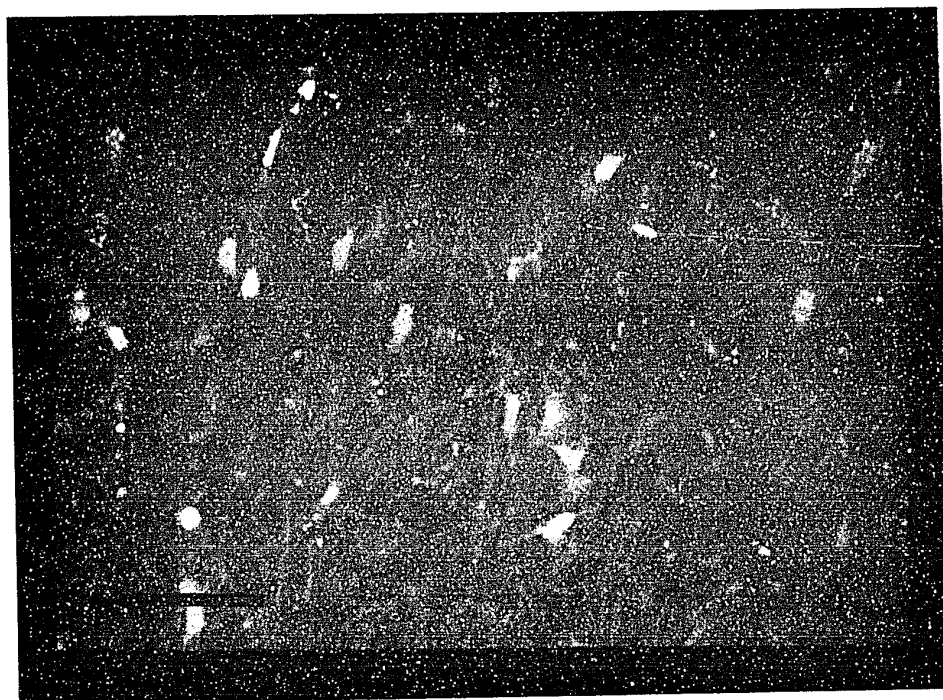
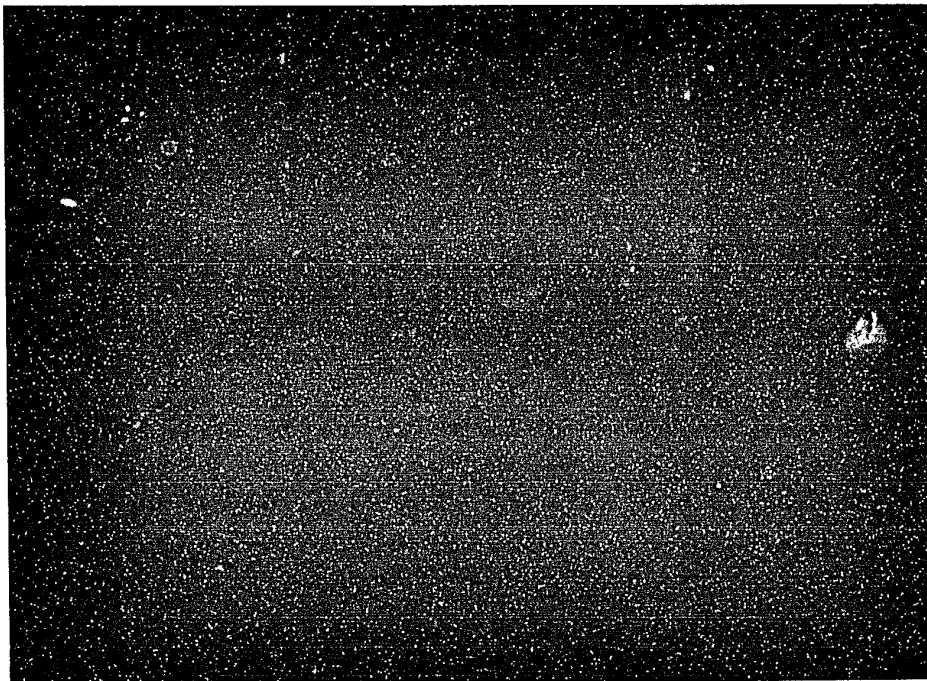
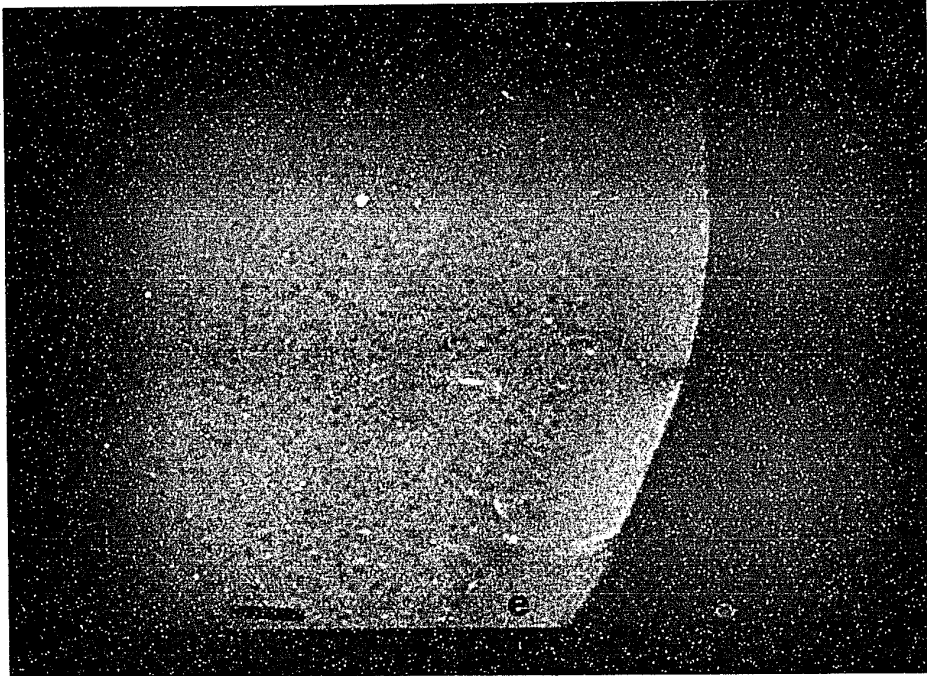


FIGURE 5e: Fluorescence micrograph of an area without BBB breakdown of normal cat cerebral cortex. The penetrating vessels are seen as "negative images". The greenish autofluorescence displayed by the cerebral tissue is interrupted by yellow dots of lipofuscin pigment. The subpial layer of the cortex does not show any special features. (bar = 100 μm , 20 μm thick section).

FIGURE 5f: Cortical penetrating vessel as seen under the fluorescence microscope in an area without BBB breakdown. The cortex surrounding the perivascular space presents normal aspect. (bar = 100 μm , 20 μm thick section).

52-F



layers exhibited the same aspect as the deep layers under the fluorescence microscope, in areas without any BBB breakdown (Fig. 5e). The penetrating cortical vessels were seen as "negative" spaces extending from the surface into the brain parenchyma (Fig. 5f).

2. Group II

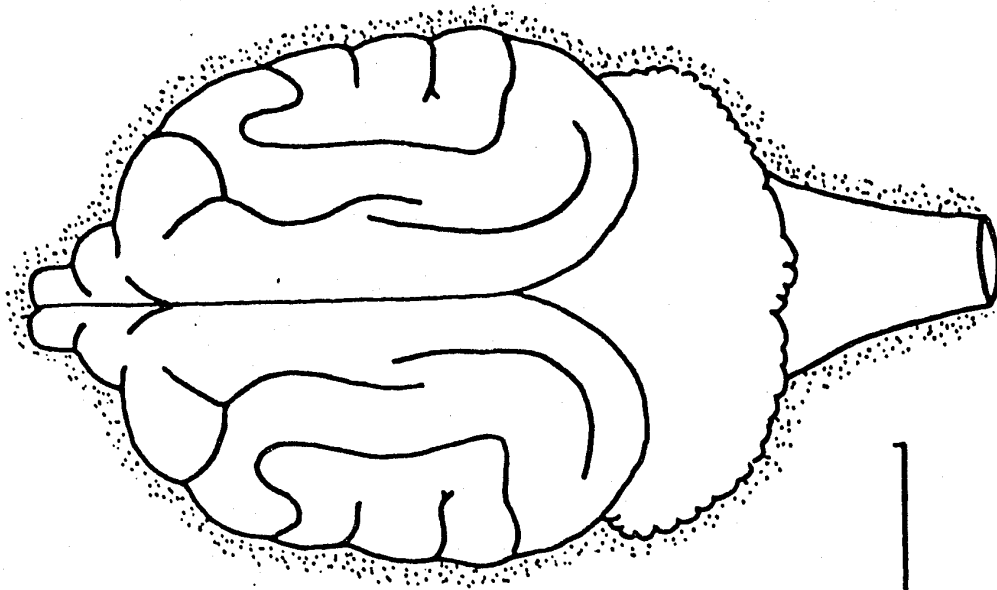
Animals included in Group II underwent a subarachnoid injection of blood, followed by intravenous injection of tracers (Fig. 6). They didn't undergo any hypertensive insult. The blood was injected over a 4 minute period causing an elevation of intracranial pressure shown in Fig. 9. The ICP fell slowly to values close to the baseline. The changes in the BP due to the SAH are represented in Fig. 11. The subarachnoid injection of blood lead invariably to an increase of BP and ICP. As previously noted, the ICP was prevented from reaching values beyond 40 mm below the diastolic arterial blood pressure.

Under macroscopic examination (including gross inspection of the brain surface and sections, and examination under the operating microscope) no blue discoloration was seen, except for areas without BBB (Fig. 7a, 7b and 7c). Therefore, no BBB breakdown was present as evidenced by protein tracers. The amount of blood covering the hemispheres showed wide individual variations, in spite of the same volume being injected in each instance (compare Fig. 7a and 12a). It was noticed that a 45° flexion of the cat's head about the transverse (biauricular) axis of the stereotaxic head-holder facilitated the even

53-A

FIGURE 6

FIGURE 6: Diagrammatic representation of the main steps and results
from animals in Group II of Experiment A.



GROUP II

SAH



30 min | **15 min**

FIG. 6

53-C

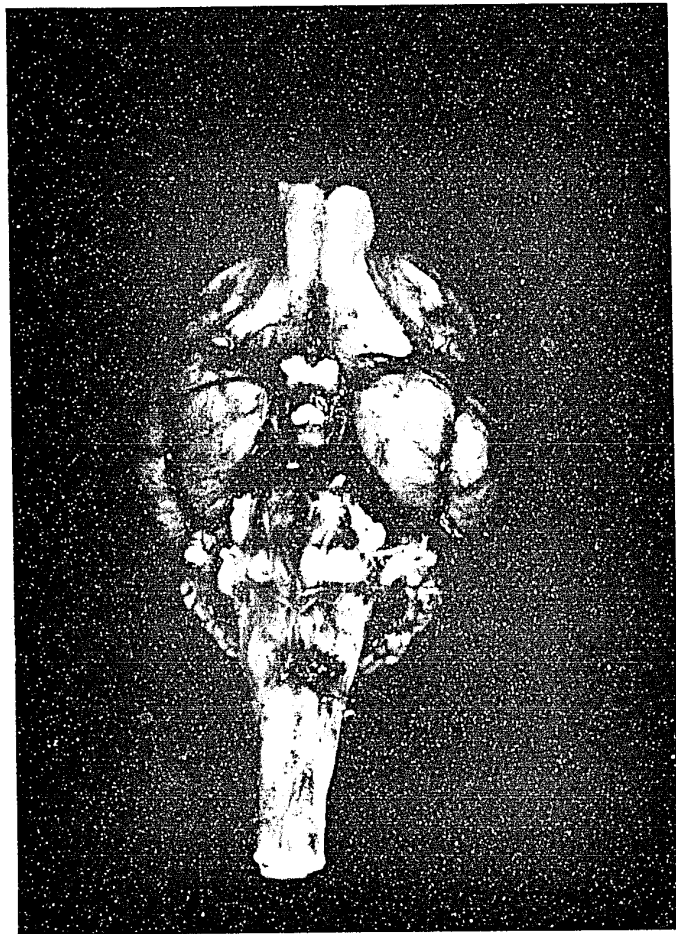
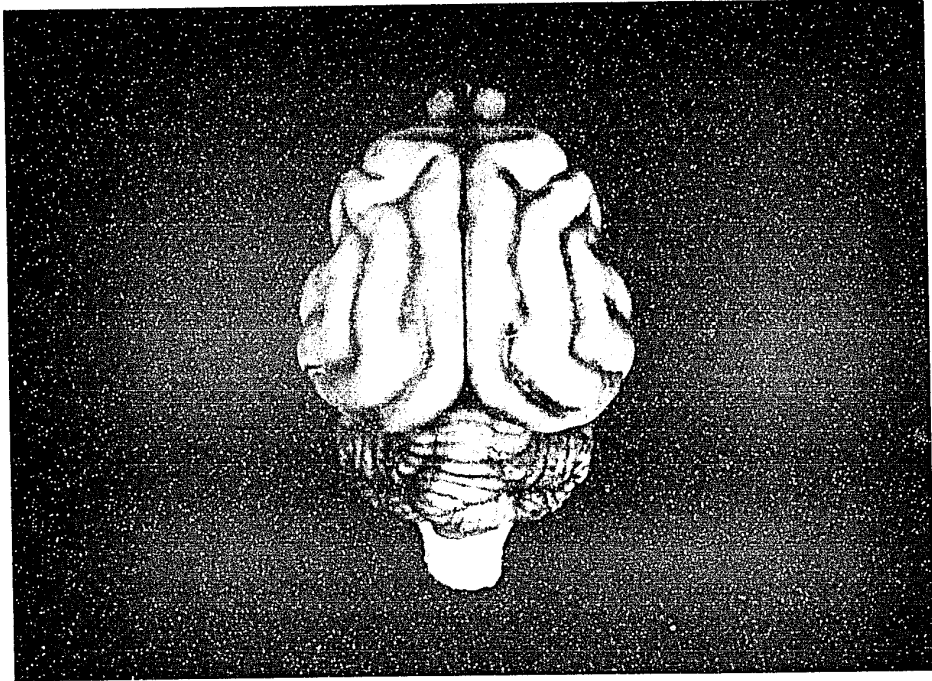
FIGURE 7

Fig. 7: Photographic documentation of macro and microscopic specimens from Group II animals.

Fig. 7a: Macroscopic view of the external surface of the superior aspect of a cat's brain to demonstrate the absence of superficial blue discoloration. Photography taken after formalin immersion. It shows the even spread of blood over cerebral and cerebellar hemispheres.

Fig. 7b: Basal view of another specimen from Group II to show the accumulation of blood in the basal cisterns, plus the absence of superficial BBB breakdown.

53 D



53-E

FIGURE 7c: Coronal section exhibiting the absence of deeply located BBB breakdown.

FIGURE 7d: Fluorescence photomicrograph from a cortical area. No Evans blue-induced fluorescence is seen. The yellow fluorescence is due to artifacts of lipofuscin pigment, normally exhibited by the nervous tissue. (bar = 100 μ m, 20 μ m thick section).

53-F

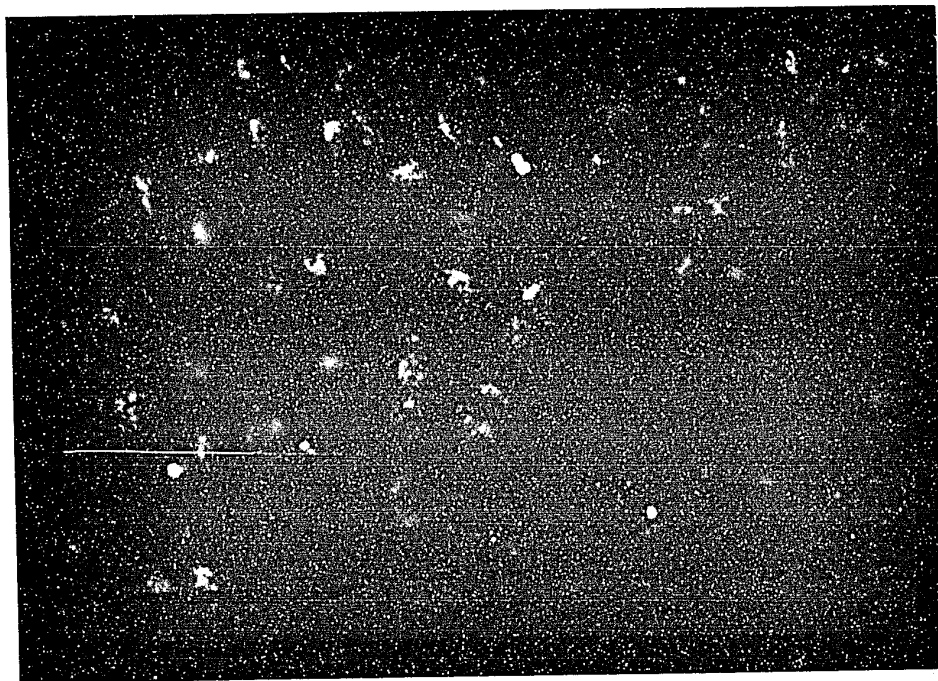
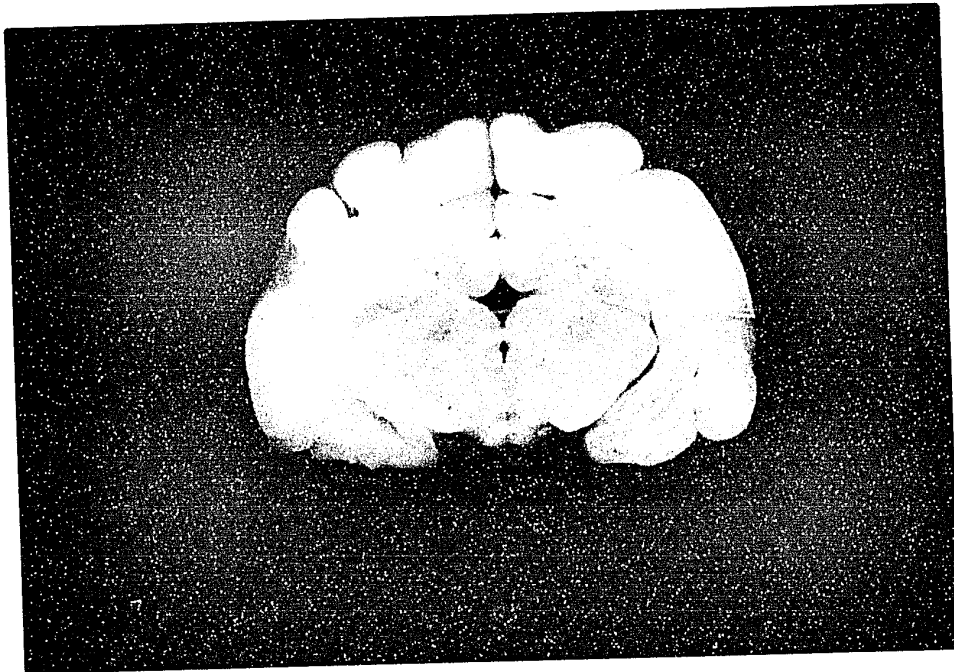
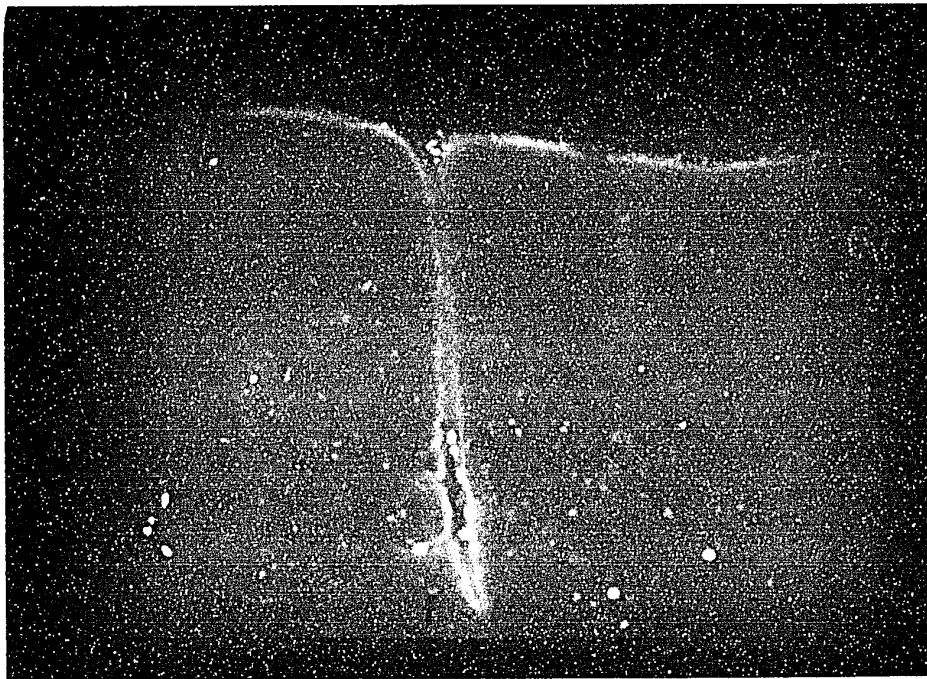
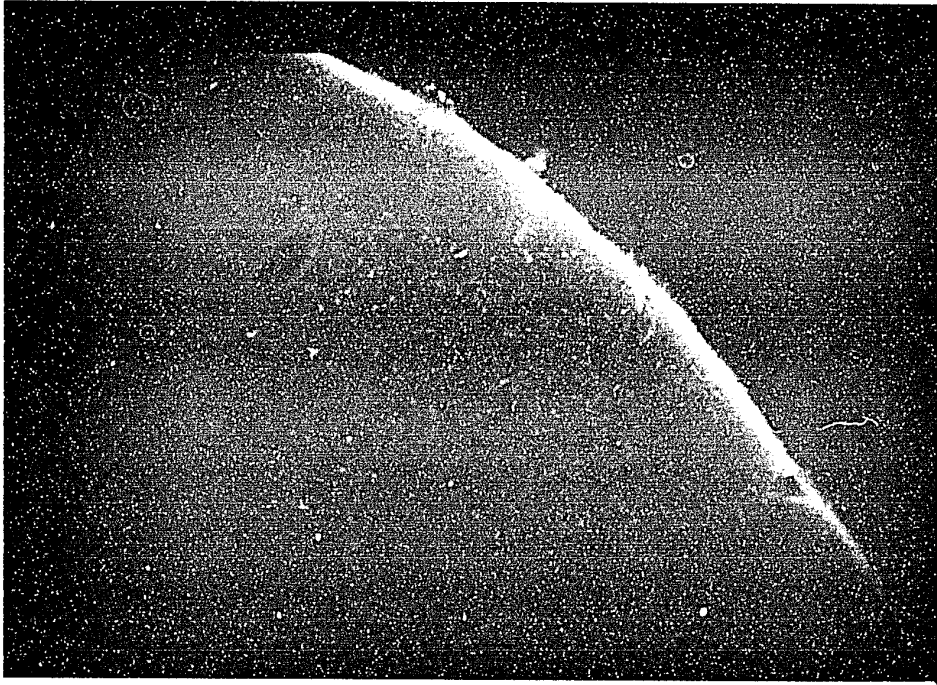


FIGURE 7e: Fluorescence micrograph of the cortex of a Group II animal. A narrow strip of tissue underneath the pia-glial membrane exhibits a yellow autofluorescence distinct from the usual fluorescence displayed by the normal nervous tissue (compare with Fig. 5e).
(bar = 100 μm , 20 μm thick section).

FIGURE 7f: Cortical penetrating vessel seen as negative image. The Virchow-Robin perivascular space is surrounded by nervous tissue exhibiting the same fluorescence as that of the outermost cortical layer. The continuity of the subarachnoid space into the perivascular space can be appreciated.
(bar = 100 μm , 20 μm thick section).

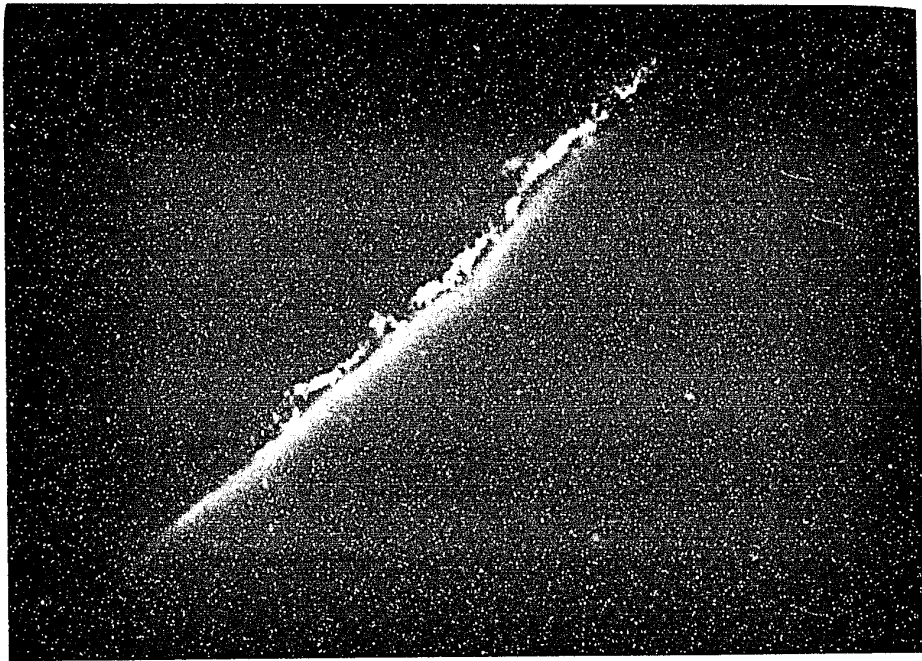
53-H



53-I

FIGURE 7g: Fluorescence micrograph demonstrating the presence of blood in the subarachnoid space. The autofluorescence displayed by the red blood cells is similar to that seen in the subpial area.
(bar = 100 μm , 20 μm thick section).

53-J



distribution of the blood in the subarachnoid space. In all animals, the injection of 4 ml of blood was sufficient to cause spread of blood over the hemispheric convexities, cerebellar hemispheres and in the median longitudinal fissure. The blood was more heavily localized in the basal cisterns (Fig. 7b).

The absence of BBB damage was confirmed under fluorescence microscopic examination. In no instance was any red fluorescence seen. Sections were taken randomly from the cerebrum and cerebellum. The dark greenish autofluorescence displayed by the nervous tissue (Fig. 7d) was similar to that described for Group I animals, except for the outermost layer of the cortex and for the tissue surrounding the perivascular space of large penetrating vessels (Fig. 7e and 7f). These areas exhibited an intense yellow fluorescence which was similar to the fluorescence displayed by the blood elements in the subarachnoid space (Fig. 7g). These areas of yellow fluorescence were confined to a narrow peripheral strip.

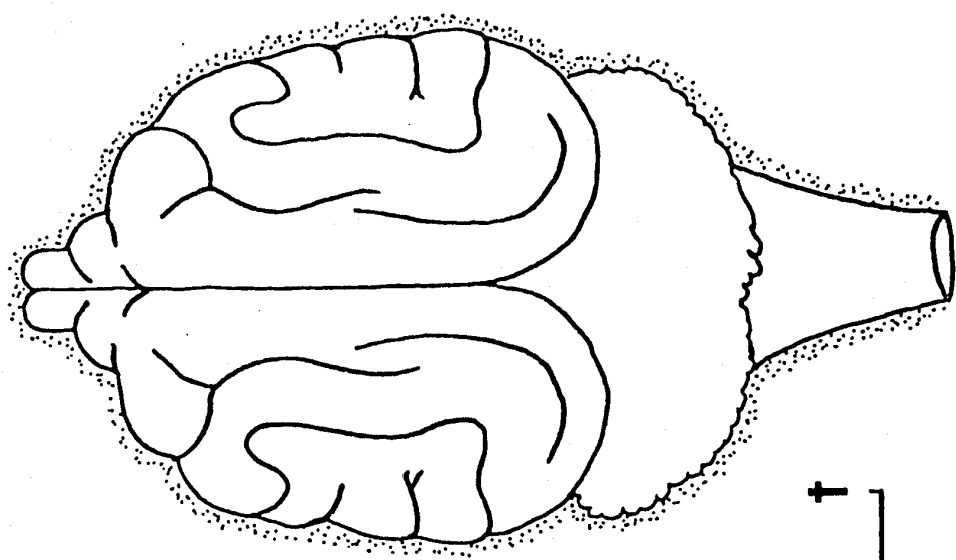
3. Group III

Group III comprises animals that underwent a subarachnoid hemorrhage followed by acute arterial hypertensive episode 30 minutes later (Fig. 8). The changes in the ICP and mean BP caused by subarachnoid injection of blood, are represented in Fig. 9 and Fig. 11, respectively. The mean and standard error figures for the metaraminol-induced BP rise were 105.1 ± 5.0 mm Hg for the mean arterial blood

54-A

FIGURE 8

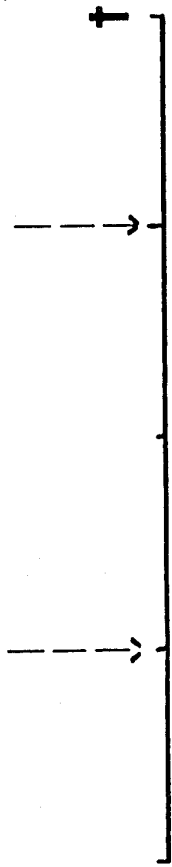
FIGURE 8: Diagrammatic representation of the main steps and results of animals in Group II of Experiment A.



GROUP III

SAH

HYPERT.



30 min

15 min

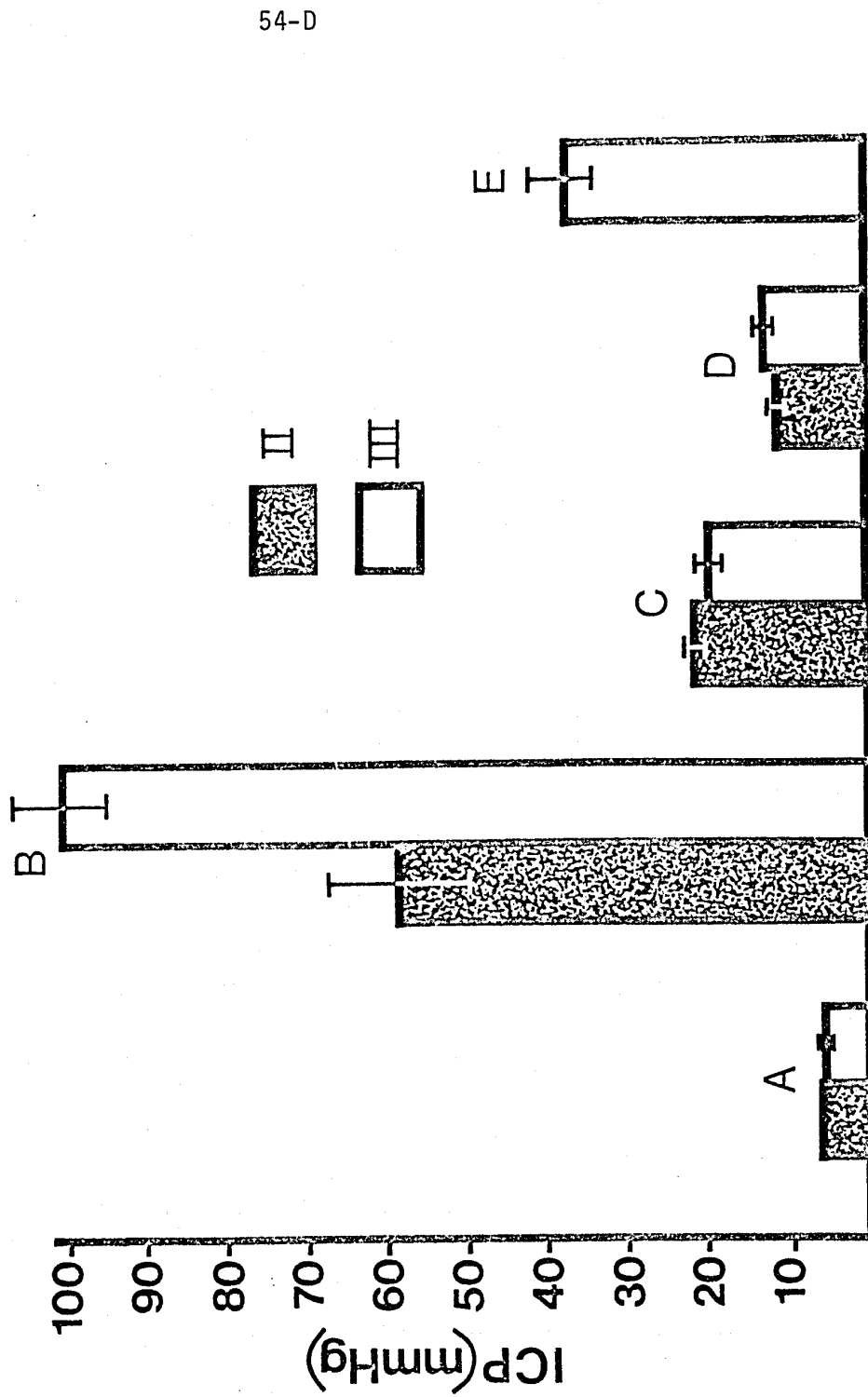
FIG. 8

54-C

FIGURE 9

FIGURE 9: Changes in ICP caused by subarachnoid injection of blood in cats from Groups II and III. The initial baseline values for ICP (A) were 6.0 ± 0 mmHg for Group II and 5.8 ± 0.31 for Group III (average and standard error). The pressure immediately after end of blood injection (B) was 59.75 ± 9.2 mmHg for Group II and 100.8 ± 7.8 mmHg for Group III. Three minutes after end of SAH (C) the ICP went down to 23.0 ± 1.8 mmHg for Group II and 20.3 ± 2.5 mmHg for Group III. The lower values (D) observed after SAH (which correspond closely with the post-SAH baseline values) were 13.9 ± 1.1 mmHg for Group II and 15.6 ± 0.8 mmHg for Group III. The ICP was found to elevate during the metaraminol-induced arterial hypertension (E) in Group III. It went up from 15.6 ± 0.8 mmHg to 38.6 ± 4.8 mmHg.

Changes In ICP Induced By SAH



Experiment A

FIG. 9

54-E

FIGURE 10

FIGURE 10: Average value with standard error for the increase in systolic and mean arterial blood pressure induced by metamizol in Groups I and III. The average increase in mean pressure was 74.4 ± 1.9 mmHg for Group I and 105.1 ± 5.0 mmHg for Group III. The systolic pressure rise was 101.5 ± 3.5 mmHg for Group I and 124.7 ± 5.4 mmHg for Group III. The increase in blood pressure in Group III was significantly higher than the correspondent in Group I for mean blood pressure. ($t = 3.05$, $df = 11$, $p < 0.01$).

Average Values & Standard Errors

for Metaraminol-Induced Increase In BP

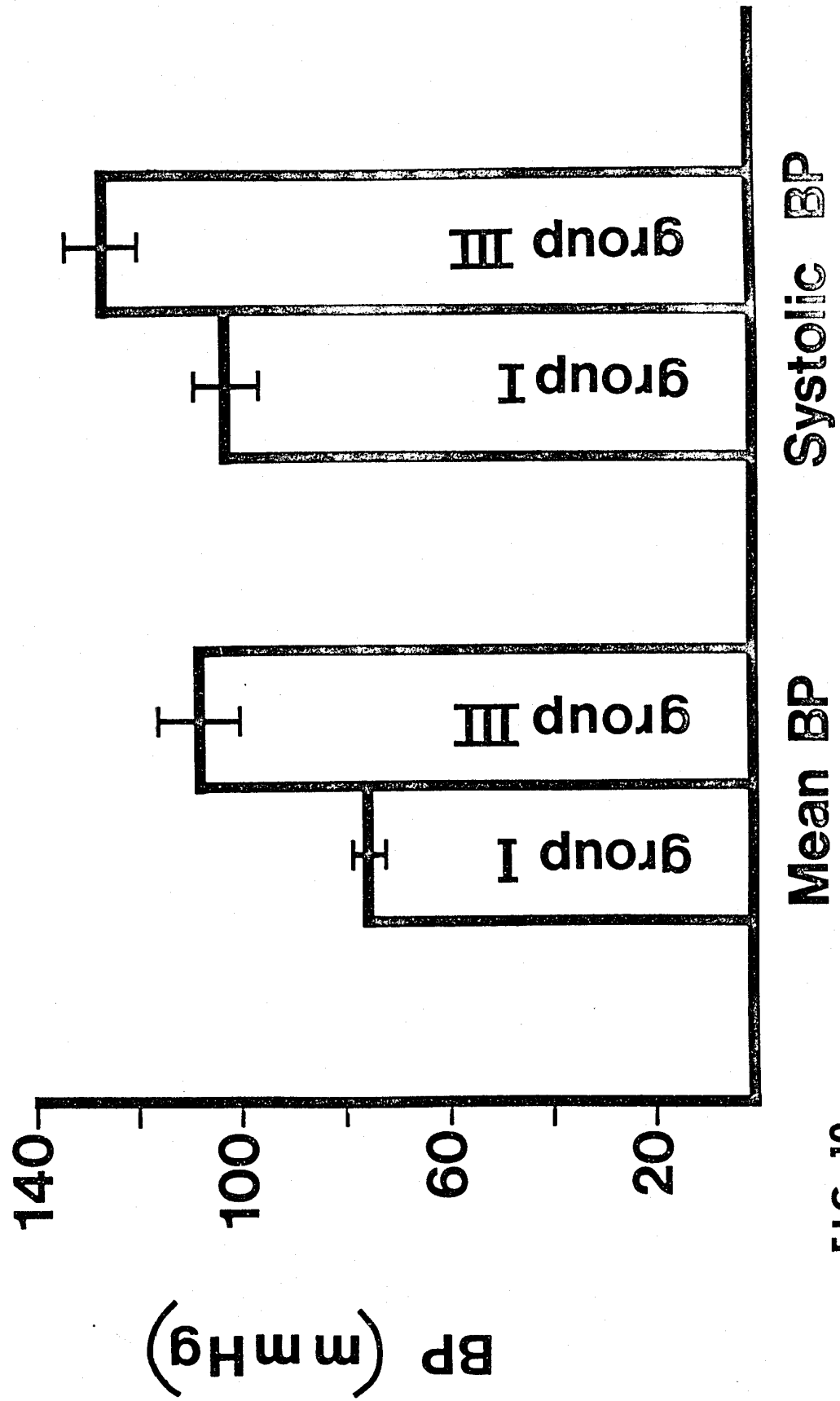


FIG. 10

54-G

FIGURE 11

FIGURE 11: The subarachnoid injection of blood led to a significant rise of the mean arterial blood pressure in Experiment A ($t = 3.39$ for Group II and 1.02 for Group III). The animals in Group II had an average mean blood pressure and standard error of 158.0 ± 4.7 mmHg thirty seconds before the start of blood injection, 204.5 ± 5.0 mmHg for the maximum level reached during the 4 minute period of injection, and 176.0 ± 4.7 mmHg thirty seconds after the end of blood injection. The correspondent values for Group III animals were 155.4 ± 4.2 mmHg before injection, 173.6 ± 7.8 mmHg during injection, and 151.4 ± 4.6 mmHg after blood injection.

Experiment A BP Alterations Induced By SAH

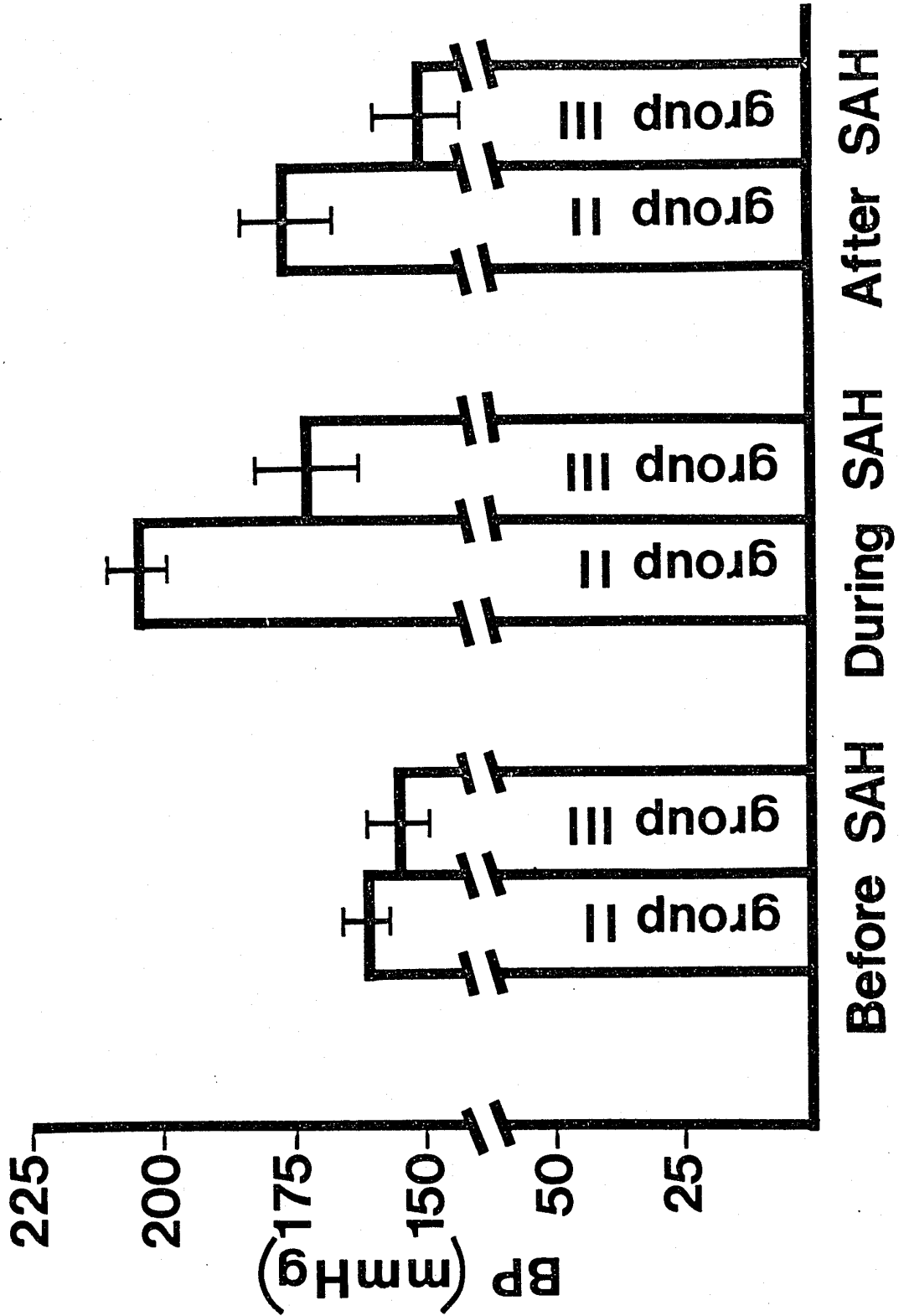


FIG 11

pressure and 124.7 ± 5.4 mmHg for the systolic blood pressure (Fig. 10). When compared with the corresponding figures found for animals in Group I, the mean BP increase in animals of Group II was significantly larger ($t = 3.05$, $df = 11$, $p < 0.01$) than in Group I). We believe that these values have ruled out the possibility of absence of lesions in Group III due to ineffective BP increase. The mean and standard error of the time required for blood pressure rise were 7.45 ± 0.69 seconds, which were close to the figures found for Group I. The aspect of the curve representing the increase in blood pressure was similar to that shown for Group I (Fig. 4). So was the duration of time during which BP stayed elevated and the occurrence of cardiac arrhythmias.

The results of macroscopic and microscopic examination of the brains of animals in Group III were exactly similar to the ones described for Group II. No evidence of BBB breakdown was seen (Fig. 12a and 12b). The distribution of blood in the subarachnoid space and the findings under fluorescence microscopy (Fig. 12c) did not show any difference from the previous findings described for Group II.

In summary, the results regarding pathological changes of the cerebral vascular permeability are as follows: 1) acute arterial hypertension induces BBB breakdown to proteins (Fig. 3), as previously described, 2) SAH does not cause BBB breakdown to proteins, during its acute stage (Fig. 6), 3) during the acute stage following SAH, acute arterial hypertension fails to induce BBB breakdown (Fig. 8).

55-A

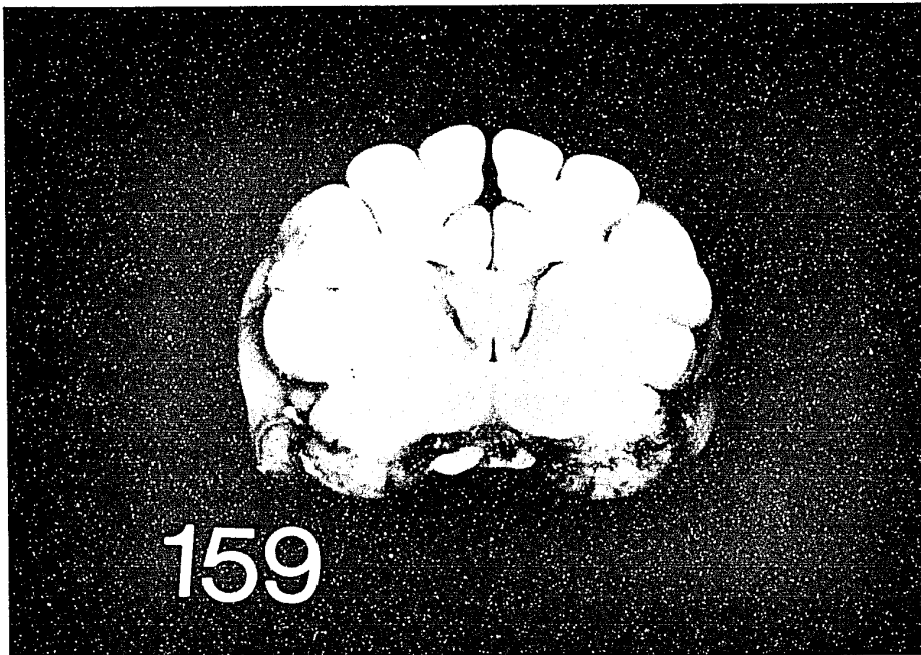
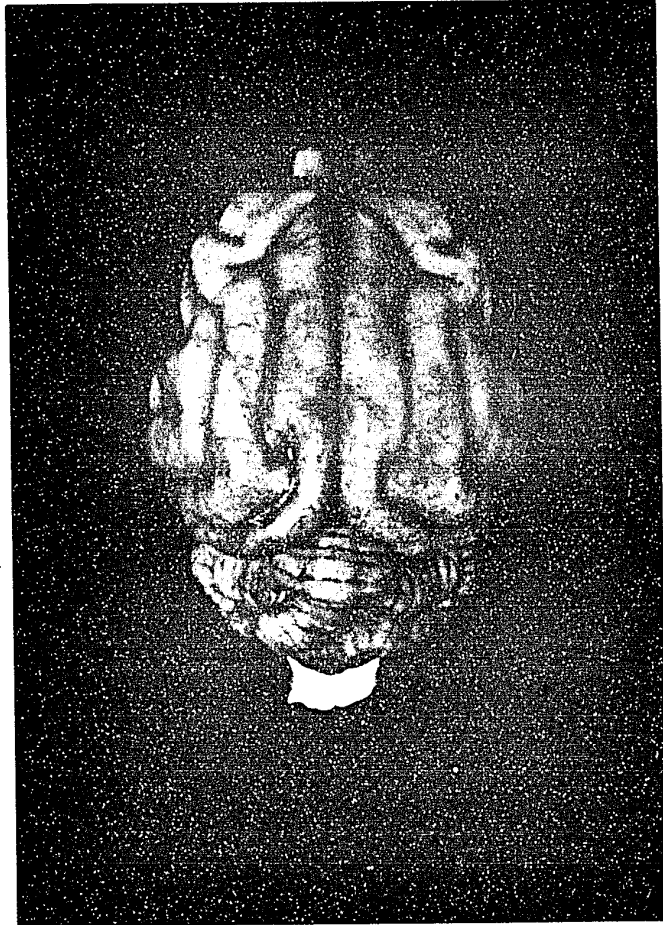
FIGURE 12

FIGURE 12: Photographic documentation of macro and microscopic specimens from Group III.

FIGURE 12a: Macroscopic view of the superior aspect of a cat's brain. There is no superficial blue discoloration. Note the intense and regular distribution of blood in the subarachnoid space.

FIGURE 12b: Coronal section to illustrate the absence of deep abnormal blue discoloration.

55 B



55-C

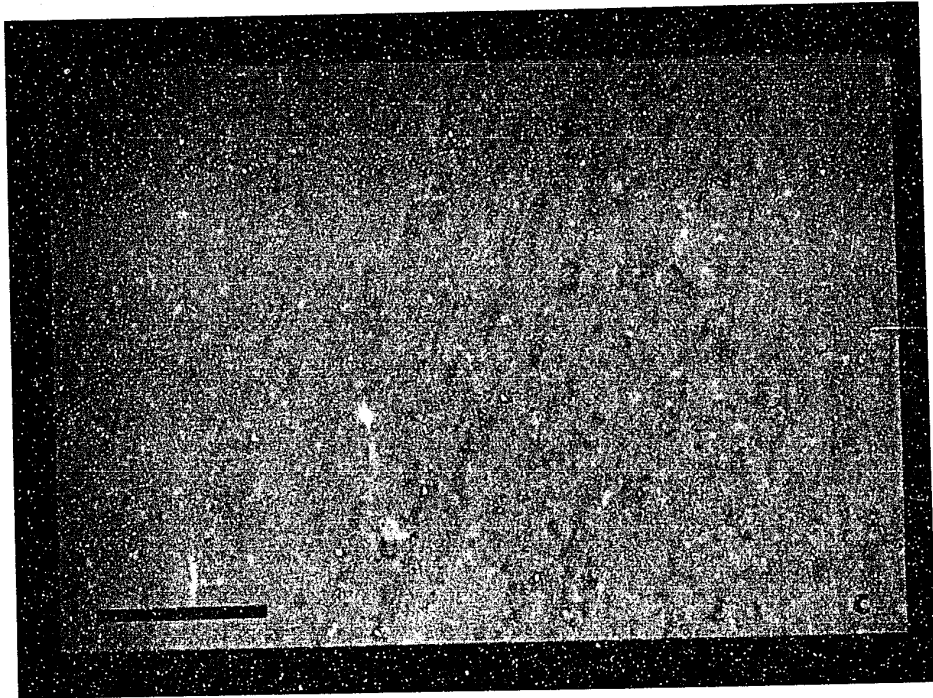
FIGURE 12c: Fluorescence photomicrograph from the cortex.

No Evans blue fluorescence is demonstrable.

The nervous tissue exhibits the usual background autofluorescence.

(bar = 100 μ m, 10 μ m thick section).

55 D



B. EXPERIMENT B

The animals were divided in two experimental groups:

Group A: Control Group

Group B: Test Group

Both groups went through the same experimental procedure except for the subarachnoid injection in Group A animals. During the initial attempts for stabilizing the experimental models, subarachnoid injection of mock CSF was tried in Group A animals to resemble more closely the situation with the test group. Small contamination with blood during cannulation of the subarachnoid space led us to abandon this procedure. So the ICP was not recorded in the control group.

The results of mean values and standard errors for arterial acidemia and gases for all animals included in Experiment B were:

pH - 7.268 ± 0.009

PaCO₂ - 31.975 ± 0.441

PaO₂ - 123.058 ± 2.363

The number of animals included in each group plus a summary of the main results are shown in Table II. The arterial blood pressure was maintained quite constant throughout the experiment. There was a slight increase in BP induced by the left carotid artery cannulation (average 10.3% increase). The average blood pressure measured in the distal segment of the carotid artery just after cannulation was 56.41% of the simultaneous systemic blood pressure values.

56-A

TABLE II

TABLE II: RESULTS OF EXPERIMENT B

Group	Number of Animals	Animals with BBB Breakdown Total %	Lesion Characteristics
A HgCl ₂	5	5 100	small multifocal cortical
B SAH + HgCl ₂	10	1 10	diffuse sharp limits central

TABLE II

56-C

FIGURE 13

FIGURE 13: Diagrammatic representation of the experimental set-up used for the Experiment B:

- (1) Blood bases sampling from the abdominal aorta through the right femoral artery
- (2) Rectal probe for temperature monitoring
- (3) Intracarotid injection of HgCl_2 under controlled injecting pressure
- (4) Intracisternal injection of blood with concomitant pressure monitoring
- (5) Pressure transducers
- (6) Endotracheal cannulation for artificial ventilation
- (7) Cannulation with proximal ligation of the left carotid artery
- (8) Cranial screws for bipolar EEG recording
- (9) Craniectomy for inspection during HgCl_2 injection
- (10) Temperature recording
- (11) Inferior vena cava catheterization through the right femoral vein for injection of drugs

BP: Continuous arterial blood pressure monitoring

EEG: Intermittent bipolar electroencephalogram monitoring

ICP: Continuous intracranial pressure monitoring

IP: HgCl_2 injecting pressure monitoring

R: Recorder

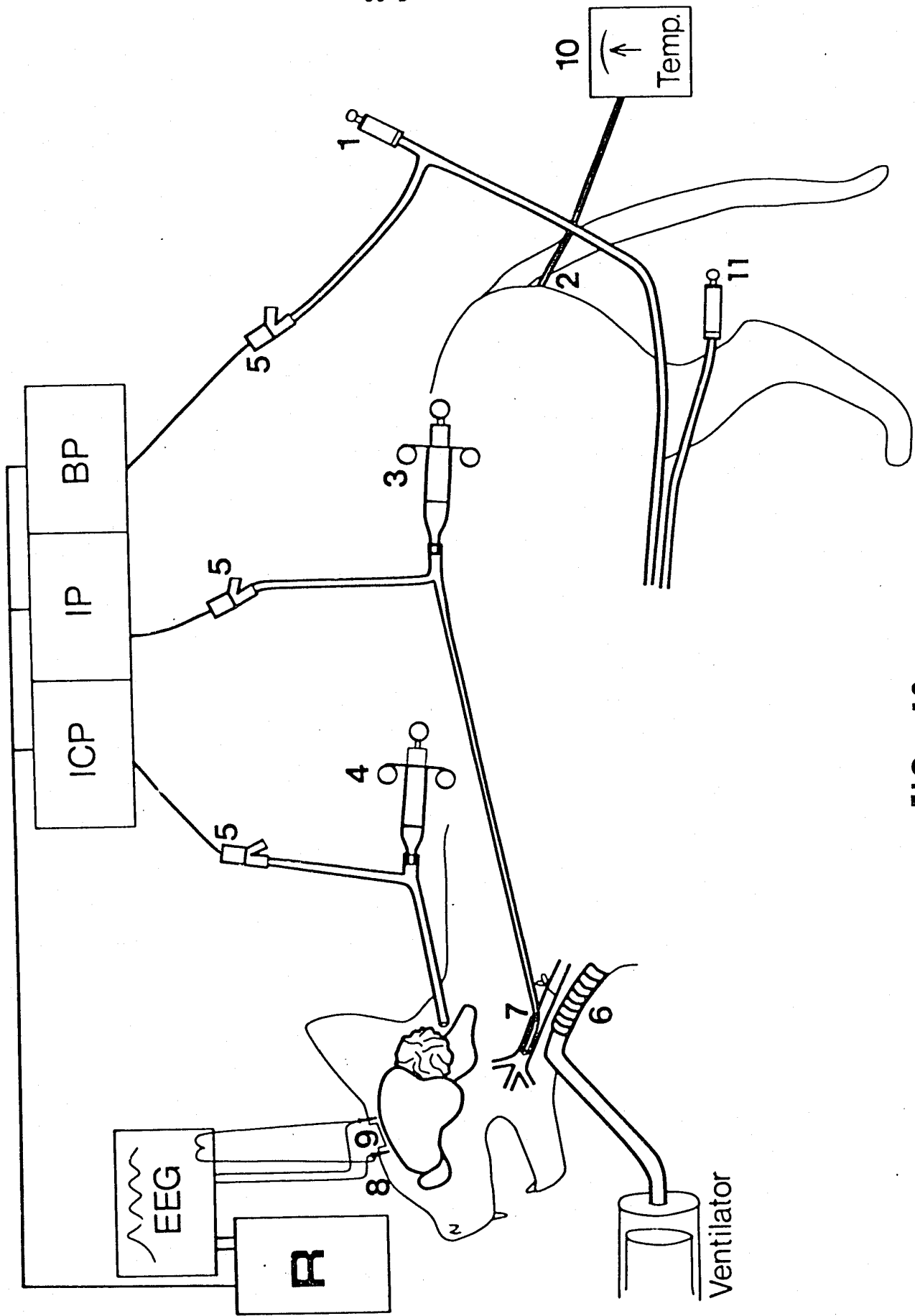


FIG. 13

1. Group A

The left carotid artery ligation did not cause any significant EEG changes in this group. A slight EEG slowing and reduction in amplitude were observed following the injection of HgCl_2 , without relation with the injected hemisphere. The recording from one animal is shown in Fig. 15.

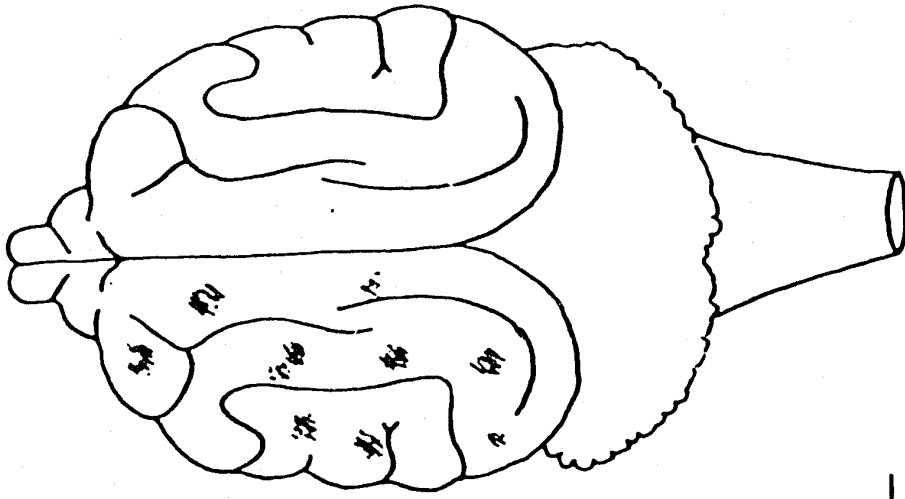
All the animals belonging to Group A displayed BBB breakdown in the hemisphere ipsilateral to HgCl_2 injection (Fig. 14). The intensity of lesions varied widely individually (compare Fig. 16a and 16c). Under macroscopic examination (gross inspection and visualization under the operating microscope) the stained areas were easily identified, well delineated, with multifocal distribution, varying in size from punctate lesions to extensive confluent staining. They were mostly confined to the peripheral grey matter, but in animals with more intense lesions, they could also be seen in the white matter (Fig. 16b). Under the fluorescence microscope, the Evans blue-protein complex displayed a red fluorescence with the filter combination used here. The fluorescence was seen designing the silhouette of the vessel wall (Fig. 16e) which was not seen in unstained areas (Fig. 21c). Perivascular globules of intensely dense fluorescence were frequently seen (Fig. 16f and 16g). It has been suggested that they represent high intracytoplasmic concentration of tracer in pericytes (Olsson and Hossman, 1970a). A diffuse spread of tracer in the neuropil could also be visualized (Fig. 16e). In these instances, the usual light greenish background autofluorescence exhibited

57-A

FIGURE 14

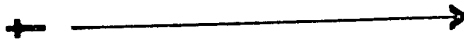
FIGURE 14: Diagram representing the main events and results of
Group A in Experiment B.

57-B



GROUP A

HgCl_2



30 min

FIG. 14

57-C

FIGURE 15

FIGURE 15: Simultaneous blood pressure and EEG recordings from one animal in Group A during three different stages of the experimental procedure. The initial EEG was characterized by 9 Hz synchronous activity (as in most of the other animals) superimposed on a mixed background of slow (3 Hz) and faster (15 Hz), low amplitude activity.

The left common carotid cannulation did not cause any significant changes in the EEG, but induced a slight increase in BP.

During the resting period following the left hemispheric perfusion of HgCl_2 , a slight reduction in amplitude and EEG slowing was present in the right side. Similar change was seen sometimes in the left side or bilaterally in other animals.

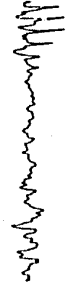
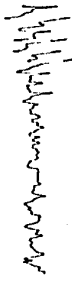
GROUP A

AFTER CAROTID
ARTERY LIGATION

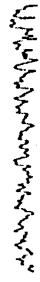
AFTER HgCl₂
PERFUSION

INITIAL

BP mmHg
300
200
100
0



50µV [



1 SEC

FIG. 15

57-E

FIGURE 16

FIGURE 16: Photographic documentation of macro and microscopic specimens belonging to Group A animals.

FIGURE 16a: Gross view of the superior aspect of a cat's brain. The entire hemisphere in the side injected with HgCl_2 displays intense superficial discoloration, due to BBB breakdown and intraparenchymal extravasation of Evans blue protein complex.

FIGURE 16b: Coronal section of the same specimen above to demonstrate the preferential distribution of lesions in the gray matter.

57 F

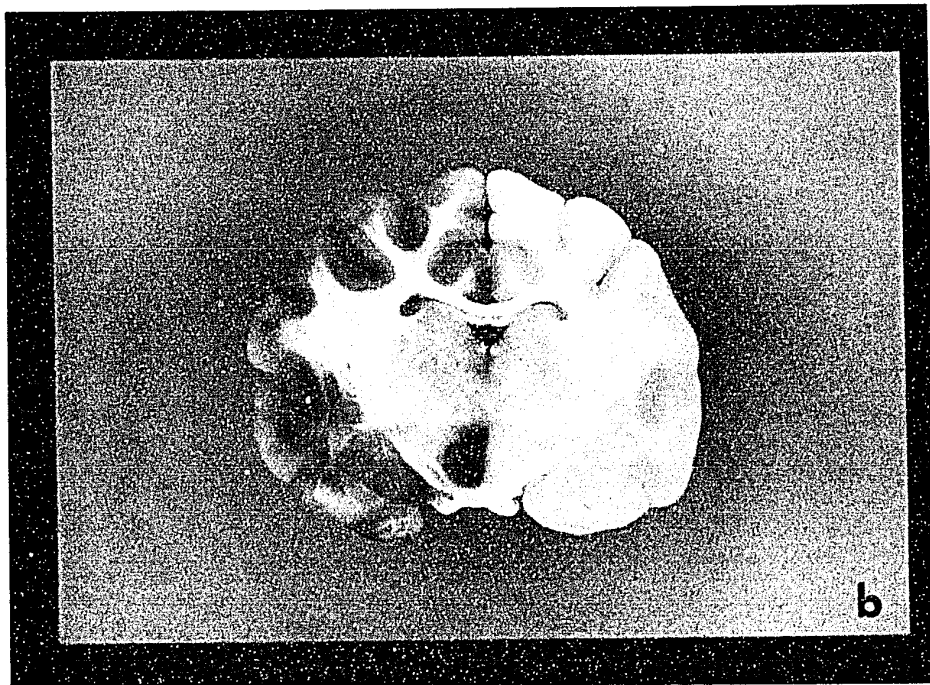
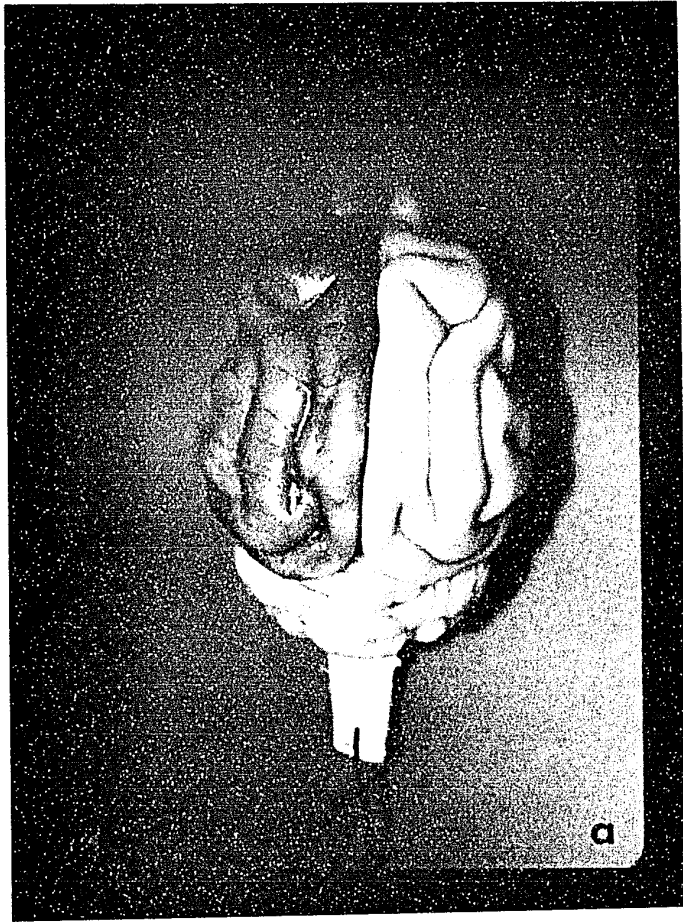


FIGURE 16c: Photograph of the hemispheric surface of another animal, taken under visualization with the operating microscope. This represents the most common pattern of superficial discoloration seen in this group. There is a moderate discoloration constituted by conglomerates of multifocal stained lesions.

FIGURE 16d: Fluorescence photomicrograph from a blue stained area. The red fluorescence displayed by the Evans-blue protein complex is seen in the cytoplasm of neurons and in the neuropil. There can also be seen a diffuse spread of red fluorescence in the nervous tissue.
(bar = 20 μ m, 20 μ m thick section).

57 H

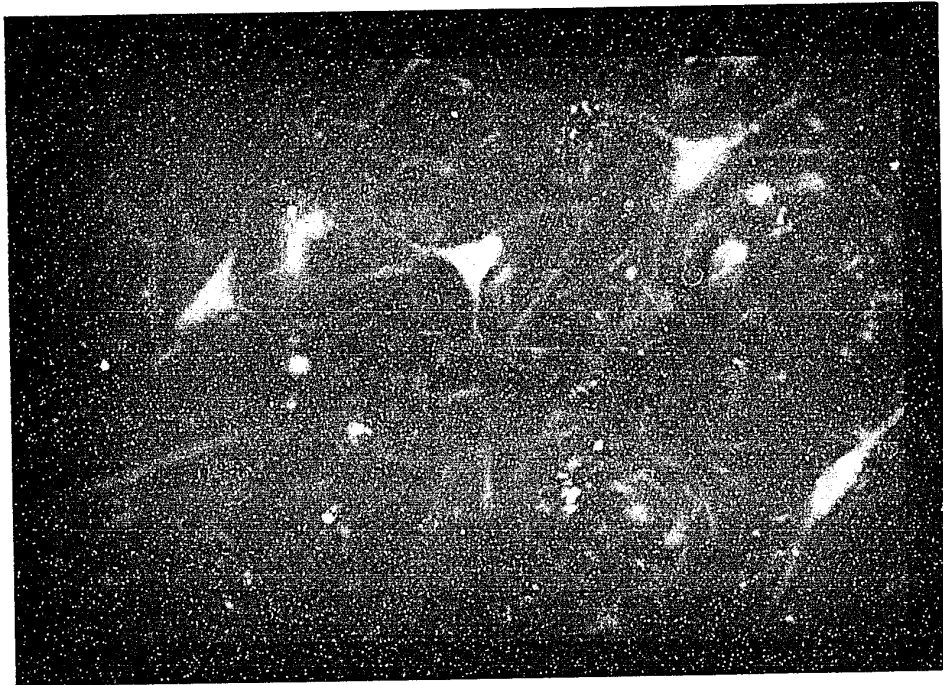
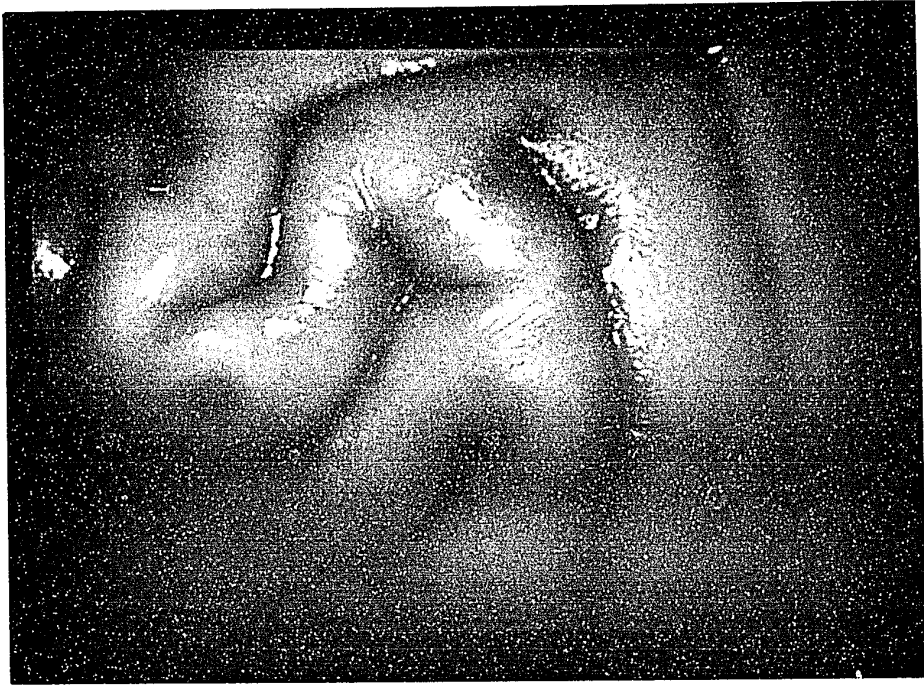


FIGURE 16e: Photomicrograph showing a cortical blood vessel in a stained area and extravascular extravasation of tracer. The vessel wall is traced by a deposit of red fluorescent material. A "globule" of fluorescence is seen outside the vascular space. There is also present a diffuse infiltration of red fluorescence in the tissue surrounding the vessel.

(bar = 20 μ m, 30 μ m thick section).

FIGURE 16f: Fluorescence photomicrograph showing the extravascular leakage of red fluorescent material in form of "globules" surrounding the vessels.

(bar = 100 μ m, 10 μ m thick section).

57-J

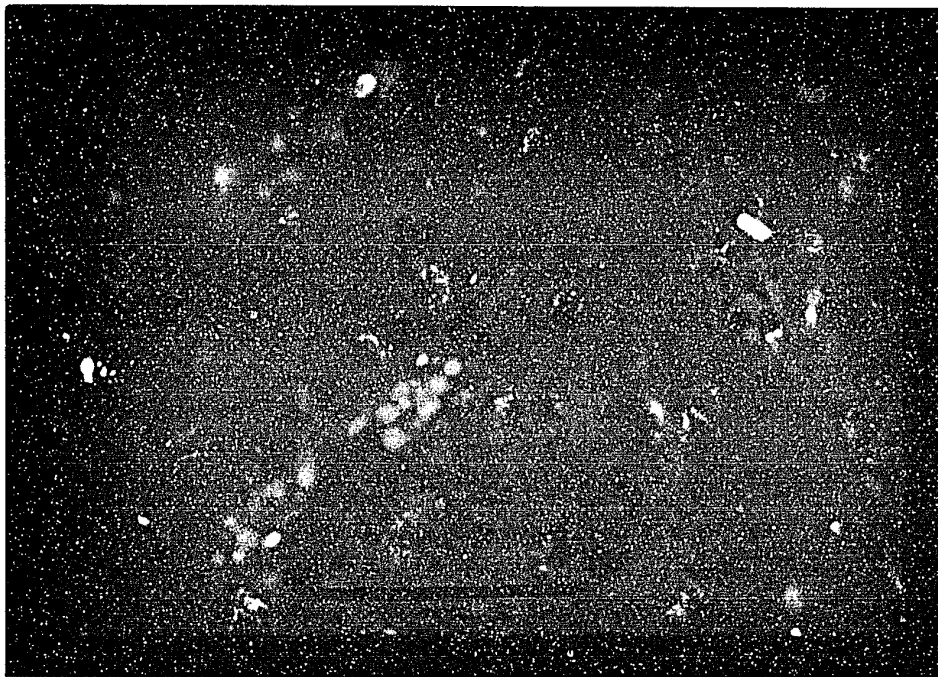
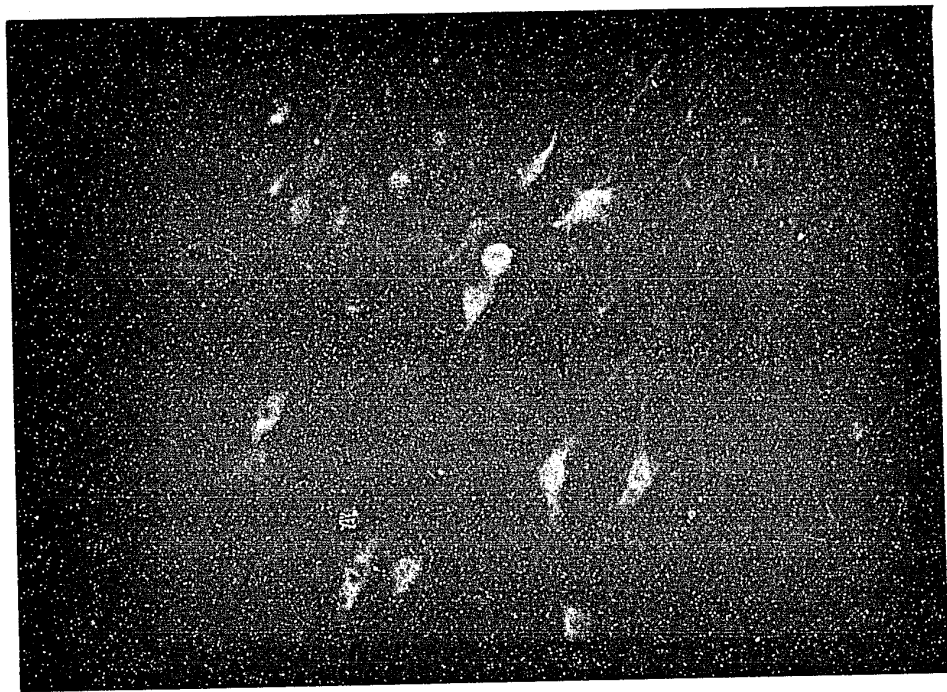
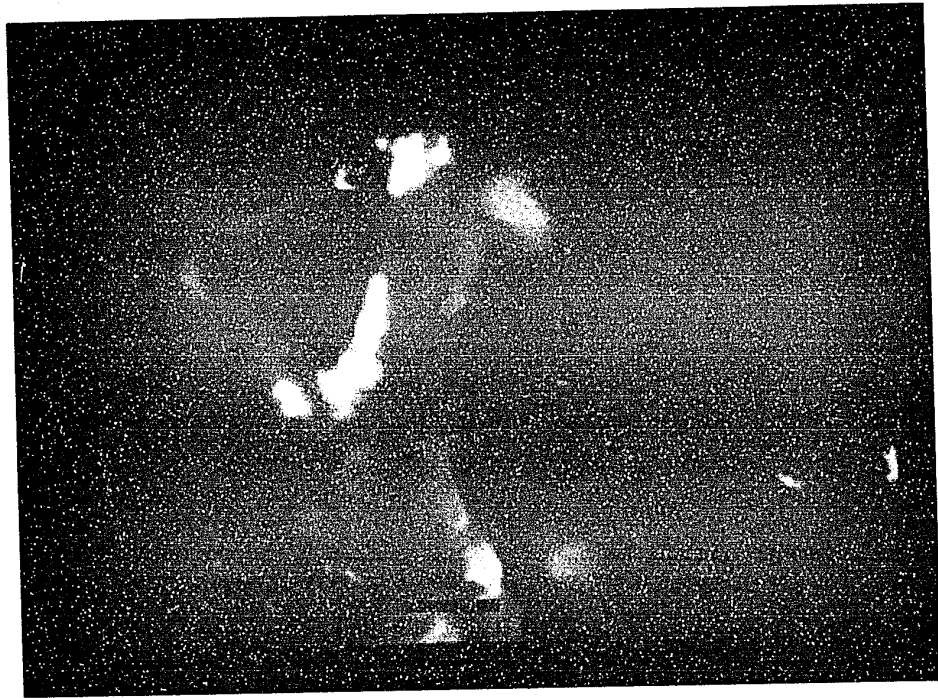


FIGURE 16g: Photomicrograph exhibiting a blood vessel in a stained area. The red fluorescence is located in the vessel wall plus outside the vessel limits. The positive design of the vessel wall is due to the extravasation of tracer from the intravascular space into the structures of the vessel wall.
(bar = 20 μm , 20 μm thick section).

FIGURE 16h: Photomicrograph of an area with BBB breakdown. The extravasated tracer is located diffusely in the cerebral tissue, giving a reddish tonality to the usual green autofluorescent background. It is also taken up by nervous cells, localizing in the perikarion and processes.
(bar = 100 μm , 10 μm thick section).

57 L



by the normal brain tissue (Fig. 16h) was darkened and less evident. The extravasated fluorescent material was usually taken up by the neurons; resulting in a strong intracellular red fluorescence (Fig. 16d and 16h). Microscopic sections from areas without gross blue extravasation always failed to show any red fluorescence. The aspect of the outer layers of the cortex was similar to that described for animals in Group I (Experiment A).

The most evident differences between the fluorescence microscopic findings between areas with BBB damage caused by HgCl_2 perfusion and acute hypertension were: 1) the "vascular labelling", due to deposit of tracer in the vessel wall, was much more evident in the HgCl_2 treated animals, 2) the extravasation of fluorescence material in the form of perivascular globules were seen only in HgCl_2 treated animals, 3) the intensity of fluorescence displayed by HgCl_2 induced lesions was stronger than that displayed by hypertensive lesions.

2. Group B

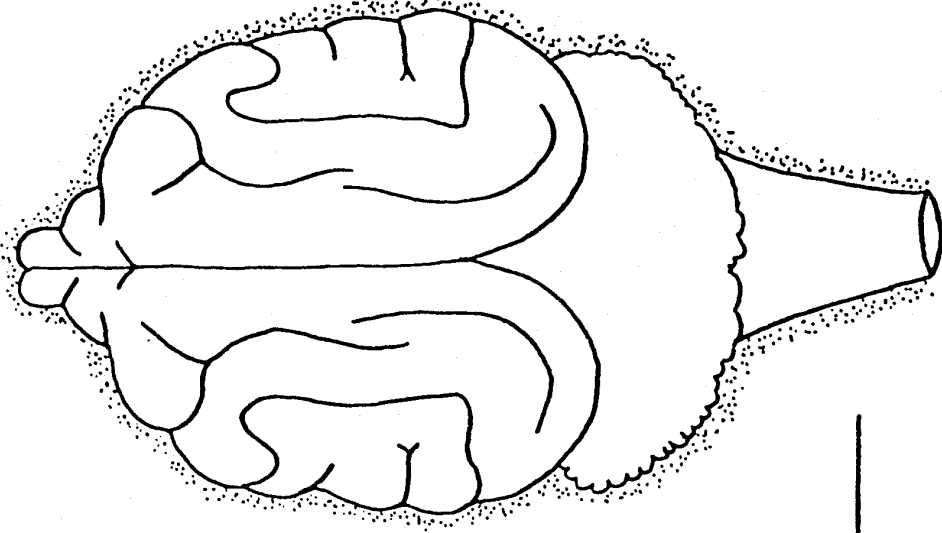
Animals in Group B underwent a subarachnoid injection of blood followed, after 30 minutes, by an intracarotid perfusion of HgCl_2 (Fig. 17). The changes in arterial blood pressure caused by subarachnoid hemorrhage, are shown in Fig. 19. The average values for ICP before SAH, the maximum value reached during SAH and the average value 30 seconds after end of subarachnoid injection of blood are shown in Fig. 20. The ICP recording was discontinued just prior to left carotid artery catheterization because the animal had to be turned on its right side

58-A

FIGURE 17

FIGURE 17: Diagram with the main events and results of Group B
in Experiment B.

58-B

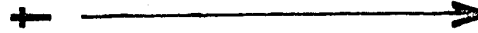
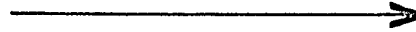


GROUP B

SAH



HgCl₂



30 min

30 min

FIG. 17

58-C

FIGURE 18

FIGURE 18: Simultaneous blood pressure, EEG and intracranial pressure recordings from one animal in Group B. The recordings after SAH and HgCl₂ perfusion were taken several minutes (resting situation) after each of these events.

The simulated SAH induced temporary EEG amplitude decrease which returned in a few minutes (10 min.) to a resting rhythm similar to the initial one. The present animal also exhibited a slight decrease of the BP and a ICP increase after SAH.

The presence of cardiac arrhythmias following HgCl₂ perfusion, which occurred with this animal was not a constant finding. Subsequent to the HgCl₂ perfusion there was a bilateral decrease in EEG amplitude.

The ICP was relatively unaffected by the carotid artery ligation and HgCl₂ perfusion in this particular animal.

58-D

GROUP B

INITIAL

AFTER SAH

AFTER CAROTID
ARTERY LIGATION

AFTER HgCl₂
PERFUSION

BP mmHg
300
200
100
0

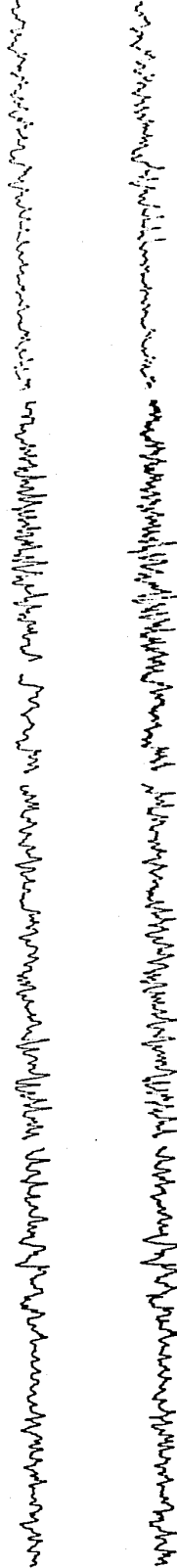


RT

50µV

LT

ICP mmHg
150
100
50
0



sec

FIG. 18

58-E

FIGURE 19

FIGURE 19: The injection of blood into the cisterna magna caused an increase of the mean arterial blood pressure in animals of Group B. The average and standard error for the blood pressure values were 154.9 ± 2.8 mmHg thirty seconds before the start of the injection, 174.5 ± 5.1 mmHg for the maximum value reached during the injection and 159.5 ± 4.0 mmHg thirty seconds after the end of the subarachnoid blood injection.

Experiment B BP Variations Caused By SAH

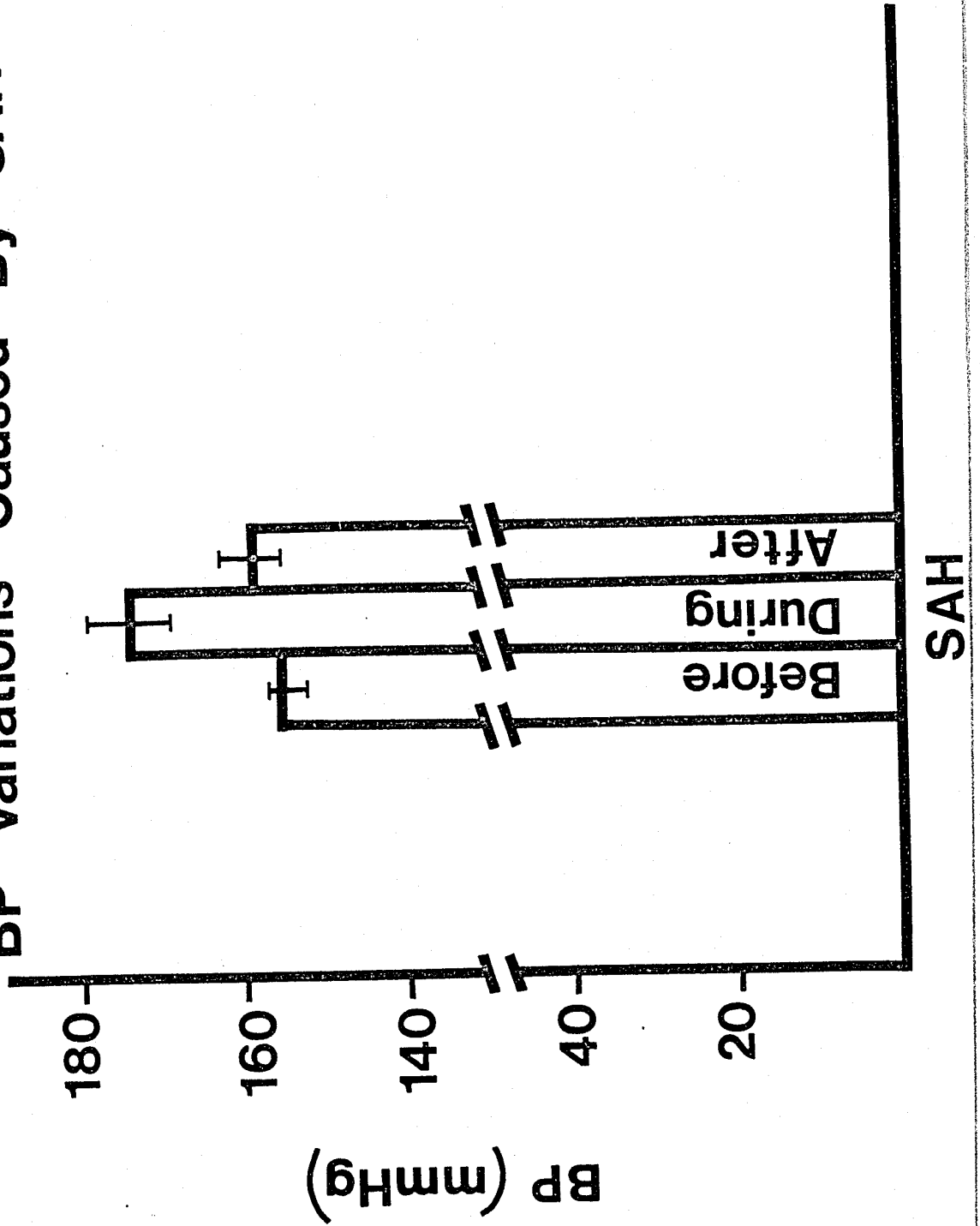


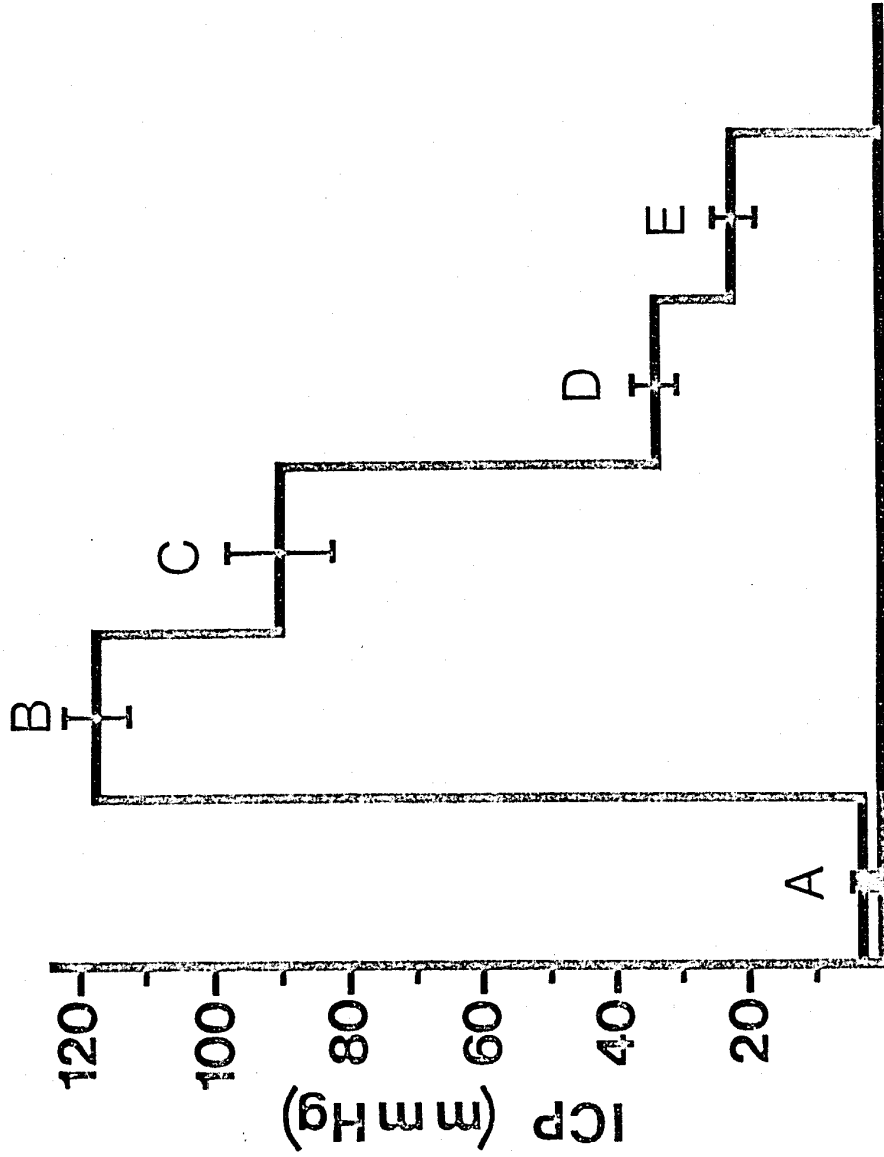
FIG. 19

58-G

FIGURE 20

FIGURE 20: Changes in ICP caused by subarachnoid injection of blood in cats from Group B. The initial baseline values (A) for ICP (average and standard error) were 3.86 ± 0.37 mmHg. The maximum level reached during the blood injection (B) was 119.83 ± 4.63 mmHg. The pressure immediately after end of injection (C) was 90.30 ± 6.54 mmHg and at 3 minutes after end (D) 34.12 ± 3.17 . Usually the ICP would return to a resting value (E) higher than the initial one. The average values found just prior to HgCl_2 injection were 23.33 ± 2.51 mmHg. The ICP was not measured after HgCl_2 injection due to repositioning of the animal.

Changes in ICP Caused By SAH



Experiment B

FIG. 20

for arterial ligation and cannulation. This manoeuver was usually accompanied by blockage of the cisternal catheter.

The injection of blood in the subarachnoid space was generally followed by an initial bilateral amplitude decrease, often more marked on the left side, with amplitude recovery occurring in the few minutes following the end of the injection. There was no significant changes in the frequency. Subsequent to the injection of HgCl_2 , there was a bilateral decrease in EEG amplitude. No asymetry was verified though. Figure 18 represents an example of changes of BP, ICP and EEG during the different stages of Experiment B.

Lesions were completely absent in 90% of the animals in Group B (Fig. 21a and 21b). In all animals the injection of 4 ml of blood was sufficient to cause diffuse spread of blood over the convexities. A weak blue discoloration was seen in areas devoid of BBB (such as choroid plexus, area prostroma, tuber cinereum). The injected blood was evenly distributed in the subarachnoid space with a slight increase in concentration in the basal cisterns. In just one animal of this group, BBB breakdown was seen (Fig. 21d and 21e). It was mostly localized deep in the brain. It displayed an intense spread and well demarked discoloration (Fig. 21f) and included the thalamus, hypothalamus, basal ganglia and optic tract. There were also present small patchy lesions in the left occipital grey matter and left olfactory bulb. Under the

59-A

FIGURE 21

FIGURE 21: Photographic documentation of macro and microscopic specimens from Group B animals.

FIGURE 21a: Gross view of the superior aspect of the brain. Diffuse SAH. No superficial BBB breakdown.

FIGURE 21b: Coronal section of the cerebral hemispheres to demonstrate the absence of deep blue staining.

59-B

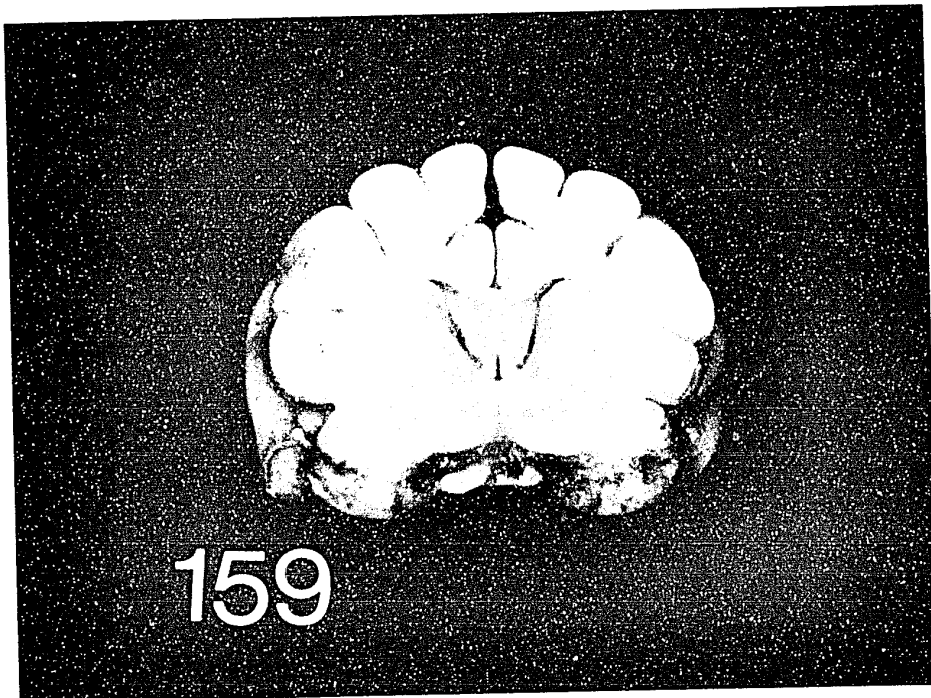
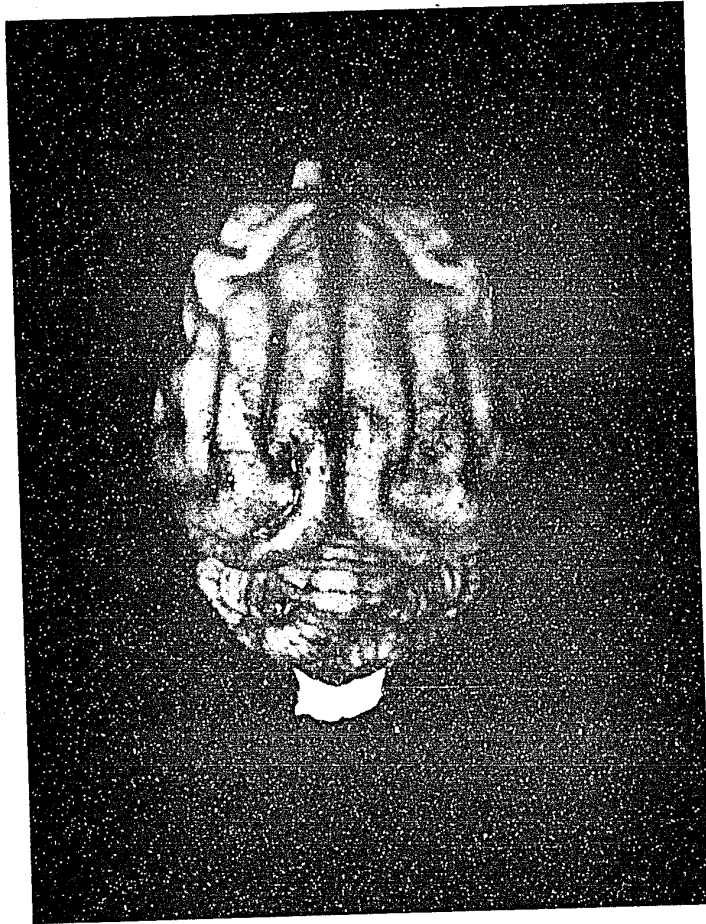
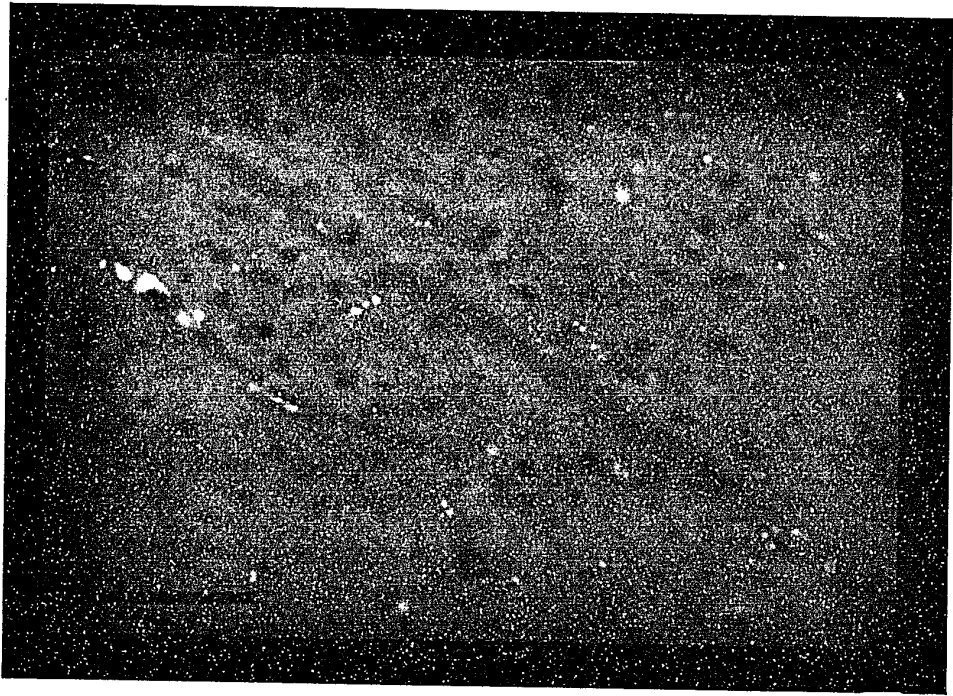


FIGURE 21c: Fluorescence photomicrograph from the cortex demonstrating the absence of abnormal fluorescence. The normal autofluorescence displayed by the cerebral tissue is divided by an oblique "negative" space represented by a blood vessel. There is no red fluorescent deposit in the vessel wall.
(bar = 100 μm , 20 μm thick section).

59 D



59-E

FIGURE 21d: Gross view of the inferior aspect of the brain from the only animal with BBB breakdown in Group B. The blue discoloured areas were mostly located on the basal surface (left olfactory bulb, supraorbital cortex, left optic tract and infundibulum).

FIGURE 21e: Close-up view of the same specimen as in Figure 21d, demonstrating faint diffuse discoloration in the left temporal cortex.

59 F

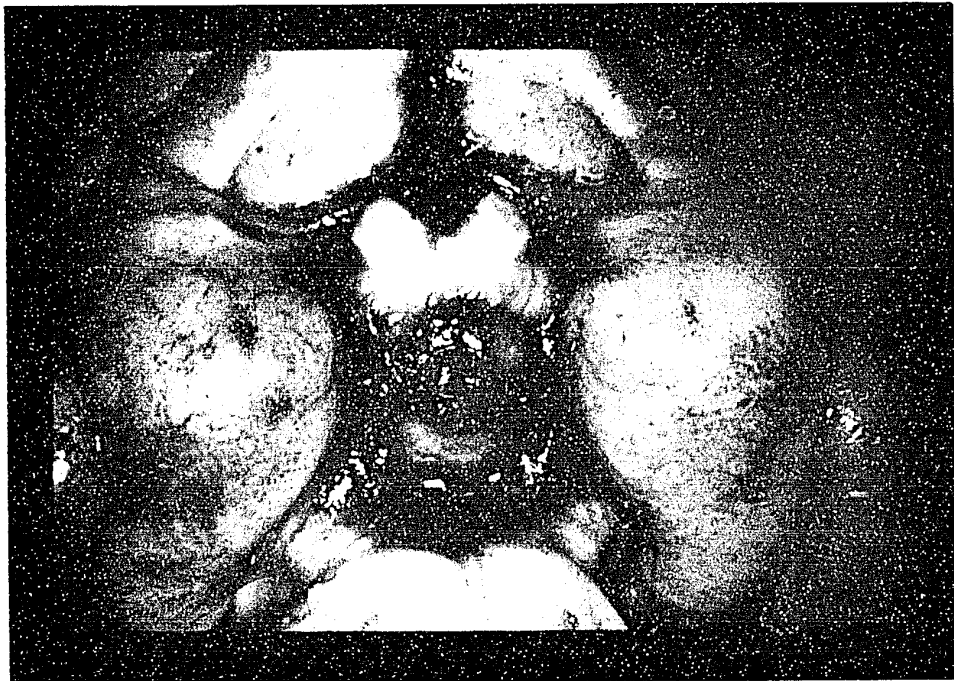
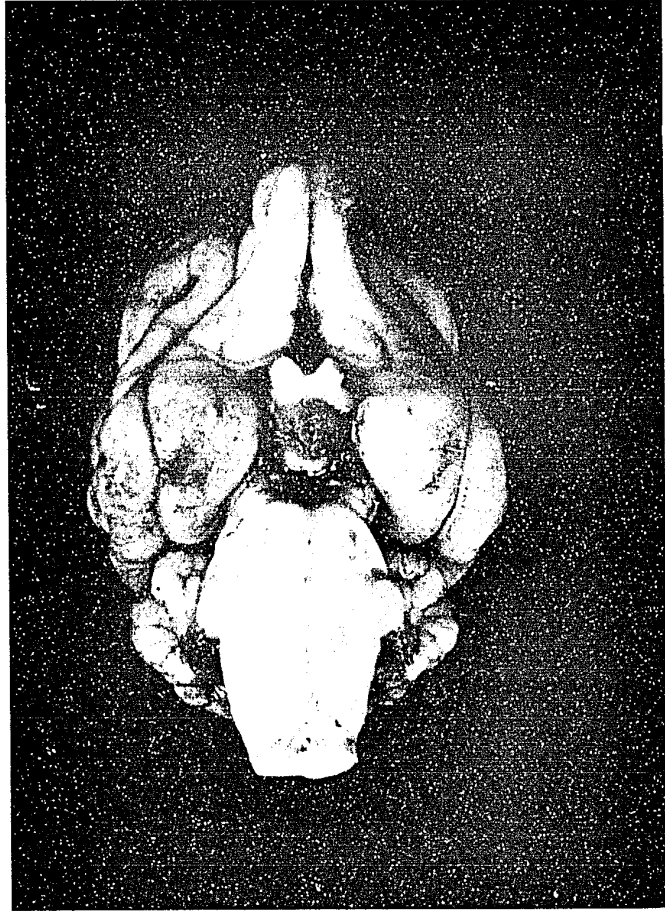
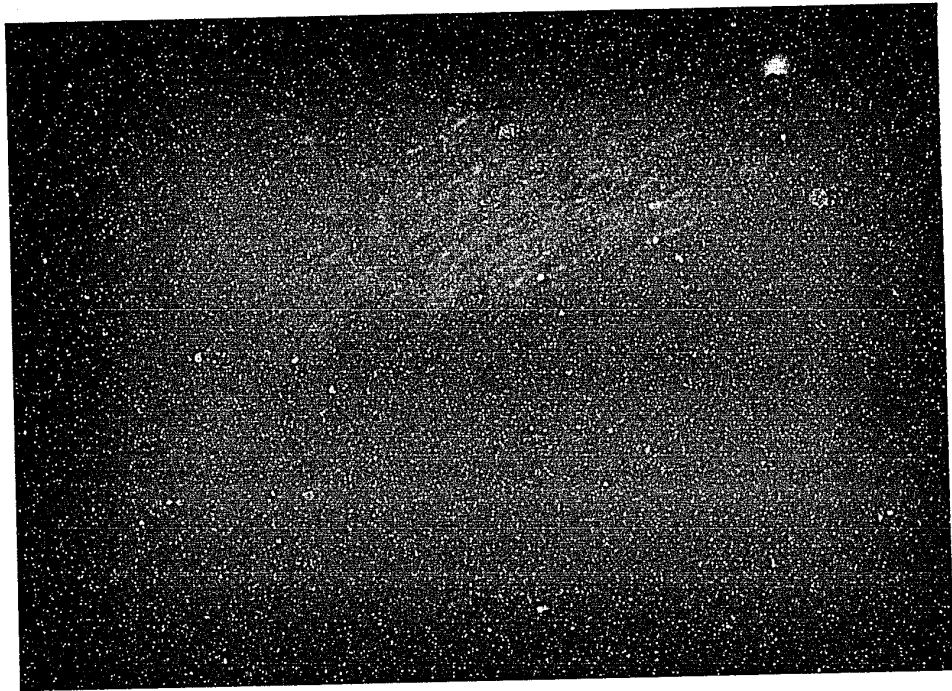
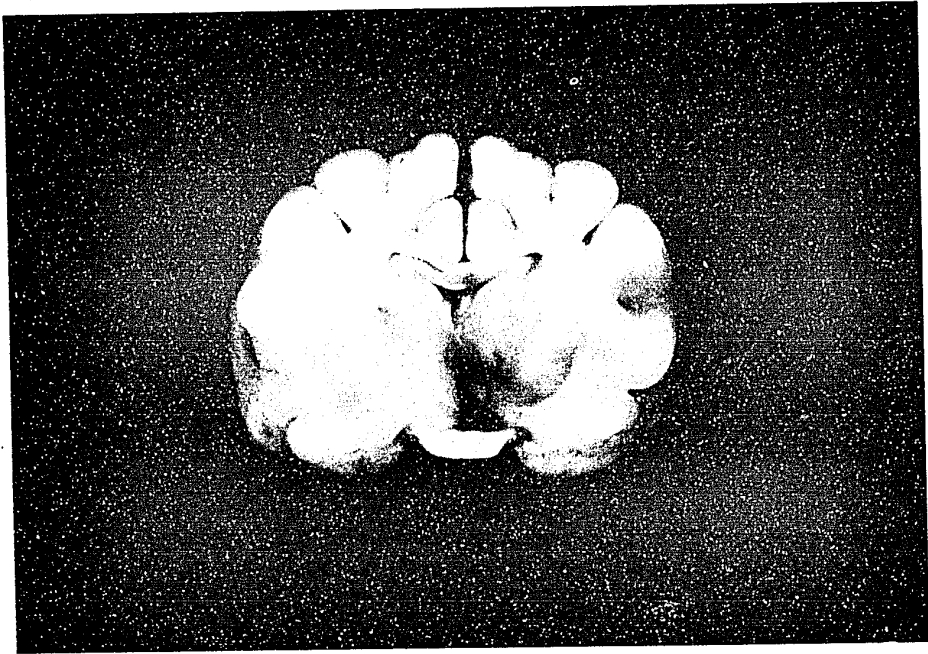


FIGURE 21f: Coronal section of the same specimen shown in Figures 21d and 21e. The BBB breakdown was predominantly deeply localized in the left hemisphere. Some cortical discoloration can also be appreciated. There is an intense diffuse leakage of tracer into the left thalamus, hypothalamus, basal ganglia and optic tract. The white matter displays lesser degree of staining.

FIGURE 21g: Fluorescence photomicrograph showing the well defined limits between an area with BBB damage and normal brain tissue from the same specimen in Figures 21d, 21e, and 21f. The upper half of the picture shows the intense extravasation of fluorescent tracer into the neurophil and cell bodies, due to BBB breakdown.

(bar = 100 μ m, 10 μ m section).

59 H



fluorescence microscope the aspect of stained areas was similar to that found for damaged areas in Group A, except for the sharply defined limits of tracer extravasation in Group B (Fig. 21g). No cerebellar or brain stem lesions were found.

The findings regarding fluorescence microscopic appearance of brain tissue and subpial region were similar to those described for Groups II and III (Experiment A). They were characterized by a narrow subpial band of yellowish fluorescence, probably due to blood infiltration in the brain parenchyma. The inner layers of cortex, as well as the white matter, displayed appearance similar to normal brain tissue.

IV. DISCUSSION

A. COMMENTS ON THE EXPERIMENTAL MODEL

The design of an experimental model to resemble a clinical SAH faces many problems. These are due to the inability to reproduce in animals some of the conditions which closely resemble the clinical counterpart, plus a poor knowledge of some variables occurring during a spontaneous subarachnoid hemorrhage. The standardization of some of the variables is very important for investigations concerning alterations during the acute stage following SAH. The most common cause of spontaneous SAH in patients is, by far, the leakage of an arterial aneurysm. It has not been possible to reproduce aneurysm dilatation in animals. Techniques such as arterial puncture with a needle (Yamaguchi and Waltz, 1971; Simeone et al., 1972; Asano and Sano, 1977), and injection of blood into the subarachnoid space (Petruk et al., 1974; Martins et al., 1975; Fein, 1976) have been used to replace aneurysmal rupture. The needle puncture technique is used preferentially for investigations related to vasospasm, because it involves both bleeding and vessel damage, which approximates more closely the clinical phenomenon.

1. Speed of Blood Injection

Indirect clinical evidence obtained from continuous CSF pressure measurements during re-bleeding of an aneurysm, suggests that a massive hemorrhage occurs in a few seconds, and that CSF pressure rises sharply to values between the systolic and diastolic arterial

blood pressure (Hayashi et al., 1977). It is possible, though, that the decreased or abolished pressure gradient across the aneurysmal wall acts as a hemostatic factor (Nornes, 1973). This situation is very close to that used in experimental models for studies of the effect of increased ICP on the CBF (see Introduction). It has been demonstrated that under these experimental circumstances, cerebral ischemia appeared, due to decreased CPP, followed by a "reactive hyperemia" with breakdown of autoregulation (Kjallquist et al., 1969; Zwetnow, 1970) and increased cerebrovascular permeability (Petersen, 1967). Reported differences of CBF measurement immediately after experimental SAH (Hashi et al., 1972a; Petruk et al., 1972; Martins et al., 1975), may be due to the duration of blood injection. A fast injection can cause a marked elevation of ICP. The intracranial hypertension could presumably lead to BBB breakdown by itself. For purposes of the present study, focused on the cerebral vascular permeability properties due to the presence of blood in the subarachnoid space, a sharp increase of ICP was avoided. Controlled subarachnoid injection of blood, with continuous CSF pressure monitoring was used in the present experimental series. The duration of blood injection was determined by the CSF pressure. It was found that injection of 4 ml of blood during a 4 minute period was always effective in preventing intense elevations of CSF pressure. This was in agreement with other authors (Hashi et al., 1972a; Martins et al., 1975).

2. Amount of Injected Blood

It is difficult to establish the amount of blood to be

injected in the subarachnoid space of an animal to mimic the clinical situation. Autopsies from clinical material suggest the existence of a large variation in the amount of blood, from localized hemorrhage in the basal cisterns to widespread distribution over the cerebral hemispheres (Walton, 1976). For adult cats, injection of 4 ml of blood led to an even spread in the subarachnoid space, associated with a denser concentration of clotted blood in the basal cisterns. This volume was comparable to that used by other investigators for animals of similar size (Denton et al., 1971; Petruk et al., 1972a; 1974; Martins et al., 1975).

3. Timing

The choice of the 30 minute period for the induction of hypertension or perfusion of $HgCl_2$ following subarachnoid injection was arbitrary. It was based on findings that acute changes after experimental SAH such as vasospasm, CBF changes, metabolic disturbance, take place during the first few minutes after SAH and last for hours (Hashi et al., 1972a; Petruk et al., 1972; Martins et al., 1975).

4. Unilateral Internal Carotid Ligation in Cats

Experimental models for intracarotid perfusion with BBB damaging agents have been extensively used (see Introduction). Work has been reported in rabbits (Steinwall, 1958; 1968; Flodmark and Steinwall, 1962), cats (Hossman and Olsson, 1971a; 1972), and monkeys (Rapoport et al., 1972; 1973a). Cerebral changes secondary to unilateral carotid ligation seemed to vary with the species studied. Flodmark and

Steinwall (1962) reported no significant effect on the EEG following unilateral occlusion of the common carotid artery in rabbits. No EEG abnormality was found even after bilateral carotid clamping combined with lowering of the blood pressure to about 50% of basal values. The authors postulated that a well developed circle of Willis in rabbits was sufficient to provide adequate collateral circulation. Rapoport et al., (1972; 1976b) have studied an experimental model of BBB breakdown in monkeys by intracarotid injection of hypertonic solutions. They observed that unilateral carotid occlusion did not produce any measureable brain damage because of autoregulation of cerebral vessels to preserve a constant CBF. But, under these circumstances, the reserve adjustments for autoregulation were depleted (Sengupta et al., 1971/72) and brain damage did occur after minimal insults such as arterial hypotension, bradycardia or apnea, which can accompany intracarotid injection of hypertonic solutions. It was found that a similar BBB breakdown occurred after temporary clamping (20 seconds injection time) or permanent ligation of one internal carotid artery (Rapoport and Thompson, 1973; Rapoport, 1976b). However, brain injury was seen only after permanent ligation (Rapoport et al., 1972).

In the present investigation, a similar experimental model was used for the injection of HgCl_2 into the carotid artery of cats. However, due to special characteristics of the cat's carotid arterial system, a modification of the previously described techniques was made, according to Hossman and Olsson, (1971a; 1971b; 1972). The branches coming off the common carotid artery in cats are too small in diameter

for catheterization by a cannula sufficiently large to deliver an adequate flow of HgCl_2 perfusate. Additionally, the internal carotid artery of the cat is not patent in the great majority of animals (Davies and Story, 1943; Holmes et al., 1958). The blood from the common carotid artery reaches the brain through the carotid plexus (rete externum) formed by anastomotic channels entering the base of the brain through the orbital fissure. Other important anastomotic arteries include the internal maxillary and the ascending pharyngeal arteries (Waldron et al., 1974; Kamijyo and Garcia, 1975). These two special features imposed a modification of the carotid artery perfusion for cats, namely the cannulation of the common carotid artery.

B. POSSIBLE HEMODYNAMIC ALTERATIONS AND BIOCHEMICAL INTERACTION

The results of this investigation indicate that during the first hour following experimental subarachnoid hemorrhage in cats, there was no increase in the cerebral vascular permeability to proteins, as detected by the Evans blue-albumin complex. Furthermore, animals subjected to SAH 30 minutes before an acute arterial hypertensive episode, failed to display the usual blood-brain barrier breakdown seen after hypertension alone. The theories regarding pathophysiological mechanisms for BBB breakdown caused by arterial hypertension have been presented in the Introduction. The interaction of SAH with these mechanisms can be considered from two viewpoints: hemodynamic alterations and biochemical interaction.

1. Hemodynamic Alterations

The most likely hypothesis to explain BBB damage following acute hypertension is based on the proposed enhancement of luminal pressure in the cerebral capillary tree (MacKenzie et al., 1976). Vasospasm of major cerebral blood vessels, which follows SAH, could interfere with this mechanism. It seems clear that during the first hour following a SAH, spasm of major conductive vessels and dilatation of small intraparenchymal arteries and arterioles take place (Hashi et al., 1972a; Heros et al., 1976). Vasospasm has been demonstrated in several species (reviewed by Heros et al., 1976) including the cat (Kapp et al., 1968; Mahaley, Jr. and Kapp, 1970; Yamaguchi and Waltz, 1971), within the first minutes after experimental SAH. It has been confirmed by cerebral angiography under the present experimental conditions, in animals not included in the experimental series (Figs. 23a and 23b).

Studies with large arteries such as the femoral artery or the origin of the internal carotid artery have demonstrated the existence of an intraluminal pressure gradient between the proximal and distal portions of a severely constricted segment. At least a 60% reduction of the luminal area is required to cause a blood pressure drop and decreased blood flow in the distal segment (Tindall et al., 1962; May et al., 1963; DeWeese et al., 1970). The pressure drop is also proportional to the length of the stenosis (Brice et al., 1964). The data obtained from these publications have only limited value for

comparison with the spasm of cerebral arteries in the present model due to the difference in vessel calibre. Furthermore, the degree and length of vasospasm under the present experimental conditions was not determined. Simon's (1967) study of traumatic spasm of the middle cerebral artery in baboons provided hemodynamic data which were probably closer to those obtained under our experimental conditions. He always found a pressure drop in the distal branch of the middle cerebral artery after traumatic spasm of the proximal portion of that artery, although the length and percentage of constriction were not measured. If the above considerations are applicable to the present model, the results of Experiment A might suggest that the brain is relatively protected, as far as BBB breakdown is concerned, from the harmful effects of acute hypertension because of the spasm of the basal vessels.

2. Biochemical Interaction

An experiment based on chemical BBB damage was designed to attempt to rule out vasospasm as a factor responsible for the decreased susceptibility of the BBB to damaging agents after SAH (Fig. 22).

The increased cerebrovascular permeability which follows HgCl_2 perfusion is thought to be due to inhibition of enzymes of the endothelial cells (Chang and Hartmann, 1972). Two variables are expected to remain constant for the control and experimental groups during the chemical reactions that take place upon perfusion with HgCl_2 : (1) the time of exposure to the test substance, and (2) the concentration of

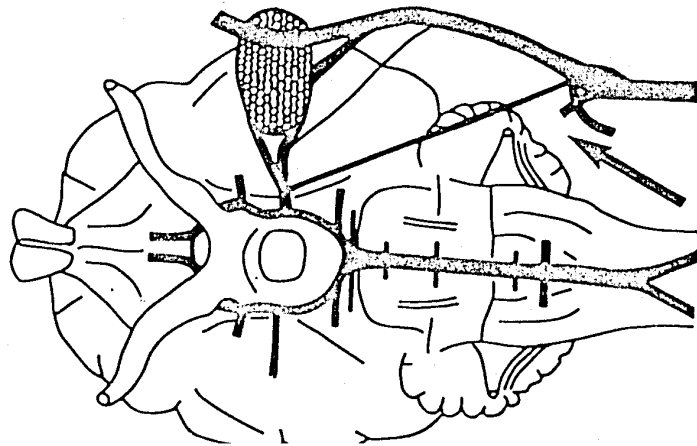
67-A

FIGURE 22

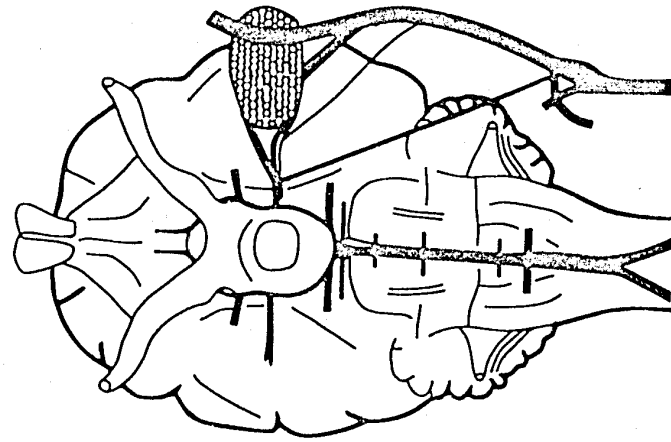
FIGURE 22: Diagrammatic representation of the main hemodynamic events taking place during Experiment B. Some special features of the cat's cerebral circulation are illustrated in the first diagram. Note the absence of a patent internal carotid artery (arrow), plus the existence of a rete caroticum between the external carotid circulation and the Circle of Willis.

After SAH there was spasm of the major intracranial vessels (Figure 23). The left common carotid artery was then cannulated and HgCl₂ injected under controlled conditions. Disregarding the presence of vasospasm, HgCl₂ solution was still provided to react with the endothelium distal to the constricted area, during the desired length of time.

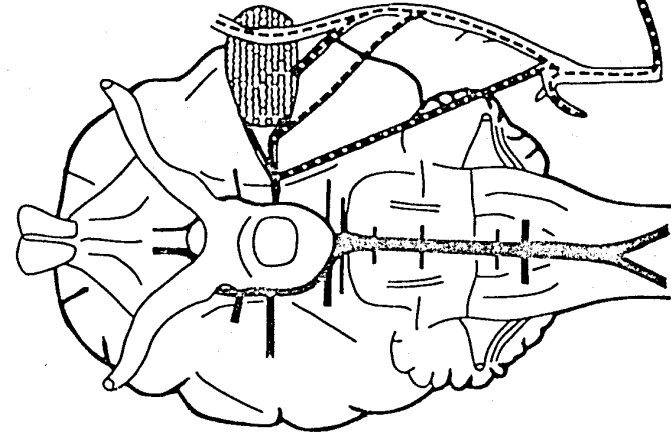
Before SAH



After SAH



HgCl₂ Perfusion



45-60 sec.

≡ blood pressure

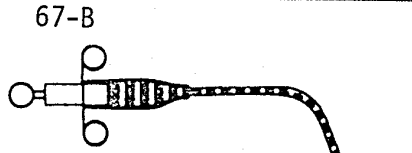


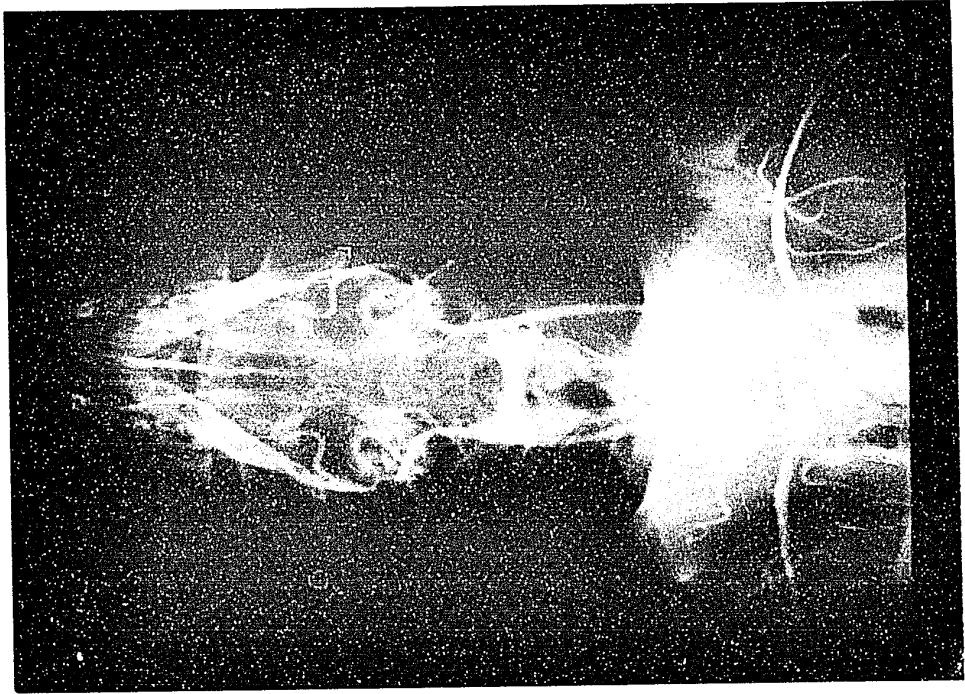
FIG. 22

67-C

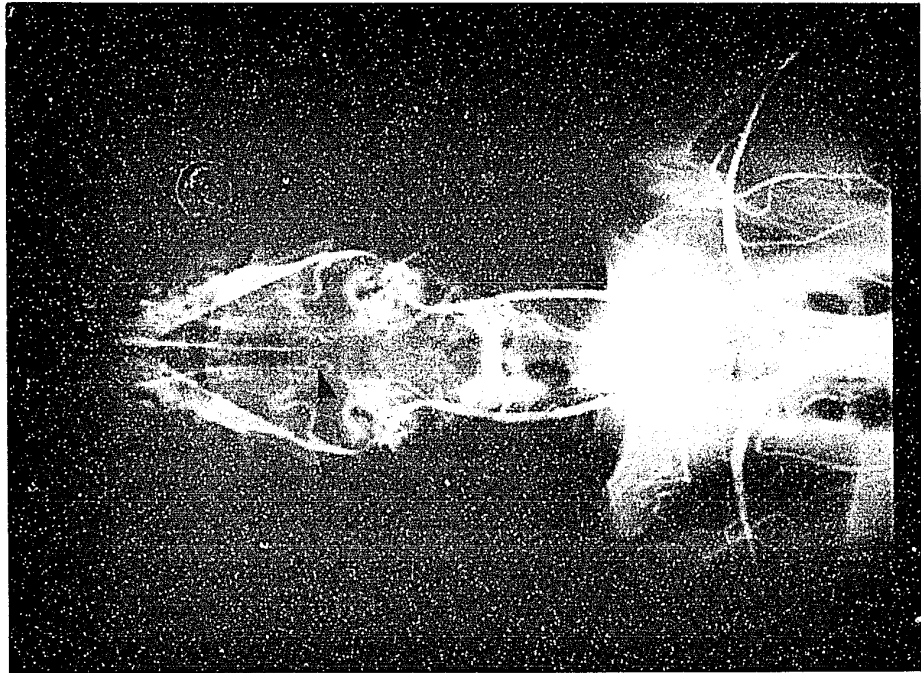
FIGURE 23

FIGURE 23: Cerebral angiography of a cat before and after SAH to demonstrate the spasm of major intracranial vessels after SAH. The arteries forming the circle of Willis (arrow) are well visualized during the filling with the radiopaque material (Hypaque 60%) before SAH (A). A second angiography performed 30 minutes after SAH (B) demonstrates the decreased diameter of the circle of Willis' vessels leading to poor radiographic visualization of these vessels.

67 D



B



A

the solution. The amount of injected solution does not change the final result, providing the solution is given at the same concentration, and kept in contact with the endothelial cells for the same length of time (Steinwall, 1968). The amount of the solution injected should depend on the injecting pressure and the amount lost in the extra-cranial vasculature. In fact, in our experiments, there was no appreciable difference in HgCl_2 volume injected into both groups. Therefore, despite any intracerebral flow change caused by spasm of major vessels and possible dilatation of the microcirculation after SAH, conditions were thought to be adequate for similar chemical reactions in both groups. The perfusion time was checked through the craniectomy by timing the blood being expelled from the pial vessels during the HgCl_2 perfusion.

The results of the HgCl_2 experiments indicate that the increased vascular permeability induced by intracarotid perfusion of a toxic agent is modified during the first hour following subarachnoid hemorrhage. From previous considerations, it seems reasonable to assume that the possible role played by vasospasm in preventing hypertensive-induced BBB breakdown through the interference with hemodynamic variables was ruled out. This second group of results (Experiment B) seems to indicate that the alterations responsible for such results take place at the level of the microcirculation. Theoretically, the explanation for these events would be two-fold: there could be either a direct interaction of blood substances with the metabolism of endothelial

cells, or this interaction would be mediated by a previous interaction of those substances with pre-capillary vessels, which would then lead to a secondary repercussion on the endothelial responses.

a. Primary Interaction of Blood Substances with Endothelial Cells

There is evidence suggesting that the BBB response to damaging agents is modified by certain chemical agents (Dinsdale et al., 1976; Flamm, 1976). The explanation for these phenomena is not yet known. For instance, Dinsdale et al., (1976) failed to find BBB breakdown after acute hypertension in adrenalectomized rats. However, the BBB leakage would take place if the adrenalectomized animals were previously injected with physiological doses of glucocorticoids. Another example is the action of dexamethasone in preventing the damage to endothelial cells caused by X-ray exposure (Blomstrand et al., 1975b). In our experiment, blood substances or possible products liberated during clotting in the subarachnoid space could have interacted with the endothelial cells to prevent HgCl_2 action. The possible selective barrier properties for blood substances by the pial-glial membrane can be speculated as a factor to determine which substances would be allowed to diffuse into the brain parenchyma and interact with the vessel wall. It is known that the membranes surrounding the CNS represent a barrier to certain substances and are permeable to others. For instance, the pial lining is impermeable to proteins injected in the subarachnoid

compartment (Klatzo et al., 1964) but not to amino acids (Lajtha and Toth, 1961) and electrolytes (Pape and Katzman, 1972; Cserr, 1974). The route followed by a substance after crossing the pial membrane will depend on its chemical nature. It can be taken up by the brain cells or travel through the ECS of the brain, cross the vessel walls and reach the blood circulation (Lajtha and Toth, 1961; Fenstermacher et al., 1970).

It has been shown that several substances, when applied to the pial surface, permeate through the pia-glia membrane and the brain tissue and interact chemically with the BBB structures (Rapoport et al., 1972; 1973). It has been also shown that certain conditions, such as ischemia, cause swelling of the endothelial cells and delayed response to BBB damaging agents (Hossmann and Olsson, 1971a; Leaf, 1973; reviewed by Rapoport, 1976a). These conditions are thought to inhibit the metabolism of the endothelial cells. There is clinical and experimental evidence indicating that SAH causes a general depression of brain metabolic processes (Parkes and James, 1971; Fein, 1975; 1976). The endothelial cell participation in this phenomenon is just speculative. This possible metabolic inhibition by blood could account for our results. There is no information available in the literature regarding the morphological state of endothelial cells following SAH.

It was initially thought that serotonin, which is presumably released during blood clotting in the subarachnoid space, could cause increased cerebrovascular permeability after SAH, or perhaps only an

increased susceptibility of the BBB to damaging agents. Our results did not support this hypothesis. Three different explanations could account for the discrepancy. The first possibility involves a dose related phenomenon: Westergaard (1975) found that the BBB breakdown was variable for different animals injected with the same dose. In addition, animals injected with the lowest (50 ug) or the highest (800 ug) dose could fail to respond or present BBB leakage. Moreover, the intensity of the leakage was not dose-related. The CSF serotonin levels after SAH are not known. Presumably, the levels are lower than those used by Westergaard. This presumption is based on the estimation of blood and platelet levels (Contractor, 1964; Zervas et al., 1973). Authors have worked with values around 10 ug in studies regarding possible levels following SAH (Raynor et al., 1961; Hashi et al., 1972a). The second possibility refers to the possible interaction of serotonin with other blood substances which would antagonize the specific action of serotonin on the endothelial cells. A third factor that could account for the difference between ours and Westergaard's results could be the injection site. He has injected serotonin intraventricularly while we have injected blood into the subarachnoid compartment.

b. Primary Action of Blood Substances on the Precapillary Vessel Reactivity

Another hypothesis to explain our results deals with the possibility that blood or clotting products could be affecting primarily the precapillary vessel reactivity and, therefore, causing secondary

changes in the endothelial cells. This hypothesis is based on clinical and experimental findings. Focal ischemic areas are frequently seen during autopsies in the cortex of patients who died after SAH not associated with the presence of vasospasm of major vessels (Birse and Tom, 1960; Crompton, 1964a). Smith (1963) has stressed the curious selectivity of ischemic changes for the cortex. She suggested the possibility of anomalous reactivity of peripheral small arteries to vasospastic substance liberated during the clotting process. Autopsied specimens were found to display circumscribed patchy areas of ischemia which were not seen in the white and deep grey matter. Asano and Sano (1977) described a curious phenomenon taking place after experimental SAH in dogs: multifocal areas of circulatory defects were found localized in the cortical and central grey matter. These areas failed to stain after intravascular perfusion of carbon black. It is interesting to note that the CBF was enhanced in the same animals after SAH. It was postulated that the paradoxical findings of increased CBF along with non-perfused areas were due to the low pressure head in arterial boundary zones and long penetrating arteries. Supposedly those areas would not participate in the post-SAH "reactive hyperemia". As far as the circulatory defects in the cortical grey matter are concerned, Asano and Sano's results seem to corroborate Smith's autopsy findings. If both results were due to the diffusion of blood substances into the brain tissue, and not to abrupt changes in the ICP, then a similar phenomenon could

be playing a role in the results found in our investigation, regarding the BBB response to HgCl_2 after SAH. The non-perfused areas could be preventing the contact of HgCl_2 with the capillary endothelial cells, or the localized patchy cortical ischemia could be responsible for the absence of lesions after HgCl_2 perfusion. It is worth noticing that in one animal in Group B with BBB breakdown after SAH, the dye was located mostly in the deep structures (basal ganglia, thalamus, hypothalamus and optic tract) in contrast to the usual cortical BBB lesions displayed by the control group (Fig. 21f).

C. ISCHEMIA

It has been demonstrated that immediately after the release of total cerebral ischemia, acute hypertension and HgCl_2 perfusion also fail to cause BBB breakdown, but the phenomenon only takes place when the ischemic insult is intense enough to cause EEG flattening and suppression of pyramidal electrical responses (Olsson and Hossman, 1970b; Hossman and Olsson, 1971b; 1972). It was later shown that the modified BBB response after ischemia was present only in the initial periods following release of total ischemia (Spatz et al., 1976). More prolonged observation, from one hour to one week, following ischemia release, revealed spontaneous BBB breakdown (Westergaard et al., 1976; Ito et al., 1976). The length of time required for BBB leakage following ischemia release was inversely proportional to the intensity (duration) of the ischemic injury. This was named "maturation phenomenon" and

has been demonstrated for metabolic and histological injury following brain ischemia (reviewed by Klatzo, 1975; Mrsulja et al., 1976). The stained lesions due to spontaneous BBB breakdown during the longer periods of time after total ischemia were specifically localized in the basal ganglia, hippocampus and, less intensely, in the cerebral cortex (Ito et al., 1976). Microscopic evaluation performed at the end of one hour following post-ischemic recirculation revealed that the intensity of BBB leakage was proportional to the duration of the ischemic insult (Westergaard et al., 1976). Under these circumstances, transport of proteins was demonstrated to depend only on increased vesicular transport by the endothelial cells, with no demonstrable alteration of the tight junctions.

In the present experiments, the association of common carotid ligation and SAH could be causing some degree of ischemic injury to the brain tissue, but it was not enough to cause major EEG changes. In no instance was EEG asymmetry seen following common carotid ligation. It seems that our results cannot be attributed to a degree of brain ischemia which is possibly present after SAH and carotid artery ligation in the present model. Previous work has demonstrated that a total ischemia, lasting for about 9 minutes and sufficient to abolish EEG and pyramidal responses, is required to prevent BBB breakdown caused by HgCl_2 (Hossmann and Olsson, 1972). Lesser degree of ischemic injury does not interfere with the BBB response (Rapoport et al., 1972;

1976b). The mechanisms responsible for BBB behaviour after total ischemia have not been clarified yet. The concomitant endothelial cells swelling was believed to play a role in the pathophysiology (Hossmann and Olsson, 1971), but the alteration underlying this cell swelling has not been clarified yet. The similarity of results regarding BBB behaviour after SAH and total ischemia, plus the close resemblance of lesions seen during longer observations after ischemic release (Ito et al., 1976) and those shown by the animal in Group B with BBB breakdown suggest a common mechanism underlying the BBB dysfunction displayed by the two pathological entities.

D. BRAIN EDEMA

The BBB breakdown which takes place after some pathological circumstances, such as X-ray irradiation (Miguel and Haymaker, 1965; Blomstrand et al., 1975a), ischemia (Ito et al., 1976) and infarct (O'Brien et al., 1974) is a late event (hours or days). Some of the post-SAH alterations, potentially related with BBB damage are also late changes. For instance, the aseptic inflammatory reaction of sub-pial vessels reaches its maximum in 48-72 hours after experimental SAH (Hammes, 1944). Most likely, an assessment during the first hour after SAH would fail to reveal a possible BBB breakdown eventually caused by such inflammatory process. Similarly, the absence of increased vascular permeability to proteins during the first hour after SAH does not rule out a vasogenic mechanism for the formation of brain edema during the

course of a SAH, because (1) BBB breakdown could still happen later in the course of a SAH, or (2) brain staining with Evans blue was not measuring the BBB to other elements, for instance, the sodium exchange rates between blood and brain (Lajtha, 1962; Cervos-Navarro et al., 1976). In a brain infarct for instance, edema formation precedes the vascular leakage of proteins. This is due to the leakage of small particles such as electrolytes (O'Brien et al., 1974). A similar phenomenon could take place after SAH.

The results regarding BBB breakdown during the acute stage following SAH cannot be extrapolated to the chronic stage. The present investigation is the initial step to approach cerebral vascular permeability after SAH. Further investigation with longer periods of observation will be carried out.

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SUMMARY

The present investigation aimed at studying the state of cerebrovascular permeability to proteins during the acute stage after SAH. It was believed that the results could help to elucidate some yet unclear aspects of the clinical picture which develops after a SAH.

The experimental procedures were designed to investigate the possible BBB alterations in the presence of SAH and the possible interaction of SAH with some BBB damaging agents. The experiments were carried out in anesthetized cats, kept as close as possible to physiological conditions. For this purpose, they were monitored, during the entire procedure, for blood pressure, temperature, respiration and acid-base equilibrium. SAH was simulated by cisternal injection of autologous blood. The BBB breakdown was traced with Evans blue-protein. The brain was further examined under the fluorescence microscope for confirmation of macroscopic findings and search of possible BBB breakdown not seen under gross examination. The animals were divided into two experimental groups since different methods were used in each experiment.

The first experimental group was composed of animals which underwent SAH alone and animals which had a pharmacologically-induced acute arterial hypertension following the SAH. In addition, a control group was exposed only to acute hypertension. This control group displayed BBB breakdown according to previous descriptions.

Animals which had a SAH did not show any BBB breakdown. Furthermore, the SAH prevented the BBB caused by acute hypertension. In an attempt to differentiate between hemodynamic and biochemical alterations caused by SAH which would be responsible for the results found with that model, a second experimental model was designed to exclude the interference that hemodynamic factors could be playing in the first model. Thus, a second experimental group was used to investigate the interaction between SAH and the intracarotid injection of HgCl_2 . Control animal had a controlled carotid perfusion of HgCl_2 according to previous descriptions. Ipsilateral BBB breakdown was observed in this control group. The large majority of animals which had the HgCl_2 perfusion performed after a SAH failed to display any BBB breakdown. These results seemed to indicate that the prevention of BBB breakdown observed after SAH was due to a biochemical mechanism involving the interaction between blood substances and BBB structures.