CEREBROSPINAL FLUID ENZYMES
IN ACUTE BRAIN INJURY

To my mother
To the memory of my father

This thesis is based upon the following papers:

Maas A.I.R. Cerebrospinal fluid enzymes in acute brain injury

I.   Dynamics of changes in CSF enzyme activity after acute experimental brain injury (1977).
      Journal of Neurology, Neurosurgery and Psychiatry, (in press).*

II.   Relation of CSF enzyme activity to the extent of brain injury (1977).
      Journal of Neurology, Neurosurgery and Psychiatry, (in press).*

III. Effect of hypotension on the increase of CSF enzyme activity after cold injuries in cats (1977).
      Journal of Neurology, Neurosurgery and Psychiatry (in press)

Maas A.I.R., de Lange S.A. Effect of moderate arterial hypotension combined with repeated withdrawal of cerebrospinal fluid on the development of increased ventricular fluid pressure after cold injuries in cats (1977).
Clinical Neurology and Neurosurgery 79: 91-106.

* These papers are presented as they were submitted to the Journal of Neurology, Neurosurgery and Psychiatry for publication. Before acceptance they have been condensed by the Editor.

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ABBREVIATIONS

ChE	Pseudocholinesterase
CPK	Creatine Phosphokinase
CSF	Cerebrospinal fluid
D	Diffusion coefficient
ECF	Extracellular fluid of the brain
α-HBDH	α-Hydroxybutyrate dehydrogenase
GOT	Glumatic oxalacetic transaminase
GPT	Glumatic pyruvic transaminase
LDH	Lactate dehydrogenase
MABP	Mean arterial blood pressure
$t_{\overline{D}}$	Mean transit time
VFP	Ventricular Fluid Pressure

CHAPTER 1

1.1. Prognosis in severe brain injury

> *One ought to know that on the one hand pleasure, joy, laughter and games, and on the other hand grief, sorrow, discontent and dissatisfaction arise only from the brain.*
>
> On the Sacred Disease, Hippocrates

Severe brain injury is a major cause of death, especially in young men. In 1972, over 20% of all deaths occurring in England and Wales in men aged 15-25 years were due to head injury (Field, 1976).

The mortality rate after severe brain injuries is high. Jennett et al. (1977) reporting on a large international study comprising 700 patients describe a 53% mortality rate. In this study patients with brain injuries were studied in whom consciousness was suppressed for a period of at least six hours to a degree that inability to obey commands, to speak, or to open the eyes existed. Studies reported from different parts of Europe show a similar mortality rate (table 1).

Not only do severe brain injuries call a large death toll, but many of the patients who survive remain disabled, often for life. Of 65 patients who were admitted to the University Hospital of Rotterdam in the period 1974-1976 with severe brain injury and were still alive after 6 months, 24 (37%) were disabled at that time. Disability may be due to physical sequelae, to disturbances of mental function, which are especially common following head injuries, or to difficulties arising when social reintegration is attempted.

The potential outcome of the individual patient suffering from a severe brain injury is dependant on the site and extent of primary brain damage, as well as on the degree of secondary brain damage resulting from ischaemia, deranged cerebral metabolism, and other intracranial complications such as intracranial haema-

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Table 1

Mortality rate after severe brain injuries

Author(s)	Year of publication	Total number of patients	Mortality
Carlsson et al.	1968	496	35%
Heiskanen and Sipponen	1970	204	50%
Vapalahti and Troupp	1971	50	36%
Vigouroux et al.	1972	150	37%
Overgaard et al.	1973	136	44%
Pagni	1973	1091	52%
Frowein et al.	1975	830	57%
Pazzaglia et al.	1975	282	50%
Jennett et al.	1977	700	53%

toma or brain edema. The pre-traumatic condition and the presence of extra-cranial lesions must also be taken into account. Whether the potential outcome may be attained will depend on adequate therapeutic measures, rehabilitation facilities, and later on when social reintegration is attempted, on the strength and support of relatives and friends.

Establishing a reliable prognosis for the individual patient with severe brain injuries is notoriously difficult. Yet many clinical decisions are based on the clinician's assessment of the patient's chances of recovery.

Prospective studies searching for parameters which may have prognostic value as to the outcome in patients with severe brain injuries have been based on elementary clinical signs (Overgaard et al., 1973; Jennett et al., 1976), but also on more elaborate investigations such as measurement of intracranial pressure (Vapalahti and Troupp, 1971). Only a few studies have concentrated on estab-lishing a definite prognosis for the individual patient. Preliminary results of a large internationally conducted cooperative study indicate that a confident prediction based on elementary clinical signs alone may be made in 41% of severely brain injured patients within 24 hours after injury, and in 52-61% within 2-3 days (Jennett et al., 1976).

These results are very promising, yet also a need is felt for more objective parameters, especially for assessing the extent of brain damage, for this is indeed one of the main determinants of outcome. Objective parameters are also re-quired in order to compare results of various centres and to be able to evaluate the efficacy of different (new) methods of treatment.

An objective index for the extent of brain damage was sought.

In modern medicine enzyme determinations play an important part in the diagnosis and evaluation of diseases of many organs, for enzymes are part of the cellular proteins released into the extracellular space after tissue damage.

1.2. Enzyme determinations as index of tissue damage.

Generally speaking enzyme determinations provide a sensitive and objective index of tissue damage.

Since Ladue et al (1954) and Wroblewski and Ladue (1956a and b) demonstrated increased activity of the enzymes aspartate- and alanine aminotransferase (GOT and GPT) in the serum of patients with hepatic and cardiac disease, determination of these enzymes has become daily routine practice, so much so that in the Netherlands GOT and GPT determinations are — erroneously — termed "liver function tests".

The diagnosis of myocardial infarction is frequently made on determinations of the enzymes Creatine Phosphokinase (CPK), α-Hydroxybutyric acid dehydrogenase (α-HBDH), Lactate dehydrogenase (LDH), and aspartate aminotransferase (GOT) (Ladue et al., 1954; Elliot and Wilkinson, 1961; Forster and Escher, 1961; Konttinen, 1961; Colombo et al., 1962; Konttinen and Halonen, 1962; Konttinen and Halonen, 1963; Vincent and Rappaport, 1965; Warburton et al., 1965; Smith, 1967; Sobel and Shell, 1972).

However serum enzyme determinations have proved to be of only limited value in the diagnosis of intracranial pathology. Raised values of many enzymes have been demonstrated in the serum of patients with various neurological disorders (Acheson et al., 1965; Schiavone and Kaldos, 1965). Not all neurological patients have raised serum enzyme levels.

In patients with severe brain injuries also, raised levels of many enzymes have been demonstrated in the serum (Dubo et al., 1967; Kaltiala et al., 1968; Lindblom and Aberg, 1972; Rabow, 1972; Klun, 1974; Thomas and Rowan, 1976). However, studies attempting to relate the height or duration of raised serum enzyme levels to the outcome have offered conflicting results (table 2).

These conflicting results may in part be explained by the fact that many patients with severe brain injuries have also sustained bruises and trauma to skeletal muscle, which may in itself cause a considerable increase of serum enzyme activity. Dubo et al. (1967) demonstrated with isoenzyme studies that the observed elevation of CPK activity in the serum after brain trauma was mainly the result of extracerebral damage; Kaltiala et al. (1968) observed a

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Table 2

Studies reporting on the relation between serum enzyme activity and outcome
of severe brain injury

good correlation	some correlation	no correlation
Rabow (1972)	Rabow, Hebbe and Liedén (1971)	Dubo et al. (1967)
	· Klun (1974)	Kaltiala et al. (1968)
	Thomas and Rowan (1976)	Lindblom and Aberg (1972)

better correlation of serum enzyme elevation with peripheral contusions than
with the clinical severity of brain trauma.

*Serum enzyme analysis has not been shown to be of great value in the
diagnosis or assessment of intracranial pathology.*

Little result was expected of further investigations on the value of serum
enzyme determinations for assessing the extent of brain damage. It was con-
sidered that a more specific line of research should be adopted, either by
undertaking serum isoenzyme studies, or by focussing attention on the other
fluid with which the brain is in intimate contact: The Cerebrospinal Fluid (CSF).
Results of recent serum isoenzyme studies do appear promising (Lindblom
and Aberg, 1972; Rabow, 1972; Somer et al., 1975; Thomas and Rowan, 1976).
Our interest was concentrated on the CSF.

1.3. CSF enzyme levels indicative of the extent of brain injury?

It was thought to be worthwhile to investigate whether *CSF* enzyme levels
would be more indicative of the extent of brain injury than *serum* enzyme levels
on the following considerations:
The CSF is shielded from the blood by the presence of the blood − CSF and
blood − brain barriers. These prevent passage of large molecules − as enzymes
are − from blood to CSF (Davson, 1970). Consequently the height of CSF
enzyme activity may be expected to be relatively independant of serum enzyme
activity, and thus little influenced by the presence of extracranial lesions.
The volume of CSF (in man 140 ml.; Davson, 1970) is considerably less than
the total blood volume, so increase in enzyme activity after brain injury may be
expected to be much more marked in the CSF than in the serum, and thus more
sensitive and accurate.

Go et al. (1976) have demonstrated that after brain injury enzymes are released from damaged or necrotic brain cells into the Extracellular fluid of the brain (ECF). Hypothetically the amount of enzymes released may be expected to be related to the extent of tissue damage. Enzymes released into the ECF need to be transported away from there, eventually into the blood. Transport from healthy brain tissue directly into the blood stream is improbable, because of the presence of the blood — brain barrier. In injured areas of the brain, however, the blood — brain barrier is damaged, and it is conceivable that enzymes may then enter into the blood directly from the ECF. The importance of this for removal of enzymes from the ECF of injured brain tissue is not known, but the low value of serum enzyme determinations in patients with brain injuries may be considered evidence that it is not functionally of great importance.

It was considered not unlikely that transport would be towards the CSF, and that CSF enzyme levels might consequently show some correlation to the extent of brain injury. This hypothesis is based on recent insight into the close relation between CSF and brain ECF, on the concept of bulk flow of ECF towards the CSF, and on the possibility that the CSF may function as the lymph of the brain (Milhorat, 1975; Editorial The Lancet, 1975). Interest in the CSF was further initiated by the realisation that analysis of various CSF parameters has been shown to be of value in assessing brain metabolism and condition (*CSF acid base status*, Gordan and Rossanda, 1968; Katsurada et al., 1969; Zupping, 1970; Gordon, 1971; King et al., 1974; *CSF lactate and pyruvate*, Salaymeh et al., 1971; Schnabert and Schubert, 1974; Crockard and Taylor, 1972; *Metabolites of neurotransmittors*, Johansson and Roos, 1965; Moir et al., 1970).

The aspects of CSF physiology, which are particularly relevant to our interest, will now be considered before further introducing the studies investigating changes in CSF enzyme activity after brain injuries.

THE CEREBROSPINAL FLUID

*Thus we see that the CSF, long regarded as
little more than a watery cushion for the brain,
is a dynamic and circulating medium, whose
flowing characteristics and biological functions
justify its description as:*
"The third circulation".

Th. Milhorat (1975)

1.4.1. Relation of CSF to Brain Extracellular Fluid

*Present opinion is that the CSF is in direct communication with the
extracellular fluid of the brain (ECF).*

The interface between the CSF and the ECF is formed in the ventricles by the
ependyma, and in the subarachnoid space by the pia mater.

The junctions of ependymal cells have been shown to be formed by "gap
junctions", between which intercellular clefts exist, that permit passage of
molecules as large as Ferritin (mol. mass 400.000) (Brightman and Reese, 1969).
Passage of horse radish peroxidase (mol. mass 43.000) and microperoxidase
(mol. mass 2000) has also been demonstrated to occur relatively freely between
ependymal cells from ECF to CSF (Brightman and Reese, 1967, 1970; Bright-
man et al., 1970). Klatzo et al. (1962, 1964) have demonstrated uptake of
fluorescent protein by the ependymal cells after ventriculocisternal perfusion
with fluoresceine labelled protein. Fluoresceine was not taken up by the subpial
tissue when the subarachnoid space was perfused.

These studies indicate that a relatively free movement of large molecules may
occur over the ependyma.

Restriction of passage of substances between ependymal cells will probably be
caused by the limitations imposed by the diffusion process, as well as by the fact
that substances diffused into the ECF cannot be taken up by the blood directly
owing to the presence of the blood brain barrier (Davson, 1970).

Whether large molecules may move across the pia mater is still controversial.
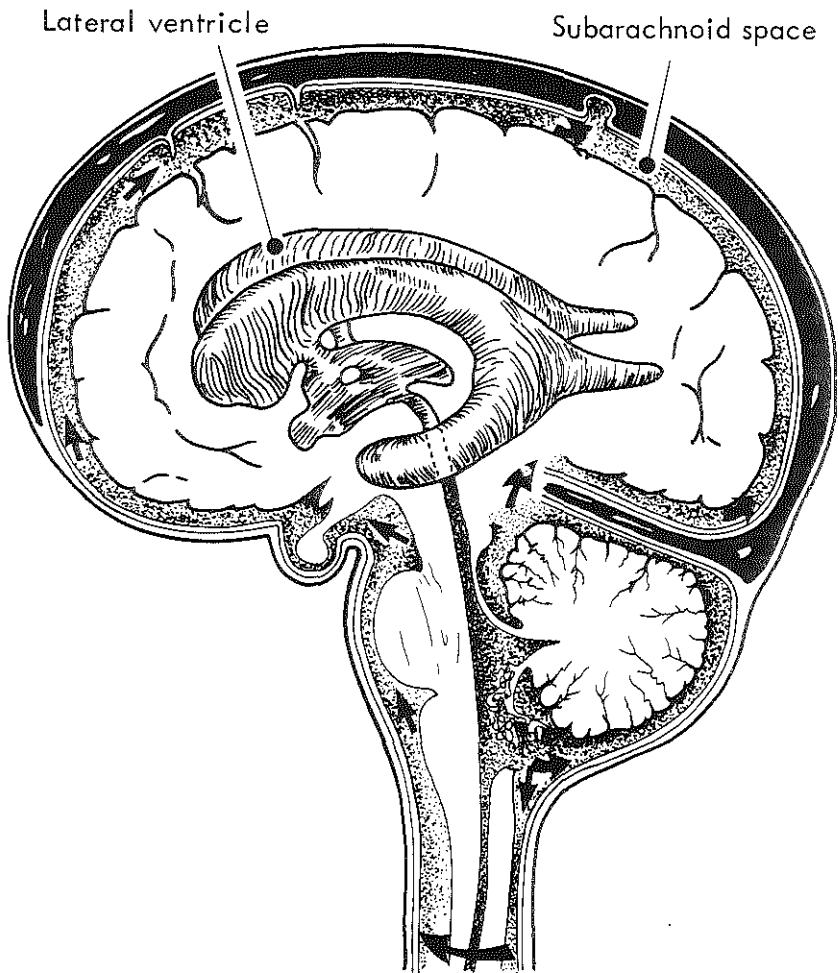
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Lateral ventricle   Subarachnoid space

Fig. 1. The Cerebrospinal fluid system.

1.4.2. Site of CSF production – bulk flow of ECF

The CSF is formed mainly in the brain ventricles (Cserr, 1971; Katzman and Pappius, 1973; Milhorat, 1975). Recent data suggest a possibility of production and resorption of CSF occurring also in the spinal region (Sato et al., 1972). This is not relevant to the studies described in this thesis and will therefore not be discussed.

For a long time the view has been held that the choroid plexus was the sole site of CSF production. Few investigators questioned the classic experiments of Dandy. In these studies Dandy (1919) blocked the foramina of Monro bilaterally and removed the choroid plexus on one side only. An enlarged ventricle resulted on the side that still contained choroid plexus, whilst the plexectomized ventricle collapsed. This was considered irrefutable evidence that the choroid plexus was the sole source of CSF.

These experiments however were only successful in one or two cases, and no adequate control studies were conducted. Nearly 15 years later Flexner (1933) argued that leakage of CSF through the operative wound was by no means inconceivable. Hassin et al. (1937) emphasized that considerable scar formation may occur after plexectomy, and that this scar formation could prevent the ventricle from expanding. Repeating Dandy's experiments, Hassin et al. (1937) were unable to duplicate the results.

Recently Milhorat (1969) also failed to do so. Milhorat demonstrated that after blocking the foramina of Monro and performing plexectomy, marked dilatation of the ventricle resulted within three weeks, thereby indicating that "hydrocephalus can occur rapidly and progressively in the plexectomized ventricular system and that the choroid plexus is not essential, either as a source of ventricular fluid or as a pulsatile mechanism for expanding the ventricles".

Additional evidence for the existence of extrachoroidal CSF production has been afforded by experiments utilising the perfusion technique of isolated ependyma, or by experiments in which ventriculo-cisternal perfusion was performed after plexectomy.

Bering and Sato (1963) estimated that 40% of the CSF formed in dogs is from an extrachoroidal site.

Pollay and Curl (1967) perfused the isolated aqueduct in rabbits and calculated that approximately 30% of the CSF is formed by secretion over the ventricular ependyma.

Milhorat, Hammock et al. (1971) performed bilateral plexectomy in monkeys and determined the rates of CSF formation after obstruction of the fourth ventricle. After correcting for the contribution of the third ventricle they estimated that the total formation of CSF was still 60 to 70% of normal.

On basis of these summarized results it seems probable that a major part of the CSF, probably 30 to 40%, may be produced as extrachoroidal sites, at least under the pathologic circumstances described.

The most probable source of extrachoroidal CSF is the brain ECF. Trans-ependymal flow of ECF has been postulated.

Definite evidence for bulk flow of ECF towards the CSF in the normal situation has not yet been provided, but Cserr and Ostrach (1974) have shown that Blue Dextran 2000 injected into the caudate nucleus of rats is transported towards the CSF.

Reulen et al. (1977) have provided strong evidence for bulk flow of edema fluid towards the CSF when vasogenic brain edema is developing.

CHAPTER 2

2.1. Introduction to experimental studies

On basis of the data presented in the previous chapter on the close relation between the CSF and the brain ECF, it seemed likely that the CSF composition may reflect changes in ECF contents.

It was hoped that the CSF enzyme activity would be indicative of the extent of brain damage, and consequently be of prognostic value in patients with severe brain injuries.

The results of preliminary clinical studies however, were not quite as promising as had been hoped for (see Chapter 3.1.). It was then decided to suspend clinical studies until the potential value of CSF enzyme levels as a parameter for the extent of brain damage had been established in the experimental situation.

This was decided because obtaining specimens of spinal fluid from patients with severe brain injuries via lumbar or cisternal taps is not without risk. These patients are prone to develop increased intracranial pressure, and withdrawing CSF from below the tentorium may provoke the formation of intracranial pressure gradients, and promote the development of brain stem herniation. Placement of ventricular catheters which would permit sampling ventricular CSF is not routine for patients with severe brain injuries in the Neurosurgical Department from which these studies originated.

The aim of the studies to be described in this thesis is to investigate in the experimental situation the value of CSF enzyme levels in the assessment of the extent of brain damage.

A model for producing experimental brain injuries was sought and chosen. This model had to meet the following demands:
1. the injuries produced must be reproducable, and of standardized severity.
2. Induction of different, but standardized degrees of injury must be possible.

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3. The resulting injuries must be comparable to those seen in the clinical situation.

Various methods were contemplated.

Experimental brain injury can be produced by banging the animals head, or dropping standardized weights on the skull from a specified height. Apart from being somewhat brutish, these methods have the disadvantage that varying degrees of actual brain damage are produced, as large interindividual variations can occur in the thickness of the skull.

Gently abrading or scraping the cortex with blunt instruments (Smith et al., 1960) is neither reproducable, nor is it possible to produce injuries of standardized severity.

One of the most widely used methods for producing cerebral injury and edema is focal freezing of the cortex (Clasen et al., 1953; Klatzo et al., 1958; Pappius and Gulati, 1963; Bakay and Haque, 1964; Beks et al., 1965). The inflicted damage can be titrated as surface, duration and depth of freezing can be standardized. The damage resulting from a cold injury consists of necrosis of brain tissue, and the development of cerebral edema remaining confined to the ipsilateral hemisphere. The edema formed is of the vasogenic type, which clinically is also the most common and important and the same as that which is seen in patients with severe brain trauma (Klatzo, 1972).

For these reasons induction of a cold lesion was chosen as method for producing brain injuries.

The relation between the extent of brain damage and the height of the CSF enzyme activity was studied. Experimental brain injuries were induced in cats. The experiments were conducted on cats as much knowledge on CSF physiology has been obtained from studies on cats, and as many studies on cold injury edema have also been conducted on cats.

Changes in the activities of various enzymes in the CSF were studied after induction of cold lesions. Which enzymes were studied and why, are items discussed in the following paragraph.

2.2. Choice of enzymes to study

The choice which enzymes to study is closely related to the clinical aim of these studies. It was considered that should CSF enzyme determinations ever be put to clinical use, the enzymes studied should, apart from being present in brain tissue, preferably belong to the routine laboratory determinations. This was the main reason for studying the enzymes chosen. No attempt was made to study the various enzymes peculiar to specific subcellular locations.

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The following enzymes were studied:

E.C. Number*	Name	Abbreviation
1.1.1.27	Lactate dehydrogenase	LDH
2.6.1.1	Glutamic oxalacetic transminase = Aspartate aminotransferase.	GOT
2.6.1.2	Glutamic pyruvic transaminase = Alanine aminotransferase.	GPT
2.7.3.2	Creatine Phosphokinase	CPK
3.1.1.8	Pseudocholinesterase = Acylcholine acylhydrolase	ChE
	α-hydroxybutyrate dehydrogenase	α-HBDH

α-HBDH is not a separate enzyme, but a chemical estimation of the rapidly migrating isoenzymes of LDH, as the affinity of the anodic LDH components for 2-oxobutyrate is greater than that of the cathodic fractions (Rosalki and Wilkinson, 1960 and 1964; Dubach and Margreth, 1965).

The relative activity of α-HBDH to the fractions of LDH is shown in table 3.

Table 3

Relative activity of α-HBDH to the isoenzyme fractions of LDH

	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
α-HBDH/LDH	0.92	0.78	0.55	0.39	0.25

from Methods of Enzymatic Analysis, edited by H.U. Bergmeyer, 2nd Edition. Verlag Chemie Weinheim, Academic Press Inc., New York and London, 1974.

The fast migrating isoenzymes of LDH occur predominantly in heart and brain tissue (van der Helm et al., 1962; Gerhardt et al., 1963; Lindblom et al., 1967; Lowenthal et al., 1961; Pfleiderer and Wachsmuth, 1961; Rabow, 1977; Wieme and van Maercke, 1961) (Table 4).

* Enzyme Commission Number

Table 4

Percentage of LDH isoenzymes in muscle and in different parts of cat and rabbit brain

Origin	Animal	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5	Author
Cerebral hemisphere	rabbit	31 ± 12	17 ± 4	32 ± 7	16 ± 7	4 ± 2	Rabow (1977)
brain stem	rabbit	61 ± 9	17 ± 4	7 ± 4	4 ± 3	6 ± 4	Rabow (1977)
Brain	cat	52	27.2	11.8	6.9	2.0	Lindblom et al. (1967)
Muscle	cat	0	0	5.5	7.3	87.2	Lindblom et al. (1967)
Heart	cat	75	21.4	3.6	0	0	Lindblom et al. (1967)

The content of the various enzymes studied in different tissues, is shown in table 5, the subcellular location of these enzymes in table 6.

Table 5

Enzyme content of muscle and brain

Author	Species	Enzyme	Muscle	Brain
Lindblom et al. (1967)	cat	LDH	407.000^+	40.000^+
Lindblom et al. (1967)	cat	GOT	46.000^+	9.000^+
Lindblom et al. (1967)	cat	GPT	8.000^+	600^+
van Schalkwijk (unpubl.)	cat	CPK	340.000^+	28.200^+
Rabow (1977)	rabbit	HBDH		2.000^o

+ = Activity in I.U./gram of tissue

o = Activity in I.U./gram of protein .

Table 6

Subcellular location of the enzymes studied

Enzyme	Cellular location			
	Cytosol	microsomal	mitochondrial	nuclear
CPK[o]	+++		+	
LDH[+]	+++			
GOT[+]	+++		+	
ChE[+]		++	$\pm$	$\pm$

+ : From The Enzymes, Ed. by M. Dixon and E.C. Webb, Longmans, London, 1965.

o : H. Somer 1975.

CHAPTER 3

Prelude

The studies reported in the papers collected in this chapter, investigate the value of CSF enzyme levels as a parameter for the extent of brain damage.

The first paper describes the dynamics of changes of CSF enzyme activity developing after cold injuries in cats. The second paper concentrates on the relation between the changes in CSF enzyme activity and the extent of brain damage. In the third paper studies are reported concerning the effect of hypotension on the development of increased CSF enzyme activity after induction of cold injuries in cats. This paper concentrates solely on the effect of hypotension on CSF enzyme levels. However, it is the author's opinion that these studies cannot be seen in their proper perspective without more data on the effect of hypotension on the ventricular fluid pressure.

For this reason the fourth paper – although beside the main theme of this thesis – is also appended. In the fourth paper the same series of experiments are described as in the third paper, but attention is focussed on the effect of hypotension on the development of increased ventricular fluid pressure after cold injuries in cats.
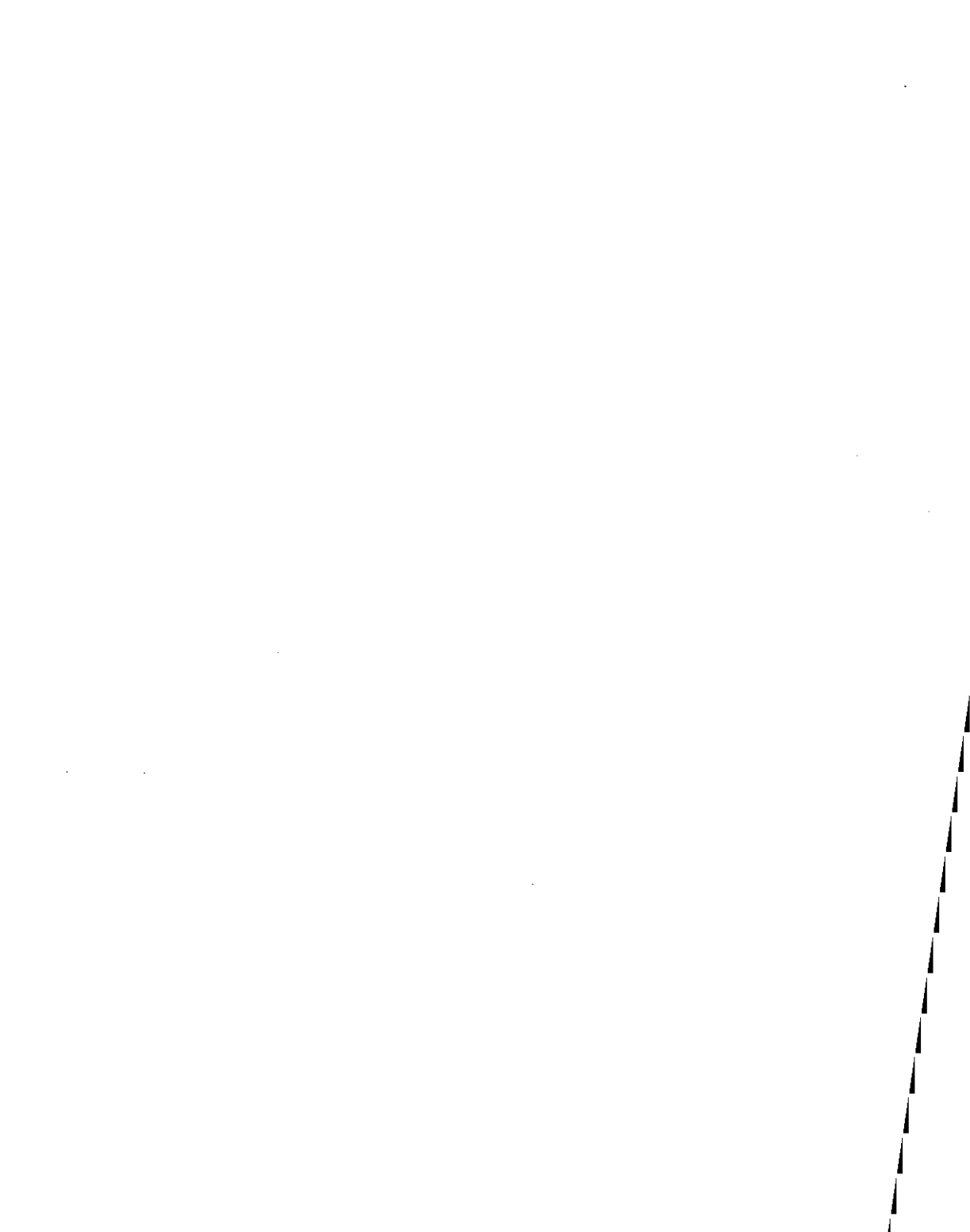
PURPOSE OF THE PRESENT STUDIES

To investigate the value of CSF enzyme levels as a parameter for the extent of brain damage.

CEREBROSPINAL FLUID ENZYMES IN ACUTE BRAIN INJURY.

I.: Dynamics of changes in CSF enzyme activity after acute experimental brain injury.

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SUMMARY

Changes in CSF enzyme activity were studied after brain trauma. It was thought that an increase in CSF enzyme activity might be a parameter for the extent of brain damage and therefore have prognostic value in patients with severe brain injuries. Raised values of CPK and α-HBDH were demonstrated in the CSF of patients with severe brain injuries.

The dynamics of changes in the CSF enzyme activity were studied in the experimental model. Standardized cold lesions of the brain were induced in cats. The activities of the enzymes CPK, α-HBDH, LDH, GOT, GPT and pseudocholinesterase were studied at half hour intervals in the Cerebrospinal Fluid (CSF) and at hourly intervals in the serum. A statistically highly significant ($P < 0,01$) increase of all enzymes studied developed in the CSF.

The greatest changes occurred within four hours of freezing. Great increases could occur per half hour. Isoenzyme studies demonstrated CPK and LDH to be of cerebral origin. No consistantly significant changes could be demonstrated in the serum enzyme activity. It is concluded that after brain injuries, enzymes are released into extracellular fluid of the brain (ECF) and transported to the CSF. The limited value of a single enzyme determination is emphasized. It is further postulated that the results described provide indirect evidence for transependymal flow of ECF in brain edema.

Patients with severe head injuries present many difficult clinical problems. One of these is how to estimate the extent of brain injury and how to establish a reliable prognosis in the individual patient. The prognosis will be dependant on many factors. Two important factors are the site and degree of brain injury (Jennett, 1972). Searching for an objective and early obtainable parameter for the extent of brain injury, it was decided to evaluate the prognostic value of the Cerebrospinal Fluid (hereafter to be abbreviated as CSF) enzyme activity in the acute phase after brain injury, i.e. within the first 24 hours. It was considered likely that enzymes released from damaged or necrotic brain cells would be transported towards the CSF. Raised values of various enzymes have been reported in the CSF in many neurological disorders including severe brain injury (Sherwin et al., 1967; Kaltiala et al., 1968; Hildebrand and Levin, 1973; Navarro, 1973; Klun, 1974; Nordby et al., 1975).

Prognostic value has been attributed to the height of the CSF enzyme activity in patients with severe head injuries (Smith et al., 1960; del Villar et al., 1973). More recently Nordby et al. (1975) demonstrated higher concentrations of CPK and LDH in the CSF of patients with more severe brain injuries, but no close relationship between the severity of a moderate brain injury and the social rehabilitation or late effects could be shown. Florez et al. (1975) describe a general correlation between the severity of brain damage and enzyme activity in the CSF, but state that the prognostic value of these changes is only limited.

The first results of preliminary clinical studies conducted by us were also somewhat disconcerting. In 20 patients with head injuries of variable severity the activity of the enzymes Creatine phosphokinase (CPK) and α-hydroxy butyric acid dehydrogenase (α-HBDH) was studied in the CSF within the first 24 hours of trauma. The results are summarized in table I, showing the enzyme activities in the group of patients who either died within a month after injury, or were still in a vegetative state at that time and those who were still alive one month after injury.

Although the highest values were obtained in the most severely injured patients, those dying within 24 hours, a few patients who were clinically in a very serious condition and who also died of the cerebral damage, either had normal values or only slightly raised enzyme activity in the CSF.

These findings might be explained by local brain stem lesions without extensive cortical necrosis. It was felt, however, that too little was known about the changes in enzyme activity of the CSF after brain injury and that the dynamics of these changes in enzyme activity needed evaluation in the experimental situation before further clinical research could be attempted. Therefore it was decided to conduct a series of animal experiments using cats in which cold injuries of the brain of standardized severity were produced and the changes of

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Table 1

CPK AND α-HBDH IN THE CSF OF PATIENTS WITH SEVERE BRAIN INJURIES*

Outcome 1 month	Dead or VS **		Alive	
	CPK U/l	α-HBDH U/l	CPK U/l	α-HBDH U/l
	267	—	75	125
	167	203	55	—
	150	224	21	93
	117	190	19	70
	102	—	11	33
	60	—	6	17
	53	88	3	19
	42	25		
	32	207		
	16	72		
	14	—		
	11	63		
	3	21		

* CSF samples obtained within 24 hours after injury.
** VS = vegetative state.

enzyme activity in the CSF studied.

In this paper the dynamics of changes in enzyme activity that occur in the CSF in the acute phase after a cold lesion to the brain are described. Isoenzyme studies were also conducted to determine the origin of the enzymes. The twin partner of this paper reports on the relation between the CSF enzyme activity and the extent of brain lesions. In future studies it is hoped to report on the effect of artificial hypotension on the changes in enzyme activity of the CSF, which will be described in this paper, and ultimately we hope to continue our clinical studies and to report on these.

MATERIAL AND METHODS

21 Healthy adult cats, weighing 2800-6400 grams, otherwise unselected for age or sex, were sedated with phenobarbital intramuscularly (50 mg/kg. weight) and anaesthetized with pentothal intraperitoneally (25 mg/kg. weight). Atropine (0,5 mg) was given to all animals. Anaesthesia was maintained with a mixture of N_2O and O_2 (2.5 : 1), administered via an endotracheal tube.

The animals breathed spontaneously throughout the experiment. Respiratory frequency varied considerably between the individual animals but arterial blood gases were maintained at a reasonable level. The mean pCO_2 averaged 31.3 ± 4.5 mm.Hg. and the pH 7.35 ± 0.04 during the first eight hours of the experiments.

The animals were placed in a stereotaxic frame (David Kopf Instruments). A burr hole was made in the left frontoparietal region and a cooling thermode screwed into this burr hole over the ectosylvian and suprasylvian gyri. Arterial blood pressure, End expiratory CO_2, ECG, ventricular and cisternal fluid pressure were measured continuously. Ventricular fluid pressure was monitored via a stereotactically placed cannula in the cella media of the right lateral ventricle. Cisternal fluid pressure was measured via a lumbar needle, placed in the cisterna magna after the atlanto-occipital membrane had been exposed. Samples of CSF (0,35 ml) were drawn every half hour from the cisternal needle, which was connected to the transducer via a three way stop cock. Blood samples were taken every hour. The samples were centrifuged at 3500 r.p.m. and stored in the dark at $4°C$. until the following day, when they were analyzed for the activity of the enzymes: Creatine Phosphokinase (CPK), α-Hydroxybutyric acid dehydrogenase (α-HBDH), Aspartate amino transferase (GOT), Alanine amino transferase (GPT) and pseudocholinesterase (ChE). The enzyme determinations were performed utilizing the standard optimated Merck-I-Tests ® (E. Merck Darmstadt, Germany).

The first three samples of CSF obtained were dicarded so as to eliminate faulty estimations either due to contamination of the first samples with muscle enzymes picked up during placing of the cisternal needle, or due to dilution of the CSF samples with physiologic saline remaining in the cisternal catheter after filling the transducer.

Control values were obtained per experiment by averaging the values measured in samples 4 - 6 (i.e. in the period 1 - 2½ hours after beginning of cisternal taps). Just prior to the withdrawal of CSF every half hour the following parameters were also measured: arterial blood pressure, ventricular and cisternal fluid pressure, respiratory frequency, heart rate and end-expiratory CO_2. For these parameters also, control values were established in the period 1 - 2½ hours after beginning of cisternal taps.

After the control period, experimental brain injuries were produced by induction of cold lesions. This was done in 14 of the 21 experiments conducted. The remaining seven animals served as a control group.

Cold lesions were induced according to the method of Beks and coworkers (1965). Cooled methanol (temp.: $-40°C$) from an Ultra Cryostate (Lauda Ultra Cryostate) was led through the thermode for 6 minutes exactly. After freezing the tubes connecting the thermode to the ultra cryostate were disconnected but the cooling thermode left in situ, so that the actual duration of freezing was longer.

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Samples of CSF were taken every half hour, and blood samples every hour until herniation of the brain stem occurred. If this did not occur within 7¼ hours of induction of the lesion the experiment was terminated at the arbitrarily chosen time limit of 7¼ hours, except in a few animals which were studied for a longer period. In one case ventricular fluid was collected for one hour after herniation had occured at 7¾ hours. Herniation of the brain stem was considered to have occurred if all of the following symptoms were present:

a. a progressive gradient developed between the ventricular and cisternal fluid pressure.

b. CSF could no longer be obtained from the cistern in quantities sufficient for analysis.

c. slowing of the repiratory frequency occurred.

After termination of the experiments the animals were sacrificed with an overdose of intravenous pentothal. The brains were removed immediately after death and fixed in a 4% formalin solution for two weeks. After fixation photographs were taken of the macroscopical aspect of the brain as seen on a frontal section taken through the maximal diameter of the lesion.

Statistical analysis

The differences between the values measured at the various time levels (i.e. ¼ - 7¼ hours after induction of the cold lesion) and the control values per individual experiment were calculated. Statistical analysis was performed on these calculated differences between the control group and the group with the cold lesion at every time level (i.e. every half hour) using Wilcoxon's ranking test.

Isoenzyme studies

Creatine kinase isoenzymes were studied in CSF and serum in six experiments. The isoenzymes were separated electrophoretically and visualized with the Nitro Blue Tetrazolium (NBT) method (van der Veen and Willebrands, 1966).

LDH isoenzymes were also visualized with the NBT method. The isoenzyme patterns of tissue-extracts were determined from brain, heart and and skeletal muscle. Tissue samples were obtained directly after death and immediately frozen in liquid nitrogen. A 10% extract was made in a medium containing 0.24 M sucrose, 10 mMol Tricine, 1 mMol EDTA (isotone, pH 7.3).

Control experiments — "no cold lesion":

Ventricular Fluid Pressure and CSF and serum enzyme activity.

The mean Ventricular Fluid Pressure (hereafter to be abbreviated as VFP) measured at the different time levels in the control experiments before withdrawal of CSF samples ranged from -3.5 to 8.4 mm.Hg. All animals in the control group showed slight activity of all enzymes measured in the CSF. The range of values observed is summarized in table 2.

Table 2

CONTROL GROUP
Range of enzyme activities measured
in CSF and serum

Enzyme	CSF values (U/l)	Serum values (U/l)
CPK	3-70	330-2130
α-HBDH	3-26	51-170
LDH	4-43	140-617
GOT	2- 9	6-32
GPT	1- 3	6-17
ChE	2-44	540-2300

There was no consistent rise or fall in enzyme activity of the CSF during these control experiments. In four of the seven animals the enzyme activity of the CSF stayed at the same level or fell as compared to the individual control value per experiment. In three animals a slight transitory elevation of the activity of all enzymes studied in the CSF was noted, the most obvious for CPK. This is illustrated in fig. 1 showing the pattern of CPK activity in the CSF in animal No. 23, as well as the median CPK values for the control group.
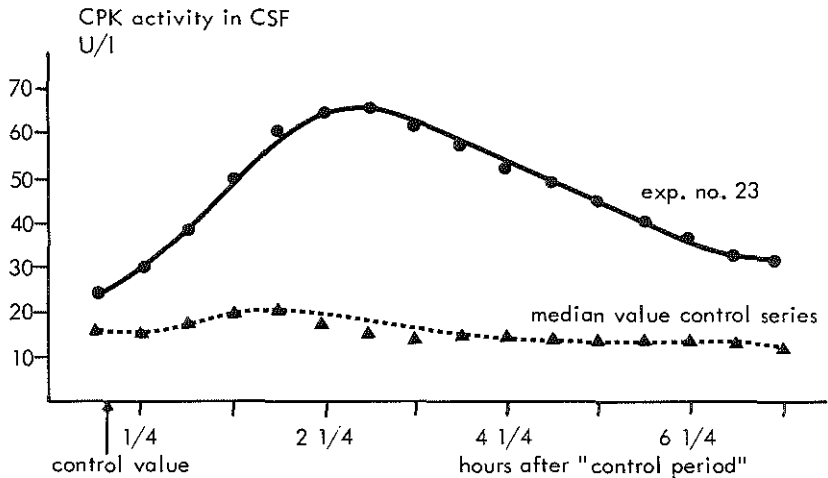
33

Fig. 1. CSF CPK activity in the control series. This graph shows the median values of the levels of CPK observed in the Cerebrospinal fluid of the animals belonging to the control series, and also shows the transient rise of CPK activity as occurred in Exp. No. 23 (control experiment).

Serum enzyme activity showed a wide variation between the individual animals. The range of values observed is also given in table 2.

Experiments in which a cold lesion was induced

Extent of cold lesion

Induction of cole lesions according to the method described resulted in a large zone of focal necrosis and formation of extensive cerebral edema, which remained confined to the ipsilateral hemisphere. The microscopical aspect of cold injuries has been well documented by others (Klatzo et al., 1958; Clasen et al., 1962; Go et al., 1967) and will not be further described here.

An example of the extent of the induced cold lesion is shown in fig. 2.

Effect of freezing on VFP and CSF enzymes

All 14 animals developed a rise in Ventricular Fluid Pressure, starting between 15 and 25 minutes after induction of the cold lesion. Five of these animals developed a rapidly progressive rise in VFP and brain stem herniation occurred
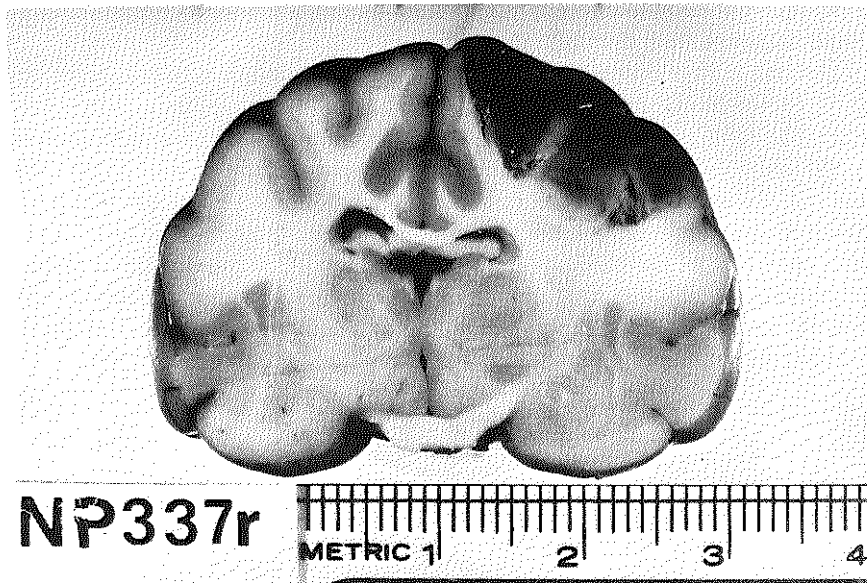
34

Fig. 2. Macroscopical aspect of the extent of a typical cold lesion.

within 2½ hours after induction of the lesion. These five animals did not exhibit any change in enzyme activity in this period. The enzyme activity of the last obtainable specimen is shown in table 3.

Table 3

CPK and GOT in the CSF of five animals developing
brain stem herniation within 2½ hours of induction
of the cold lesion

Enzyme:	Enzyme activity before freezing		Enzyme activity of last obtained specimen	
	CPK (U/l)	GOT (U/l)	CPK (U/l)	GOT (U/l)
Exp. No.:				
24	8	22	44	49
27	10	—	3	—
36	2	3	20	11
42	2	—	19	—
81	—	5	—	15

We did not analyse these results further as in these animals no information could be gained from the CSF enzymes.

The other nine animals in the group with a cold lesion survived longer and they, except in one case, developed great changes in the enzyme activity of the CSF. The results reported here will therefore concentrate on these nine animals.

The VFP rose to values considerably higher than in the control group. This rise was statistically significant ($p < 0,01$). A significant (Wilcoxon: $p < 0,01$) rise of all enzymes studied developed. This rise started between 1¼ and 2¼ hours after induction of the lesion. All enzymes, except GPT showed a similar pattern of increase. Even when measured at half hourly intervals great changes in CSF activity occurred from sample to sample. The results are illustrated in fig. 3, showing the median values of the CSF enzyme activity at each time level.
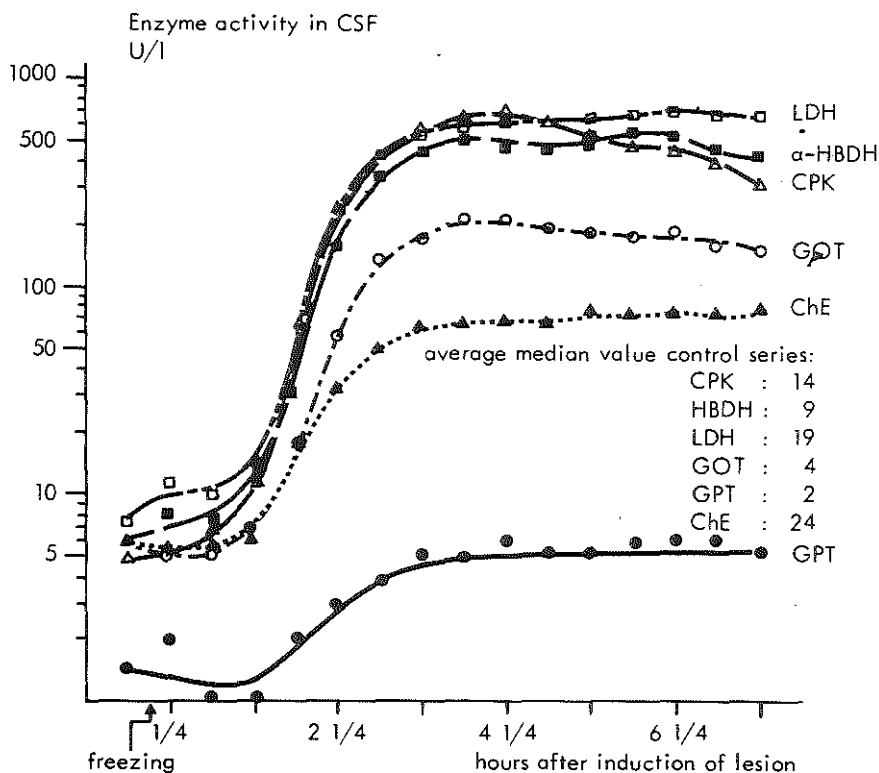

Fig. 3. Increase in CSF enzyme activity after induction of a cold lesion of the brain in cats. The median values of the activities of the enzymes LDH, α-HBDH, CPK, GOT, Cholinesterase and GPT in the Cerebrospinal fluid are plotted.

As can also be seen in fig. 3 it even appears that a peak value is reached within the first seven hours of freezing. However, a peak value was not reached in each experiment and a wide variation existed in the increase of enzyme activity in the CSF between the individual animals. This is demonstrated by the scatter diagram fig. 4.
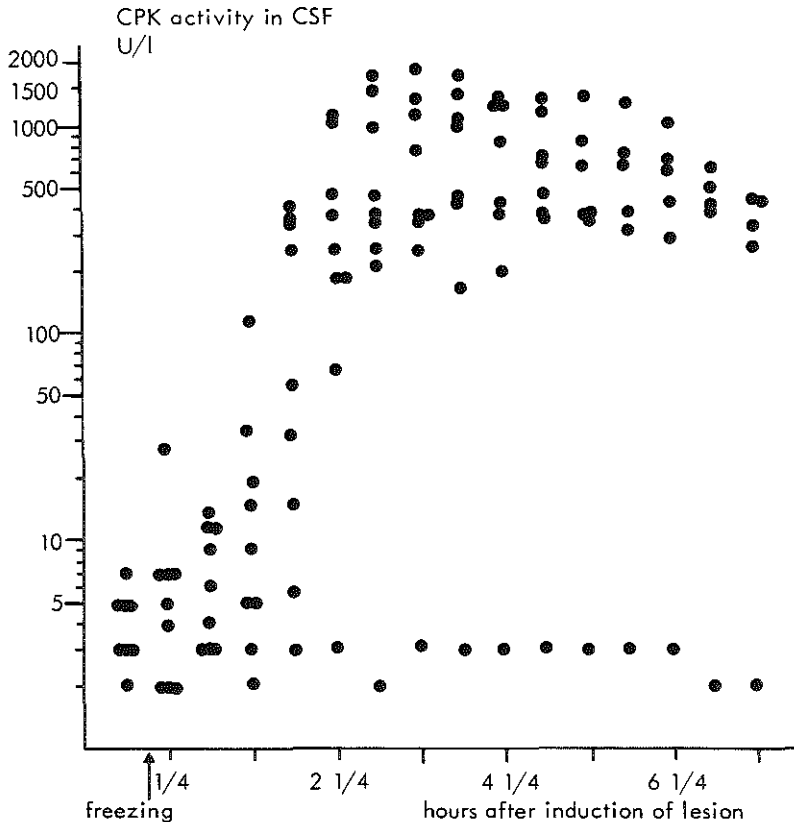

Fig. 4. Scatter diagram, showing the changes in CPK activity observed in the CSF of 9 animals after induction of a standardized cold lesion to the brain. This diagram illustrates the similar pattern of response occurring in all animals but for one and also shows the great interindividual variability of response.

Fig. 4 also shows that one of the animals (nr. 39) did not respond at all to the lesion with a change of CSF enzyme activity in this period. Nevertheless the VFP did rise to values of 15 mm.Hg. (therefore to a higher level than observed in the

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control group (up to 8.4 mm.Hg.) but lower than in any of the other animals in the group with a cold lesion.

This animal was observed during an additional 8 hours. In this period a progressive increase of the VFP ensued, so much so that towards the end of this period brain stem herniation occurred. '

Even in this latter period hardly any changes in CSF enzyme activity occurred. The lack of changes in CSF enzyme activity in this animal is demonstrated in table 4, comparing the median values of the group with the cold lesion to the values observed in Experiment 39. At post mortem examination the expected cold lesion was present. It was, however, slightly smaller than in the other experiments. No evidence or indications existed at all to support any suspicion of an experimental failure.

Table 4

Lack of changes of CSF enzymes in Exp. No. 39

Hours after induction of cold lesion	Median values CSF enzymes in animals with cold lesion					CSF enzyme values in Exp. No. 39				
	CPK	HBDH	GOT	VFP	MABP	CPK	HBDH	GOT	VFP	MABP
Control	5	6	6	3.2	140	3	6	55	3.2	132.5
3¾	560	480	170	24.5	142.5	3	6	4	10.6	107.5
6¼	505	540	185	28.3	142.5	3	5	4	14.8	95
9						2	5	5	19.0	100
12						8	22	11	18.0	125
15						11	37	17	40.0	135

Serum enzymes

No statistically significant differences of serum enzyme levels could be demonstrated between the control group and the series with a cold lesion. This failure to attain significancy is in part due to the high serum enzyme levels occurring in the control series (see table 2) and the large variation between the individual animals of this group; the high levels of serum enzyme activity are probably caused by both the anaesthesia and operative trauma.

Correlative studies

The correlation was studied between the activity of the various enzymes measured at the same time levels as well as between the enzymes and the ventricular

fluid pressure. All enzymes were intercorrelated. The analyses were performed at each time level separately. The strongest correlation was obtained – as would be expected – between α-HBDH and LDH.

We wil not report the actual values of the calculated correlation coefficients as although a significant correlation was present, the accuracy of the calculated correlation coefficients is small owing to the limited number of observations, especially at the latter time levels, when some of the experiments had been terminated due to herniation of the brain stem.

Correlation CSF enzymes – Ventricular Fluid Pressure

All enzymes studied showed only a poor correlation with the increase in VFP. This correlation did not improve when the enzyme levels were correlated with VFP measured ½, 1 or 1½ hours earlier. This was examined because the VFP obviously reacted sooner to the lesion than the CSF enzymes, and also because the sample obtained at a certain time level really shows part of the activity of an earlier time level, due to the "dead space" in the cisternal catheter.

The correlation between the VFP and CPK activity of the CSF both at the same time level, as well as after introduction of a time lag is shown in table 5.

Table 5

Correlation between ventricular fluid pressure and CSF CPK activity

Hours after induction of cold lesion	Correlation coefficients at: Time lag:			
	0	1	2	3
1¼	0.49	0.80	0.70	0.62
2¼	0.64	0.59	0.55	0.48
3¼	0.18	0.66	0.64	0.49
4¼	0.64	0.38	0.41	0.47
5¼	0.27	0.32	0.44	0.77
6¼	0.80	0.85	0.84	–
7¼	0.65	–	–	–

Time lag 0 = VFP + CPK at same time level
Time lag 1 = VFP + CPK ½ hour later.
Time lag 2 = VFP + CPK 1 hour later.
Time lag 3 = VFP + CPK 1½ hrs. later.

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There was no correlation whatsoever between the CSF enzyme activity and the serum enzyme activity in the animals with a cold lesion.

Isoenzyme studies

CPK-isoenzymes

The CPK isoenzyme patterns in the tissue extracts of brain, heart and skeletal muscle were completely in accord with the literature (Burger et al., 1964; Dawson and Fine, 1967). The brain extract showed almost exclusively a fast migrating fraction towards the anode (BB-fraction). Skeletal muscle exhibited a slow moving fraction that stayed in the region of the cathode (MM-fraction). The tissue extract of heart showed two bands, one similar to the MM-fraction and the other smaller fraction exactly intermediate between the MM- and the BB-fractions (MB-fraction). These fractions are shown in fig. 5.
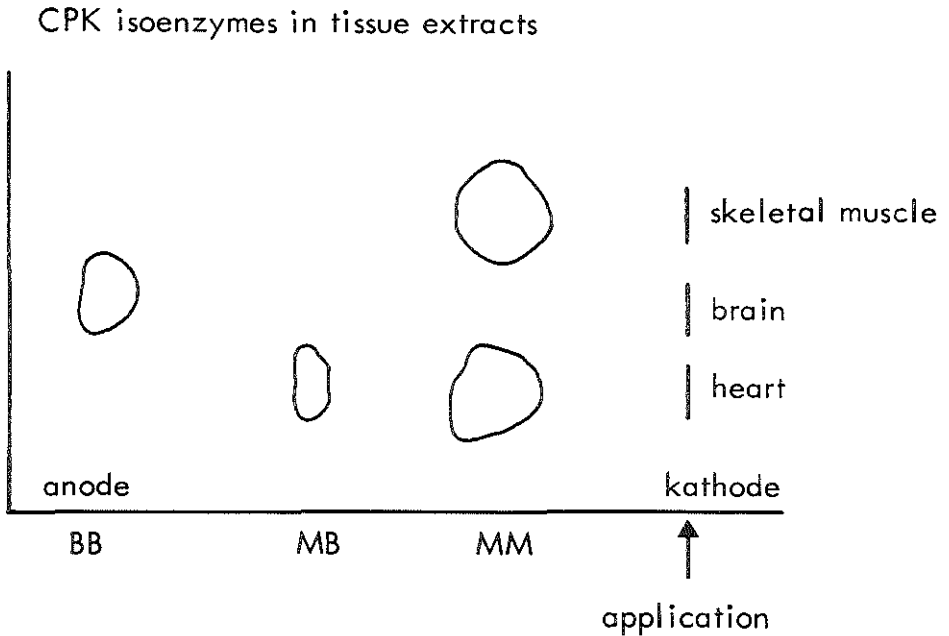

Fig. 5.   CPK isoenzyme pattern of extracts of skeletal muscle, brain and heart, obtained from cats.

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In six of the nine experiments, isoenzyme studies of the CSF were conducted at various time levels. In all samples studied the predominant activity was in the region of the BB-fraction. Only slight activity was noted in the MM-region. In serum, however, more than 90% of the activity was localized in the region of the MM-fraction. A typical example of the isoenzyme pattern obtained in CSF and serum is shown in fig. 6.
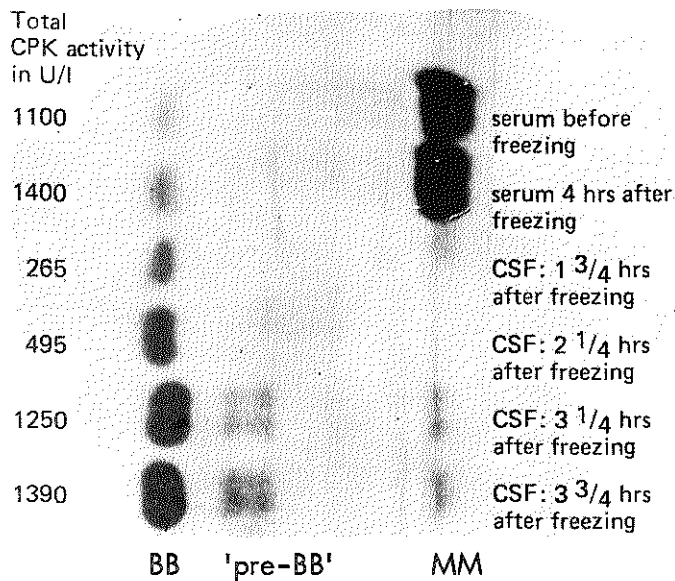

Total
CPK activity
in U/l

1100 — serum before freezing

1400 — serum 4 hrs after freezing

265 — CSF: 1 $3/4$ hrs after freezing

495 — CSF: 2 $1/4$ hrs after freezing

1250 — CSF: 3 $1/4$ hrs after freezing

1390 — CSF: 3 $3/4$ hrs after freezing

BB   'pre-BB'   MM

Fig. 6. CPK isoenzymes in CSF and serum after induction of a cold lesion. Raised CPK levels in the CSF are mainly due to the brain type isoenzyme and in the serum mainly due to the muscle type.

It is evident from the picture that as time progresses the BB-activity becomes more intense and increases in density as total CPK activity rises. An interesting phenomenon to note is that when CPK levels were very high, another band — sometimes a double band — appeared intermediate between the MB and the BB-fraction. This new fraction was termed the "pre-BB" fraction. It appeared in all experiments during the height of CPK activity and was most obvious in those experiments that showed a high total CPK activity. A more detailed report on this apparently new isoenzyme is in preparation (Van Schalkwijk et al., 1977).

The isoenzyme pattern of the tissue extracts of brain and skeletal muscle are shown in fig. 7.
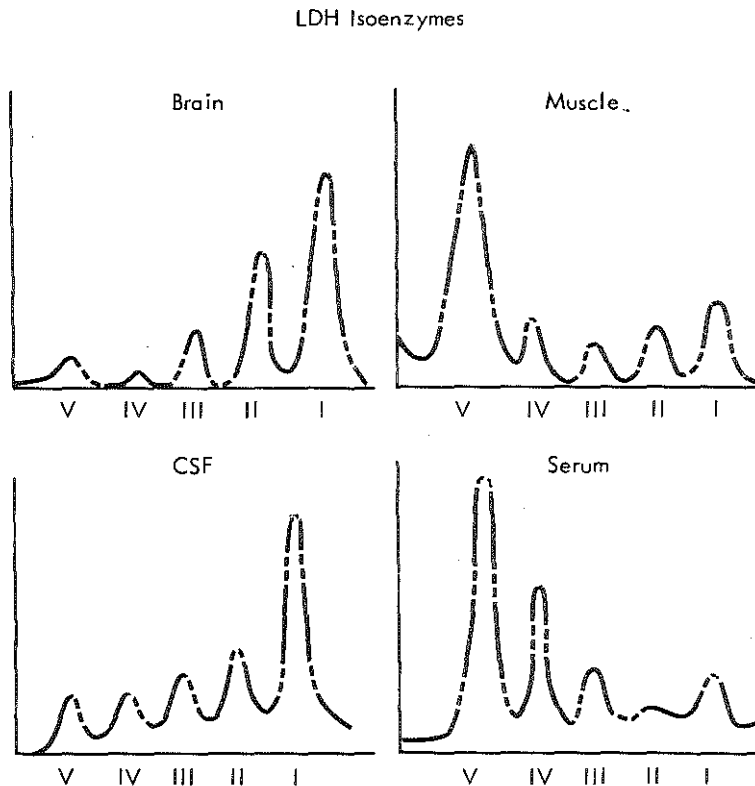
LDH Isoenzymes

Fig. 7. LDH isoenzymes of cat brain and cat skeletal muscle. The fast migrating isoenzymes LDH 1 and 2 (brain types) are found in the CSF after cold lesions, while the slowly migrating isoenzymes LDH 4 and 5 (muscular origin) were demonstrated in the serum.

Brain and heart contain mainly LDH 1 activity, while skeletal muscle predominantly exhibits the slower migrating fractions LDH 4 and 5 (Lindblom et al., 1967). Isoenzyme studies of LDH were done in four experiments. In the CSF the LDH 1 activity was greatest in all samples studied. In serum, however, mainly LDH 4 and 5 activity was seen. These findings are also demonstrated in fig. 7.

The predominance of the fast migrating isoenzymes LDH 1 and 2 in the CSF is also shown by the α-HBDH/LDH ratio. α-HBDH is a chemical estimation for the fast migrating LDH-isoenzymes 1 and 2 (Rosalki and Wilkinson, 1964; Dubach and Margreth, 1965). In CSF the ratio α-HBDH/LDH varied from 0.6 to 1.0 and in the serum from 0.3 to 0.5, therefore demonstrating a far higher content of the fast migrating LDH isoenzymes, which occur in brain and heart.

DISCUSSION

Our studies have demonstrated an increase in enzyme activity of the CSF in the acute phase after brain injury. The idea of studying CSF enzymes in acute brain injury stems from the hypothesis that intracellular substances, such as enzymes, will be released from damaged or necrotic brain cells into the extracellular fluid of the brain (ECF). Go et al. (1976) have indeed demonstrated an increase of LDH in the ECF of cats with a cold lesion of the brain. It was presumed that enzymes released into the ECF would be transported into the CSF.

This hypothesis touches on one of the more controversial aspects of brain physiology: Does a transependymal secretion of CSF exist?

Weed in 1914 already suggested a dual origin of CSF. Bering and Sato (1963) estimated that 40 per cent of the CSF formed in dogs came from an extrachoroidal site. Pollay and Curl (1967) calculated that approximately 30 per cent of the CSF in rabbits is formed by secretion over the ventricular ependyma. Milhorat (1969) demonstrated that hydrocephalus could occur rapidly and progressively in monkeys even after plexectomy.

Milhorat, Hammock et al. (1971) performed bilateral plexectomy in monkeys and determined rates of CSF formation after obstruction of the fourth ventricle. After correcting for the contribution of choroid plexus of the third ventricle they estimated that the total formation of CSF was only 60 to 67 per cent of normal.

Cserr and Ostrach (1974) have demonstrated a net flow of brain interstitial fluid towards the ventricle, possibly due to developing cerebral edema.

We presume that enzymes, released from damaged or necrotic brain cells into the extracellular fluid of the brain, are transported by the spreading edema and by way of simple diffusion and bulk flow of ECF towards the CSF. It might also be supposed that enzymes are transported from the supracortical CSF towards the cisternal CSF.

Matsen and West (1972) have demonstrated a release of albumin from brain into the subarachnoid compartment of the CSF within 45 minutes of cold injury.

In one of the experiments conducted by us 0,5 ml. of ventricular CSF was collected after tentorial herniation had occurred and CSF could no longer be obtained from the cisternal catheter. Beks and coworkers (1973) have demonstrated that in tentorial herniation, developing in cats after a cold injury to the brain, the flow of CSF from the ventricle to the cistern is interrupted. In that case enzymes from the ventricular CSF would not be able to reach the cisternal CSF. The values of enzyme activity in the last cisternal sample and in the ventricular CSF are shown in table 6.

Table 6

Cisternal and ventricular CSF enzyme activity

Hours after induction of cold lesion	Cist. CSF 8¼	Ventr. CSF 8¼	Ventr. CSF 9¼	Ventr. CSF 9½
Enzyme:				
CPK	230	380	740	680
α-HBDH	580	700	1500	1400
LDH	660	840	1900	1750
GOT	224	269	687	578

The fact that the higher values of enzyme activity are found in the ventricular CSF would support the hypothesis that the enzymes are transported through the cerebral parenchyma towards the ventricles and from there towards the cisternal CSF. We believe that these results support the views held by early investigators, including Cushing (1914), Weed (1914), and Flexner (1933), that a steady bulk flow of extracellular fluid exists towards the CSF pathways.

This study was not designed to provide definite evidence for this, but it is nevertheless felt that the results obtained probably do provide evidence for this bulk flow of ECF in developing edema.

The question arises whether the raised enzyme activities demonstrated in the CSF after brain injury, are indeed the result of brain tissue destruction, or whether they may possibly be caused by blood enzymes, seeping through a severely damaged blood-brain barrier. The origin of the enzymes may be differentiated by isoenzyme studies. The brain contains a CPK isoenzyme with a different electrophoretic mobility than muscle. Although reports on isoenzyme studies conducted in the CSF demonstrate a cerebral origin for these enzymes (Frick, 1967; Sherwin, 1967), we were not quite convinced that this would also be valid in the presence of such extreme destruction of the blood-brain barrier as must have been inflicted in the experiments conducted. However, results of both CPK and LDH isoenzyme studies indicated a cerebral origin.

The ratio of α-HBDH/LDH also provided evidence for the cerebral origin.

The elevation of enzyme activity in the CSF only started between 1¼ and 2¼ hours after induction of the lesion. In stating this, however, it must be kept in mind that the "dead space" of the cisternal catheter was approximately 0,25 ml., so that also incorporated in this time delay is an artificial delay of approximately ½ hour (= 1 sample). The observed delay of 1¼ - 2¼ hours until appearance of the enzymes could explain why only slight changes of CSF enzyme activity were observed in the group of five animals that developed tentorial herniation within 2½ hours after induction of the cold lesion. We do not consider herniation a suddenly occurring event, but rather as a more gradual displacement of brain tissue, resulting in the progressive interruption of CSF flow from ventricle to cistern. This is evidenced by an increasing gradient between ventricular and cisternal CSF pressure. The flow of enzymes from ventricular to cisternal CSF becomes interrupted before the enzymes have had time to appear in the cisternal CSF.

The fact that not all animals respond in a uniform way to freezing lesions has also been observed by other authors. Beks and coworkers (1965) found a variable response of ventricular fluid pressure to cold injuries induced in cats. Kaste and Troupp (1972) produced a lethal cold lesion in rabbits and found that in about half of the animals the injury resulted in a quick rise in cerebral sinus pressure and in its relation to the blood pressure, while in the other half of the animals the cerebral sinus pressure and the ratio to the blood pressure rose more slowly.

We not only observed this variable reaction of intracranial pressure to cold injury, but were also surprised to observe a great variability in height of elevation of enzyme activity in the CSF. This may be due either to biological variation or to experimental differences. In all experiments the surface of cerebral cortex frozen as well as the duration and depth of freezing were standardized. The cooling thermode was always positioned according to stereotactical coordinates by the author. The only variability that could have occurred is the pressure exerted by the cooling thermode on the cortex. However, we do not believe that this — only possible — variability can explain the wide scatter of enzyme activities in this group. Other explanations failing, it is concluded that the observed scatter is, in part anyway, due to an individual physiologic variability in response.

A very interesting experiment was no. 39, an animal that did not develop any changes in CSF enzyme levels in the first 7½ hours after induction of the lesion. In the second eight hours of this experiment the ventricular fluid pressure steadily increased and at the end of this period brain stem herniation occurred. Even in these latter hours hardly any increase in CSF enzyme activity was noted. At post mortem examination the expected lesion was present, being only slightly smaller than in the other experiments. There were no reasons or

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indications to suspect an experimental failure. The only factor that distinguished this animal from the other in his group was a lower blood pressure. The median values of the mean arterial blood pressure in this group ranged from 132.5 to 145 mm.Hg. The mean blood pressure of experiment No. 39 varied from 95-135 mm.Hg. with a median value of 110 mm.Hg.

These observations prompted further research into the role of arterial blood pressure in the development of changes in CSF enzyme activity after a cold lesion. Preliminary results indicate that the transport of enzymes towards the CSF in the acute phase after brain trauma can be delayed, or even inhibited by lowering the arterial pressure.

In the control experiments a slight rise of enzyme activity of the CSF was seen in three of the seven experiments. There are two possible explanations for this:
1. Enzymes are freed from muscle trauma during exposure of the atlanto-occipital membrane and contaminate the cisternal CSF.
2. The increased enzyme levels result from cerebral tissue damage caused by puncturing the ventricle for continuous pressure monitoring.
No isoenzyme studies of CPK were conducted in the control experiments to detect the origin of this enzyme as the method used for visualizing CPK isoenzyme fractions was not sensitive enough to demonstrate these low activities. The ratio of α-HBDH/LDH in the CSF of these control experiments (0.5 to 0.8) points towards a cerebral origin. In the histological preparation sometimes quite a large destruction of tissue was seen to be the result of ventricular puncture; possibly this may have caused the slight increase of CSF enzyme activities observed.

The idea of studying enzymes in the CSF as a parameter for brain injury is not new. However, only relatively little research has been conducted into the changes of enzyme activity in the CSF after experimental brain injury. Wakim and Fleischer (1956) produced experimental brain infarction in dogs by injecting vinyl acetate into the internal carotid artery. They described an increase of GOT activity in the CSF which reaches a peak value after appr. 100 hours. The augmentation of transaminase was proportional to the severity and extent of infarction. Smith et al. (1960) produced experimental brain injury in dogs by lacerating or bluntly abrading the cerebral cortex with a guarded scalpel. These workers describe a great increase of GOT in the CSF up to values of 700 U/l, with a maximal value between 2 and 4 hours after laceration. They found higher values in the more severely lacerated animals. Akashi (1966) found increased CSF GOT levels 3 to 4 hours following a vibration trauma. A slight increase of GOT and GPT activity was noted in the CSF about 12 hours after carotid ligation. In guinea pigs the GOT, GPT and LDH levels of frozen grey matter were lower than those of the non-frozen side at 2 and 3 hours. Rasmussen and Klatzo (1969) described a slight elevation of LDH but not of GOT in the CSF of cats

with a cold lesion. They, however, only studied a few animals and not in the acute stage, but in the first week following injury.

Klun (1974) also reported on the GOT, GPT and aldolase activity of CSF one to three days after cold injury in cats. He only described a slight increase of GOT in the CSF after brain injury.

Of these studies only those of Smith et al. and of Akashi have concentrated on the acute phase after injury.

Our studies — as also those of Smith et al. do — indicate that the greatest increase of enzyme activity in the CSF after brain injury occurs within a couple of hours after induction of the lesion. These studies further indicate that from hour to hour great changes in the enzyme activity of the CSF can occur.

CONCLUSIONS

CSF enzyme activity is greatly increased in the acute phase after brain injury and CSF enzyme levels may therefore be of value as a parameter for the extent of primary brain damage. A single determination will only yield very limited information. Serial enzyme determinations in ventricular CSF of patients receiving a ventricular drain soon after brain trauma may provide useful information.

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CEREBROSPINAL FLUID ENZYMES IN ACUTE BRAIN INJURY.

II: Relation of CSF enzyme activity to the extent of brain injury.

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SUMMARY

The value of CSF enzyme levels as a parameter for the extent of brain damage was studied in the experimental situation. Standard cold lesion of different severity were induced in cats. The activities of the enzymes CPK, α-HBDH, LDH, GOT and ChE were studied at half hour intervals in the cerebrospinal fluid (CSF). The ventricular and cisternal fluid pressure, as well as the arterial blood pressure were monitored continuously. These studies were conducted to investigate the value of CSF enzyme levels as a parameter for the extent of brain damage.

Higher enzyme levels were found in the animals with more severe injuries of the brain. Differences were statistically significant.

It is concluded that CSF enzyme activity is related to the extent of cerebral damage, and should therefore have prognostic value in patients with severe head injuries.

INTRODUCTION

This is the second paper in a series describing the changes in cerebrospinal fluid (CSF) enzyme activity after induction of a cold lesion of the brain in cats. These studies were conducted to determine the value of CSF enzyme levels as a parameter for the extent of cerebral damage in acute brain injury.

It was considered likely that enzymes released from damaged or necrotic brain cells into the extra cellular fluid of the brain (ECF) would be transported towards the CSF.

Results earlier described in the first paper (Maas, 1977) demonstrated a great increase in the activity of various enzymes in the CSF during the first 7 hours after induction of a cold lesion to the brain in cats. Although prognostic value has been attributed by various authors to the level of CSF enzyme activity in patients with severe head injuries (Smith et al., 1960; del Villar et al., 1973), only few studies whether clinical or experimental, have concentrated on the relation between the changes in CSF enzyme activity in the acute phase after brain trauma and the extent of brain damage (Smith et al., 1960; Akashi, 1969 and Florez et al., 1975). It was therefore decided to investigate further whether such a relation existed between the changes in CSF enzyme activity and the extent of brain damage in the acute phase after experimental brain injury. This was done by producing standardized cold injuries of different severity in three series of cats. In one series a deep lesion was induced, in the second series a more shallow lesion by freezing at a less deep temperature, and in the third series a smaller area of cortex was frozen. CSF enzymes were studied at half hour intervals and differences analyzed between the various series.

MATERIALS AND METHODS

38 anaesthetized cats, weighing 2800-6400 grams, otherwise unselected for age or sex, were studied. The experimental model as well as anaesthetic and surgical methods were identical as that described in earlier studies (Maas, 1977). Experimental brain injury was effected by induction of cold lesions to the brain.

Before and after freezing CSF samples of 0.35 ml. were withdrawn from a cisternal catheter every half hour and analyzed for the activity of the enzymes: Creatine Phosphokinase (CPK), Alpha hydroxybutyric acid dehydrogenase (α-HBDH), Lactate dehydrogenase (LDH), Aspartate-amino transferase (GOT), and Pseudocholinesterase (ChE). Enzyme estimations were performed utilizing the standard optimated Merck-I-test kits® (E. Merck Darmstadt, Germany).

Arterial bloodpressure, end expiratory CO_2, ECG, ventricular and cisternal fluid pressure were monitored continuously and measured every half hour prior to withdrawal of CSF samples. Respiratory frequency and heart rate were also calculated.

After the control period as described in the first paper, a cold lesion was induced in 31 of the 38 experiments conducted. The other 7 animals served as a control series. This series is the same as described in the first paper. Cold lesions were induced according to the method of Beks and coworkers (1965).

The experiments were divided into four series:
Series 1:
Control series (7 animals). These animals received a complete sham operation, but no cold lesion was induced.

Series 2:
In 14 animals an extensive cold lesion of the brain was induced by freezing
1.34 cm^2 of cortex for 6 minutes exactly at $-40°$C.
Series 3:
In this series less extensive lesions of the brain were induced.
3a: In series 3a (9 animals) this was done by freezing the same area (1.34 cm^2)
    for the same length of time, but at a less deep temperature: $-30°$C.
3b: In series 3b, (8 animals) a smaller lesion was induced by freezing a smaller
    area (0.7 x smaller) of 0.97 cm^2 for 6 minutes exactly at $-40°$C. After
    freezing, samples of CSF (0.35 ml) continued to be withdrawn every half
    hour.

The experiments were usually terminated at an arbitrarily chosen time limit of
7¼ hours after induction of the cold lesion, unless brain stem herniation
developed, in which case the experiments were terminated prematurely as CSF
could then no longer be obtained from the cisternal catheter in quantities
sufficient for analysis. The criteria for brain stem herniation have been described
(Maas, 1977).

After termination of the experiments, the animals were sacrificed with an
overdose of intravenous pentothal and the brains removed. The brains were fixed
in a 4% formalin solution for two weeks and paraffin sections cut through the
maximal diameter of the cold lesion. These sections were stained with haema-
toxylin and eosin (HE), Periodic acid Schiff (PAS), Trichome and PAS, and
according to the method of Klüwer-Barrera.

Statistical analysis

The differences between the values measured at the various time levels (i.e. every
half hour up to 7¼ hours after induction of the cold lesion), and the control
values per individual experiment were calculated. Statistical analysis of these
calculated differences was performed at every time level (i.e. every half hour)
using Wilcoxon's ranking test.

The following series were compared:
A: Control animals versus animals with cold lesions:
    Control — series 2
    Control — series 3a
    Control — series 3b
B: Series with extensive cold lesions versus series with less extensive lesions.
    Series 2 — series 3a
    Series 2 — series 3b.

Difference in occurrence of tentorial herniation between the series with more
severe and the series with less severe injuries was analyzed with Fisher's test.

RESULTS

Development of brain stem herniation

Eight of the fourteen animals in the series with a severe cold injury (series 2) developed brain stem herniation within 7¼ hours of induction of the cold lesion. Five of the animals developing herniation did so within 2½ hours of induction of the lesion. These five animals were excluded from further enzyme analysis as the survival time was too short for an increase in CSF enzyme activity to occur. One experiment in this series was terminated prematurely, because mechanical obstruction of the cisternal catheter had rendered it impossible to obtain further samples of CSF. The number of animals developing brain stem herniation within the 7¼ hours standard study period in series 2 and in the series 3a and 3b are summarized in table I.

Table I

Number of animals developing brain stem herniation after severe and less severe cold injuries

Series	No herniation	Brain stem herniation	Mechanical obstruction of cisternal cathether	Total No. exp.
Control	7	0	0	7
Series 2	5	8	1	14
Series 3a	5	3	1	9
Series 3b	6	2	0	8

Statistical analysis using Fisher's test revealed that the probability of brain stem herniation occurring less often in the series 3a or 3b (less severely injured) than in series 2 (more severely injured animals) was $p = 0.80$ and $p = 0.90$ respectively. These probabilities are not statistically significant, but they do show a definite tendency for brain stem herniation to develop less often in the series with the less severe injuries (series 3a and 3b) than in the series with more extensive lesions (series 2).

Differences in CSF enzyme activity and in the Ventricular Fluid Pressure (VFP) between the control series and the series with cold lesions.

All three series (2, 3a and 3b) of animals with cold lesions developed a statistically significant increase in the activities of the enzymes CPK, α-HBDH,

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LDH and GOT in the CSF, when compared with the control series. Cholinesterase was significantly increased in the series 2 and 3b but not in series 3a ($-30°C$). The Ventricular Fluid Pressure (VFP) was elevated in all animals with a cold lesion.

Differences in CSF enzyme activity and in VFP between the series with more severe and with less severe injuries.

A statistically significant (Wilcoxon: $p < 0.05$) difference of enzyme activity in the CSF developed between the series with severe injuries (series 2) and the series with less severe injuries (3a and 3b) from approximately 2¾ hours after induction of the cold lesion onwards. All enzymes studied demonstrated this difference (CPK, α-HBDH, LDH, GOT and ChE). These differences are summarized in the figures 1 - 4 showing the median values of CSF enzyme activity and the median VFP in the three series.

In 23 of the 26 experiments conducted, either a maximal value of the activities of the enzymes in the CSF was reached, or otherwise a plateau level (a more or less constant raised enzyme activity) was reached. This "maximal – or plateau – value" was defined as the level of enzyme activity, after which the activity of the enzymes studied in the CSF did not increase by more than 10%. Fig. 5 shows that this "maximal value" is highest in the series with the more severe injuries.

One might expect this 'maximal value' to be reached later in the animals in series 3a or 3b than in those in series 2 (severely injured). This however was not the case as is illustrated in fig. 6.

These findings lead us to conclude that higher enzyme activities are encountered in the CSF of the more severely injured animals, but that the time span in which the "maximal activity" is reached does not differ.
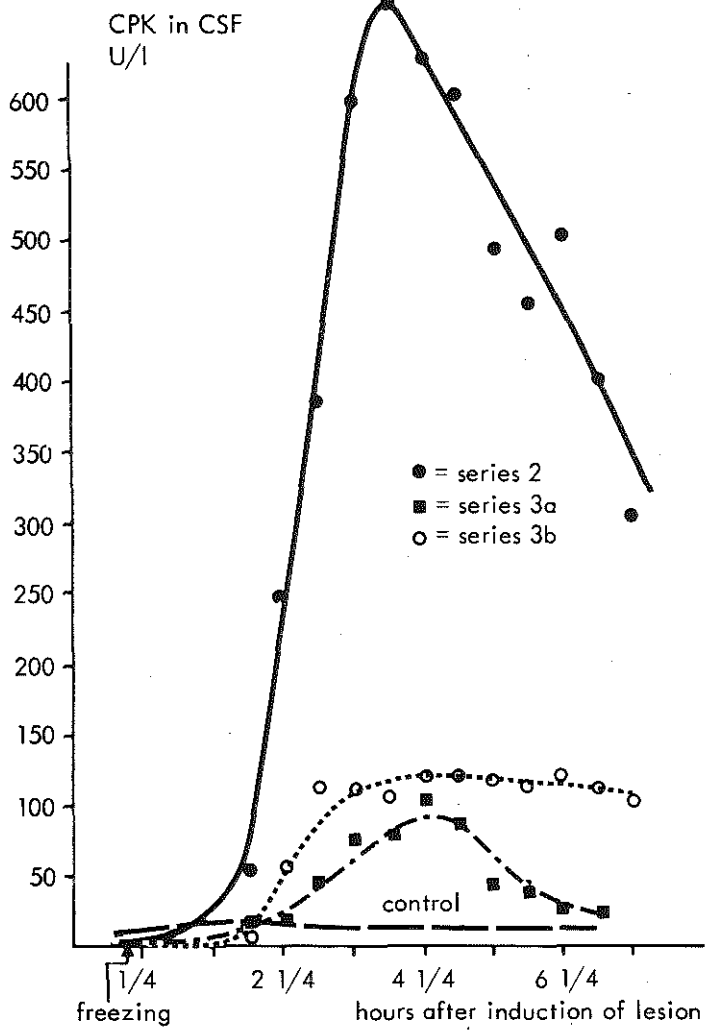
Fig. 1. Median values of CPK activity in the cerebrospinal fluid after induction of cold lesions of different severity in cats. The animals in series 2 were severely injured, the animals in series 3a and 3b less severely.
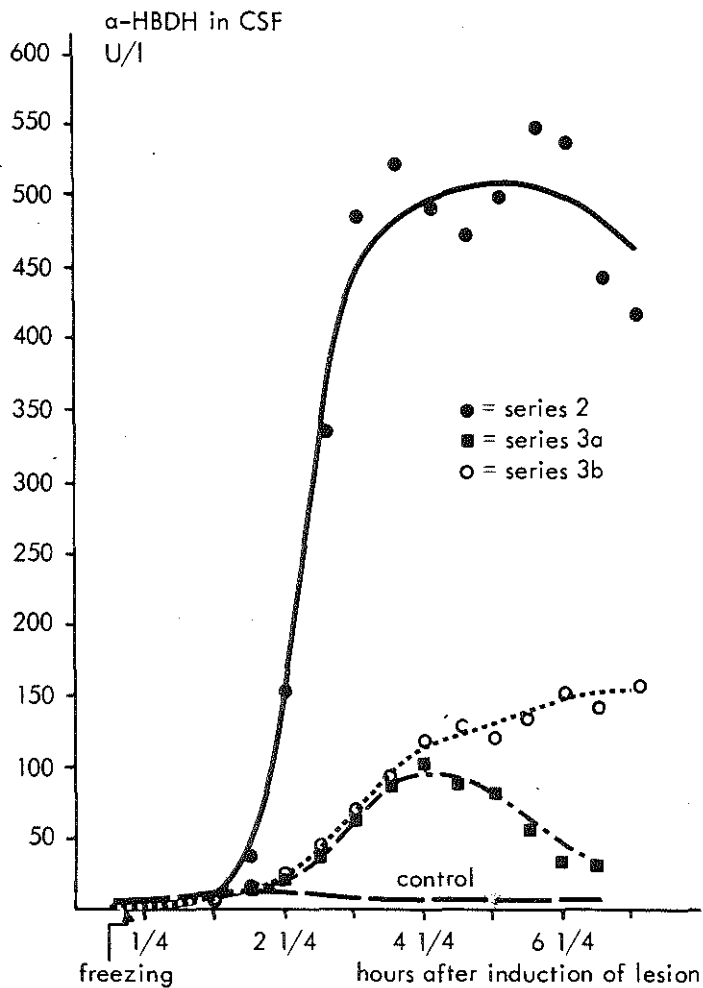
Fig. 2. Median values of α-HBDH acitivity in the CSF after induction of cold lesions of different severity in cats. The animals in series 2 were severely injured, the animals in 3a and 3b less severely.
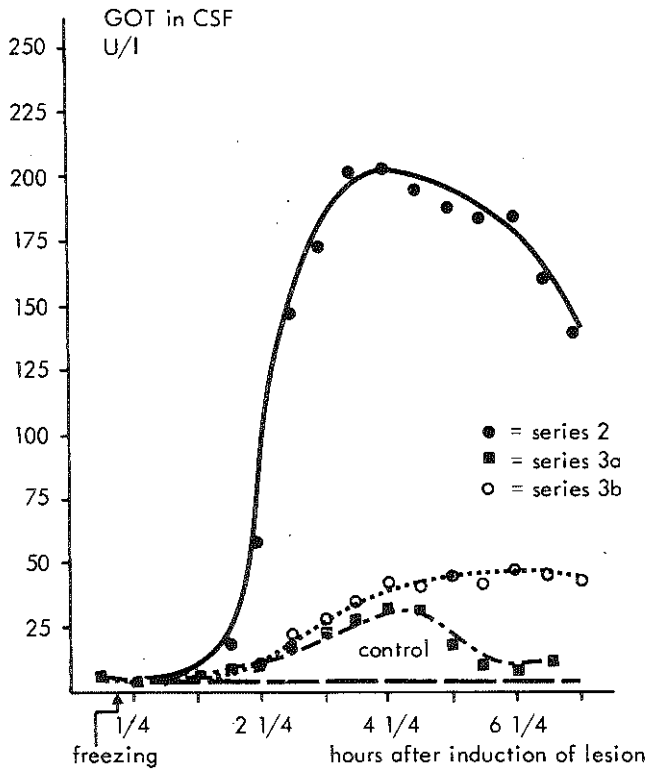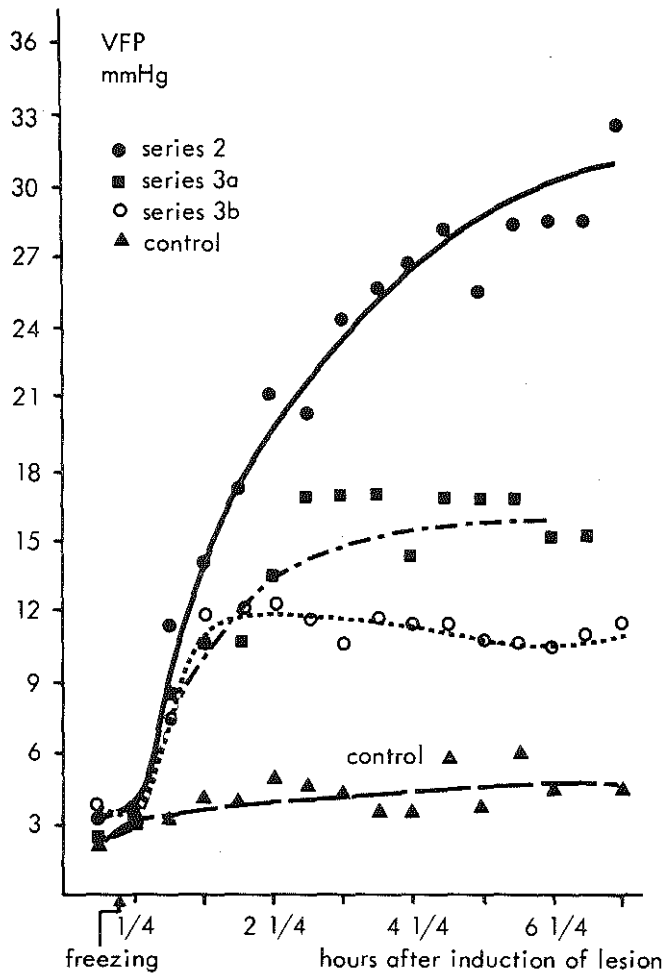
Fig. 3. Median values of GOT activity in the CSF after induction of cold lesions of different severity in cats. The animals in series 2 were severely injured, the animals in series 3a and 3b less severely.

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Fig. 4. Median values of the ventricular fluid pressure after induction of cold lesions of different severity in cats. The animals in series 2 were severely injured, the animals in series 3a and 3b less severely.

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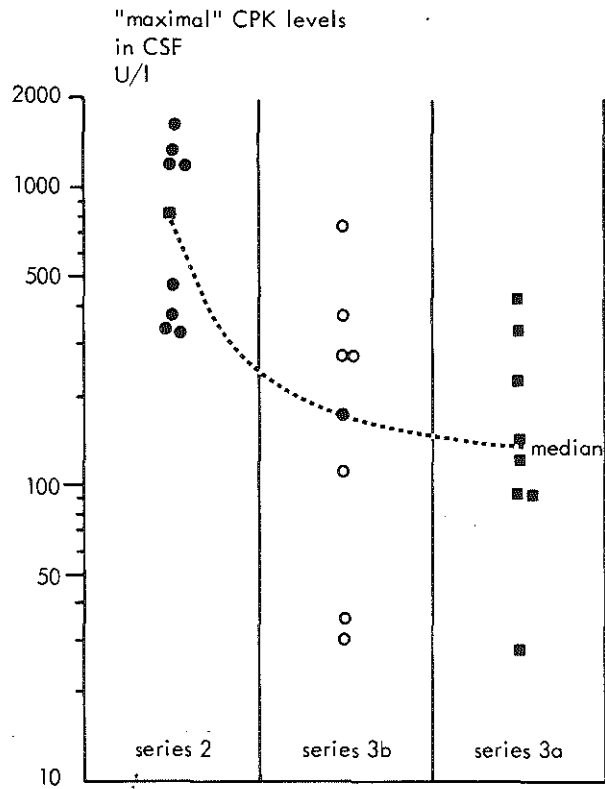
Fig. 5. 'Maximal values' of CPK activity observed in the CSF after induction of cold lesions to the brain in cats. The highest levels are seen in the more severely injured animals (series 2).

Fig. 6. Time-span before maximal CPK activity is reached in the CSF after induction of cold lesions of different severity. No difference exists between animals with severe (series 2), and animals with less severe lesions (series 3a and 3b).

The value of CSF enzyme activity and Ventricular Fluid Pressure related to the severity of brain injury.

The VFP was more elevated in series 2 than in 3a or 3b. The difference was statistically significant (Wilcoxon: $p < 0.05$). However in series 3a $(-30°C.)$ this statistical significancy was only transitory and not present later than four hours after induction of the lesion.

The question arose whether the CSF enzyme activity, although obviously offering different information than the VFP, was not also more valuable in discriminating between the groups with injuries of different severity. In fig. 7 and 8 the observed values of the CPK activity in the CSF and the VFP are shown at hourly intervals for the series 2 and 3a.
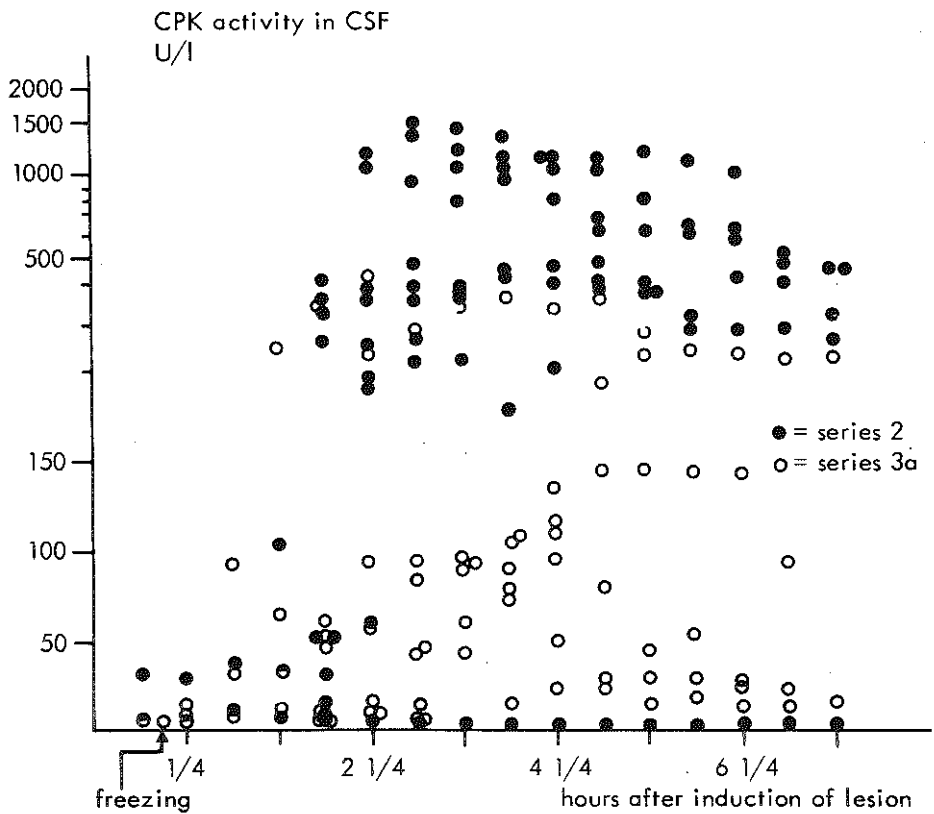
60

Fig. 7. Scatter diagram showing the levels of CPK activity observed in the CSF of cats with severe injuries (series 2) and less severe injuries (series 3a).
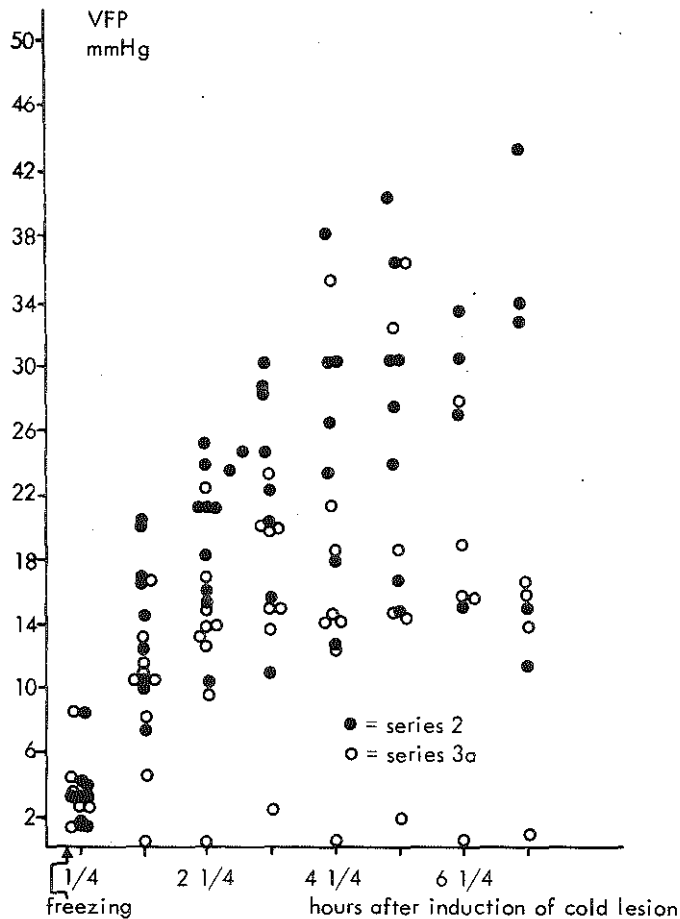
61

Fig. 8. Scatter diagram showing the ventricular fluid pressure measured in cats after induction of cold lesions of different severity. The animals in series 2 were severely injured, the animals in series 3a less so.

It becomes evident that the CSF CPK levels differentiate better between the two series than the VFP does. This is also illustrated in table 2, showing the development of changes in CPK activity in the CSF and in the VFP in two experiments, one in series 2 and one in series 3a. The VFP reaches higher levels in the animal that is less severely injured, while the CPK activity is more elevated in the animal with the larger lesion. Apparently the development of raised VFP

is not linearly related to the extent of brain damage. This phenomenon is also known in the clinical situation: Patients — especially children — with relatively minor brain damage can develop explosive cerebral edema.

Table 2

CSF CPK activity and ventricular fluid pressure in two animals with a cold lesion, one severely injured and one less severely injured

		Control value	¼	1¼	2¼	3¼	4¼	5¼	6¼	7¼
	Hours after induction of cold lesion									
Exp. No. 35	VFP	3.2	5.0	16.3	21.3	28.0	30.0	30.0	26.5	33.5
series 2	CPK	5	4	5	1040	1100	830	610	600	430
Exp. No. 30	VFP	2.1	2.5	10.6	16.8	24.5	34.0	100		
series 3a	CPK	2	2	5	230	340	340	290		

Histologic studies

All animals with a cold lesion exhibited cortical necrosis and cerebral edema. Macroscopically a slightly swollen, darkly discoloured area of cortical necrosis was present with evident hyperemia. The hemisphere in which the lesion had been induced was swollen, with displacement over the midline, and compression of the ventricle on the side of the lesion. Microscopically the lesions were essentially the same as described by other authors (Klatzo et al., 1958; Clasen et al., 1962; Go et al., 1967). The borders of the necrotic lesion were sharply defined in the cortex, but the extent of the necrosis into the white matter was more difficult to delineate. Edema was evident by less intense staining of the white matter in the hemisphere with the cold lesion, and by dispersion of the myelin fibres. Occasionally a complete status spongiosus resulted. The edema was confined to the ipsilateral hemisphere, sparing the corpus callosum. In the necrotic zone all symptoms of cellular destruction were present. The cell bodies of the neurons were shrunk, exhibiting pycnosis, their nuclei being hyperchromatic with a triangular deformation; karyorrhexis and occasionally total cell lysis were present. The cytoplasm was eosinophilic and homogenisation of the Nissl substance was seen. The destruction of glial elements was less complete, but the white matter often showed dissolution. The necrosis proceeded to a greater depth in the length of the nerve fibres. In the necrotic zone hyperemia of veins and capillaries was present, with erythro- and leukodiapedesis, and often haemorrhages, in a few experiments so extensive as to produce a haemorrhagic

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necrosis. It was difficult to quantitatively estimate the extent of brain damage, but it was possible to trace the extent of the necrotic area under the microscope at the constant magnification. The average extent of the lesions in the three series is shown in fig. 9.

Extent of necrotic zone in cats with cold injuries of different severity
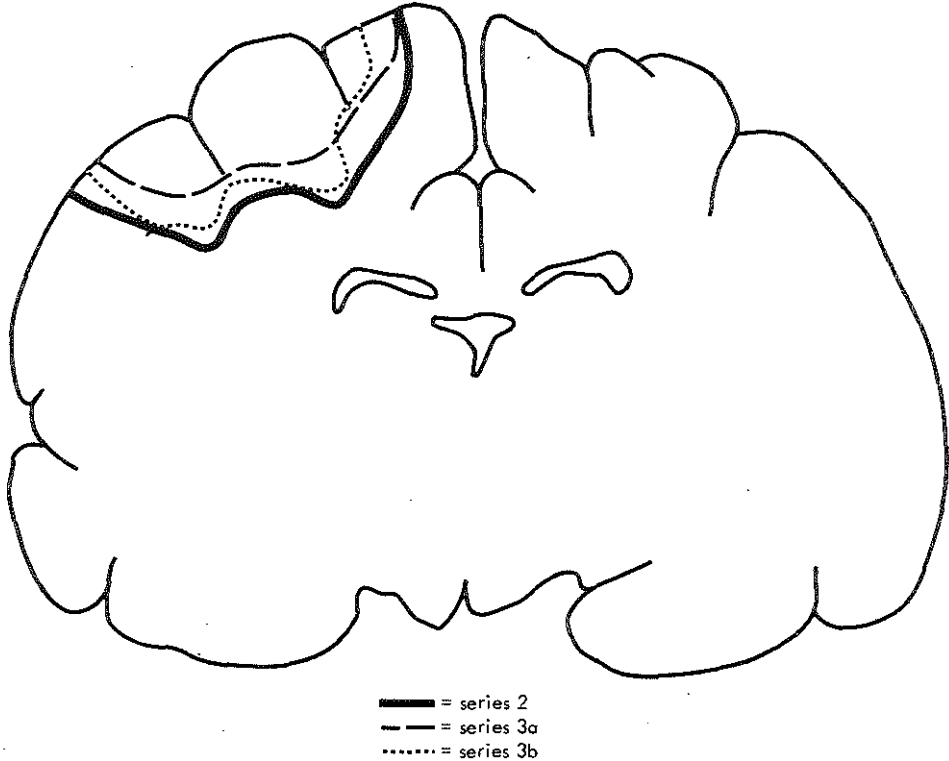

= series 2
= series 3a
= series 3b

Fig. 9. Average extent of cold lesion as seen on a frontal section of the brain through the maximal diameter of the cold lesion. The area of necrosis and bleeding is obviously more shallow in series in 3a and smaller in diameter in series 3b than in series 2 (more severely injured animals).

As could be expected the largest area of destruction was observed in the series frozen at $-40°$C. (series 2). In the series frozen at $-30°$C. (series 3a), the average lesion was obviously more shallow, but the depth of the lesion in series 3b (0.97 cm^2) was similar to that of series 2. The average diameter of the lesion in this series however was obviously smaller than in series 2.

Establishing a reliable prognosis in patients with severe head injuries can be very difficult.

The prognosis will depend on many factors of which site and degree of brain damage are of great importance (Jennett, 1972). What we should wish for as a prognosticon is an easily obtainable and objective parameter for the extent of brain tissue damage. Enzymes are released from damaged tissue into the extra-cellular fluid (Go et al., 1976) and the amount of enzymes released could be expected to be proportional to the extent of tissue damage. Enzymes will be transported from the extracellular fluid towards serum and CSF. Serum enzyme determinations would — if of prognostic value — fulfill the requirements of being both objective and easily obtainable. Results of research into the prognostic value of serym enzyme determinations in patients with severe brain injuries have been disappointing and often conflicting.

Dubo et al. (1967) reported raised values of CPK in the serum of all patients studied with head injuries, as well as in 17 out of 28 patients suffering from stroke or meningitis. However, no definite clinical correlations or prognostic inferences could be drawn from the serum CPK levels; isoenzyme analysis of CPK demonstrated the CPK to be mainly derived from muscle.

Kaltiala et al. (1968) studying GOT, LDH and GPT in the CSF and serum in 30 patients with head injuries only found GOT levels raised in the serum, but this rise correlated in the first place to peripheral contusions and no definite correlation was observed between the GOT activity in serum and CSF and the clinical severity of the lesion. Lindblom and Aberg (1972) found no correlation, again in clinical studies, between the clinical severity of the brain injury and the occurrence of enzyme abnormality.

Rabow, Hebbe and Lieden (1971) demonstrated raised levels of LDH 1 more often in patients with cerebral contusion than in those with cerebral concussion. Rabow (1972) demonstrated in a series of 70 patients that the maximal LDH 1 activity in the serum in the acute phase after head injury, shows a very good correlation to the prognosis in the individual patient. Klun (1974) demonstrated increasing levels of GOT and GPT in the serum of patients dying after head injury, while in the surviving patients decreasing values were noted. Thomas and Rowan (1975) demonstrated an increase of the LDH isoenzymes 1 and 2 in the serum following head injury, but also remark that prognostic application of serum LDH isoenzyme estimations is not definite as a wide range of LDH isoenzymes existed within the head injured group, so that findings for those who died and those who were left disabled overlapped.

These conflicting results may be explained by the high incidence of peripheral contusions and muscle damage in patients with severe head injuries causing raised levels of serum enzyme activity. Recent research of Somer et al. (1975)

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into the brain isoenzyme of CPK in the serum of these patients seems in addition to the LDH isoenzyme studies as conducted by Thomas and Rowan, and Rabow a more specific line of research.

The CSF is in far more intimate contact with the brain E.C.F. than the blood is. CSF may well in fact serve as the lymph of the brain. Previous studies have demonstrated high levels of especially CPK, α-HBDH, LDH and GOT in the CSF of cats in which a cold lesion of the brain had been induced. Isoenzyme studies on CPK and LDH confirmed these enzymes to be of cerebral origin (Maas, 1977).

It was considered likely that the enzyme changes in CSF after severe brain injuries would yield more consistent results than serum enzyme analysis. This view seems to be supported by the results obtained by other workers in this field.

Smith et al. (1960) found that patients with either persisting or greatly elevated values of GOT in the CSF were those with also the most extensive brain damage. In these patients it was predictable that neurological defects would persist. These authors stated that GOT in the CSF is indicative of the severity of a craniocerebral injury and may be helpful in prognosticating end results in the early stages of injury to the head.

Kaltiala et al. (1968) found the most pronounced increase of GOT in the CSF of patients with cerebral contusion, not in those with only cerebral concussion. Klun (1974) however, found no difference in GOT or GPT activity of the CSF in patients dying or surviving after head injury. Florez et al. (1975) reporting on CSF and serum enzyme studies conducted on 35 patients with head injuries showed the GOT activity of both serum and CSF to correlate well with the severity of brain lesion, but could find no such correlation for the CPK activity. They conclude that the prognostic value of the changes in CSF and serum enzyme activity is only limited. Del Villar et al. (1973) demonstrated a benign prognosis in the majority of patients with craniocerebral injuries without CPK activity in the CSF and a bad prognosis when CPK was very elevated in the 37 patients studied. However, low CPK values in the CSF were also seen in three of the eight patients with serious neurological symptoms. The authors do not mention at what time after injury the specimens of spinal fluid were obtained, and our studies have demonstrated that rapid changes of CSF enzyme activity can occur especially in the acute phase after trauma. Still the observation that some patients can be clinically in a very serious condition and still have low activities of the enzymes in the CSF, also struck us during preliminary clinical studies (Maas, 1977). Although the assumption that the CSF enzymes would be related to the extent of brain damage seems logical, the fact that low enzyme activities may also occur in the CSF of patients with severe head injuries decreases the prognostic value of CSF enzyme levels.

Only very little experimental work investigating the relation between the

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enzyme activity of the CSF and the extent of brain damage has been done (Smith et al., 1960; Wakim and Fleischer, 1956). Smith et al. (1960) established this relation in a limited number of dog experiments.

The results of our studies also definitely establish a relation between CSF enzyme activity and the extent of brain damage. This is evidenced by the higher levels of CSF enzyme activity occurring in the series with severe injuries (series 2) than in the two groups with less injuries (3a and 3b) and corroborated by the histological studies showing more extensive damage in series 2 than in series 3a or 3b. Our studies would also indicate that CSF enzyme levels would offer more information than the VFP alone, being of a more discriminative nature as to the severity of the injury. CSF enzyme determinations provide information on the extent of brain damage, while ventricular fluid pressure measurement does not clearly do so. The height of the intracranial pressure is not necessarily related to the extent of brain damage. Brain edema can be out of proportion compared to the actual brain damage.

Although the increase in CSF enzyme activity occurring after brain trauma has been show to be related to the extent of brain damage, it must be remembered, that even in the standardized experimental model described, great variations in the response of CSF enzyme activity to brain trauma were demonstrated in the individual animals. Further research into the cause of this variability of individual response is required. Nevertheless, it is believed that a potential application of CSF enzyme determinations exists for patients with severe brain injury.

Possibly low enzyme activities in the CSF of patients in the acute stage after brain injury in combination with a high intracranial pressure, could, after exclusion of a haematoma, indicate severe brain edema in a not so severely damaged brain and the possibility of a potentially treatable syndrome. The results reported here indicate that the CSF enzyme activity is related to the extent of cerebral damage, and may therefore be of value in estimating the outcome in individual patients with severe head injuries. It must, however, be borne in mind that prognosis is not only determined by the degree, but also by the site of brain damage, while pre-traumatic factors and the presence of extracerebral lesions must also be taken into account.

We do not believe that a definite prognosis for the individual patients can be established on basis of the enzyme activity of the CSF alone. It must also be remembered that the prognostic value of CSF enzyme levels depends on the consistency with which enzymes released into the ECF will be transported towards CSF. It is conceivable that transport of enzymes into the CSF may be influenced by a number of factors, for instance the height of the bloodpressure. More research into this aspect is in progress.

CONCLUSIONS

It is concluded that CSF enzyme activity is related to the extent of cerebral damage, and should therefore have prognostic value in patients with severe head injuries. Only the height of the enzyme activity is related to the extent of the lesion, not the speed with which changes occur in the activity of the enzymes in the CSF.

CSF enzyme levels discriminate better as to the severity of brain damage than ventricular fluid pressure monitoring does.

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CEREBROSPINAL FLUID ENZYMES IN ACUTE BRAIN INJURY.

III: Effect of hypotension on the increase of CSF enzyme activity after cold injuries in cats.

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SUMMARY

The influence of Trimetaphan induced hypotension was studied on the increase in the activities of various enzymes in the Cerebrospinal Fluid after cold injuries of the brain in cats. Hypotension was induced immediately after freezing, and in a second series after a delay of 45 minutes. It was shown that induction of hypotension may inhibit the appearance of enzymes in the CSF after cold injuries in the first seven hours after freezing. Histologic studies revealed less pronounced edema in the hypotensive animals.

The results suggest that hypotension retards the transport of enzymes released from necrotic areas through the extra-cellular fluid towards the CSF.

INTRODUCTION

Previous work has demonstrated increased activities of the enzymes CPK, α-HBDH, LDH, GOT and ChE in the Cerebrospinal Fluid (CSF) after induction of cold lesions to the brain in cats, the increase being related to the severity of the injury (Maas, 1977a and b). The practical value of CSF enzyme determinations as an indication of the extent of brain damage will be greatly dependant on the consistency of enzyme transport from the Extracellular fluid (ECF) to the CSF. Should transport be hampered in any way, then fallacious low CSF enzyme

activity may result even in the presence of severe brain damage.

Not unlikely brain edema could promote transport. It was decided to investigate this.

MATERIALS AND METHODS

The experimental set-up has been described in detail (Maas, 1977a). In summary: In healthy, adult, anaesthetized cats weighing 2800-6400 grams, otherwise unselected for age or sex, a cold lesion of the brain was induced according to the method of Beks and coworkers (1965).

Before and after freezing, CSF samples of 0.35 ml. were withdrawn from a cisternal catheter every half hour. Samples were analyzed for: Creatine phosphokinase (CPK), α-hydroxybutyric acid dehydrogenase (α-HBDH), Lactate dehydrogenase (LDH), Aspartate aminotransferase (GOT), and Pseudocholinesterase (ChE). Enzyme determinations were performed utilizing the optimated Merck-1-test kits [®] (E. Merck Darmstadt, Germany).

Arterial blood pressure, End expiratory CO_2, ECG, Ventricular and Cisternal fluid pressure were monitored continuously and measured every half hour. Respiratory frequency and heart rate were calculated.

The first three samples of CSF were discarded to eliminate false high enzyme levels due to contamination during placement of the cisternal catheter. Control values were established per experiment by averaging the values obtained in the period 1-2½ hours after beginning of cisternal taps (i.e. CSF samples 4-6). Thereafter a cold lesion of the brain was induced in 29 of the 36 experiments. Seven animals served as a control group. In fifteen animals artificial hypotension was induced using Trimetaphan (Arfonad[®]) in a concentration of 0.5-1.0 mg/ml. The mean arterial blood pressure was reduced to 75-90 mm.Hg.

The experiments were divided into four series:
Series 1: Control series (7 animals). These animals received a complete sham operation, without cold lesion or hypotension.
Series 2: In 14 animals cold injuries were induced by freezing 1.34 cm^2 of cortex in the left fronto-parietal region for 6 minutes exactly at $-40°C$. No hypotension was effected.
Series 3: In these series (15 animals) cold lesions were induced as in series 2, and then hypotension induced.
3a. In series 3a (6 animals) the blood pressure was lowered immediately after freezing.
3b. In series 3b (9 animals) lowering of the blood pressure was delayed until 45 min. after induction of the cold lesion.

The experiments were terminated after an arbitrarily chosen time limit of 7¼ hours after induction of the cold lesion, or earlier if brain stem herniation

occurred. One animal in each series was studied longer.

After termination of the experiments the animals were sacrificed, the brains removed and fixed in a 4% formalin solution for two weeks. Paraffin sections were cut through the maximal diameter of the lesion and stained with haematoxylin-eosin (HE), periodic acid Schiff (PAS), Trichrome PAS; and according to the method of Klüwer-Barrera.

Statistical analysis

The differences between the values at the different time levels (i.e. in the period ¼ to 7¼ hours after induction of the lesion) and the control values per individual experiment were calculated. Statistical analysis was performed on these at every time level (i.e. every half hour) using Wilcoxon's ranking test. The following series were compared:

A. Control *versus* cold injury
    control — series 2
    control — series 3a
    control — series 3b

B. Cold injury *versus* cold injury + hypotension
    series 2 — series 3a
    series 2 — series 3b

C. Immediate *versus* delayed hypotension
    series 3a — series 3b

RESULTS

The systemic effects of Arfonad ® and the effect of hypotension on the development of increased ventricular fluid pressure after cold injuries in cats have been described elsewhere (Maas and de Lange, 1977). The results reported in this paper concentrate on the effect of hypotension on the changes in CSF enzyme activity.

CSF enzymes after cold injury — no hypotension (series 2).

Five of the fourteen animals in this series developed brain-stem herniation within 2½ hours of induction of the cold lesion. The survival time in these animals was too short for changes in CSF enzyme activity to occur. Hence they were excluded from further analysis.

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A significant increase (Wilcoxon $p < 0.01$) of the activity of all enzymes studied in the CSF was demonstrated in the remaining nine animals. All enzymes showed a similar pattern of response. The dynamics of these changes have been described previously (Maas, 1977a). The results obtained are illustrated in fig. 1 for CPK.
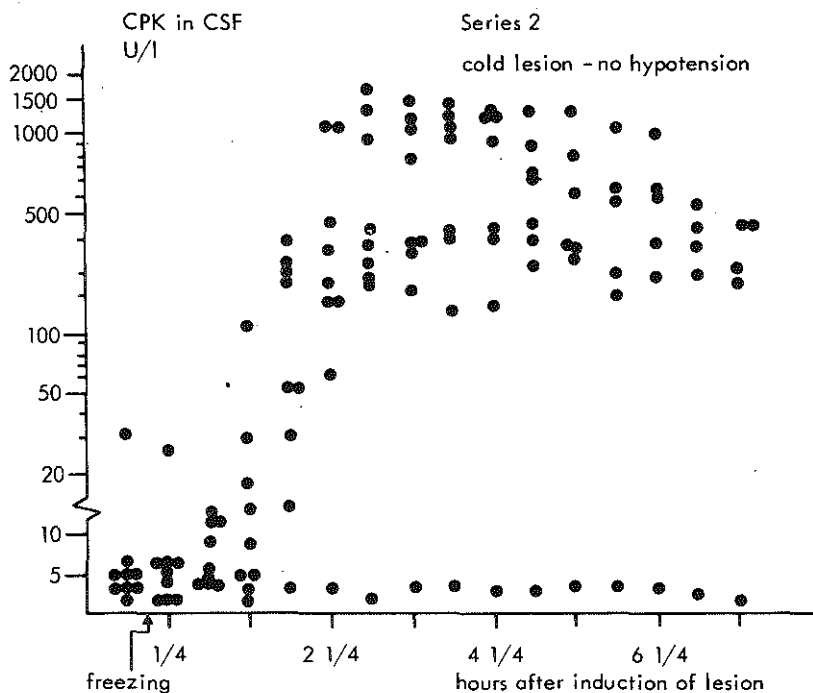

Fig. 1. Scatter diagram showing the levels of CPK activity observed in the CSF of cats after induction of a cold lesion to the brain. These animals were not treated with hypotension.

All animals, surviving the cold injury for more than 2½ hours, excepting one developed increased CSF enzyme activity.

CSF enzymes after cold injury + immediate hypotension (series 3a).

No appreciable changes in the activity of the enzymes studied in the CSF were noted in three of the six animals in this series. Three animals developed a slight increase in CSF enzyme activity, but this rise occurred later and was far less pronounced than in the series without hypotension. The results are illustrated for CPK in fig. 2. The other enzymes exhibited a similar pattern.
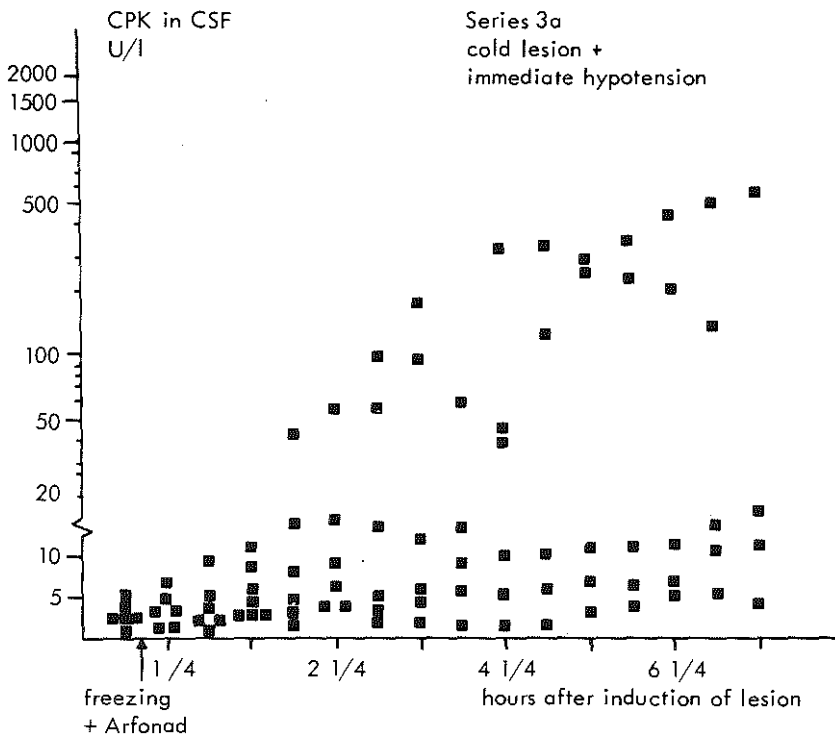
Fig. 2. Scatter diagram showing the levels of CPK activity observed in the CSF after induction of a cold lesion to the brain in cats, in which hypotension was effected immediately after freezing. The effect of the induction of hypotension on the development of raised CPK levels in the CSF after freezing is evident when figures 1 and 2 are compared.

CSF enzyme values in series 3a did not differ significantly from the control series. The levels of all CSF enzymes studied in series 3a were significantly less elevated (Wilcoxon p < 0.01) than in series 2.

CSF enzymes after cold injury + delayed hypotension (series 3b).

In series 3b induction of hypotension was delayed until 45 min. after induction of the cold lesion. Within these first 45 min. the Ventricular Fluid Pressure (VFP) had already risen to values of 7.6-17.5 mm.Hg. (control values 0.8-5.8 mm.Hg.). One animal developed a progressive gradient between the ventricular and cisternal fluid pressure shortly after induction of hypotension, and was excluded from further analysis as an early interruption of flow from ventricle to cistern seemed probable.

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Of the remaining eight animals, five did not develop any appreciable changes in CSF enzyme activity at all. One experiment was terminated prematurely, because of mechanical difficulties with the cisternal catheter. Two animals did develop a considerable increase in activity of all CSF enzymes. One of these was studied for an additional 6 hours under hypotension. The pattern of changes in the activities of the enzymes CPK and α-HBDH in the CSF are given in fig. 3.
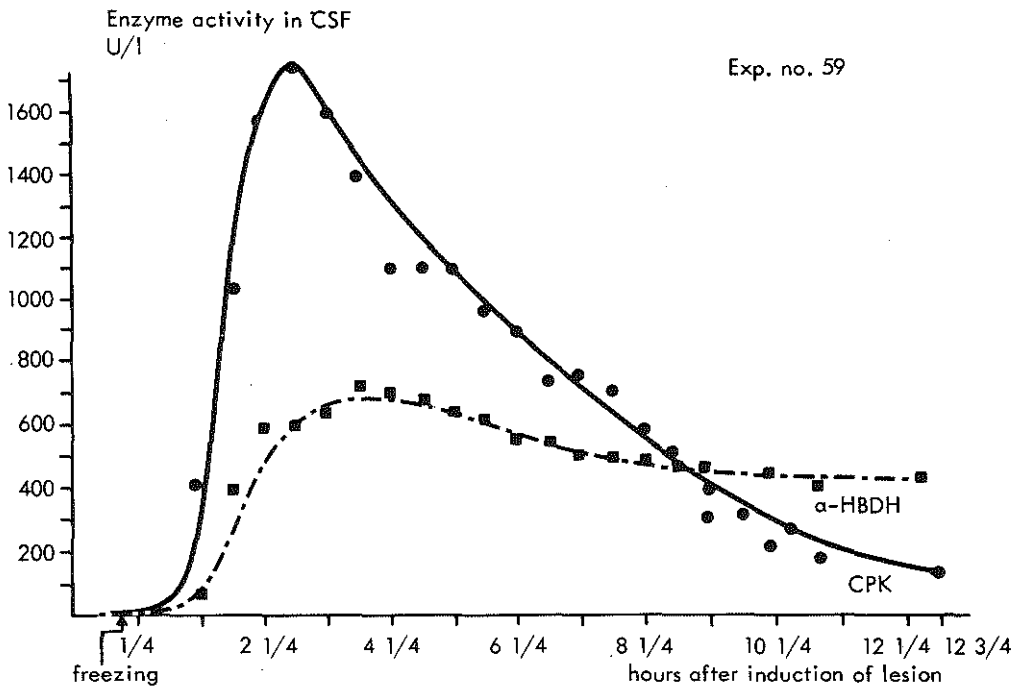

Fig. 3. Changes in the activities of the enzymes CPK and α-HBDH in the CSF of animal No. 59. This animal demonstrates a considerable rise in the activities of both enzymes, although hypotension was effected ¾ hour after induction of a cold lesion to the brain.

The rapid increase and fall of CSF enzyme activity observed in this animal suggests that the greatest changes occur within 12 hours after injury.

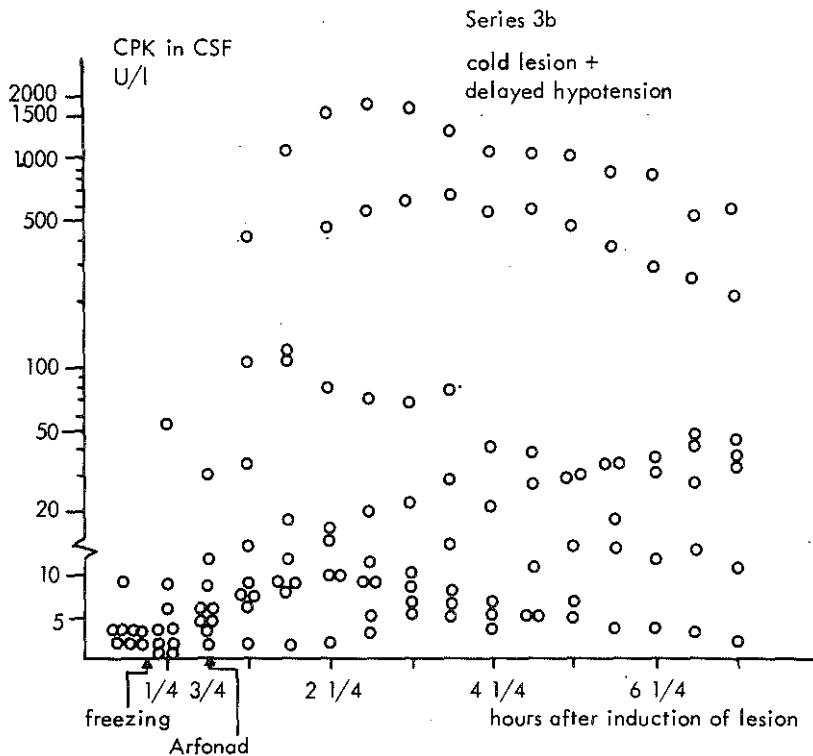The over-all results of series 3b are illustrated for CPK in fig. 4.

Fig. 4. Scatter diagram showing the levels of CPK activity observed in the CSF after induction of a cold lesion to the brain in cats, in which hypotension was effected ¾ hour after freezing. The effect of delayed hypotension on the development of raised CPK levels in the CSF after induction of cold lesions to the brain is evident when figures 1 and 4 are compared.

The enzymes CPK, α-HBDH, LDH, GOT were significantly raised (Wilcoxon $p < 0.05$) in the CSF above the control series. ChE was not. Statistical analysis of the enzyme values between series 2 and 3b revealed at most a tendency for the lower enzyme levels, to occur in the animals with hypotension. This lack of significancy was due mostly to the presence of the non-responding animal in series 2. When this animal was excluded from further analysis, the difference becomes significant for all enzymes (Wilcoxon $p < 0.05$). No statistical difference existed between the series with delayed and the series with immediate hypotension.

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Histologic studies

The histologic changes in the brain after induction of a cold lesion have been well documented (Klatzo et al., 1958; Clasen et al., 1962; Go et al., 1967). The lesion induced in our studies resulted in a relatively large zone of focal necrosis and development of extensive cerebral edema, confined to the ipsilateral hemisphere. Edema was evident by less intense staining and dispersion of the myelin fibres.

The induction of hypotension resulted in two marked differences: less edema, and fewer haemorrhages in the necrotic zone. Only four of the fifteen animals in the series with hypotension (series 3a and 3b) exhibited extensive brain edema. These four animals had developed increased CSF enzyme levels.

DISCUSSION

It was thought that the amount of enzymes released from injured brain tissue into the CSF would be related to the extent of brain damage, and so could be of prognostic value (Smith et al., 1960; del Villar et al., 1973). However, prognostic value of CSF enzyme levels will also depend on the consistency with which enzymes released from damaged or necrotic brain cells are transported to the CSF.

Previous studies on changes in CSF enzyme activity after a cold lesion to the brain in cats, demonstrated a considerable rise of the enzymes CPK, α-HBDH, LDH, GOT and ChE in the CSF of eight of nine animals surviving more than 2½ hours after induction of the lesion (Maas, 1977a). One animal did not respond to the injury with any rise of CSF enzyme activity at all. The only factor that distinguished this animal was a lower blood pressure. It was considered possible that this might be the cause of the lack of CSF enzyme response. The same phenomenon was also noted in another series of experiments (Maas and Beks, 1974). Further research into the relation between arterial blood pressure and CSF enzyme levels after brain injuries seemed indicated.

The results reported here suggest that lowering the arterial blood pressure delays or even inhibits the appearance of enzymes in the CSF in the acute phase after brain injury (Table I).

Although no changes were observed of CSF enzyme activities after the cold lesion in more than half of the animals with hypotension, the expected cold lesion was present. Necrosis was marked. In our opinion it is not conceivable that no enzymes are then released into the ECF. However, these enzymes did not appear in the CSF. Probably the transport of enzymes towards the CSF was delayed or inhibited.

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Table I

Number of animals developing changes in CSF enzyme activity after a cold
injury in the series with and without hypotension

Series	No change	Evident increase
No hypotension (series 2)	1	8
Immediate hypotension (series 3a)	3	2
Delayed hypotension (series 3b)	5	2

Transport through the ECF towards the CSF is in theory possible by way of
simple diffusion, by bulk flow of ECF, by flow of edema fluid, or by a
combination of these possibilities. Klatzo et al. (1967) have demonstrated that
the spreading of edema can be slowed by inducing hypotension. Our studies also
revealed less edema in the animals with hypotension. In four hypotensive
animals edema was more obvious. In these animals the activity of CSF enzymes
was considerably raised.

Probably enzymes are transported more slowly through the ECF towards the
CSF in the absence of brain edema. Apparently the process of diffusion, or of
normal bulk flow of ECF is not sufficient to transport the enzymes from the
necrotic site of the brain towards the CSF within the first 7 hours after injury.

Cserr and Ostrach (1974) have demonstrated a bulk flow of ECF towards the
CSF in the presence of brain edema. Our results seem to confirm this, but would
also indicate that bulk flow of ECF towards the CSF is either slow or absent
when brain edema is slight.

CONCLUSIONS

Studies on the changes in CSF enzyme activity following cold lesions of the
brain in cats made hypotensive indicate that no increase of the enzyme levels
occurs in the CSF in the first seven hours after injury, in the absence of
extensive brain edema, even although extensive cortical necrosis is present. This
entails that early determination of CSF enzymes in patients with severe brain
injuries will only be of value as a prognosticon when a combination of brain
damage and extensive brain edema is present.

ACKNOWLEDGEMENTS

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EFFECT OF MODERATE ARTERIAL HYPOTENSION COMBINED WITH REPEATED WITHDRAWAL OF CEREBROSPINAL FLUID ON THE DEVELOPMENT OF INCREASED VENTRICULAR FLUID PRESSURE AFTER COLD INJURIES IN CATS

*A. I. R. Maas and S. A. de Lange**

SUMMARY

The influence of Trimetaphan induced hypotension was studied on the development of increased ventricular fluid pressure after induction of cold injuries of the brain in cats. Hypotension was induced in two series of animals: in one immediately after freezing and in another series after a delay of three quarters of an hour.

In the animals not treated with hypotension ventricular fluid pressure increased considerably according to three types of response. In both series treated with hypotension the ventricular fluid pressure and elastance were significantly lower than in the animals not treated; also brain stem herniation tended to occur less frequently in the animals treated. No deleterious effect of the hypotension was seen on cerebral metabolism as measured by the lactate content in the cerebrospinal fluid.

INTRODUCTION

The most frequent and often serious complication in patients with severe head injury is brain-edema. It acts as a space occupying lesion as well as a disrupting factor of brain function. It may be a major cause of death in patients with severe head injuries (RANSOHOFF, 1972). Through the years many different kinds of treatment for traumatic brain edema have been devised. The great variety of therapies, however, might be an indication of the fact that none of them can be regarded as a panacee.

Surgical as well as pharmaceutical measures have been advocated, directed at reducing dangerously high intracranial pressure, e.g. decompression and osmotherapy. Controlled hyperventilation causes a decrease in arterial PCO_2 which results in cerebral vasoconstriction, and thereby also reduction of intracranial pressure. Hyperosmolar agents, such as mannitol, urea and glycerol, withdraw water from brain tissue with an intact blood-brain barrier. These therapies are directed at a reduction of intracranial pressure, but the results, useful though they can be, are not obtained by reducing brain edema. After initial reports on the use of corticosteroids in the treatment of brain edema (GURDJIAN and WEBSTER, 1958, GALICICH and FRENCH, 1961, FRENCH and GALICICH, 1964, FRENCH, 1966) it was hoped that trau-

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matic brain edema could be influenced favourably by these hormones as well. Extensive animal experiments, however, provided evidence that corticosteroids given after brain trauma had only little influence on the quantity of edema that was formed (SANO et al., 1972).

Neurosurgeons, using controlled hypotension in brain surgery, got the impression that their patients tended to develop less brain edema than patients operated upon under normotension. KLATZO et al. (1967) provided proof that, lowering the arterial bloodpressure reduces the formation of brain edema after cold injuries induced in cats, while hypertension accelerates the rate of edema formation. CLASEN et al. (1968) demonstrated diminished water and dye uptake after freezing lesions induced in cats, which were subsequently treated with hypotension or hypothermia.

Only very little research has been done, either clinical or experimental, to evaluate the possible beneficial effect of induced hypotension on the treatment of brain edema. A drawback of hypotension could be, that it may jeopardise the cerebral blood flow increasing the risk of brain hypoxia. Still, in view of the lifethreat posed by massive cerebral edema, it was considered worthwhile to investigate the therapeutic effect of moderate arterial hypotension on the development of increased intracranial pressure in the experimental situation. The effect of moderate arterial hypotension induced by intravenous infusion of Arfonad on the increase of ventricular fluid pressure after cold injuries was studied in cats. Hypotension was combined with the repeated withdrawal of cerebrospinal fluid.

MATERIAL AND METHODS

Thirty-six healthy adult cats, weighing 2800-6400 grammes, otherwise unselected for age or sex, were sedated with phenobarbital intramuscularly (50 mg./kg. weight) and anaesthetized with Pentothal intraperitoneally (25 mg./kg. weight). Atropine (0.5 mg.) was given to all animals. Anaesthesia was maintained with a mixture of N_2O and O_2 (2.5 : 1) administered via an endotracheal tube. The animals breathed spontaneously throughout the experiment. After cannulating the femoral vessels the animals were placed in a stereotaxic frame (David Kopf Instr.). A burr hole was made in the left front-parietal region and a cooling thermode screwed into this burr hole over the ectosylvian and suprasylvian gyri.

Arterial blood pressure, end expiratory CO_2, ECG, ventricular and cisternal fluid pressure were continuously monitored and values measured every half hour, just prior to withdrawal of CSF samples. Arterial blood pressure was measured via a cannula in the right femoral artery. Ventricular fluid pressure was monitored via a stereotactically placed cannula in the cella media of the right lateral ventricle.

Cisternal fluid pressure was measured by a lumbar needle, placed in the cisterna magna after the atlanto-occipital membrane had been exposed. Brain elastance was calculated according to the equation $\dfrac{\Delta P.,}{\Delta V.}$ in which ΔP stands for the fall in VFP

caused by the withdrawal of CSF samples ($\Delta V = 0.35$ ml.) Brain elastance is a measure for the reserve capacity of the brain to compensate for an increase in intracranial volume (MILLER *et al.* 1973). Samples of CSF (0.35 ml.) were withdrawn every half hour from the cisternal catheter, which was connected to the transducer via a three-way stop cock. The samples of CSF were analysed for the activities of various enzymes. The results of these studies have been reported elsewhere in detail (MAAS, 1977). The samples of CSF were also analysed for the lactate content; lactate was estimated according to the enzymatic method of Boehringer, Mannheim (HOHORST, 1970).

After the beginning of cisternal taps an equilibration period of $1^1/_2$ hours was allowed. After this equilibration period, controlvalues were established by averaging the values measured in the next $1^1/_2$ hours. After this controlperiod a cold lesion of the brain was induced in 29 animals. In 7 animals no cold lesion was induced; this group served as a control series. The cold lesions were induced according to the method of BEKS and coworkers (1965). Cooled Methanol (-40° C) from an ultracryostat (Lauda) was led through the thermode for 6 minutes exactly. The surface of the thermode in contact with the brain was 1.34 cm^2. After freezing, the connecting tubes were disconnected, but the cooling thermode left in situ, so that the actual duration of freezing was longer.

After freezing, hypotension was induced in 15 animals. Fourteen animals were not treated with hypotension.

Hypotension was induced rapidly by intravenous infusion of Trimetaphan (Arfonad ^{R}) in a concentration of 0.5-1.0 mg./ml. 0.5-1 cc of Arfonad was sufficient to reduce the mean arterial blood pressure to 75-90 mm Hg. Hypotension was maintained by slow intravenous infusion of Trimetaphan. The amount of Arfonad needed to maintain the blood pressure at this level varied considerably per animal.

The experiments were divided into 4 series:

Series 1. Control series. This series consisted of 7 animals. They received a complete sham operation. CSF was withdrawn every half hour, but no cold lesion was induced, nor was hypotension effected.

Series 2. In this series of 14 animals a cold lesion of the brain was induced according to the method described, CSF samples were withdrawn every half hour, but no hypotension was effected.

Series 3. In this series, comprising a total of 15 animals the same cold lesion was induced as in series 2, CSF samples were also withdrawn, but after freezing, hypotension was induced. This series was divided into 2 subgroups:

3a. In series 3a, consisting of 6 animals, the blood pressure was lowered immediately after freezing.

3b. In series 3b, consisting of 9 animals, lowering of the blood pressure was delayed until 45 min. after induction of the cold lesion. This delayed hypotension was considered to be more relevant to the clinical situation, when obviously some delay must occur between ictus and possible induction of hypotension in a therapeutic attempt to limit formation of brain edema.

The reported results concentrate on the effect of hypotension on the development of increased ventricular fluid pressure in the first 7 hours and 15 min. after induction of the cold lesion. Most experiments were terminated after this 7 hour and a quarter study period. The experiments were terminated earlier if brain stem herniation occurred. Brain stem herniation was considered to have been developed if all the following symptoms were present:

a. a progressive gradient developed between the ventricular and the cisternal fluid pressure, the first being more elevated.

b. CSF could no longer be obtained from the cistern, although the cisternal needle was still in place (block of CSF flow from ventricle to cistern).

c. Slowing of the respiratory frequency occurred.

After termination of the experiments the animals were sacrificed with an overdose of intravenous Pentothal and the brains were removed. The brains were fixed in a 4 per cent formalin solution for two weeks, and paraffin sections cut through the maximal diameter of the lesion and stained with Haemotoxalin-Eosin (HE), Periodic-Acid-Schiff (PAS), Trichrome PAS and according to the method of Klüwer-Barrera.

STATISTICAL ANALYSIS

The differences between the values measured at the various timelevels (i.e. every half hour after freezing) and the control values per individual experiment were calculated. Statistical analysis were performed on these calculated differences at every time level (i.e. every half hour) using Wilcoxon's ranking test. The following series were compared:

control   – series 2
control   – series 3a.
control   – series 3b.
series 2   – series 3a.
series 2   – series 3b.
series 3a. – series 3b.

Differences in occurrence of tentorial herniation in the series with and without hypotension were analyzed with Fisher's test.

RESULTS

Development of increased intraventricular pressure after cold injury – no hypotension (series 2)
All fourteen animals in this series developed an increase in ventricular fluid pressure (hereafter to be abbreviated as VFP) after induction of the cold lesion. Three types of response were noted:

I. Rapidly progressive increase of VFP, resulting in the development of brain stem herniation within a few hours of injury.

II. A continuous but less rapid increase of VFP, or an initially rapidly increasing VFP, flattening off at a sort of plateau level and only increasing gradually thereafter.

III. A slow, gradual increase of VFP.

Type I. Response was noted in 5 cases, type II in 7 and type III in only 2 animals.

The three types of response are illustrated in fig. 1, showing the development of increased VFP in the animals not treated with hypotension.
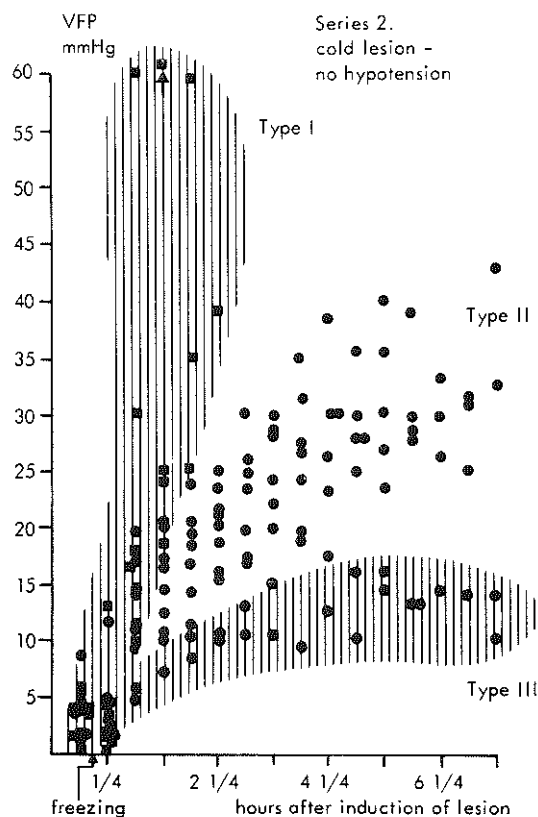

Fig. 1. Development of increased ventricular fluid pressure in the first 7 hours and 15 min. after induction of a cold lesion in cats not treated with hypotension (series 2). This graph illustrates the three types of response of the ventricular fluid pressure to freezing lesions.

Systemic effect of Arfonad

The average mean arterial blood pressure before induction of hypotension was 132.5 mm.Hg.; by infusion of Arfonad the mean arterial blood pressure was lowered to 75-90 mm Hg. and maintained at this level. It was not possible to effect a gradual decrease of the blood pressure. In all cases a fairly sudden fall was produced. However, once lowered, it was not very difficult to maintain the blood pressure at the desired level. Arfonad resulted not only in a fall of the mean arterial blood pressure,

TABLE 1
Heart rate before and after induction of hypotension

	heart rate (beats/min.)	
	before induction of hypotension	30 min. after induction of hypotension
	210	150
	225	170
	200	150
	190	130
	155	140
	215	165
	155	150
	190	155
	260	170
	210	160
	220	180
	225	170
	205	165
	240	240
	150	120

but also caused a decrease of the heart rate by 15-25 per cent of the individual control value in 13 of the 15 animals studied with hypotension. This is illustrated in table 1.

TABLE 2
VFP and Elastance before and after induction of hypotension.

Exp. nr	Before induction of hypotension		Immediately after induction of hypot.		2 hrs. after induction of hypot.	
	Elastance	VFP	VFP	Cushing	VFP	Elastance
			Series 3a.			
46	12.1	4.1	8	—	7.1	18.5
47	3.5	2.3	2.5	—	1.8	3.8
48	4.7	2.5	2.8	—	7.9	19.7
52	8.6	3.4	5.3	—	8.6	20.3
53	4.9	2.5	8.1	—	1.3	2.6
64	7.1	1.8	2.3	—	4.8	10.9
			Series 3b.			
54	23.4	7.5	23	+	3.3	11.1
55	29.4	8.5	29	+	6.0	18.3
56	25.7	13.8	23.5	—	13.5	—
59	29.1	7.0	17.5	+	12.0	33.4
60	21.1	18.8	20	—	14.0	27.1
65	16.6	3.5	11	—	10.0	18.3
70	28.6	12.5	25	+	16.8	—
71	11.4	7.5	12.5	—	13.3	8.6
73	19.7	7.5	17.5	—	10.8	17.1

Range Control values:
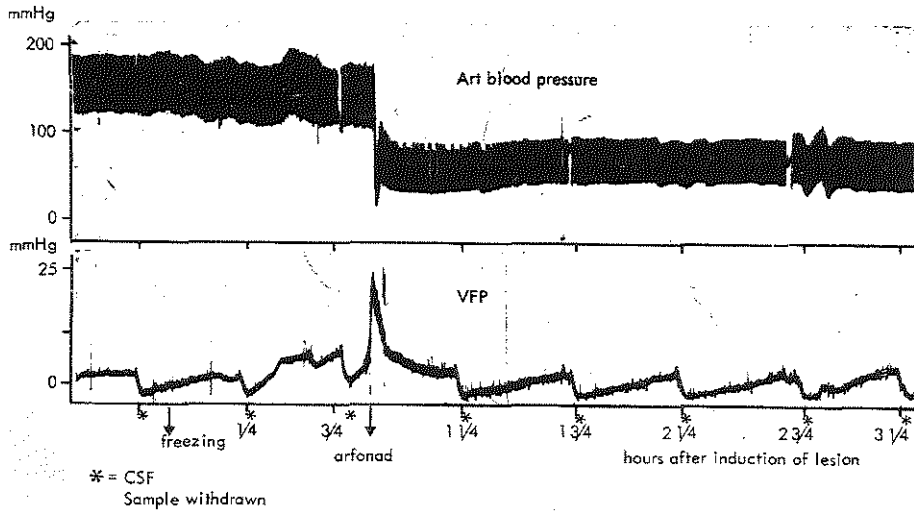Elastance: 1.8 - 15.7 mmHg/ml.
VFP   : 0.8 - 6.6 mmHg.

Fig. 2. Ventricular fluid pressure and arterial blood pressure recording of animal no. 54, illustrating the acute increase of the ventricular fluid pressure due to induction of hypotension. This recording also illustrates the effective control of VFP by lowering the arterial blood pressure and maintainnig it in a steady state at lower levels.

Acute effect of induction of hypotension on VFP

Induction of hypotension with Arfonaud caused a transient rise of VFP in 12 of the 15 animals treated with hypotension. This occurred in three of the 6 animals in which hypotension was induced immediately after freezing and in all animals in which Arfonad infusion was delayed until 45 min. after freezing. In this series (series 3b.) VFP and particularly the elastance had already risen considerably within these first 45 min. after freezing as is shown in table 2.

The transient rise of VFP during induction of hypotension was most marked in the animals with the highest elastance. A high elastance indicates a tight brain situation in which the brain cannot compensate properly for a further increase in intracranial volume. Most probably the induction of hypotension with Arfonad[R] causes a slight increase in cerebral blood volume, thereby resulting in an increase of VFP. In one case the VFP increased to 30-40 mm Hg., for cats a dangerously high level at which transtentorial herniation may occur (BEKS, 1973). In response to the acute rise in VFP a Cushing response was observed in four animals: the blood pressure increased in spite of Arfonad[R] infusion to compensate for the rise in VFP. An example of this transient rise of VFP and Cushing response is given in fig. 2.

The acute increase in VFP only lasted a few minutes after which both VFP and blood pressure stabilised at lower levels.
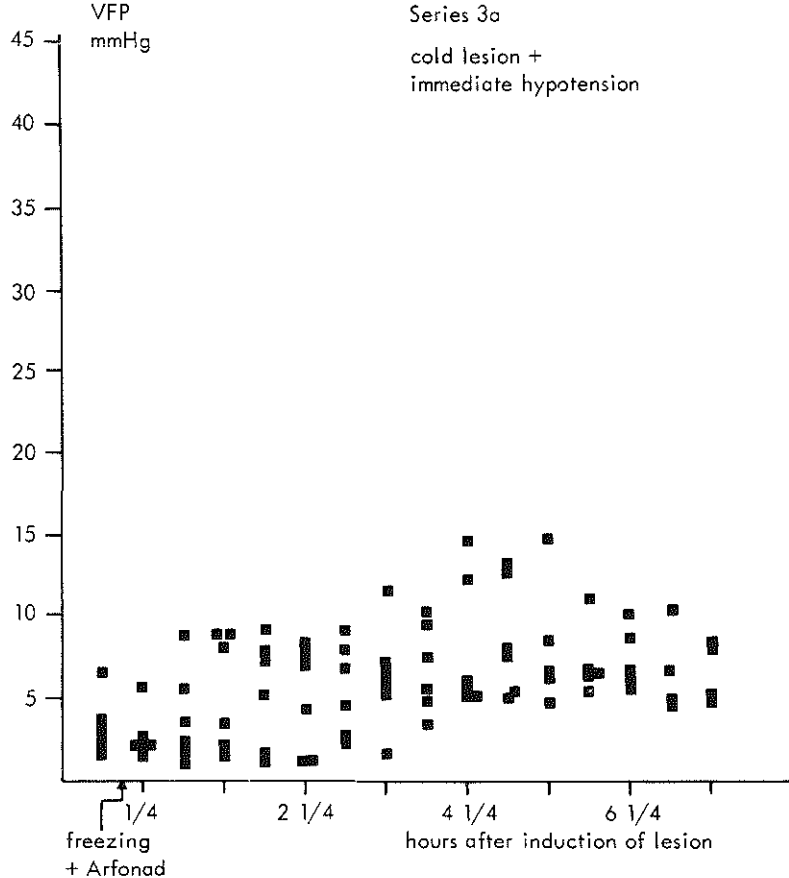
Fig. 3

Effect of hypotension on the development of increased VFP

Lowering the arterial blood pressure, had a most pronounced effect on the development of increased ventricular fluid pressure after induction of a cold lesion. The increase of VFP as occurred in the series not treated with hypotension is given in fig. 1. The effects of treatment with hypotension on the increased ventricular fluid pressure are summarized in fig. 3 and 4, showing the values of VFP observed in the animals of series 3a and 3b.

The effect of hypotension on the development of increased VFP in an individual animal is demonstrated by comparing fig. 2 and 5, giving the changes in ventricular fluid pressure after induction of a cold lesion in an animal treated and an animal not treated with hypotension.

Immediate hypotension

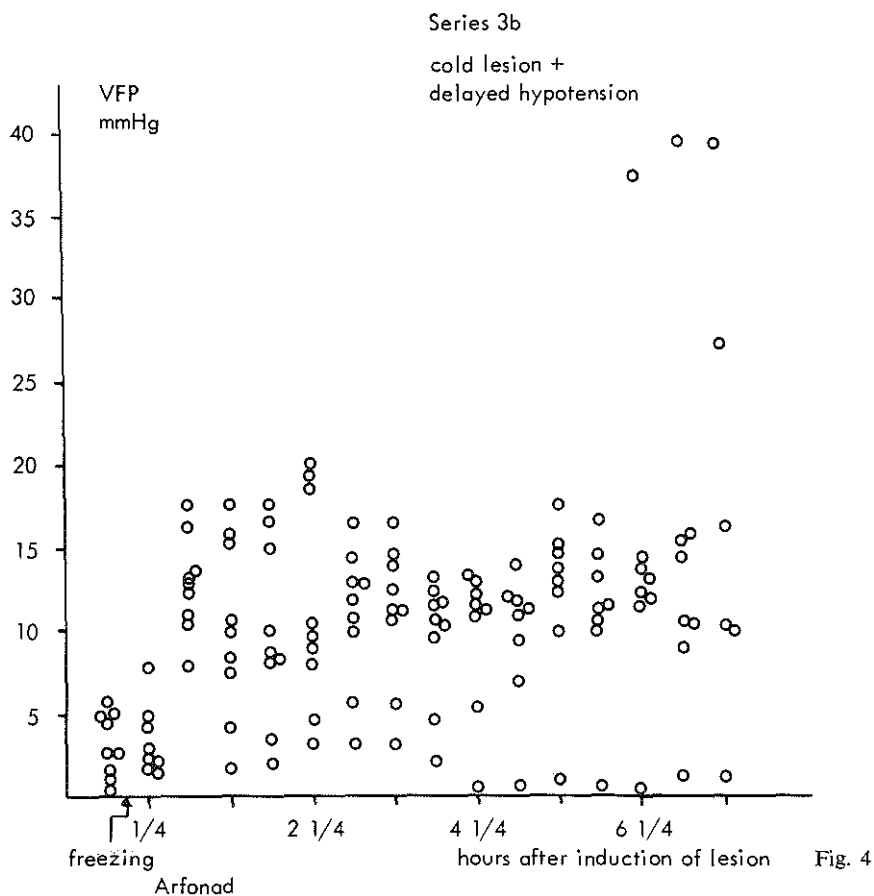In 4 of the 6 animals in series 3a. no rise of VFP had at all occurred in the first 7 hours

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Fig. 3 and 4. Ventricular fluid pressure measured at half hour intervals after induction of a cold lesion in series 3a and 3b. The animals in series 3a were treated with hypotension immediately after freezing, the animals in series 3b. after a delay of 45 min. These graphs illustrate the control of rising VFP by the induction of hypotension.

and 15 min. after freezing. One of these animals was studied for an additional 10 hours. Even then no increase of VFP developed whatsoever. No statistical difference existed between the control group and series 3a as far as VFP and elastance are concerned. Both VFP and elastance were significantly lower (Wilcoxon: p<0.01) in the series 3a than in the series without hypotension. Also elastance was significantly lower, indicating not only lower levels of VFP but also a more favourable volume pressure situation.

Delayed hypotension

Lowering the arterial blood pressure after a delay of 45 min., was also effective in controlling VFP. VFP increased in all animals before induction of hypotension.
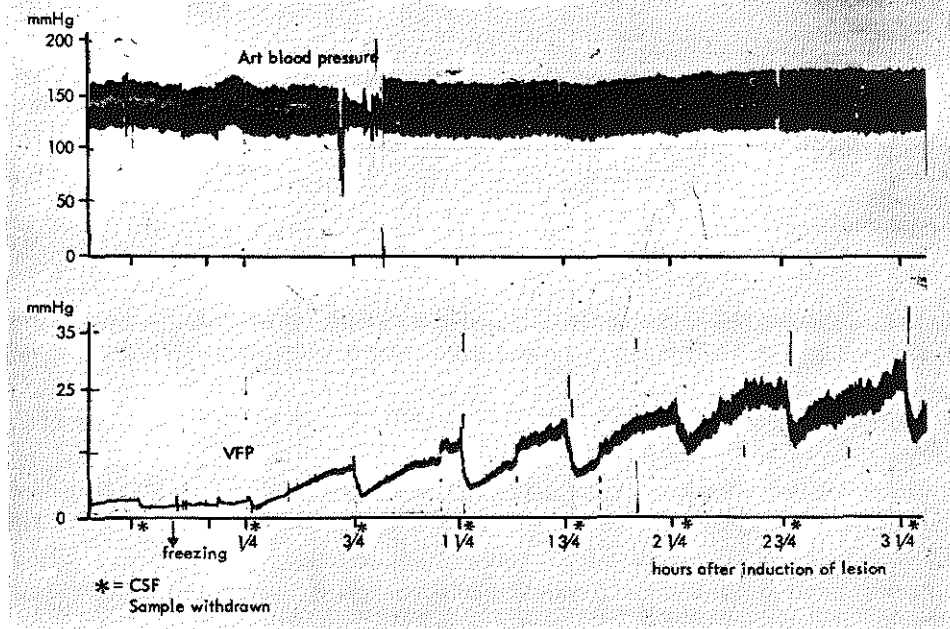
88

Fig. 5. Ventricular fluid pressure and arterial blood pressure recording of animal No. 29, illustrating the increase of ventricular fluid pressure following cold injury in an animal not treated with hypotension.

Delayed induction of hypotension resulted in one animal in a decrease of the already increased VFP to normal or even subnormal levels, which were maintained during the 7 and a quarter hour study period. In another three animals a temporary decrease below the value measured at 45 min. after freezing was produced. In the other animals VFP only increased very gradually (pressure response type III). The induction of hypotension prevented the development of pressure responses type I and II. In two animals a series of plateau waves (Lundberg type I) developed towards the end of the 7 hour and a quarter study period, resulting in the development of tentorial herniation. Both VFP and elastance were significantly lower in series 3b. than in the animals without hypotension.

Effect of hypotension on the development of tentorial herniation

Brain stem herniation developed in 8 of the 14 animals in series 2 (no hypotension). One animal developed a mechanical obstruction of the cisternal catheter and was excluded from further analysis. The remaining 5 animals in this series did not develop brain stem herniation within the 7 hour and a quarter study period.

In series 3a. (immediate hypotension) only 2 of the 6 animals developed herniation of the brain stem and in series 3b. only one of the 8 animals, while in this series obstruction of the cisternal catheter occurred in one case. These results are summarized in table 3.

TABLE 3
Occurrence of brain stem herniation in the series with and without a cold lesion.

	Herniation	No herniation	Mechanical obstruction of cisternal catheter
Control series	0	7	0
Series 2	8	5	1
Series 3a	2	4	0
Series 3b	1	6	1

On basis of these results the probability, calculated with Fisher's test, that brain stem herniation occurs less frequently in the series treated than in the series not treated with hypotension, is:

series 2 − series 3a. : p = 0.79
series 2 − series 3b. : p = 0.95

However, just after the 7 hour and 15 min. study period brain stem herniation developed in another 2 animals in series 3b. and in one more animal in series 2. This reduces the probability to p = 0.80. Nevertheless, it remains evident that a distinct tendency exists for brain stem herniation to occur less frequently in the acute phase after freezing lesions in the series treated with hypotension.

CSF Lactate

The median values of CSF lactate observed in the control series and in the series with and without hypotension are summarized in table 4.

TABLE 4
Median CSF lactate values in cold injured cats treated and not treated with hypotension.

Hours after freezing	CSF lactate in mMol/1.			
	Control series	Series 2	Series 3a	Series 3b
before	1.24	1.36	1.20	1.02
1/4	1.42	1.35	1.26	0.92
1 1/4	1.67	1.37	1.66	1.04
2 1/4	2.01	1.46	1.83	1.36
3 1/4	1.81	2.08	2.14	1.59
4 1/4	1.88	2.35	2.04	1.55
5 1/4	1.87	2.88	1.74	2.04
6 1/4	2.04	2.99	1.94	2.19
7 1/4	2.09	3.02	2.12	2.35

No statistical differences were found between the control series and the series 2, 3a. or 3b. Neither did any difference exist between the cold injury series with and without hypotension.
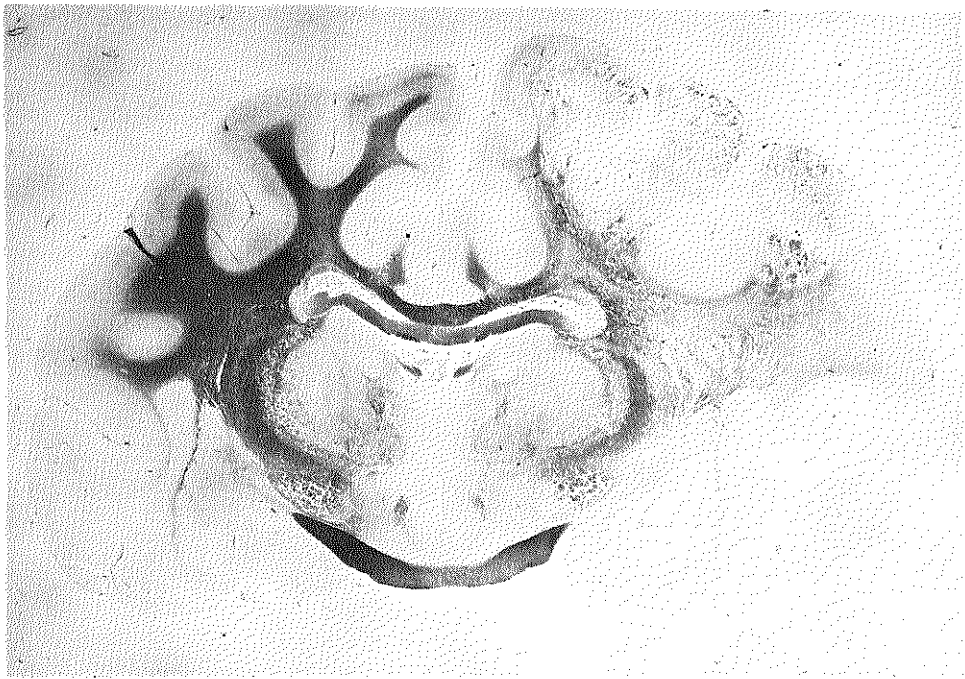
Fig. 6.   Section through maximal diameter of cold lesion in an animal not treated with hypotension.

Histologic studies

The cold lesions induced in the described studies resulted in the series which had not been treated with hypotension in a fairly large zone of focal necrosis and formation of extensive edema in the ipsilateral hemisphere. Edema was evident by less intense staining of the edematous white matter and dispersion of the myelin fibres. In the series in which hypotension was effected edema was much less marked and fewer hemorrhages were present in the necrotic zone. No ischaemic lesions were present in the animals which were not treated with hypotension and in only one case, in which immediate hypotension had been effected; the patchy ischaemic lesions in this animal occurred in the immediate surrounding of the necrotic zone, which was subject to severe edematous changes in those animals which had not been treated with hypotension.

The effect of hypotension on the development of the freezing lesions is illustrated in fig. 6 and 7, showing the microscopical aspect of the lesion developed in an animal that had not and in an animal that had been treated with hypotension.

Fig. 7. Section through maximal diameter of cold lesion in an animal treated with hypotension, starting 45 min. after induction of a cold lesion.
Note: the lesser extent of edema as compared with fig. 6.

DISCUSSION

Brain edema may result from noxious influences on the brain. Four types of brain edema are recognised: vasogenic, cytotoxic, osmotic and hydrostatic. Edema consecutive to brain trauma is mainly of the vasogenic type, both in the clinical and experimental situation.

Vasogenic brain edema results from a local or diffuse breakdown of the blood brain barrier. Proteins, mainly albumen, and water leak through the capillaries into the extracellular fluid (ECF) of the brain (KLATZO, 1972). Because of the resulting raised colloid osmotic pressure of the ECF, more water is attracted and an increase of ECF volume results: brain edema.

Although this is undoubtedly an oversimplification of the actual mechanism, it is illustrative to visualize this process as a leaking sieve through which protein molecules are pressed. The amount of transsudate will be related to the equation of forces proximal and distal of the sieve. The driving forces in the brain will be:
pressure in the capillary bed – tissue pressure – colloid osmotic pressure (blood).

In the normal brain the mechanism of autoregulation keeps the pressure in the brain capillaries within constant levels, in spite of wide variations of arterial blood

pressure. However, local brain damage with acidosis of the perivascular ECF and raised potassium content of the ECF causes impaired autoregulation. CHIGASAKI *et al.* (1970) and ISHII *et al.* (1975) also postulate on basis of cat experiments in which acute brain swelling was produced by various means, that a neural mechanism exists, which also impairs autoregulation, causing engorgement of the cerebrovascular bed, and enhancing the development of brain edema. Due to the impaired autoregulation the capillaries receive the full brunt of the arteriolar blood pressure. In these cases the quantity of brain edema that will be formed after brain damage is dependent on the mean arterial blood pressure. It has been shown that hypertension accelerates the formation of brain edema after cold injury (KLATZO *et al.* 1967; SCHUTTA *et al.* 1968) KLATZO *et al.* (1967) also demonstrated that induction of hypotension can greatly limit the spreading of brain edema after a cold injury in cats. GO *et al.* (1974) have also demonstrated less edema developing after cold injuries in cats, when the blood pressure was low. These authors further suggest that the variable response of VFP observed after cold injuries in cats may be due to a variable degree of vasomotor disturbance.

Obviously the height of the blood pressure is one important factor that determines the quantity of edema in a damaged brain. Lowering the arterial blood pressure may be expected to have a beneficial effect in preventing or limiting formation of brain edema. However, it is conceivable that lowering the arterial blood pressure could influence also the cerebral blood flow in a detrimental way, depending on the degree of hypotension and the level of the intracranial pressure.

Ischaemic lesions are a prominent feature in brain injuries (ADAMS and GRAHAM 1972) and if induced hypotension in brain damaged patients would be considered, cerebral metabolism and blood flow should be monitored. In our experiments a pronounced beneficial effect of moderate hypotension on the development of increased VFP in cats with cold injuries was found. During these experiments we lacked the facilities to monitor cerebral blood flow. However, the lactate content of the CSF was determined as an indicator of cerebral metabolism (SALAYMEH, *et al.* 1971). CSF lactate rises during hypoxia when aerobic glycolysis is insufficient and anaerobic glycolysis is stimulated. In our experiments higher levels of CSF lactate were found in animals with a cold lesion than in the control series, but the differences were not statistically significant. In our experiments the respiratory frequency varied considerably per animal, this probably could explain the failure to attain statistical significancy as SCHUBERT *et al.* (1973) have emphasized the effect of respiratory changes on CSF lactate, demonstrating higher levels when the arterial PCO_2 was lowered.

The effect of hypotension on CSF lactate levels has been studied also. MAGNESS *et al.* (1973) demonstrated raised CSF lactate levels in dogs who where made hypotensive. It was demonstrated that the lactate levels were clearly more raised when hypotension was effected by bleeding, than in drug-induced hypotension (Trimetaphan). In our experiments no difference in CSF lactate was seen between the cold injured animals with and without hypotension. Lower levels of CSF lactate were even observed in the series treated with hypotension. On the basis of these results it may be con-

cluded that moderate arterial hypotension induced by Trimetaphan does not significantly alter CSF lactate after cold injuries in cats. Care must be taken, however, to translate these findings uncritically into a clinical situation. Moreover, KOGURE *et al.* (1975) have demonstrated that metabolites in the CSF may not always indicate the condition of the underlying cerebral tissue.

In summary, the described studies have demonstrated an obviously beneficial effect of moderate drug induced arterial hypotension on the development of raised intracranial pressure after cold injuries in cats. No deleterious effect of the hypotension was seen on cerebral metabolism, as measured by CSF lactate content. Further experiments are necessary to ensure that in cases of high intracranial pressure induced hypotension does not impair the cerebral blood flow in a way that may damage the brain even more. A further point to consider is the fact that during induction of hypotension a transient rise of ventricular fluid pressure may occur, which may be dangerous, especially in a 'tight brain' situation.

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CONCLUSION

The activity of the enzymes CPK, α-HBDH, LDH, GOT and ChE in the CSF is related to the extent of brain damage in the acute phase after experimental brain injury when extensive brain edema is present.

Chapter IV

DISCUSSION

The results of the studies described in Chapter 3 have already been extensively discussed in the various papers.

In this chapter it will be attempted to discuss the combined results of the four papers with respect to two items. The ultimate object of these studies, namely what the clinical implications of the experimental results are, will be considered, but before doing so the transport mechanism of enzymes from the extracellular fluid of the brain to the CSF is discussed. This is done, because it is felt that the results described offer some evidence for bulk flow of edema fluid towards the CSF after cold injury edema. The implication that edema fluid may drain into the CSF is considered to be of importance.

4.1. Transport mechanism of enzymes from ECF to CSF

Transport of molecules from the extracellular fluid of the brain (ECF) to the CSF may in theory be possible by way of:

    diffusion
    bulk flow of ECF
    flow of edema fluid,

or by a combination of these possibilities. In the vasogenic type of brain edema, as results from cold injuries, the edema fluid is located mainly in the extracellular compartment (Klatzo, 1972); Edema fluid may consequently be regarded as (an increase in) extracellular fluid, and flow of edema fluid as essentially the same as flow of ECF.

Diffusion is an important transport mechanism on cellular level, and can occur within fractions of seconds from the interstitial fluid into the cell and vice-versa, but becomes a very slow process when large molecules and greater distances are concerned (table 7).

Table 7

Calculated mean transit time for diffusion of sucrose and albumin through
brain tissue

Tissue thickness in cm.	t_D in hours	
	sucrose	albumin
0.1	0.6	4.4
0.2	2.5	17.6
0.3	5.6	39.7
0.4	9.9	70.6
0.5	15.4	110.2
0.6	22.2	158.7
0.7	30.2	216.1
1.0	61.7	440.9

Mean transit time (t_D) calculated according to: $t_D = x^2/3D$ (Cserr, 1971)

x = tissue thickness in cm.

D = diffusion coefficient

D for sucrose in periventricular white matter: 1.5×10^{-6} cm^2/sec.

D for albumin in periventricular white matter: 0.21×10^{-6} cm^2/sec. (Reulen et al., 1977)

Mol. mass sucrose: 342

Mol. mass albumin: 69.000

Molecules may enter the CSF from the ECF either across the ependyma into
the ventricles, or across the pia mater into the subrachnoid space. The transport
mechanism towards the ventricles will be considered first.

In order to consider whether diffusion is a feasible mechanism of transport of
enzymes released from necrotic sites towards the ventricular CSF, the shortest
distance from the necrotic site to the brain ventricle must be known. This
distance was calculated from tracings made of frontal sections through the
maximal diameter of the cold lesions. The method of measuring this distance is
illustrated in Fig. 2. The borders of the lesion in the cortical layers were sharply
defined and easy to trace (fig. 3), but the extent of the cold lesion into the
depth (white matter) was less sharply demarcated, and more difficult to judge
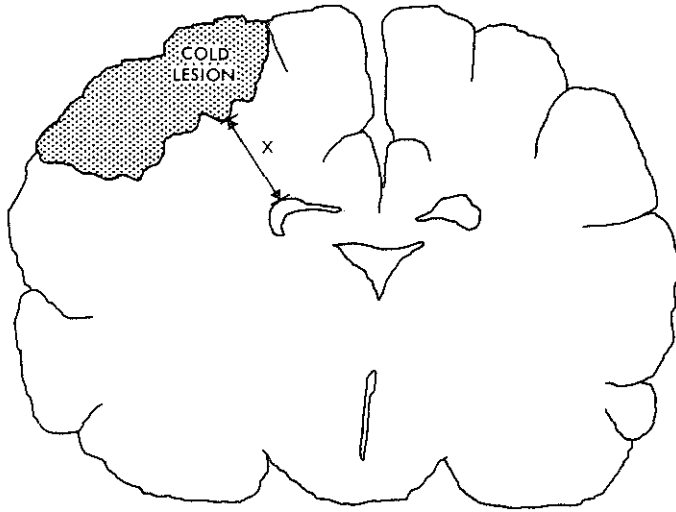exactly.

Fig. 2. Schematic drawing illustrating estimation of the minimal distance (x) from the necrotic area to the ipsilateral ventricle.
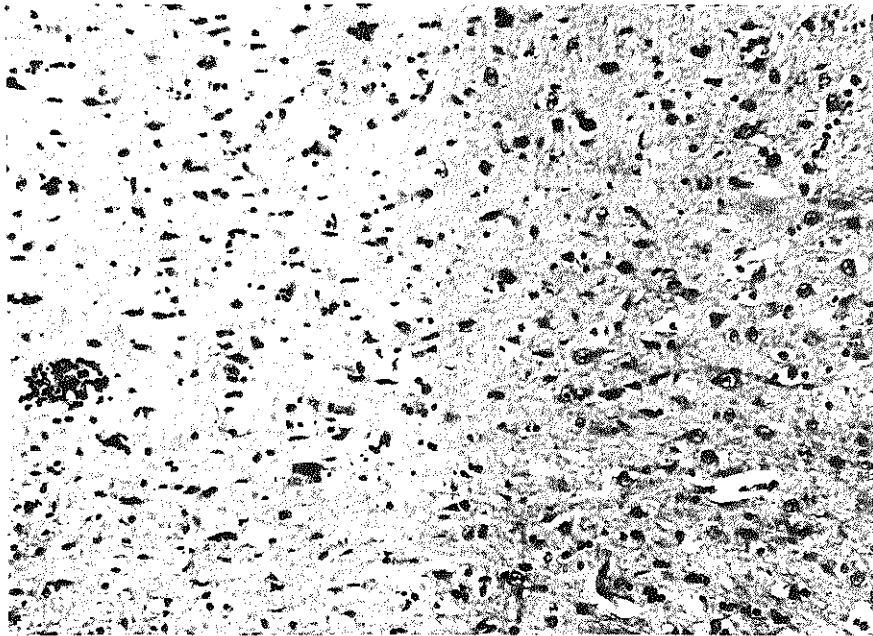

Fig. 3. Detail of border of cold lesion of the brain (HE 150x); the borders of the lesion in the cortical layers are sharply defined.

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The resulting measurements are consequently only an approximation and cannot be considered as more than a rough estimate of the actual minimal distance from the necrotic lesion to the ipsilateral ventricle. Moreover these measurements were taken from the formalin fixed preparation, in which some shrinkage had inevitable occurred, so the estimated distances probably tend to be shorter than in vivo.

Table 8 shows the distance from the necrotic site to the ipsilateral ventricle as measured in eight animals in which extensive cold lesions of the brain were induced (freezing for 6 min. at $-40°C.$). The mean transit time theoretically required for albumin to diffuse across these distances was also calculated according to the equation mentioned by Cserr (1971). For comparison the actual time before raised levels of CSF enzyme activity were seen is also given.

Table 8

Estimated distance from necrotic site to ventricle, calculated time for albumin to traverse this distance, and actual time until enzymes appeared in CSF

Exp. No.	Distance necrotic site to ventricle	t_D estimated in hrs. for albumin	actual time before CSF enzymes increased
26	1.1	5.3	2
28	2.9	37.1	2½
29	3.9	67.1	1½
32	2.0	17.6	2
35	2.9	37.1	1¾
39	3.7	60.4	no enzyme ↑
43	3.5	54.0	1¾
44	2.9	37.1	1¼

The expected transit time for the enzymes studied was not calculated as no data were available on the diffusion coefficients in brain tissue. But as the diffusion coefficient is inversely related to the root of the molecular mass (Netter, 1969), and as the mol. mass of the enzymes studied is greater than that of albumin (table 9), the transit time may be expected to be longer for the enzymes studied than for albumin.

On basis of these data it is tempting to conclude that in theory anyway diffusion is too slow a process to transport enzymes released from the necrotic site into the ECF towards the ventricular CSF within the relatively short time period in which the animals were studied.

Table 9

Molecular mass of albumin and of the enzymes studied

	mol. mass
Albumin	69.000
CPK	80.000
GOT	90.000
GPT	115.000
LDH	160.000
ChE	300.000

From: Th.E. Barman, Enzyme Handbook I: Springer Verlag, Berlin, Heidelberg, New York 1969.

However, only the *mean* transit time (= average transit time of all molecules) for diffusion of albumin was calculated according to Cserr's equation. It is quite possible that a smaller fraction of the enzymes present in the extracellular fluid of the necrotic site reaches the ventricle at an earlier moment.

The concentration resulting from diffusion at a point x cm. distant from the point of origin relative to the concentration at the point of origin (i.e. the concentration gradient C_x/C_0) may be calculated according to the following equation (Netter, 1969):

$$C_x/C_0 = \text{erfc}\ \frac{x}{2\sqrt{Dt}}$$

C_x the concentration in the extracellular space at a point x cm. distant from the point of origin.
C_0 the concentration at the point of origin.
D   the effective diffusion coefficient in brain tissue
t   the time in seconds.
erfc denotes the complementary error function, calculated according to the formula:

$$\text{erfc}\ x = 1 - \frac{2}{\sqrt{\pi}} \int_0^x e^{-t^2}\ dt$$

Table 10 shows the calculated concentration gradients (C_x/C_0) as would be expected to result from diffusion of albumin through brain tissue at various time levels and distances from the point of origin.

Table 10

Calculated concentration gradients (C_x/C_0) for albumin per millimeter resulting from diffusion at hourly intervals

Distance from necrotic site	Hours after induction of cold lesion						
	1	2	3	4	5	6	7
in mm.							
1	0.01	0.069	0.138	0.198	0.250	0.294	0.331
2	0.26×10^{-6}	2.7×10^{-4}	0.0029	0.010	0.021	0.036	0.052
3		4.7×10^{-8}	8.4×10^{-6}	0.0001	0.00036	0.0016	0.0035
4				2.7×10^{-7}	4.2×10^{-6}	3×10^{-5}	0.00010
5							1.17×10^{-6}

The activity of LDH in the extracellular fluid of the necrotic region resulting from cold injury in cats (produced by freezing 1.34 cm^2 for 5 min. at -30 to $-40°$C.) has been shown to increase to a mean level of 2070 U/l (Go et al., 1976).

For a significant increase in the LDH activity of the CSF above control levels to be observed, an increase of at least 20 to 30 U/l is required; this is approximately 1/100 of the activity in the ECF of the cold lesion.

On comparing tables 8 (distance from necrotic site to ventricle) and table 10 (theoretical concentration gradients) it may be concluded that diffusion is not a likely mechanism for transport of enzymes from the necrotic site to the ventricular CSF within the time period in which the animals were studied.

Diffusion directly from the necrotic site into the subarachnoid space is conceivable, but may be assumed to be difficult for large molecules as — apart from the limitations imposed by the process of diffusion — no gap junctions are known to exist in the pia mater.

Evidence against diffusion as transport mechanism is further provided by the following observations:

1. When enzyme elevations developed in the CSF after cold injuries, the activities of all enzymes studied increased rapidly at the same time after induction of the lesions, irrespective of the different molecular mass. If diffusion

were the main method of transport, the increase would be expected to be more gradual, and the larger molecules would be expected to appear in the CSF at a later time (i.e. ChE or LDH later than CPK).

2. Induction of hypotension prevented elevation of CSF enzyme activity in 8 of 15 animals studied with hypotension in the first seven hours after injury. It is not probable that the process of diffusion can be greatly influenced by the height of the arterial blood pressure, but the issue is still in discussion. But Klatzo et al. (1967) and Go et al. (1974) have demonstrated that induction of hypotension can slow the spreading (i.e. bulk flow) of edema fluid. In our studies also, the ventricular fluid pressure was significantly lower, and less edema was seen in the animals treated with hypotension. This is clearly demonstrated in the picture shown in the fourth paper (Chapter 3.4.).

On basis of these considerations it seems probable that transport of enzymes through the ECF to the CSF in the acute phase after brain injury is by way of bulk flow of edema fluid.

Conclusive evidence for this concept is not provided by these experiments, but they were not designed to do so. Investigations simultaneously studying the spreading of edema fluid, and the changes in enzyme activity of the ECF and CSF in cold injured animals, treated and not-treated with hypotension seem indicated.

Reulen et al. (1977) have provided strong evidence that bulk flow of ECF is an important mechanism for spreading of brain edema. These authors further postulate that besides reabsorption into the blood and phagocytosis, one of the major mechanisms for the removal of edema fluid is bulk flow into the CSF.

The results obtained from our studies support this concept. They further suggest that enzymes, released into the ECF after brain injury, are transported with the edema to the CSF. Likewise it is probable that other substances in the ECF, released from damaged or necrotic brain cells or resulting from transsudation through the damaged blood brain barrier, are also transported to the CSF by this route.

Thus brain edema should perhaps not only be regarded as a harmful complication of brain injury, but also — perilous though the process may be — as an attempt of the brain to hasten restoration of homeostasis in the brain tissue. Further research into this concept seems indicated.

4.2. Clinical implications of the results obtained

The potential value of CSF enzyme levels as a parameter for the extent of brain injury has been demonstrated in the experimental situation.

A significant increase of enzyme activity in the CSF was already observed three to four hours after induction of cold lesions in cats. Although this does not necessarily mean that CSF enzyme levels will also be elevated in patients with severe brain injuries within a few hours of injury, it does show that CSF enzyme levels are an *early* obtainable objective parameter for the extent of brain damage.

Great increases in CSF enzyme activity were shown to develop in the acute phase after brain injury, consequently enzyme determinations of a single CSF sample are of limited value. It is not considered justified to obtain CSF for enzyme analysis by cisternal or lumbar taps in patients with severe brain injuries. The risk of promoting brain stem herniation is considered too great, whilst results of enzyme determinations performed on one sample only may lead to erroneous conclusions.

Sampling ventricular CSF is a procedure not wrought with the dangers of promoting brain stem herniation. Placing a ventricular catheter in patients with severe brain injuries has been advocated by many authors, because it affords the possibility of monitoring intraventricular pressure, thus offering an objective and reliable rationale for institution of therapy or further diagnostic measures (Johnston et al., 1970; Vapalahti and Troupp, 1971; Jennett and Johnston, 1972; Blaylock et al., 1974; Troupp, 1974).

Although in theory placement of ventricular catheters in patients with severe brain injuries is a procedure to be advocated, in practice it is not so simple. Due to the presence of brain edema the ventricles may be compressed to a small size, displaced across the midline, and consequently often very difficult to find. Especially if, before placing the catheter, the depth of the ventricle is first "sounded", a jet of CSF may escape, making it wholly impossible to find it again.

It is proposed that when placing a ventricular catheter in patients with brain injuries, it either be attempted to insert a catheter cut to the estimated correct length directly without measuring the required length of catheter, or otherwise to measure the length of catheter required with a closed system during pressure monitoring, not allowing any CSF to escape.

Placement of a ventricular catheter has the advantage above other methods of monitoring intracranial pressure, such as epidural pressure recording, that its presence permits aspirating CSF in an attempt to lower intracranial pressure. Ensuring an adequate CSF drainage may be an important therapeutic measure. Disadvantages of the use of a ventricular catheter are the risk of infection, that it may cease to function properly owing to further displacement of the ventricle, or because the openings become clogged with tissue débris or blood clots.

When a ventricular catheter is in situ, serial enzyme determinations of the ventricular CSF are possible, and it is felt that these may afford information on the extent of brain damage. It seems highly probable from the results of the experimental studies, that greatly elevated CSF enzyme levels in patients with severe brain injuries will indicate extensive brain damage, and consequently carry a poor prognosis. Indeed in the preliminary clinical studies conducted (see Chapter 3.1.) all patients with high CSF enzyme levels died of the brain injuries.

In other cases with fatal head injuries, however, low values of CSF enzyme activity were seen. In the animal experiments too, no elevation of CSF enzyme activity developed in eight of fifteen animals which were studied with hypotension. These observations decrease the prognostic value of CSF enzyme determinations.

It was concluded that the height of CSF enzyme activity is only indicative of the extent of brain damage in the acute phase after injury when extensive brain edema is present.

The conclusion that transport of enzymes from the necrotic lesion to the CSF is dependant on the formation of brain edema, infers that determination of CSF enzymes in the brain injured patient offers information on the presence and extent of brain edema, and consequently may also be of some diagnostic value.

Low CSF enzyme levels observed in patients with clinically severe brain injuries may theoretically be seen in the following situations:

1. No extensive brain damage is present, and only little release of enzymes occurs from cerebral tissue. The clinical symptoms may theoretically be caused either by local brain stem damage, producing severe functional disturbances, or by greatly elevated intracranial pressure due to the presence of intracranial haematoma or massive cerebral edema.

2. Extensive damage of the brain is present, but the transport of enzymes released into the brain ECF towards the CSF is insufficient, i.e. brain edema is not (yet) extensive. This may occur when the blood pressure is low.

These theoretical possibilities may be differentiated by taking the intracranial pressure into account. The potential possibility of differential diagnosis on basis of intracranial pressure monitoring and CSF enzyme determinations is illustrated in table 11. Whether this will be of any practical value appears doubtful as similar information may be gained from Computerized Axial Tomography, which is a less invasive investigation.

Establishing a reliable prognosis for patients with severe head injuries on basis of CSF enzyme levels alone is not considered feasible.

The large inter-individual variation of CSF enzyme response observed after cold injuries in cats, infers that CSF enzyme determinations provide no more

Table 11

Possible diagnostic value of CSF enzymes and intracranial pressure
in severe brain injury

	High pressure	Low pressure
CSF enzymes ↑↑	severe diffuse brain damage + edema	severe injury + edema, pressure not yet increased, or low due to decompression.
Low CSF enzymes	1. haemorrhage 2. massive edema little necrosis	1. localized brain stem lesions 2. little edema 3. metabolic coma?

than a rough estimate of the extent of brain damage.

Moreover it must be borne in mind that prognosis in patients with severe head injuries is not only determined by the extent, but also by the site of brain damage, whilst the presence of extracranial lesions and the pre-traumatic condition of the patient must also be taken into account.

Establishing prognosis should be attempted in the simplest way possible. In this respect the studies reported by Jennett and coworkers (1976) are very promising. These authors were able to make a confident prediction of the outcome of patients with severe head injuries, based on elementary clinical signs alone in 41% of severely brain injured patients within 24 hours of injury, and in 52-61% within two to three days.

CSF enzyme determinations are not indicated when a confident prediction may be arrived at on basis of elementary clinical signs alone. But it is felt that a potential application of CSF enzyme determinations exists for those cases in which a confident prediction is not attained on basis of clinical signs.

It is the author's opinion that the results of the experimental studies reported in this thesis afford sufficient grounds for commencing serial CSF enzyme studies in patients with severe brain injuries receiving a ventricular catheter for pressure monitoring.

Only clinical research will be able to tell the ultimate practical prognostic and diagnostic value of CSF enzyme determinations in patients with severe brain injuries.

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SUMMARY

In this thesis studies are described investigating the value of CSF enzyme levels as a parameter for the extent of brain injury. These — mainly experimental — studies are reported in four papers, collected in Chapter 3.
The first two chapters are introductory.

In the first two chapters the clinical interest and the theoretical considerations leading to these studies are described.

Establishing a reliable prognosis in patients with severe brain injuries is notoriously difficult. Prognosis is dependant on a number of factors, of which the extent of brain damage may be considered to be of importance. A need was felt for more objective parameters for assessing the extent of brain damage.

For various reasons it was considered worthwhile to investigate if CSF enzyme levels might show some correlation to the extent of brain injury, and so be of prognostic value in patients with severe brain injuries.

Results of preliminary clinical studies were somewhat disappointing (see Chapter 3.1). Studies investigating the relation of CSF enzyme levels to the extent of brain injury in the experimental situation were then instituted.

In chapter 3 the papers reporting on these experimental studies are collected. The dynamics of changes of CSF enzyme activity after experimental brain injury in cats were studied, the relation of CSF enzyme levels to the severity of the injury, and the effect of induced hypotension on the development of increased CSF enzyme levels and increased ventricular fluid pressure.

To this end experimental cold injuries of the brain were induced in cats, and samples of CSF withdrawn every half hour and analyzed for the activities of the enzymes CPK, α-HBDH, LDH, GOT, GPT and ChE.
The ventricular fluid pressure (VFP), the cisternal fluid pressure, the arterial blood pressure, the End expiratory CO_2, and the ECG were monitored continuously. The experiments were terminated at an arbitrary chosen time limit of 7¼ hours after freezing, or earlier if brain stem herniation developed.

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In all, 53 experiments were conducted. The experiments were divided into the following series:
Control series (7 animals)
Series with severe cold injuries (14 animals)
Two series with less severe injuries (9 + 8 animals)
Two series with hypotension (induced immediately in 6, and after a delay of ¾ hour after freezing in 9 animals).

The results may be summarized as follows:

Chief results:

— After induction of cold lesions to the brain in cats the activities of the enzymes CPK, α-HBDH, LDH, GOT and ChE increase considerably in the CSF, starting 1¼ to 2¼ hours after freezing.
— Considerable increase of CSF enzyme activity was seen in samples taken at ½ hour intervals.
— Isoenzyme studies conducted on CPK and LDH demonstrated the increased enzyme activity in the CSF to come from the brain.
— No consistently significant changes could be demonstrated in the *serum* enzyme activity.
— Higher CSF enzyme levels were found in the animals with more severe injuries of the brain. Differences were statistically significant.
— Induction of hypotension prevented increase of enzyme activity in the CSF after cold injuries in the first seven hours after freezing in 8 of the 15 animals studied with hypotension.

Additonal results:

— Isoenzyme analysis of CPK in the CSF of cats after cold injuries demonstrated a "new" isoenzyme fraction intermediate between the MB and BB bands.
— During induction of hypotension with Arfonad ® a transient rise of the ventricular fluid pressure may develop, being most marked in the "tight brain situation".
— In the cold injured animals treated with hypotension the ventricular fluid pressure was significantly lower than in the animals not treated.
— Histologic studies revealed less pronounced brain edema in the cold injured animals treated with hypotension.

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Conclusion:

The height of the activity of the enzymes CPK, α-HBDH, LDH, GOT and ChE in the CSF is related to the extent of brain damage when extensive brain edema is present.

In the fourth chapter the combined results of the four papers are discussed with respect to the following two items:

1. Transport mechanism of enzymes from ECF to CSF. This may in theory be possible by way of diffusion, bulk flow of ECF, flow of edema fluid, or by a combination of these possibilities. On basis of theoretical considerations and experimental data, it is concluded that transport of enzymes through the ECF towards the CSF is probably by way of bulk flow of edema fluid in the acute phase after brain injury. Likewise it seems probable that other matter is also transported from the ECF to the CSF by the same route after brain injury.

2. Clinical implications of the results obtained. The potential value of CSF enzyme levels as a parameter for the extent of brain damage has been demonstrated in the experimental situation.

CSF enzyme levels may constitute an early obtainable and objective parameter for the extent of brain damage in patients with severe brain injuries.

The limited value of analysing a single CSF sample is emphasized, and it is concluded that it is not justified to perform lumbar or cisternal taps in patients with severe brain injuries in order to obtain CSF for enzyme determinations. Serial studies of ventricular CSF would be necessary.

The practical value of CSF enzyme levels as a parameter for the extent of brain damage is decreased by the observation that hypotension may retard the transport of enzymes from ECF to CSF. It is concluded that determination of CSF enzymes is only of value in the acute phase after brain injury, when extensive brain edema is present.

The theoretical possibilities of differential diagnosis with aid of CSF enzyme levels and monitoring intracranial pressure are discussed.

Establishing prognosis in brain injured patients should be attempted in the simplest way possible. Determination of CSF enzymes becomes superfluous when a confident prognosis may be attained on basis of elementary clinical signs.

It is not felt feasible to determine prognosis on basis of CSF enzyme levels alone. The large inter-individual variation of response noted in cats with similar injuries, the uncertain transport to the CSF are factors diminishing the value of CSF enzyme levels as a "hard parameter" for the extent of brain damage, whilst prognosis in patients with severe brain injuries is dependant on too many other factors.

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Nevertheless it is the author's opinion that the results of the studies reported in this thesis afford sufficient grounds for commencing serial enzyme determinations in all patients receiving a ventricular catheter soon after brain injury for pressure monitoring.

Only clinical research will be able to tell the ultimate practical, prognostic and diagnostic value of CSF enzyme levels in patients with severe brain injuries.

SAMENVATTING

Dit proefschrift beschrijft onderzoek naar de relatie tussen de hoogte van de aktiviteit van enzymen in de liquor en de ernst van traumatisch hersenletsel. Het behelst voornamelijk resultaten van experimenteel werk. Deze worden in hoofdstuk 3, waarin vier publikaties zijn gebundeld, beschreven.

De eerste twee hoofdstukken verschaffen de inleiding tot hoofdstuk 3.

In de hoofdstukken 1 en 2 worden de klinische problematiek en de gedachtengang toegelicht, die tot het onderzoek geleid hebben.

Het bepalen van de prognose bij patiënten met ernstig schedelhersenletsel is moeilijk. De prognose is afhankelijk van een aantal faktoren, waaronder de ernst van het letsel van belang is. Voor de kliniek van deze patiënten bestaat behoefte aan meer objectieve parameters voor het bepalen van de ernst van het letsel.

Op grond van een aantal overwegingen werd besloten te onderzoeken of de enzymaktiviteit in de liquor informatie zou verschaffen over de ernst van hersenletsel, en derhalve van prognostische waarde zou zijn bij patiënten met ernstig schedel-hersenletsel.

De resultaten van voorlopige klinische studies waren enigszins teleurstellend (zie hoofdstuk 3.1). Daarom werd het verband tussen de hoogte van enzymaktiviteit in de liquor en de ernst van hersenbeschadiging in het dierexperiment onderzocht.

In hoofdstuk 3 zijn de artikelen, die deze studies beschrijven, verzameld. De veranderingen in enzymaktiviteit in de liquor na het opwekken van experimenteel hersenletsel bij katten worden beschreven, de relatie tussen de enzymaktiviteit in de liquor en de omvang van het letsel, alsmede de invloed van hypotensie op het ontstaan van verhoogde enzymaktiviteit in de liquor en op het ontstaan van verhoogde ventrikeldruk.

Hiertoe werden koude-laesies van de hersenen aan katten toegebracht. Elk half uur werden monsters liquor afgenomen, waarin de aktiviteit van de enzymen CPK, α-HBDH, LDH, GOT, GPT en ChE werden bepaald.

Tevens werden de ventrikeldruk, de cisternale druk, de arteriële bloeddruk, het capnogram en het ECG voortdurend geregistreerd. De proeven werden na een willekeurig gekozen tijdslimiet van 7¼ uur na opwekken van het letsel afgebroken, of eerder wanneer het proefdier inklemde.

In totaal werden 53 proeven gedaan. De proeven werden in de volgende series verdeeld:
Controle serie (7 dieren)
Serie met ernstig letsel (14 dieren)
Twee series met minder zwaar letsel (9 + 8 dieren)
Twee series met hypotensie (bij zes dieren onmiddellijk na opwekken van het koudeletsel geïnduceerd, bij negen driekwart uur later).

De resultaten kunnen als volgt worden samengevat:

Hoofdresulaten:

— Na het toebrengen van koude-letsel aan de hersenen bij katten, stijgt de aktiviteit van de enzymen CPK, α-HBDH, LDH, GOT en ChE in de liquor sterk, vanaf ongeveer 1¼ - 2¼ uur na opwekken van het letsel.
— Zelfs tussen twee opeenvolgende monsters, ½ uur na elkaar afgenomen, kan een aanzienlijke toename optreden.
— Isoenzym studies van CPK en LDH, toonden aan dat de toename van de aktiviteit van deze enzymen in de liquor voornamelijk bestaat uit een stijging van de hersenfraktie.
— In het *serum* konden geen blijvende significante veranderingen van de aktiviteit van de onderzochte enzymen worden aangetoond.
— De stijging van de aktiviteit van de enzymen in de liquor was significant groter bij de dieren met meer ernstig letsel.
— Wanneer de bloeddruk verlaagd werd, werd in 8 van de 15 dieren geen toename van de enzymaktiviteit in de liquor gezien.

Nevenresultaten:

— Isoenzym onderzoek van CPK in de liquor van katten met ernstige koude-laesies gaf een "nieuwe" fraktie te zien gelegen tussen de MB en BB frakties.
— Het verlagen van de arteriële bloeddruk met Arfonad ® kan een voorbijgaande stijging van de ventrikeldruk tot gevolg hebben, het meest uitgesproken in de "tight brain situation".
— De ventrikeldruk was significant lager bij de dieren met koude-letsels die met hypotensie waren behandeld, dan bij de niet-behandelde dieren.

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— Bij histologisch onderzoek werd minder uitgesproken hersen-oedeem gezien in de dieren met koude-letsels die met hypotensie waren behandeld.

Conclusie:

De aktiviteit van de enzymen CPK, α-HBDH, LDH, GOT en ChE in de liquor toont een verband met de ernst van hersenletsel, wanneer uitgebreid hersen-oedeem aanwezig is.

In hoofdstuk 4 worden de verzamelde resultaten van de vier artikelen besproken ten aanzien van de volgende twee aspecten:

1. Transport mechanisme van enzymen door de extracellulaire vloeistof naar de liquor. Dit transport kan theoretisch op de volgende manieren plaats vinden: via diffusie, stroom van extracellulaire vloeistof, stroom van oedeemvloeistof, of door een combinatie van deze mogelijkheden. Op grond van theoretische overwegingen en gegevens verkregen uit het experimentele onderzoek wordt aangetoond dat transport van enzymen afkomstig uit het necrotisch gebied door de extracellulaire vloeistof naar de liquor waarschijnlijk voornamelijk via stroom van oedeem vloeistof geschiedt, althans binnen de eerste zeven uur na het trauma. Evenzo is het waarschijnlijk dat andere stoffen op dezelfde manier naar de liquor worden getransporteerd.

2. Klinische betekenis van de verkregen resultaten: De potentiële waarde van de hoogte van enzymaktiviteit in de liquor als parameter voor de ernst van hersenbeschadiging is in het experimentele model aangetoond.

Het bepalen van liquorenzymen verschaft mogelijk een objectieve parameter voor de ernst van hersenbeschadiging en is misschien al in een vroeg stadium na het trauma van waarde bij patiënten met ernstig hersenletsel.

Aangezien grote veranderingen in de aktiviteit van liquorenzymen in de acute phase na het trauma kunnen optreden heeft het bepalen van een enkele monster slechts beperkte waarde. Het wordt derhalve niet verantwoord geacht lumbaal-of suboccipitaalpuncties te verrichten bij patiënten met ernstig schedel-hersen-trauma, teneinde liquor te verkrijgen voor enzymonderzoek. Serieonderzoek van ventrikelliquor lijkt aangewezen.

De praktische waarde van de hoogte van enzymaktiviteit in de liquor als parameter voor de ernst van hersenbeschadiging wordt verminderd door de waarneming dat hypotensie het transport van enzymen naar de liquor kan vertragen. Geconcludeerd wordt dat het bepalen van liquorenzymen in de acute phase na het trauma alleen van waarde is als parameter voor de ernst van hersenbeschadiging als uitgebreid hersenoedeem aanwezig is.

De mogelijkheden van differentiaal diagnose op basis van het bepalen van liquorenzymen en het meten van de intracraniële druk worden besproken.

Het bepalen van de prognose bij patiënten met ernstig traumatisch hersenletsel dient op zo eenvoudig mogelijke wijze te geschieden. Wanneer dit kan op basis van bij eenvoudig klinisch onderzoek verkregen gegevens, dan is bepalen van liquorenzymen overbodig.

Het wordt niet mogelijk geacht een prognose te stellen alleen op basis van aktiviteit van liquorenzymen. De grote inter-individuele verschillen waargenomen bij katten met vergelijkbare laesies, het onzekere transport naar de liquor, zijn faktoren die de waarde van de aktiviteit van liquorenzymen als "harde parameter" voor de ernst van hersenbeschadiging verminderen, terwijl de prognose bij patiënten met ernstig schedel-hersenletsel afhankelijk is van teveel andere faktoren.

Desondanks is de auteur van mening dat de resultaten vermeld in dit proefschrift voldoende basis verschaffen om bij patiënten met ernstig schedel-hersenletsel, die in een vroeg stadium na het trauma een ventrikeldrain krijgen, het verloop van de aktiviteit van enzymen in de liquor te bestuderen.

Alleen klinisch onderzoek zal de uiteindelijke praktische prognostische en diagnostische waarde van bepalingen van liquorenzymen bij patiënten met ernstig schedel-hersenletsel kunnen aantonen.

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