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Intracranial Hypertension and Brain Oedema in Albino Rabbits

Part 1: Experimental Models

By

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Summary

Three models of experimental cerebral oedema in rabbits are described, one producing vasogenic oedema with a cold lesion, the other producing a cytotoxic cerebral oedema with a metabolic inhibitor, 6-aminonicotinamide (6-ANA), and finally a model employing in the same animal both vasogenic and cytotoxic injuries. The following parameters were assessed: behaviour, EEG, intracranial pressure (ICP), cerebral elastance (Em), blood brain barrier integrity, brain water, electrolyte content, and volume change. Behaviour was normal in the cold lesion group, was abnormal following the administration of 6-ANA, and pronouncedly abnormal in animals with a combined lesion. Mean ICP ($PaCO_2$ 37 ± 42 torr) in the control group was 2.7 ± 2 torr, in the cold lesion group 8.4 ± 6, in the 6-ANA group it was 8.7 ± 4, and in the combined lesion group 15.8 ± 8 torr. Em for the control group was 2.6 ± 1.3 torr, in the cold lesion group it was 5.6 ± 4 torr, in the 6-ANA group it was 8.8 ± 5 torr, and in the combined lesion group it was 8.0 ± 4 torr. The 6-ANA group manifested oedema that involved primarily the grey matter. In the control animals grey matter water content was 79.99 ± 0.8%, and in the 6-ANA group it was 81.73 ± 0.9% ($P < 0.001$). A group had both grey and white matter water content measurements under the area of a sham lesion, and this was 79.2 ± 1.3% for the left hemisphere and 79.1 ± 1.3% for the right. Following a cold lesion of the left hemisphere, the water content was 81.85 ± 1% ($P < 0.005$), and 80.25 ± 1% ($P < 0.01$) in the unlesioned right hemisphere. In those animals with combined cold lesion and 6-ANA administration, the water content of the left hemisphere increased to 82.8 ± 1% ($P < 0.05$ from vasogenic oedema alone), and in the right hemisphere to 81.1 ± 1% ($P < 0.5$ from vasogenic oedema alone).

Keywords: Experimental brain oedema.

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Introduction

Cerebral oedema and increased intracranial pressure (ICP) and their management are of major concern in clinical practice [13, 28, 29]. In an attempt to study the physiopathology and resolution of cerebral oedema, two basically different types of brain oedema have been proposed in experimental work, one vasogenic and the other cytotoxic [24]. In the former, the primary injury is to the blood vessels, with a subsequent increase in cerebral vascular permeability allowing for passage of serum constituents into the extracellular space by hydrostatic pressure, and subsequent spread through the tissues because of bulk flow [37, 38, 40, 41, 45]. In the cytotoxic oedema active ion transport is impaired by interference with cell metabolism, with subsequent intracellular fluid uptake and swelling [24, 25]. Experimental brain oedema has been produced in many ways, such as by freezing the cortex through the intact dura [1, 4, 8, 11, 18, 26, 33, 36, 46, 48], implantations [43, 44], mechanical compression and distortion [15, 19, 34], administration of triethyl tin [32, 47], interruption of the blood brain barrier [24], and metabolic inhibitors [39].

Previous research in experimental brain oedema has focussed on a limited group of parameters in the same experimental animal. The rat has been used because of its low cost, making this model easily available for a large number of experiments. However because of its small size, the parameters available for studies have been water and electrolyte content of the brain, gross pathology, microscopy and, employing certain techniques, blood flow studies. For monitoring intracranial pressure, vascular pressures, and the other parameters simultaneously with those outlined for the rat model, larger animals are needed. The monkey, dog, and cat have been the preferred models. Because of cost a large series of such animals would be very expensive. The cat is the least costly, but the albino rabbit in most areas is usually less than one third the cost of the cat. It is also easier to handle.

We describe here our work with models of cerebral oedema and intracranial hypertension in albino rabbits, demonstrating the usefulness of the animal for such research. Examples of therapeutic applications of this model to clinical practice are given.

Materials and Methods

Cold Lesion Group

Albino rabbits weighing 2–3 kilograms were anaesthetized with intravenous sodium thiopentone (20 mg/kg) and positioned in a stereotactic devise. The scalp was incised under local anaesthesia (Lidocaine 1%), and the calvarium was ex-

posed. A 12.5 mm diameter circular trephine hole was made over the left parieto-occipital cortex with a standardized technique. A stainless steel probe (area 64 mm^2), previously equilibrated with a liquid nitrogen bath, was then applied to the intact dura for 90 seconds. Sham-operated animals received no freeze lesions. The skull defect was then closed by suturing the bone remnant into its original position, and the skin incision was sutured. When the lesion was made, 1 mg of 2% Evans blue per kg was administered intravenously, and 50 mg of ampicillin per kg was given intraperitoneally as antimicrobial prophylaxis. The animals were then returned to their cages, and were allowed food and water ad libitum.

The following day the animals were briefly reanaesthetized with sodium thiopentone, tracheostomized, and continuously ventilated with a small animal respirator (Harvard Apparatus Company, Inc., Millis, Massachusetts) with a mixture of oxygen (30%) and nitrous oxide (70%). The animals were then paralyzed with pancuronium (0.5 to 1 mg), and systemic arterial and venous catheterization were performed. Systemic arterial (SAP) and central venous pressure (CVP) were continuously monitored through the appropriate strain gauge transducers, and were recorded on a multi-channel polygraph (Gilson, Model CM-8; West Coast Science Co., Oakland, California). Arterial blood gases were maintained so that the $PaCO_2$ was in the range of 37–43 torr. Animals with mean systemic arterial pressures of less than 80 torr were discarded. CVP was noted in all animals to be between 1 and 8 torr. The animals were again positioned in this stereotactic device, and a 20-gauge catheter was inserted into the cisterna magna for continuous intracranial pressure recording and elastance measurements [21, 31, 35]. The intracranial pressure (ICP) zero reference point was the interaural line. Bilateral anterior and posterior screws were placed in the calvarium for electroencephalogram (EEG) recording.

Cold Lesion Group

The animals were divided into two groups. The first group was the sham-operated population; they were monitored until the $PaCO_2$ stabilized, and were sacrificed 45 minutes after stabilization of the $PaCO_2$. In the second group (cold lesion animals) we waited until 24 hours after the freeze lesion had been made, and then they were sacrificed 45 minutes after stabilization of the $PaCO_2$.

Prior to sacrifice a measurement of cerebral compliance $\Delta V/\Delta P$ [34] was made in some animals by the injection of a 0.1 ml bolus of sterile saline through the cisternal cannula [21, 31]. The peak pressure recorded after the injection was subtracted from the preinjection baseline ICP, and the value was computed. This value was used to express the relative elastance (1/compliance) in torr [34, 3]. The animals were sacrificed by intravenous cardiac air embolization. Rapid craniectomy with removal of the brain followed. The cerebral hemisphere were then isolated and weighed. Twe samples of brain tissue, each weighing 0.63 ± 0.18 (SD) g and including white and grey matter but excluding areas of lesion necrosis or haemorrhage, or both, were taken from the regions surrounding the lesion in the left hemisphere.

Homologous samples were systematically dissected from the contralateral hemisphere outside the area of visible Evans blue extravasation. We calculated the water content after drying the tissue at 110 °C constant temperature. Tissue sodium and potassium were determined by a modification of the method of Vernadakis and Woodbury [49]. Hemispheric volume change was calculated by the formula for brain swelling of Elliott and Jasper [11]:

$$\text{Volume per cent of tissue swelling} = \frac{\Delta H_2O\% \times 100}{DW\%}$$

Where $\Delta H_2O\%$ is the difference in percentage of water content between the sham group of animals and the animals with cold lesion, and DW is the dry weight of the cold oedema tissue in percentage.

Cerebral Oedema Produced by 6-Aminonicotinamide Group

A pilot study had previously been performed to serve as the optimal dosage for brain oedema in the albino rabbits [21], and it has been concluded that 120 mg/kg was the most appropriate dose. The 6-ANA in sterile saline was administered intraperitoneally in two equal daily dosages. Behaviour was observed on a daily basis, and on Day 3 the physiological measurements were determined and the animal sacrificed. On that day the rabbits were anaesthetized, tracheostomized, paralysed, and artifically ventilated, as previously described for the cold lesion group. The animals were monitored in the same fashion with a cisternal needle for ICP, four screw electrodes for EEG, arterial and venous femoral lines, and the $PaCO_2$ was adjusted for 37–43 torr. No animals with a systemic arterial pressure of less than 80 torr were included in this study.

Following stabilization of $PaCO_2$, measurement of elastance and recording of ICP, SAP, and CVP values, the animals were sacrificed, and their brains were promptly removed as described previously. However, in these animals the pia arachnoid was dissected and samples of grey matter and white matter were taken from each hemisphere, collected in independent vials, and then used for calculations of the water content, as previously described for the cold lesion group. No electrolyte determinations were performed in this group. Evans blue was administered on the day of the initial 6-ANA injection, and prior to sacrifice on Day 3.

Combined Lesion Group

A final group of animals were prepared as described for the cold lesion group, but following the lesion they received in a single bolus 120 mg of 6-ANA intraperitoneally. These animals were then studied 24 hours following the cold lesion in a similar fashion, as previously described for the other two groups. The samples of brain tissue from the left and right hemispheres following the physiological parameters, sacrifice, and extraction of the brain were obtained by the same technique as the cold lesion group. The water content was determined as well as the tissue electrolytes, as previously described. The extent of Evans blue extravasation was also determined for blood brain barrier integrity.

Results

Behaviour

Following the cold lesion (n = 228), the animals that survived until the following day demonstrated noticeably intact behaviour. There was, however, an overall mortality of 25% on the night following the lesion. In the 6-ANA group (n = 72), there was hypoactivity and paraplegia in 67%, and a 20% mortality was noted. In the group of animals that received both the cold lesion and the 6-ANA (n = 46) there was severe hypoactivity, and paraplegia was noted in 85% of the animals, with a 30% mortality on the night of the lesion.

Gross Pathology

In the sham-operated animals, there was no extravasation of Evans blue, and no gross pathological changes were noted; in the cold lesion animals there was an area of haemorrhage and necrosis created by the injury, which averaged 13 mm in diameter at the cortical surface, and generally extended 3 to 4 mm into the underlying parenchyma. Evans blue extravasation was noted to extend within an appropriate 5 to 8 mm radius into the injured hemisphere beyond the area of haemorrhage and necrosis. Rarely this area of extravasation crossed the midline by approximately 1–2 mm on the cortical surface.

Frequently Evans blue extended deep into the white matter of the right hemisphere for approximately 3 mm. Occasionally there was a light bluish hue in both lateral ventricles. In the animals with both cold lesion and 6-ANA the areas of haemorrhage and necrosis and of Evans blue extravasation on the cortical surface and in the deep white matter of both the left and right hemispheres were equivalent to those of the animals with cold lesions only.

EEG

In the control and sham-operated groups, fast activity was present over both hemispheres. In the animals with cold lesions only there was pronounced slowing, and high voltage waves were present over the lesioned left hemisphere. Normal fast activity was present on the unlesioned right side. These findings had been previously noted by Schaul *et al.* [42].

In the animals with 6-ANA alone there was a bilaterally generalized, almost flat, tracing in 67% of the animals; 33% had bilateral slow waves. In the animals with the combined insult of 6-ANA and cold injury there was pronounced slowing, and very poor activity was noted over the left hemisphere in 90% of the animals; the right hemisphere demonstrated an almost isoelectric recording in 40%; 50% had diffuse slowing, and the remaining 10% were normal.

ICP

The mean ICP for the sham-operated animals was 2.65 ± 2.2 (SD) torr. In the cold lesion group mean ICP was 8.4 ± 6.3 torr, for the 6-ANA group 8.7 ± 4.3 torr, and for the combined cold lesion and 6-ANA group it was 15.8 ± 8 torr. The ICP difference between the experimental and the sham groups was statistically significant (Table 1).

Intracranial Elastance

The mean elastance measurement was 2.6 ± 1.3 (SD) torr in the sham-operated group, 5.6 ± 3.8 torr in the cold lesion group ($P < 0.05$ from sham), 8.8 ± 5.3 torr in the 6-ANA group ($P < 0.005$ from sham), and in the 6-ANA and cold lesion group 8.0 ± 3.7 torr ($P < 0.005$ from sham) (Table 1).

Table 1. *ICP and Elastance Measurements in 6-ANA *, Cold Lesion, and Combined Brain-Oedema Models in Rabbits*

Group	ICP	Elastance
Sham (n = 12)	2.7 ± 2.2 torr	2.6 ± 1.3 torr (n = 8)
6-ANA (n = 56)	8.7 ± 4.3 torr **	8.8 ± 5.3 torr **** (n = 16)
Cold lesion brain oedema (n = 76)	8.4 ± 6.3 torr **	5.6 ± 3.8 torr *** (n = 27)
Cold lesion and 6-ANA brain oedema (n = 39)	15.8 ± 8.0 torr ****	8.0 ± 3.7 torr **** (n = 9)

* 6-amionicotinamide.
** $p < 0.01$ from sham.
*** $p < 0.05$ from sham.
**** $p < 0.005$ from sham.

Table 2. *Comparison of Brain Oedema in the Rabbit by Vasogenic Oedema and Combined Oedema **

Group	Hemisphere	Brain water content %	Volume change %	Sodium content (mEq/kg/wet weight)	Potassium conten (mEq/kg/wet weight)
Sham (n = 16)	L	79.2 ± 1.5	—	60.7 ± 4.3	105.9 ± 3.1
	R	79.1 ± 1.2	—	60.1 ± 4.3	103.4 ± 4.7
Vasogenic oedema only (n = 12)	L	81.85 ± 1.2	14.7	67.1 ± 8.9 *****	78.8 ± 8.7
	R	80.25 ± 1.1	6.0	60.7 ± 5.5	92.0 ± 8.7
Vasogenic and 6-ANA oedema (n = 12)	L	82.8 ± 1.1 ***	20.9	76.3 ± 4.3 ****	71.8 ± 7.0 ***
	R	81.1 ± 1.4 **	10.6	66.0 ± 7.6 ***	89.7 ± 6.3

* Left hemisphere cold lesion and intraperitoneal 6-aminonicotinamide (6-ANA).
** $p < 0.05$ from vasogenic oedema.
*** $p < 0.025$ from vasogenic oedema.
**** $p < 0.005$ from vasogenic oedema.
***** $p < 0.025$ from sham.

Water Content

The mean hemispheric water content for the sham-operated animals was 79.2 ± 1.3% (SD) for the left hemisphere, and 79.1 ± 1.2% for the right hemisphere. In the cold lesion group the left hemisphere water content increased to 81.9 ± 1.2% ($P < 0.005$ from sham), and the right increased to 80.3 ± 1.1% ($P < 0.01$ from sham) (Table 2).

The mean water content of the grey matter in the control animals was 79.99 ± 0.77%, and in the 6-ANA treated group it was 81.7 ± 0.9% ($P < 0.001$ for the difference from control values). For the white matter it was 71.3 ± 2.0 in the controls and 71.9 ± 3.9 in the 6-ANA treated group (Table 3). The wide standard deviation is most likely a consequence of the small size of each sample.

Hemispheric Volume Change

In the cold lesion animals, compared to the sham-operated group, there was an increase of 14.7% in the volume of the left hemisphere and 6.0% in the volume of the right hemisphere. In the 6-ANA

Table 3. *Per Cent Water Content of Grey and White Matter in Control and in 6-Aminonicotinamide Treated Animals Mean and Standard Deviations*

Group	Gray matter (%)	Volume change * (%)	White matter (%)
Controls (n 12)	79.99 ± 0.77	—	71.94 ± 3.9
6-ANA (n 16)	81.73 ± 0.9 *	9.5	71.32 ± 2.1

* From control value (see Materials and Methods).
** $p < 0.001$ for difference from controls.

models there was an increase of 9.5% in the volume of the grey matter when compared to the control animals (Table 3). When comparing the volume change in the combined cold lesion and 6-ANA group with the sham group, there was an increase of 20.9% in the volume of the left hemisphere, and 10.6% in the volume of the right one (Table 2).

Electrolyte Changes

The concentration of sodium in the sham-operated group was 60.7 ± 4.3 (SD) mEq/kg fresh weight for the left hemisphere and 60.1 ± 4.3 for the right. The potassium content in the sham-operated group was 105.9 ± 3.1 mEq/kg weight for the left hemisphere and 103.4 ± 4.7 for the right hemisphere. The electrolyte changes in the other groups are summarized in Table 2.

Discussion

Baethmann *et al.*, in search of a model of cytotoxic oedema, had administered 6-ANA to rats and determined that there was an increase in sodium and water in brain tissue, whereas no changes were found in the myocardium of the animals [2]. Subsequently, Baethmann and Van Harreveld [3] measured electrical conductivity in the cerebral cortex of rats and found a decrease of 25% in those that had received 6-ANA, indicating a reduction of the extracellular space. Electron micrographs of the cerebellar vermis of these animals revealed an enlargement of the glial component of the tissue and a moderate extracellular space. The author sugegsted that the possible mechanism of action of 6-ANA was competitive inhibition of NADH electron receptor sites and that this decreased the tissue ATP. This in turn would lead to energy deficit, interference with sodium potassium pumping, and ultimate cell swelling due to the influx of sodium and escape of potassium. These authors had also shown that the simultaneous administration of nicotinic acid and theophylline, or nicotinic acid alon with 6-ANA, inhibited the cell swelling [2].

We felt that this model was a good example of a severe metabolic insult, and that it could have as a counterpart clinical metabolic encephalopathies. In these intracranial hypertension and cerebral oedema may be a significant problem in patient management [13, 22]. For that end we previously analyzed the response of this form of cerebral oedema in rabbits to furosemide, mannitol, and dexamethasone [21]. It was of interest that both diuretics reduced the water content in the gray matter of the oedematous brain at the moment of ICP nadir (45 minutes) in a significant fashion ($P < 0.001$). Dexamethasone, on the other hand, did not affect brain water content significantly, reduced ICP minimally, but did improve intracranial compliance, EEG, and behaviour [21]. These results tend to lend support to the theory that steroids have an affect on the oedematous brain out of proportion to the reduction of tissue water content. This same model has also been useful to analyze the differing response of pharmacological preparations of steroids in brain oedema [20]. Further experience may permit a better understanding of the mechanics of the response to therapy in cytotoxic brain oedema.

It is felt that the main event in the formation of cerebral oedema following a cold lesion is some degree of blood-brain barrier damage, and the fluid escaping from the damaged vessels has a composition closely related to plasma [25, 27]. The peak of oedema occurs 24 hours following the cold lesion [16]. The extravasation is confined to the injured area, and the blood vessels outside this area do not show an increased permeability [9, 25]. The driving force for the extravasation

of fluid seems to be the transmural capillary pressure between the intraluminal vascular compartment and the interstitial tissue pressure [12, 27, 38, 40]. Acute elevation of the blood pressure results in an increase in local oedema formation, and lowering in the blood pressure produces a marked inhibition of oedema development [27]; likewise, variations of vascular tone affect oedema formation markedly [14]. The spreading of oedema to areas of the brain more remote from the site of insult has been demonstrated to be primarily due to a mechanism of bulk flow [40] through a significantly enlarged extracellular space in the region of the lesion initially, and subsequently progressively involving areas away from the site [40]. It seems that the progressive enlargement of the extracellular space and progression of the oedema front is related to local increases of interstitial fluid pressure at the site of the oedema front (propelled by the transcapillary pressure head) and the resistance of the local tissues of the oedema [40]. A persistent spread of local interstitial pressure change is followed by expansion of the extracellular space and subsequent oedema [12, 40]. Oedema clearance will proceed by venous transcapillary reabsorption of fluid, glial uptake [6 10], or transependymal passage into the CSF pathway [40].

It is felt by Reulen [38] that vasogenic brain oedema is probably the most common form of brain oedema in clinical situations. Therapeutic modalities in this model would therefore serve to attempt to clarify the best approach in the management of clinical brain oedema. It is not difficult to understand on the basis of the physiopathology of the vasogenic oedema that therapy should have as its objective the interruption of the various stages of the disease [40]. Stabilization of the blood-brain barrier, control of the arteriolar vasoconstrictor tone, and stabilization of systemic blood pressure will eliminate the driving force. Mobilization and elimination of the oedematous interstitial fluid will help resolve existing oedema. Improvement of neuronal and glial function in the oedematous area may further improve the water and electrolyte balance between the intra- and extracellular space, as may be seen in the cytotoxic forms of oedema [21]. Control of ICP may improve regional cerebral blood flow and thus not only aid in capillary clearance of oedema fluid, but also improve cellular metabolism [32].

Such an approach has been previously initiated [16] by combining a barbiturate with a diuretic and a steroid, comparing this combination with single agent therapy; a prompt reduction of ICP was obtained with all therapeutic modalities save for acetazolamide. These responses were analyzed in reference to brain water and electrolyte content, as well as other parameters. It was felt that the

gradient of ICP fall was significant for that particular series, but that a higher initial ICP would be useful to magnify not only the ICP changes but the water and electrolyte content as well.

In both the 6-ANA and cold lesion groups a significant amount of brain oedema was obtained with the corresponding increase in volume (Table 2 and 3). The change was reflected in an increase in ICP and elastance (Table 1). It has been previously demonstrated by Reulen *et al.* that brain oedema spread can be significantly impeded by inducing a cytotoxic type of oedema [40]. Thus, by administering hexachlorophene and thus creating an intracellular oedema and then producing a cold lesion the authors demonstrated a 30% reduction in the amount of oedema when compared to cold lesion alone, and the area of oedema spread was smaller following hexachlorophene [40]. It was our desire to have an experimental group of rabbits that would demonstrate a higher ICP and elastance than that found in the 6-ANA and cold lesion groups by themselves. Because of this both lesions were made simultaneously in a third group. Indeed, the combination produced higher ICP and elastance, and this seems to be due to a significant increase in the water content of both hemispheres when compared to the cold lesion alone (Table 2). This is reflected in an increase of hemispheric volume from 14.7% in the left hemisphere to 20.9%, and from 6 to 10.6% in the right (Table 2).

Though we would have expected a decrease of cold induced oedema when superimposing this primarily intracellular oedema with 6-ANA in a similar fashion as that previously described by Reulen *et al.* [40] for hexachlorophene, this did not occur. The timing of the lesions may explain the discrepancy with the hexachlorophene group. In the present series both lesions were performed simultaneously, and therefore the "intracellular" effect had not yet established an effective reduction of the extracellular space, so as to impede vasogenic oedema spread. The increased ICP and disturbance of regional cerebral blood flow could account for the poor behaviour and higher mortality [7, 21–23, 28] in this combined lesion group.

We conclude that the albino rabbit is a practical and inexpensive model to investigate therapy for intracranial hypertension and the multiple parameters of the disease process. Its problems relate to a sensitivity to anaesthetics that may account for a high experimental mortality unil he investigator masters the animal model. For those most interested in white matter oedema there is a problem in this model, when compared to the cat; the rabbit does have a smaller volume of white matter. However we feel that this did not significantly affect our experimental work.

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