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Cerebrospinal fluid enzymes in acute brain injury

1 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 for their prognostic value. Raised values of CPK and HBDH were demonstrated in the CSF of patients with severe brain injuries. Standardised cold lesions of the brain were induced in cats. The activities of the enzymes CPK, HBDH, LDH, GOT, GPT, and pseudocholinesterase were studied at half hour intervals in the cerebrospinal fluid and at hourly intervals in the serum. A statistically highly significant increase of all enzymes studied developed in the CSF. The greatest changes occurred within four hours of freezing. Large increases could occur in half an hour. Isoenzyme studies demonstrated that CPK and LDH were of cerebral origin. No consistently significant changes could be shown in the serum enzyme activity. It is concluded that after brain injuries, enzymes are released into the extracellular fluid of the brain and transported to the CSF. The limited value of a single enzyme estimation is emphasised. The results described seem to provide indirect evidence for transependymal flow of extracellular fluid in brain oedema.

Patients with severe head injuries present many difficult clinical problems including estimation of the extent of brain injury and the prognosis in the individual patient. The prognosis depends on several factors including the site and degree of brain injury (Jennett, 1972). It was decided to evaluate the prognostic value of enzyme activity in the CSF in the acute phase after brain injury —that is, within the first 24 hours—as enzymes released from damaged and necrotic brain cells would probably be transported towards the CSF. Raised values of various enzymes have been reported in the CSF in many neurological disorders including severe brain injury (Kaltiala *et al.*, 1968; Sherwin *et al.*, 1969; Hildebrand and Levin, 1973; Navarro *et al.*, 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) found 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.

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The first results of our preliminary clinical studies were also somewhat disconcerting. In 20 patients with head injuries of varying severity the activity of the enzymes creatine phosphokinase (CPK) and α-hydroxybutyric acid dehydrogenase (α-HBDH) was studied in the CSF within the first 24 hours of trauma. The results are summarised in Table 1 which shows 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. These findings might be explained by local brain stem lesions without extensive cortical necrosis. But the enzyme dynamics are not well understood so a series of animal (cat) experiments was planned in which cold injuries of the brain of standardised severity

Table 1 *CPK and HBDH in the CSF of patients with severe brain injuries**

Dead or persistent vegetative state at 1 mo		*Alive at 1 mo*	
CPK (U/l)	*HBDH (U/l)*	*CPK (U/l)*	*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 of injury.

were produced and the changes of enzyme activity in the CSF studied.

This paper describes the dynamics of changes in CSF enzyme activity in the acute phase with isoenzyme studies to determine the origin of the enzymes.

Material and methods

Twenty-one healthy adult cats weighing 2800–6400 g, otherwise unselected for age or sex, were sedated with phenobarbitone intramuscularly (50 mg/kg weight), and anaesthetised with pentobarbitone 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), through 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 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 cooling thermode was screwed into a burr hole over the left ectosylvian and suprasylvian gyri. Arterial blood pressure, end expiratory CO_2, ECG, and ventricular and cisternal fluid pressure were measured continuously. Ventricular fluid pressure was monitored by a stereotactically placed cannula in the cella media of the right lateral ventricle. Cisternal fluid pressure was measured through 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 next day, when they were analysed for the activity of the enzymes: creatine phosphokinase (CPK: EC 2.7.3.2), α-hydroxybutyric acid dehydrogenase (α-HBDH), aspartate aminotransferase (GOT: EC 2.6.1.1), alanine aminotransferase (GPT; EC 2.6.1.2), lactic dehydrogenase (LDH; EC 1.1.1.27), and pseudocholinesterase (ChE; EC 3.1.1.8). The enzyme estimations were made with standard optimated Merck-I-Tests (E. Merck Darmstadt). The first three samples of CSF were discarded to eliminate faulty estimations caused by 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 physiological saline remaining in the cisternal catheter after filling the transducer.

Control values were obtained for each experiment by averaging the values measured in samples 4–6 (in the period one to two and a half hours after the beginning of cisternal taps). Just before 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 one to two and a half hours after the beginning of cisternal taps.

After the control period experimental brain injury was produced by a cold lesion in 14 of the 21 experiments. The remaining seven animals served as a control group. Cold lesions were induced according to the method of Beks *et al.* (1965). Cooled methanol (at −40°C) from a Lauda Ultra Cryostate was led through the thermode for six minutes exactly. After freezing, the tubes connecting the thermode to the ultracryostate were disconnected but the cooling thermode left *in situ*, so that the actual duration of freezing was longer.

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 the arbitrarily chosen time limit of 7.25 hours, the experiment was terminated except in a few animals studied for a longer period. In one case ventricular fluid was collected for one hour after herniation had occurred at 7.75 hours. Herniation of the brain stem was considered to have occurred if all of the following 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 respiratory frequency occurred.

After the experiments the animals were killed with an overdose of intravenous pentobarbitone. 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 times (0.25–7.25 hours after induction of the cold lesion) and the control values per individual experiment were calculated. Statistical analysis was performed on these differences calculated between the control group and the group with the cold lesion for each 30 min epoch 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 visualised with the nitro blue tetrazolium (NBT) method (van der Veen and Willebrands, 1966).

LDH isoenzymes were also visualised with the NBT method. The isoenzyme pattern of tissue extracts was determined from brain, heart, 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 (isotonic, pH 7.3).

Results

CONTROL EXPERIMENTS

The mean ventricular fluid pressure (VFP) measured at the different time levels in the control experiments before withdrawal of CSF samples ranged from −3.5 to +8.4 mmHg. All animals in the control group showed slight activity of all enzymes measured in the CSF. The range of values observed is summarised in Table 2.

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 with the individual control value for that experiment. In three animals a slight transient elevation of the activity of all enzymes studied in the CSF was noted, most obvious for CPK (Fig. 1). Serum enzyme activity showed a wide variation between the individual animals (Table 2).

Table 2 *Control group: range of enzyme activities (U/l) measured in CSF and serum*

*Enzyme**	*CSF*	*Serum*
CPK	3–70	330–2130
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

*Full names and enzyme numbers can be found in the Material and Methods section of text.

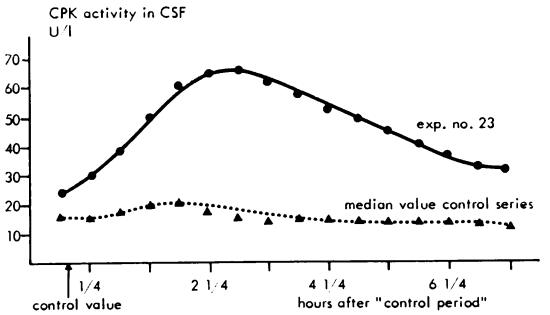

Fig. 1 *Median values of the levels of CPK observed in the CSF of the control series animals, and transient rise of CPK activity in experiment 23 (control experiment).*

EXPERIMENTS IN WHICH A COLD LESION WAS INDUCED

Extent of cold lesion

Induction of cold lesions according to the method described resulted in a large zone of focal necrosis (Fig. 2) and formation of extensive cerebral oedema 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.

Effect of freezing on VFP and CSF enzymes

Ventricular fluid pressure rose in all 14 animals, starting between 15 and 25 minutes after induction of the cold lesion. The rise was rapid in five of these animals and brain stem herniation occurred within 2.5 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. 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 all but one developed great changes in the enzyme activity of the CSF.

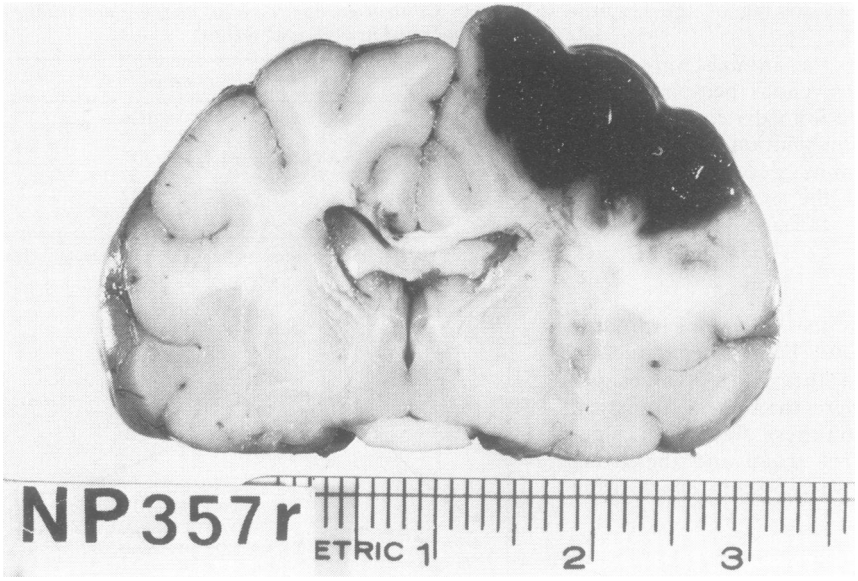

Fig. 2 *Macroscopical aspect of extent of a typical cold lesion*

Table 3 *CPK and GOT activity (U/l) in the CSF of five animals developing tentorial herniation within two and a half hours of induction of the cold lesion*

Experiment number	*Before freezing*		*Last obtained specimen*	
	CPK	*GOT*	*CPK*	*GOT*
24	8	22	44	49
27	10	—	3	—
36	2	3	20	11
42	2	—	19	—
81	—	5	—	15

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 ($P<0.01$). There was a highly significant (Wilcoxon: $P<0.01$) rise of all enzymes studied which started between 1.25 and 2.25 hours after induction of the lesion. All enzymes except GPT showed a similar pattern of increase. Even when measured at half hourly intervals, great differences in CSF enzymes activity were found from sample to sample. The results are illustrated in Fig. 3, showing the median values of the CSF enzyme activity at each time level.

A peak value appears to be reached within the first seven hours of freezing (Fig. 3) but not in every experiment, and a wide variation existed in the increase of enzyme activity in the CSF between the individual animals as demonstrated by the scatter diagram.

Figure 4 also shows that one of the animals (No. 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 mmHg (a higher level than observed in the control group (up to 8.4 mmHg) but lower than in any of the other animals in the group with a cold lesion. This animal was observed for an additional eight hours and a progressive increase of the VFP ensued with brain stem herniation towards the end of this period.

Even in this latter period hardly any changes in CSF enzyme activity occurred as shown in Table 4 which compares the median values of the group with the cold lesion to the values observed in experiment 39.

At postmortem 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.

SERUM ENZYMES

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

CORRELATIVE STUDIES

The correlation was also studied between the activity of the various enzymes measured at the

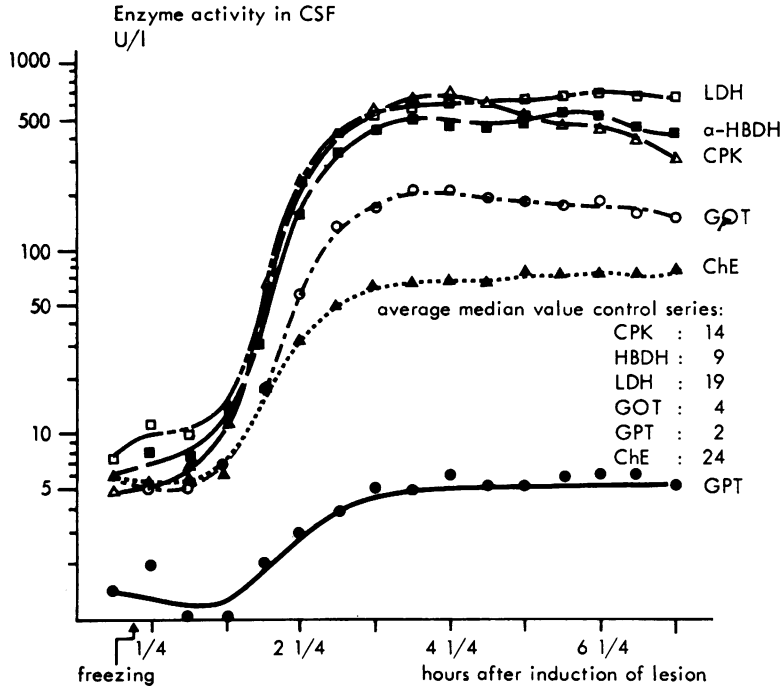

Fig. 3 *Increase in CSF enzyme activity after induction of a cold lesion to the brain in cats. Median values of the activities of the enzymes LDH, HBDH, CPK, GOT, cholinesterase, and GPT in the CSF are plotted.*

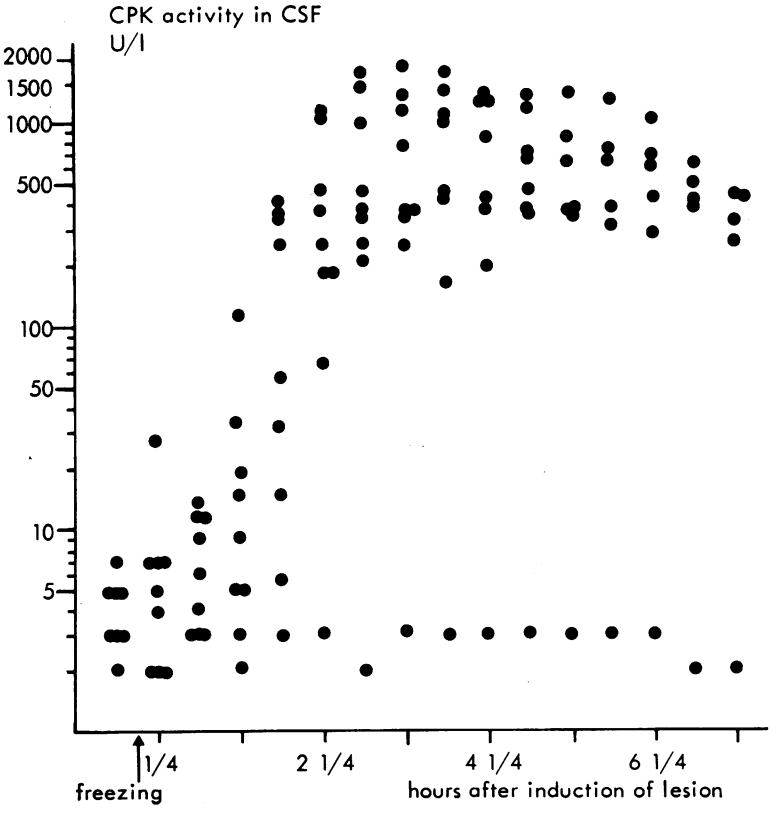

Fig. 4 *Changes in CPK activity observed in the CSF of nine animals after induction of a standardised cold lesion to the brain. This diagram illustrates the similar pattern of response occurring in all but one animal and the wide inter-individual variability of response.*

Table 4 *Lack of changes of CSF enzymes (U/l) in experiment 39*

Hours after induction of cold lesion	Median values in animals with cold lesion					Experiment 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
3.75	560	480	170	24.5	142.5	3	6	4	10.6	107.5
6.25	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

same times and all were intercorrelated. The analyses were performed at each time level separately. The strongest correlation was obtained—as would be expected—between HBDH and LDH.

I have not included the actual values of the calculated correlation coefficients as, although there was a significant correlation, the accuracy of the calculated correlation coefficients is low because of the limited number of observations, especially at the later times when some of the experiments had been terminated due to herniation of the brain stem.

Correlation between CSF enzymes and ventricular fluid pressure

All enzymes studied showed only a poor correlation with the increase in VFP which did not improve when the enzyme levels were correlated with VFP measured 0.5, 1.0, or 1.5 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 really shows part of the activity of an earlier period, due to the 'dead space' in the cisternal catheter.

The correlation between the VFP and CPK activity of the CSF both simultaneously and after introduction of a time lag is shown in Table 5.

Correlation of CSF enzymes and serum enzymes

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

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
1.25	0.49	0.80	0.70	0.62
2.25	0.64	0.59	0.55	0.48
3.25	0.18	0.66	0.64	0.49
4.25	0.64	0.38	0.41	0.47
5.25	0.27	0.32	0.44	0.77
6.25	0.80	0.85	0.84	—
7.25	0.65	—	—	—

Time lag: 0 = VFP + CPK at same time level; 1 = VFP + CPK 0.5 hour later; 2 = VFP + CPK 1 hour later; 3 = VFP + CPK 1.5 hour later.

ISOENZYME STUDIES

The CPK isoenzyme pattern in the tissue extracts of brain, heart, and skeletal muscle agreed completely with the literature (Burger *et al.*, 1964; Dawson and Fine, 1967). The brain extract showed 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 fraction (MB fraction). These fractions are shown in Fig. 5.

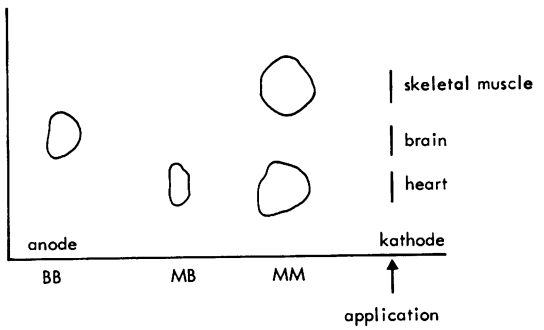

Fig. 5 *CPK isoenzyme pattern of extracts of cat skeletal muscle, brain, and heart.*

In six of the nine experiments, isoenzyme studies of the CSF were conducted at various times. 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 localised in the region of the MM-fraction (Fig. 6).

It is evident from Fig. 6 that, as time progresses, the BB-activity becomes more intense and increases in density as total CPK activity rises. An

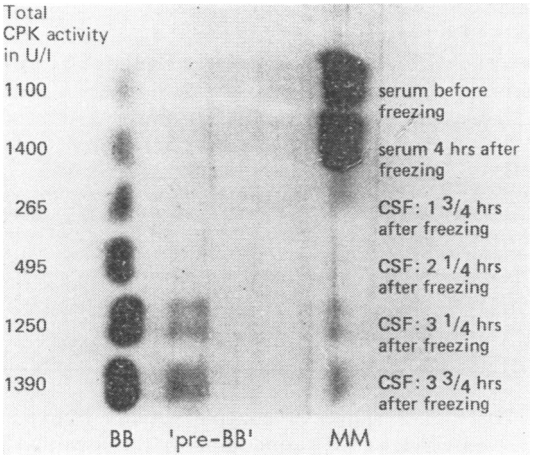

Fig. 6 *CPK isoenzymes in CSF and serum after induction of a cold lesion. Raised CPK levels are mainly due in the CSF to the brain type isoenzyme and in the serum to the muscle type isoenzyme.*

interesting phenomenon to note is that when CPK levels were very high another band—sometimes a double band—intermediate between the MB- and BB- fraction appeared. 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).

LDH-ISOENZYMES

The isoenzyme patterns of the tissue extracts of brain and skeletal muscle are shown in Fig. 7.

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. In serum, however, mainly LDH 4 and 5 activity was seen (Fig. 7).

HBDH/LDH RATIO

The predominance of the fast migrating isoenzymes LDH 1 and 2 in the CSF is also shown by the HBDH/LDH ratio. 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 HBDH/LDH

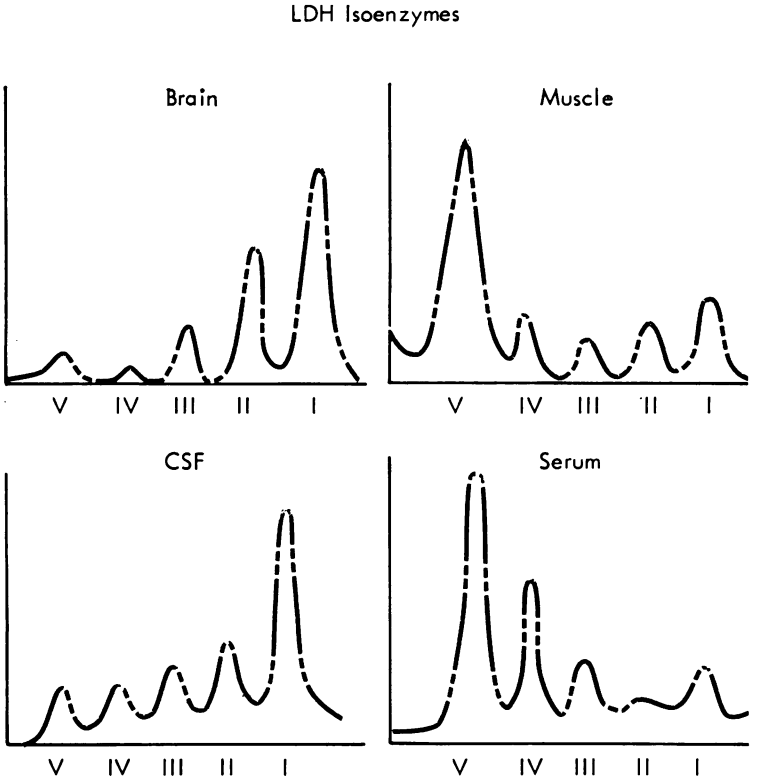

Fig. 7 *LDH isoenzymes of cat brain and cat skeletal muscle. The fast migrating isoenzymes LDH 1 and 2 (brain types) are found in the CSF after cold lesions, while the slowly migrating isoenzymes LDH 4 and 5 (muscular origin) were demonstrated in the serum.*

varied from 0.6 to 1.0 and in the serum from 0.3 to 0.5, demonstrating a far higher content of the fast migrating LDH isoenzymes which occur in brain and heart.

Discussion

These studies have demonstrated an increase in enzyme activity of the CSF in the acute phase after brain injury. Go *et al.* (1976) have found an increase of LDH in the extracellular fluid of cats with a cold lesion of the brain. It was presumed that enzymes released into extracellular fluid of the brain 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 (1914) 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 source. Pollay and Curl (1967) calculated that approximately 30 per cent of the CSF in rabbits is formed by secretion from the ventricular ependyma. Milhorat (1969) demonstrated that hydrocephalus could occur rapidly and progressively in monkeys even after plexectomy.

Milhorat *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 the 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 oedema.

We presume that enzymes, released from damaged and necrotic brain cells into the extracellular fluid of the brain, are transported by the spreading oedema and by way of simple diffusion and bulk flow of extracellular fluid 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 present experiments 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 (1973) has shown 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 (U/l)*

Hours after induction of cold lesion	*Cist. CSF 8.25*	*Ventr. CSF 8.25*	*Ventr. CSF 9.25*	*Ventr. CSF 9.5*
CPK	230	380	740	680
HBDH	580	700	1500	1400
LDH	660	840	1900	1750
GOT	224	269	687	578

The higher values of enzyme activity 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. 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 definitive evidence for this, but the results obtained probably do provide evidence for this bulk flow of extracellular fluid in developing oedema.

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 be caused by seepage of blood enzymes 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 an electrophoretic mobility which differs from muscle. Although reports on isoenzyme studies conducted in the CSF demonstrate a cerebral origin for these enzymes (Frick, 1967; Sherwin *et al.*, 1969) 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 HBDH/LDH also provided evidence for the cerebral origin.

The elevation of enzyme activity in the CSF only started between 1.25 and 2.25 hours after induction of the lesion but it must be kept in mind that the 'dead space' of the cisternal catheter was approximately 0.25 ml, so that this time incorporates an artificial delay of approximately 30 min (=1 sample). The observed delay of 1.25–2.25 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 2.5 hours after induction of the cold lesion. We do not consider that herniation is a suddenly occurring event, but 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.

Beks and coworkers (1965) also 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 the animals, the injury resulted in a quick rise in cerebral sinus pressure and in its relation to the blood pressure, while in the others the cerebral sinus pressure and the ratio to the blood pressure rose more slowly.

A great variability in the elevation of enzyme activity in the CSF was also observed in our study, due either to biological variation or to experimental differences. In all experiments the surface of cerebral cortex frozen and the duration and depth of freezing were standardised, and I always positioned the cooling thermode according to stereotactical coordinates. The only variability that could have occurred is the pressure exerted by the cooling thermode on the cortex, but this seems insufficient to explain the wide scatter of enzyme activities in this group which is assumed to be physiological variability.

Animal 39 did not develop any changes in CSF enzyme levels in the first 7.25 hours after induction of the lesion. In the second eight hours of the experiments the ventricular fluid pressure steadily increased and at the end of this period transtentorial herniation occurred but with hardly any increase in CSF enzyme activity. At postmortem examination the expected lesion was present, being only slightly smaller than in the other experiments. There were no reasons or indications to suspect an experimental failure. The only factor that distinguished this animal from the others in this group was a lower blood pressure. The median values of the mean arterial blood pressure in this group ranged from 132.5 to 145 mmHg. The mean blood pressure of experiment 39 varied from 95–135 mmHg with a median value of 110 mmHg. 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 blood 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, or (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 visualising CPK isoenzyme fractions was not sensitive enough to demonstrate these low activities. The ratio of HBDH/LDH in the CSF of these control experiments (0.5 to 0.8) indicates a cerebral origin. In the histological preparation sometimes quite a large destruction of tissue was seen from the ventricular puncture.

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 reaching a peak value after about 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. They describe a great increase of GOT in the CSF up to values of 700 U/l, with a maximal value two to four hours after laceration. They found higher values in the more severely lacerated animals. Akashi (1966) found increased CSF GOT levels 3–4 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 two and three 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 after injury. Klun (1974) also reported on the GOT, GPT, and aldolase activity of CSF one to three days after cold injury in cats. He described only a slight increase of GOT in the CSF after brain injury.

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

indicate that the greatest increase of enzyme activity in the CSF after brain injury occurs less than two hours after induction of the lesion and that great changes in the enzyme activity of the CSF can occur from hour to hour.

Conclusions

Enzyme activity is greatly increased in CSF in the acute phase after brain injury and may, therefore, be of value as an indicator of the extent of primary brain damage. A single estimation will only yield very limited information. Serial enzyme estimations in ventricular CSF of patients receiving a ventricular drain soon after brain trauma may provide useful information.

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