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# Critical Evaluation of Postmortem Changes in Human Autopsy Cisternal Fluid. Enzymes, Electrolytes, Acid-Base Balance, Glucose and Glycolysis, Free Amino Acids and Ammonia. Correlation to Total Brain Ischemia

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**ABSTRACT:** By studying early postmortem changes in cerebrospinal fluid (CSF) it is possible to draw conclusions as to premortem focal brain cell injury and terminal brain ischemia. Cisternal fluid (CF) from 40 different adult cadavers with no known neurological disorder was analyzed and compared with known in vivo values. They were divided into four groups ($n$ = 10 in each group), CF samples taken 2, 4, 10, and 24 h after death. The enzyme activity of CK and CK-BB (EC 2.7.3.2) increased linearly and statistically significantly 4–24 h postmortem ($P < 0.001$) the 2 h values being already 10 to 20 times higher than in vivo, LD and its isoenzymes 1 to 3 (EC 1.1.1.27) distinctly 10 to 24 h after death. Glucose and pyruvate concentrations in the CF declined, as did $Na^+$ and $Cl^-$. Lactate and $K^+$ increased over time. The earliest statistically significant changes between different timepoints were seen in lactate, pyruvate and $K^+$ concentrations. The GABA concentration was already more than 170 times at 2 h postmortem, and glutamate more than 20 times higher than in vivo. The concentrations of alanine, glycine, lysine, histidine, isoleucine, phenylalanine, and tyrosine were 2 to 3 times higher at 2 h postmortem than during life. The concentrations of all amino acids and ammonia increased linearly and statistically significantly ($P < 0.001$) in the CF 4 to 24 h postmortem.

**KEYWORDS:** pathology and biology, enzymes, electrolytes, glycolysis, amino acids, postmortem changes, cerebral ischemia, cerebrospinal fluid, cisternal fluid

Brain cell and tissue injury is reflected in the cerebrospinal fluid (CSF). At present very few reports [*1–6*] are available on changes in the biochemical markers of hypoxic injury in the human CSF. This is important for both clinical reasons and for the prediction of the outcome in neurological intensive care and for medicolegal purposes.

The predominant feature of central nervous system (CNS) cell injury caused by total brain ischemia (TBI) is depolarization of cell membranes and a massive efflux of $K^+$ ions [*7,8*]. The $K^+$ concentration further increases progressively in postmortem CSF [*9*].

The cellular alterations in permanent TBI ("brain death") and brain autolysis are presented in detail in previous studies [*10–13*]. The ultrastructural characteristics of neurones and glial cells in "brain death" are similar to those observed in autolysis after the (somatic) death of an individual [*14*]. TBI affects all cells in all regions of the brain

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in a simultaneous and homogeneous manner, but the progress of the autolytic changes varies somewhat in its rate in different areas [*10,11*].

In the absence of oxygen, the brain tissue can obtain energy only by spending its energy-rich phosphate reserves (creatine phosphate [PCr] ATP and ADP) and by metabolizing its glucose and glycogen reserves [*15*]. As the main result of this, lactate is produced. This causes acidosis. Certain lysosomal enzymes, which cause cell injury and tissue autolysis are activated and released by the acidic shift in pH [*7*].

Amino acids, free or as proteins, constitute over 40% of the dry weight of the brain. The concentration of certain amino acids is considerable, for example, glutamate, glutamine, γ-aminobutyrate (GABA) and glycine. Glutamate, glutamine, GABA and N-acetylaspartate are also actively produced in the brain [*16,17*].

The free amino acid pool within the nervous tissue serves as a source of residues for protein synthesis, and some amino acids serve as neurotransmitters and transmitter precursors [*18*]. Glutamate is the major excitatory amino acid [*16,19*] and GABA and glycine the major inhibitory transmitters in the mammalian CNS [*20,21*].

The maintenance of cerebral amino acids at a certain level is the result of their production and further metabolism in the brain and otherwise of their influx and efflux, largely to and from the bloodstream [*16*]. The composition of the free amino acid pool in the brain is not related to the levels of free amino acids in the plasma or the CSF [*22*].

This study is part of a larger series [*3,4,6,23*], the purpose of which is to find biochemical markers that would be prognostic for brain death and severe ischemic coma. That is why we measured the following CF components from 40 cadavers 2, 4, 10, and 24 h after death: creatine kinase (CK) and its brain-specific isoenzyme (CK-BB) (EC 2.7.3.2), lactate dehydrogenase (LD) and its isoenzymes 1-5 (EC 1.1.1.27), β-D-N-acetylglucosaminidase (NAG) (EC 3.2.1.30), acid phosphatase (AC.P) (EC 3.1.3.1), lactate, pyruvate and the ions $Na^+$, $K^+$ and $Cl^-$. In addition to this ammonia and the following free amino acids were determined: glutamate (glutamic acid) (Glu), "apparent glutamine" [glutamine (Gln) + asparagine (Asx) + threonine (Thr)], alanine (Ala), glycine (Gly), proline (Pro), aspartate (aspartic acid) (Asp), serine (Ser), γ-aminobutyric acid (GABA), leucine (Leu), valine (Val), lysine (Lys), cysteine (Cys), histidine (His), arginine (Arg), methionine (Met), isoleucine (Ile), phenylalanine (Phe) and tyrosine (Tyr).

## Patient Material

CSF samples (10 mL) were obtained from cisternal aspirates using the ordinary sterile technique of cisternal puncture with a 20 G lumbar puncture needle [*24*]. Significantly bloody CF was not studied. The samples were taken from 40 different adult cadavers with no known neurological disorder. Patient data are presented in a previous study [*23*]. The cadavers were divided into four groups ($n$ = 10 in each group). The samples were taken 2 (group A), 4 (group B), 10 (group C) and 24 (group D) h after death.

Temperature conditions: In the 2 and 4 h groups the cadavers were kept at room temperature (+18 ± 2°C) (mean ± SD). The 10 and 24 h samples were drawn from corpses kept in a cold-storage room (6 ± 2°C). Two patients in the 4 h group and one in both the 10 and the 24 h groups were febrile antemortem. All the others were normothermic before death.

### *Agonal Time*

Agonal time was defined as the period commencing from the point where irreversible decompensation of vital constants occurred, up to the moment of death [*25*]. This was measured from the beginning of unsuccessful resuscitation, when blood pressure was still measurable, or from the onset of severe hypotension (systolic arterial pressure below 65 mmHg) prior to death.

*Cause of Death*

The immediate cause of death was cardiogenic in most cases; coronary artery disease, acute myocardial infarction, cor pulmonale or pulmonary embolism in 36 cases. In greater detail, the distribution of the causes of death was as follows: Group A: 7 cases of acute myocardial infarction (AMI), 2 cor pulmonale and 1 case of mesenteric arterial thrombosis. Group B: 8 cases of AMI, 1 case of ischemic heart disease (coronary artery disease) without myocardial infarction and 1 case of dissecting aneurysm of the aorta (pericardial tamponade). In group C 7 patients died of AMI, 2 of pulmonary embolism and one patient of intoxication. In group D the cause of death was AMI in 8 cases and pulmonary embolism in one case. One patient died of pulmonary tuberculosis in this group. Pathologic anatomical diagnosis was made in 25 of 40 cases and diabetes mellitus was obvious in five patients.

*Controls*

The control group ($n$ = 10, group 1) comprised urologic ($n$ = 3) and orthopedic ($n$ = 7) patients with no known neurological disorder and is described in detail in an earlier study [6]. Spinal fluid was drawn by lumbar puncture from the interspace LII-SI. Otherwise CSF was collected from 25 patients (group 2): 22 men and 3 women by lumbar puncture from the LI-LIV interspace for analysis of acid-base balance, $pCO_2$ and $pO_2$. The diagnoses of the patients were urologic ($n$ = 20) or orthopedic ($n$ = 5). The ages varied from 32 to 86 years, with a mean of 64 ± 6 (SD) years.

## Methods

*Storage of Samples*

Collection and storage of the CF samples were uniform and the time elapsing from cisternal puncture to the freezing of the samples was 23 to 52 min; group differences were insignificant. After collection, the CSF samples of the cadavers and the samples in control group 1 were transferred to the laboratory in ice (0°C) where they were centrifuged for 10 min (+10°C, 800 g). Thereafter the clear supernatant was divided into aliquots, placed in liquid nitrogen and then stored at −70°C until analysis. In control group 2 the samples were taken for analysis immediately. The time from cisternal puncture to the moment when the samples were placed in liquid nitrogen was 32 ± 6 min (mean ± SD). The samples were taken to the laboratory in dry ice for determination.

*Experiments to Study the Effect of Autolysis During Storage of Samples*

In order to investigate the effect of freezing and storage on the activities of total CK and LD, a control group 3 comprising 10 patients (5 men and 5 women) without known neurological disease was further collected. The ages of these patients ranged from 49 to 81 years; mean of 62 ± 12 (SD) kg. CSF was drawn from the LII-LV lumbar interspace before local anesthetic was given in the treatment of inguinal hernia ($n$ = 1), varicose veins of the lower extremities ($n$ = 1), orthopedic ($n$ = 2) and urologic ($n$ = 6) diseases. The CSF samples were analyzed immediately after centrifugation.

*Analytical Methods*

CF pH, $pCO_2$, $pO_2$, BE and $HCO_3^-$ were measured with a Corning 178 pH/Blood Gas Analyzer (Corning Limited, Halstead, Essex, England 1981).

The CK (EC 2.7.3.2) activity was determined at 37°C according to the recommendations of the Scandinavian Committee on Enzymes [*26,27*]. The activity of the CK-B subunit was assayed after immunoinhibition of the CK-M moiety according to Würzburg et al. [*28*] at 37°C with the same reagents as in the assay of total CK. Total CK and CK-B were measured with a Hitachi 705 Analyzer (Oriola, Espoo, Finland).

The activity of LD (EC 1.1.1.27) was measured at 37°C according to the recommendation of the Scandinavian Committee on Enzymes [*29*], using a KONE 3000 Analyzer (Kone Ltd, Espoo, Finland). The isoenzymes of LD were fractionated electrophoretically on cellulose acetate membrane (Beckman Microzone Plus, Beckman Instruments Inc., Fullerton, CA, USA). The isoenzymes were visualized at 37°C by means of optimized concentrations of L(+)lactate (lithium salt) and NAD and coupling of the reaction with Nitro Blue Tetrazolium (NBT). Quantitation of isoenzymes was performed with a Helena AutoScanner Flur-Vis densitometer. The sensitivity of the electrophoresis was 1 to 2 U/L.

NAG (EC 3.2.1.30) activities were measured fluorometrically using a Transcon FN fluoronephelometer (Elomit Ltd, Espoo, Finland) according to Whiting [*30*].

Acid phosphatase (EC 3.1.3.1) activities were measured with a Kone C Analyzer (Kone Instruments, Espoo, Finland) with p-nitrophenol phosphate as substrate [*31*, p. 211].

For the assay of CSF lactate and pyruvate, deproteinization of CSF samples was carried out immediately after thawing prior to analysis. Lactate and pyruvate were determined by enzymatic end-point methods [*32,33*].

$Na^+$ and $K^+$ were measured with ion-specific electrodes with a Hitachi 705 E Analyzer.

*Amino acid analysis*—After thawing, proteins were precipitated by adding an equal volume of 5% sulphosalisylic acid solution. Norleucine was included as an internal standard in sulphosalisylic acid solution (13.1 mg/mL). After centrifugation amino acids and ammonia were analyzed on an ion exchange column of Waters high-performance liquid chromatograph [*34*]. Identification of amino acids was based on their known retention times. The method used is designed for analysis of protein hydrolysates at which Gln, Asx and Thr elute together. Because the Gln content in the human brain and CSF is considerable as compared to Asx and Thr the sum of these amino acids is designated as "apparent glutamine" and is calculated on the basis of reaction of 0-phtaldialdehyde with glutamine. The standard deviations in amino acid analysis were between 1 to 10%. Ammonia was determined together with the amino acids using the o-phtaldialdehyde reaction, the reliability and accuracy of its analysis being the same level as for the amino acids.

## Statistical Analysis

The group means were compared using one-way analysis of variance (ANOVA) followed by Newman-Keul's range test in cases where the means were significantly different.

Linear regression analysis was used in assessing whether CF LD5 activity or lactate and GABA concentrations were dependent on the agonal time. The effect of post mortem time was also calculated by linear regression analysis, plotting amino acid and ammonia concentrations against time.

Two-tailed Student's T-test for independent samples was used when calculating whether the means of CK and LD samples in control groups 1 and 3 were statistically different.

## Ethical Considerations

The study was approved by the Ethical Committee of Tampere University Hospital.

## Results

$pCO_2$ values increased 2 to 4 h after death and then declined ($P < 0.001$). The post mortem $pCO_2$ values at 2 h were 203 ± 23 (SD) mmHg and markedly higher than in the lumbar CSF during life. $pO_2$ decreased 4 to 24 h postmortem ($P < 0.001$) the 2 h values being 48 ± 9 mmHg. Statistically significant differences between the groups were not seen in CF, pH, BE or $HCO_3^-$.

Postmortem changes in CF enzymes are shown in Figs. 1 to 3. Changes in CF glucose, lactate, pyruvate and the ions $Na^+$, $K^+$ and $Cl^-$ are presented in Tables 1 and 2 together with the same biochemical parameters measured in lumbar CSF from 10 control patients (control group 1).

Total CK, CK-BB, LD and its isoenzymes 1-3, NAG and acid phosphatase increased linearly and statistically significantly ($P < 0.001$) after death. In LD4 only the 2 and 24 h samples differed from each other but in LD5 the differences between the groups were not statistically significant. Approximately 90% of the total CK activity was CK-BB in all groups. The LD5 activity was 3.1 (2 h), 2.5 (4 h), 2.3 (10 h) and 0.9 (24 h) % of total LD activity in the four groups studied. There was no correlation between LD5 activity and agonal time. In control group 1 no LD5 activity was found in lumbar CSF.

CK activities in control groups 1 and 3 were 2.5 ± 2 and 2.1 ± 2 U/L and did not differ statistically significantly from each other. Total LD activities were, by contrast, markedly higher ($P = 0.009$) in control group 3 (53 ± 18 U/L) than in group 1 (34 ± 9 U/L). Thus freezing and storage may reduce CSF LD activity.

Glucose and pyruvate concentrations in CF decreased and lactate increased statistically significantly ($P < 0.001$) with time (Table 1). No correlation was observed with agonal time and CF lactate concentration.

The postmortem concentrations of sodium and chloride declined ($P < 0.001$) but potassium concentration increased linearly after death (Table 2).

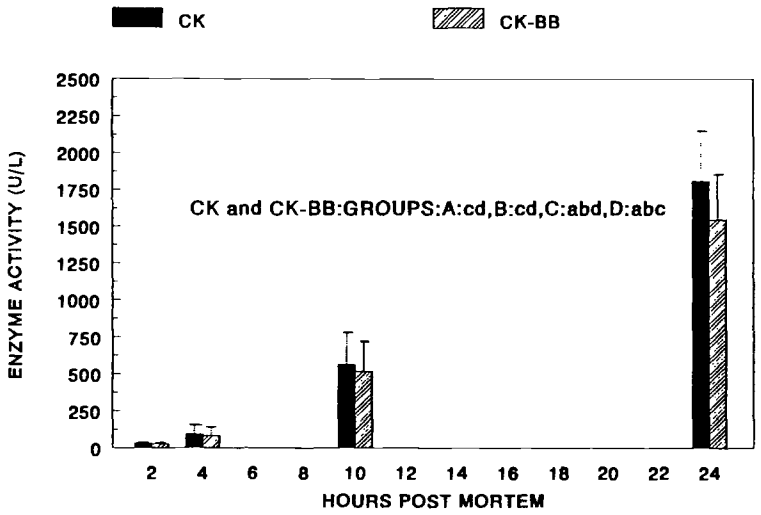


FIG. 1—*Postmortem CF activities (mean and SD) of CK and CK-BB (U/l). Statistically significant differences (P < 0.05) between the groups are marked in the following way: a = significantly different from group A (2 h), b = significantly different from group B (4 h), c = significantly different from group C (10 h), d = significantly different from group D (24 h). N.S. = non-significant. The Newman-Keul's range test was used. Reference values in vivo (control group 1) for CK 2.5 ± 2 and CK-BB 1.4 ± 1.1.*

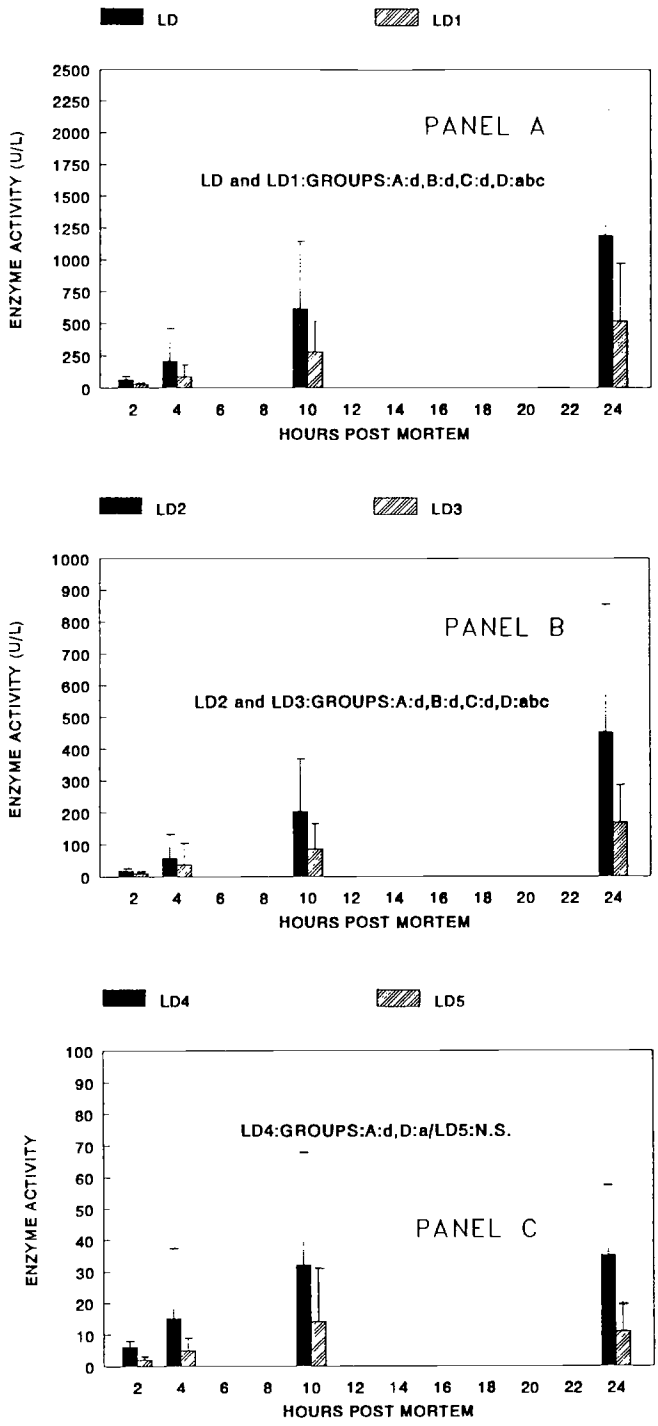


FIG. 2—*Postmortem CF activities (mean and SD) of total LD and LD1 (panel A), LD2 and LD3 (panel B), LD4 and LD5 (panel C) (U/l). Statistically significant differences* ($P < 0.05$) *between the groups as in Fig. 1. Reference values (U/l) in vivo (control group I) for LD 34 ± 9, LD1 27 ± 8, LD2 6 ± 6, LD3 1 ± 3. LD4 and LD5: not detectable.*

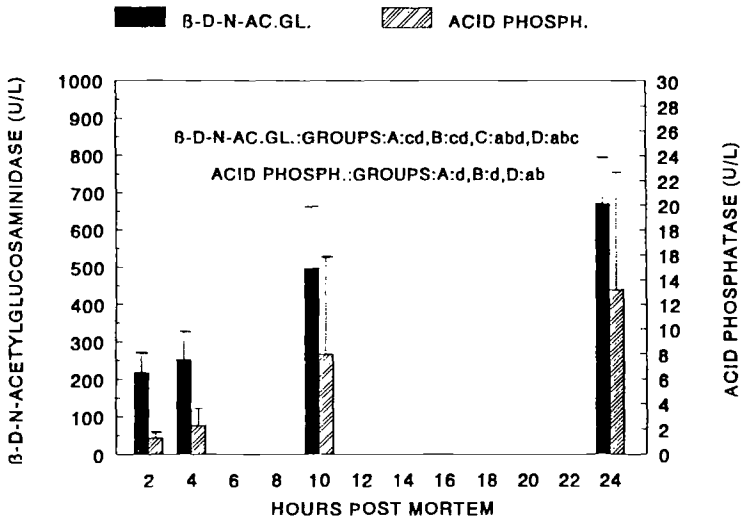


FIG. 3—*Postmortem CF activities (mean and SD) of β-D-N-acetylglucosaminidase (NAG) and acid phosphatase (U/l). Statistically significant differences (P < 0.05) between the groups as in Fig. 1. Reference values in vivo (control group 1) for NAG 368 ± 82 U/l and for acid phosphatase 0.56 ± 0.26 U/l.*

TABLE 1—*Postmortem changes (mean ± SD) in the concentrations of glucose, lactate (mmol/l) and pyruvate (μmol/l) and in vivo values in lumbar CSF from 10 patients (control group 1). Statistically significant differences (P < 0.05) between the groups are marked in the following way: a = significantly different from group A, b = significantly different from group B, c = significantly different from group C, d = significantly different from group D. The Newman-Keul's range test was used.*

| Comp | A (2h) | B (4h) | C (10h) | D (24h) | Control group 1 |
|---|---|---|---|---|---|
| Gluc. | 4.7 ± 1.8<br>c, d | 3.4 ± 2.0<br>c, d | 1.4 ± 1.8<br>a, b, d | 1.3 ± 1.5<br>a, b, c | 3.3 ± 0.4 |
| Lact. | 13.8 ± 1.2<br>b, c, d | 17.4 ± 2.8<br>a, c, d | 21.8 ± 2.7<br>a, b, d | 24.6 ± 2.9<br>a, b, c | 1.72 ± 0.26 |
| Pyruv. | 0.08 ± 0.03<br>b, c, d | 0.06 ± 0.03<br>a, d | 0.04 ± .01<br>a | 0.02 ± 0.006<br>a, b | 0.06 ± 0.02 |

TABLE 2—*Postmortem changes (mean ± SD) in the concentrations of the ions $Na^+$, $K^+$ and $Cl^-$ (mmol/l) and in vivo values in lumbar CSF from 10 patients (control group 1). Statistically significant differences (P < 0.05) between the groups are marked in the following way: a = significantly differed from group A, b = significantly different from group B, c = significantly different from group C, d = significantly different from group D. The Newman-Keul's range test was used.*

| Comp | A (2h) | B (4h) | C (10h) | D (24h) | Control group 1 |
|---|---|---|---|---|---|
| $Na^+$ | 141 ± 7<br>c, d | 139 ± 9<br>c, d | 131 ± 5<br>a, b, d | 122 ± 3<br>a, b, c | 158 ± 2 |
| $K^+$ | 14.0 ± 1.8<br>b, c, d | 21.2 ± 3.6<br>a, c, d | 30.6 ± 3.3<br>a, b, d | 35.6 ± 2.0<br>a, b, c | 3 ± 0.1 |
| $Cl^-$ | 122 ± 4<br>c, d | 122 ± 6<br>c, d | 116 ± 5<br>a, b | 113 ± 4<br>a, b | — |

The changes in CF free amino acids and ammonia are presented in Figs. 4 and 5 together with in vivo reference values of GABA [*35*] and 9 other amino acids [*36*] in lumbar CSF. The concentration of GABA was more than 170 times and that of Glu more than 20 times higher already 2 h postmortem as compared to the reference values in lumbar CSF (Fig. 4). A 2 to 3 fold increase was also seen in Ala, Gly, Lys, His, Ile, Phe and Tyr at 2 h. Pro concentration in 2 h samples as well as in vivo, was low and near zero, increasing rapidly thereafter (Fig. 5).

In all amino acids the sample means were significantly different ($P < 0.001$) in ANOVA. In cysteine the 2 and 4 h, 4 and 10 h as well as 2 and 10 h sample means did not differ statistically significantly in Newman-Keul's test. In other amino acids all the sample means were statistically different with the exception of 2 and 4 h samples. In all cases the slope of the regression line was significantly different from 0 ($P < 0.001$). When arranged according to regression line slope from the highest to the lowest the sequence was ammonia-Glu-"Apparent glutamine"-Ala-Gly-Pro-Asp-Ser-GABA-Leu-Val-Lys-Cys-His-Arg-Met-Ile-Phe-Tyr.

## Discussion

Cerebral ischemia is one of the most frequent and troubling pathological conditions encountered in neurology and neurosurgery, and it is also a fatal complication of heart failure and shock of varying etiology. In the somatic death of an individual, which often involves prolonged brain hypoxia, changes in the CSF are to be expected similar to those seen in patients with irreversible hypoxic brain injury.

Because the spinal fluid obtained from the cerebral cistern is in close proximity to brain tissue, it may more accurately reflect CNS metabolism than lumbar CSF. Furthermore, CF samples were used because of the concentration gradients, the instability or rapid metabolism of many brain substances secreted into the CSF [*17*, p. 644] [*37*].

Our reference values of CK, CK-BB, LD, lactate, pyruvate [*4*], NAG [*38*], acid phosphatase [*39*], glucose [*4*], and the ions $Na^+$, $K^+$ [*39,17*] and $CL^-$ [*4,39*] in lumbar CSF were at the same level as earlier reported.

It seems likely that brain acidosis is a component of systemic acidosis [*40*], and carbon dioxide values in post mortem CSF tend to drop with time [*41–43*]. A part of this change may be associated with the formation of lactic acid and other acids [*42*].

In barrier injuries the brain-specific isoenzymes of CK and LD (CK-BB and LDl-2) are released into both the CSF and the blood [*17,44*]. It has been shown by Paulson et al. [*41*] that CK activity is higher postmortem than in vivo. The CK and CK-BB activities in our material were already 10 to 20 times higher 2 h after death than in lumbar CSF during life. Moreover, because CK-BB represents the greater part (90%) of CK activity in postmortem CF, the enzyme must come mainly from the brain.

In brain tissue and CSF the LD-isoenzyme distribution is identical; LD1, LD2 and LD3 are predominant, whereas LD5 represents less than 1% of the total activity [*25*]. In the present study the LD5 activity was 3.1 (2 h), 2.5 (4 h), 2.3 (10 h) and 0.9 (24 h) % of the total LD activity in the four groups studied. It has been shown by Mangin et al. [*25*] that the longer the agonal duration, the higher is the LD5 activity in the CF due to increased permeability of the blood-brain-barrier. I could not, however, find any correlation between LD5 activity and agonal time.

Glucose penetrates the barriers easily [*17*] but the postmortem values of glucose in the CSF are related not only to premortem levels and rapidity of breakdown of barriers, but also to the temperature of the cadaver [*43,45*]. The temperature effect may call for attention in the 10 and 24 h groups, where the bodies were kept in a cold-storage room (+4 to +12°C). The correlation between postmortem rectal and brain temperatures has also been presented [*46*]. Moreover, 8 patients in the 2 h group, 6 in the 4 h group, 7 in

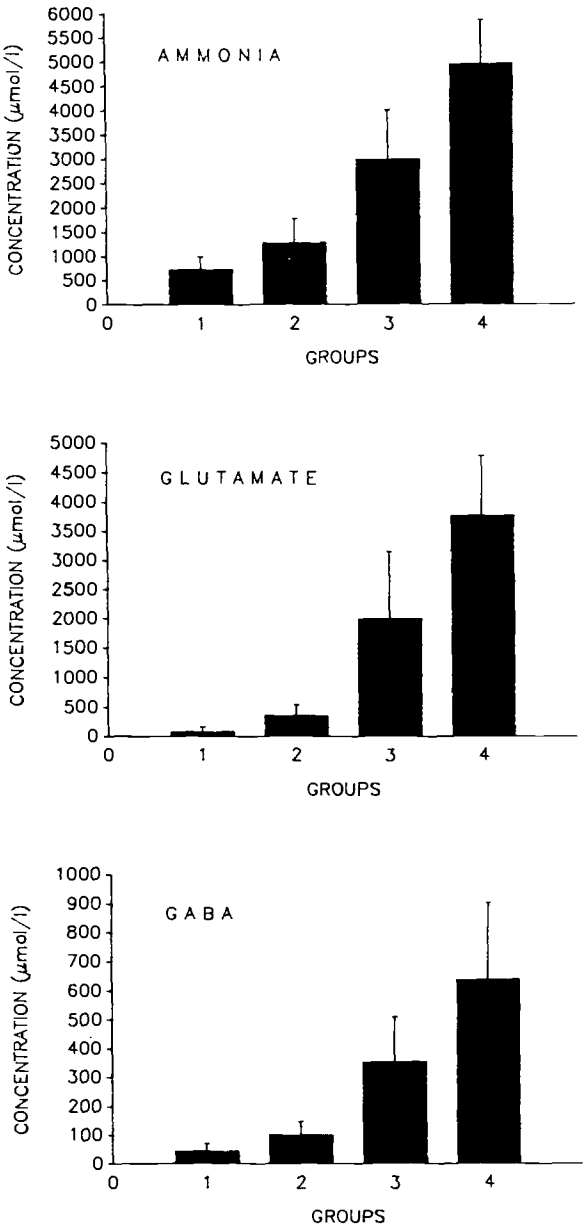


FIG. 4—*CF concentrations (μmol/l) of ammonia, glutamate (GLU) and GABA 2 h (group 1), 4 h (group 2), 10 h (group 3) and 24 h (group 4) postmortem. Mean and SD values are given. Reference values (human lumbar CSF in vivo) for GLU 0-9 μmol/l and GABA 220-300 pmol/mL (Hare et al. 1980). Ammonia: not detectable.*

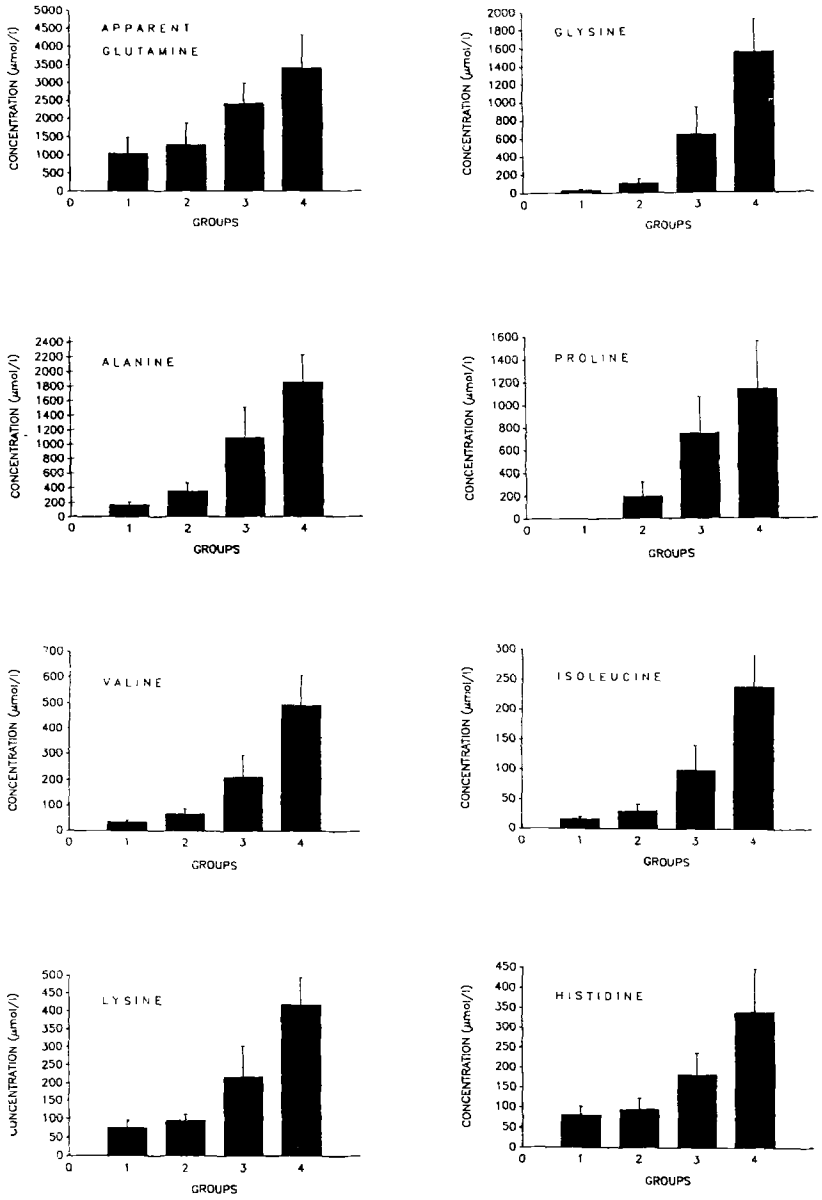


FIG. 5—*CF concentrations of "apparent glutamine" [glutamine (GLN) + threonine (THR) + asparagine (ASX)], alanine (ALA), valine (VAL), lysine (LYS), glycine (GLY), proline (PRO), isoleucine (ILE) and histidine (HIS) 2 h (group 1), 4 h (group 2), 10 h (group 3) and 24 h (group 4) postmortem. Mean and SD values are given. Reference values (human lumbar CSF in vivo): GLN 246-958, THR 6.5-56.3, ASX 1-16, ALA 10.4-49.5, VAL 4-32.2, LYS 12.2-42.8, GLY 1.3-11.5, ILE 0.3-9.3, HIS 4.6-20.8 µmol/l. PRO: not detectable.*

the 10 h group and 5 in the 24 h group were treated before death with 5 or 10% glucose infusions.

In physiological states L-lactate penetrates intact blood-brain and blood-CSF barriers only slowly by way of an active stereospecific transport mechanism, while pyruvate penetrates the barriers more rapidly. In severe systemic acidosis and anoxia, large amounts of lactate may, however, pass through the barriers and the CSF lactate level will increase [*17,47*]. Although a failing oxygen supply in the terminal phase of illness may cause a toxic rise in brain lactate concentration [*40*], no correlation between agonal time and postmortem lactate concentration was observed in the present study.

In anoxia and postmortem autolysis the ionic changes in brain tissue and CF are believed to be mediated via changes in nerve cell permeability, and glial cells may partly affect this process [*8,43*]. The sodium deficit is compensated by a potassium increase [*42*], and the postmortem rise in CSF potassium with time is well documented in previous studies [*9,41–43,45*]. The potassium concentration in the CSF of dead subjects may become 20 times greater than in vivo [*48*] and potassium moves into the CSF progressively up to 70 h after death [*45*].

The concentration of individual amino acids in the CSF will depend on the concentration in the plasma and the carrier mediated transport across the barriers of the CNS, from CSF to blood and from CSF into the brain cells. On average the concentration of most amino acids in CSF is about one third of that in plasma [*17*]. All the amino acids of the plasma are—especially in pathological conditions—capable of crossing the blood-CSF barrier, failure to detect them in the CSF being due to their use [*17*]. The agonal status also influences on the concentrations of certain amino acids in postmortem human brain tissue [*49*].

Perry (1981) found that the brain contents of 12 amino acid constituents of proteins and peptides increased steadily both after brain death and in autopsied human brain (cortical brain biopsies). These were: Thr, Ser, Pro, Ala, Val, Ile, Leu, Tyr, Phe, Lys, His and Arg [*50*]. Among these amino acids the concentration of Val and Ile as well as Asx in CSF is not dependent on their plasma concentration [*51*]. The blood-brain-barrier is also relatively impermeable to GABA, Glu, Gly, Asp, Thr, His, Lys, and Ala [*17*]. Thus the increase seen in CF, Ala, Gly, Lys, His and Ile concentrations already at 2 h may also reflect the brain metabolism of these amino acids in vivo, whereas Glu and GABA concentrations remain stable (undetectable or near zero) in cerebral ischemia [*3,15*].

The GABA content in brain tissue is stable for about 30 min, and rises to a maximum 2 to 3 h after death, after which it remains unchanged for at least 24 h [*50*]. This rise was also seen in the CF values in the present study and is thought to result from a decreased rate of oxidative metabolism resulting in decreased use through the GABA shunt [*50,52*].

Elevated $K^+$ concentrations and increased CK-BB activity in postmortem CF already at 2 h are probably due to failure in brain energy metabolism leading to cell membrane injury. Increasing lactacidosis on the other hand reflects terminal anaerobic glycolysis. These changes are seen in CF when cytoplasmic substances are released.

Glu and GABA concentrations in postmortem CF are also considerably elevated because their synthesis in the brain increases suddenly at the time of death and continues thereafter. It is, however, impossible to ascertain the final origin of the different amino acids detected in postmortem CF because of their complicated transport mechanisms between blood, CSF and brain tissue. Furthermore, there is very little reliable knowledge concerning the pathophysiology of CNS amino acid metabolism in terminal stages of life and immediately postmortem.

In conclusion, this study shows that among the CF components measured the earliest postmortem changes were seen in CK-BB, lactate, $K^+$ and certain amino acids, which

thus also could serve as markers of hypoxic brain injury in vivo. The results obtained may also be useful in forensic medicine for the determination of the postmortem interval.

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