

# The cerebrospinal fluid of newborn infants with perinatal hypoxia

M. FEKETE, Gy. SOLTÉSZ, Magdolna HORVÁTH

Department of Paediatrics, University Medical School, Pécs

Concentration of glucose, protein and amino acids in plasma and CSF was studied in 22 newborn infants suffering from perinatal hypoxia of various severity. A wide range of individual values characterized all parameters both in plasma and CSF, but a statistically significant correlation was found between gestational age and CSF protein, gestational age and CSF/plasma protein ratio, CSF and plasma protein and glucose concentration, and CSF and the plasma amino acid level. The possible explanation and clinical implications of the findings are discussed.

Despite extensive studies, the function and role of cerebrospinal fluid (CSF) are not quite clear. It seems that in addition to providing protection against mechanical trauma, it serves as a source of metabolites and various nutrients for the CNS (8, 17).

Under normal conditions, the composition of CSF is regulated and kept fairly unchanged by subtly coordinated mechanisms. The concentration of various substances in CSF depends on the rate of their penetration from blood and brain tissue, on CSF utilization by the surrounding nervous tissue, and on clearance mechanisms. Depending on the substance, one, two or perhaps all three mechanisms are operating. If any one is disturbed, remarkable distortions in CSF composition result, which may be useful in diagnostics and prognostics.

When studying pathological changes in CSF, the reliable knowledge of normal values is a fundamental

requirement. Unfortunately, this is the main difficulty in CSF studies performed on newborn infants, since normal values are, in general ill-defined. For this reason we have examined the relationships between plasma and CSF glucose, protein and amino acids in newborn infants suffering from perinatal hypoxia. The question, particularly in context with amino acids, is justified by the increasing use of various regimes of parenteral nutrition for newborn infants with increased metabolic vulnerability of their damaged central nervous system and blood-brain-CSF barrier.

## PATIENTS AND METHODS

Twenty-two newborn infants were studied. All of them had an Apgar score  $<6$  at 5 min and needed resuscitation at birth.

Supported by the Scientific Research Council, Hungarian Ministry of Health (3-23-0502-04-1/M).

TABLE I  
Clinical data of the 22 newborn infants studied

	Mean	$\pm$ S.D.	Range
Birth weight, g	2544	214	750-4150
Gestational age, weeks	35.6	1.1	26- 42
Postnatal age, hours	18.0	3.5	1- 69

As shown in Table I, mean ( $\pm$ S.E.) birth weight, gestational age and postnatal age of the infants were  $2544 \pm 214$  g,  $35.6 \pm 1.1$  weeks, and  $18.0 \pm 3.5$  hours, respectively, with a wide range of individual values in all three parameters.

Eleven of the 22 infants were preterm (gestational age  $\leq 37$  weeks), the rest were full-term (gestational age  $> 37$  weeks). Four of the 8 female and 4 of the 14 male newborn infants died, all with hyaline membrane disease associated in 5 cases with subependymal or intraventricular haemorrhage. All patients needed oxygen therapy via head-box or by CPAP or IPPV ventilation and 5-10% glucose + 4.2% bicarbonate drip infusion, right from the time of their admission to the SCBU, and were on this therapy at the time of the collection of specimens for study.

From all patients an umbilical venous blood sample was taken for measuring plasma glucose and protein concentration and performing amino acid analysis. Simultaneously, a lumbar tap was performed in order to detect or exclude intracranial haemorrhage. CSF samples were analysed

for glucose, protein and amino acids, furthermore for routine laboratory tests, culturing included. In case of blood stained CSF, the specimen was centrifuged immediately and the supernatant was used for further analysis. If the possibility of inflammation could not be excluded with certainty, the patient was excluded from the study.

Plasma glucose and CSF glucose concentration was measured by the o-toluidine method. CSF and plasma protein level was determined with turbidimetry (32) and the biuret reaction, respectively.

Blood specimens for amino acid analysis were collected in heparinized tubes and promptly centrifuged. Plasma was deproteinized by addition of four volumes of 5% sulphosalicylic acid. Protein-free supernatant corresponding to 0.5 ml plasma was immediately frozen and stored at  $-20^\circ\text{C}$  until assayed. Amino acid measurement was performed by an automatic Beckman Multichrom Liquid Column Chromatograph, using norleucine as an internal reference standard.

Regression equations were calculated by the method of least squares.

## RESULTS

Table II shows the mean ( $\pm$ S.E.) CSF and plasma concentration of total protein and glucose in the 22 subjects, furthermore the CSF/plasma ratios calculated for both substances. A wide range of individual values characterized all parameters measured, in both plasma and CSF. On further analysis, however, a statistically significant negative correlation was found between the following parameter-pairs: gestational age-CSF protein ( $r = -0.5594$ ,  $p < 0.01$ ), gestational age-CSF/plasma protein ratio ( $r = -0.6314$ ,  $p < 0.01$ ), birth weight-CSF protein ( $r = -0.6218$ ,  $p < 0.01$ ), birth weight-CSF/plasma protein ratio ( $r = -0.6519$ ,  $p < 0.001$ ). On the other hand, no remarkable relation could be observed between either CSF glucose or CSF/plasma glucose ratio and the gestational age or birth weight of the newborn infants. A highly significant reverse correlation was detected be-

tween plasma and CSF protein level ( $r = -0.6529$ ,  $p < 0.001$ ), whereas a positive relation was found between plasma and CSF glucose concentration ( $r = 0.8970$ ,  $p < 0.001$ ). A significant direct relation was observed between gestational age and plasma protein concentration ( $r = 0.6319$ ,  $p < 0.01$ ), but not between postnatal age and CSF protein or glucose level.

The mean ( $\pm$ S.E.) plasma concentrations of the free amino acids measured are shown in Fig. 1. It is seen that the plasma level of all individual amino acids measured in the hypoxic newborn infants, were closely similar to that of normal full term and preterm infants (19, 29). Figure 2 shows CSF amino acid concentration of 11 newborn infants with perinatal hypoxia, compared with that of control subjects. It has, however, to be emphasized, that in lack of normal values for CSF amino acid concentration in healthy newborn infants, values of normal subjects of various ages published in

TABLE II

Plasma and CSF concentration and CSF/plasma concentration ratio of protein and glucose in the 22 newborn infants studied

	Mean	$\pm$ S.E.	Range
<i>Protein</i>			
plasma, g/l	46.6	1.9	25.6-62.0
CSF, mg/l	1829	396	350-7600
CSF/plasma ratio	0.048	0.014	0.006-0.271
<i>Glucose</i>			
plasma, $\mu$ mol/l	4.4	0.6	0.7-12.7
CSF, $\mu$ mol/l	2.9	0.3	0.3- 6.7
CSF/plasma ratio	0.60	0.04	0.2- 1.0

the literature (9, 12, 18, 24, 27) have been used for comparison. Figure 1. shows clearly the great variation found in CSF concentrations of individ-

ual amino acids in hypoxic newborn infants. It is seen in Fig. 2 and in more detail in Table III that in hypoxic babies the CSF concentration

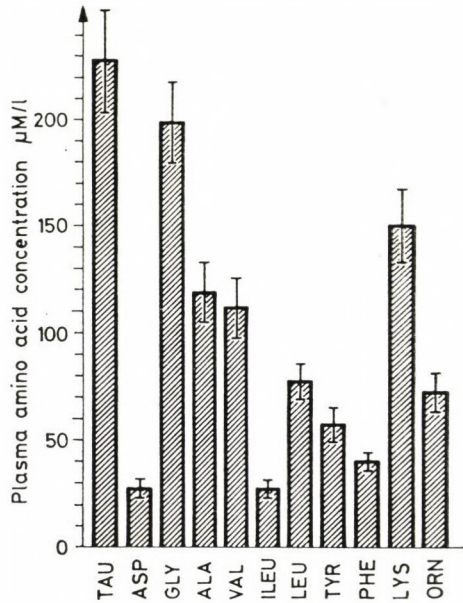


FIG. 1. Plasma concentration of individual amino acids in newborn infants with perinatal hypoxia (mean  $\pm$  S.E.)

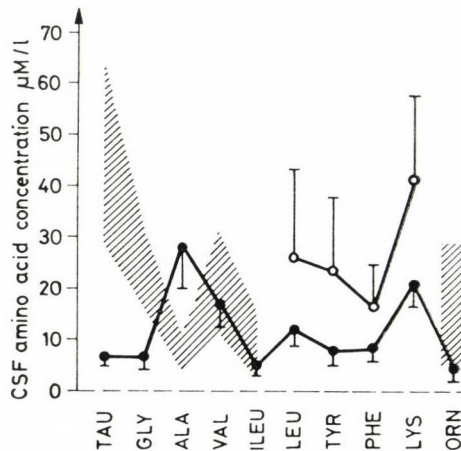


FIG. 2. CSF amino acid concentration in newborn infants with perinatal hypoxia and in control subjects (mean  $\pm$  S.D.). Empty circles, mean values of newborn infants; shaded area, range of values in newborn infants; full circles, mean of summarized control values from references Nos 9,12,18,24,27

TABLE III  
Concentration of some amino acids in CSF, mean  $\pm$  S.D.  $\mu\text{mol/l}$

	TAU	GLY	ALA	VAL	ILEU
I	7.6 $\pm$ 2.1	5.9 $\pm$ 1.8	34.8 $\pm$ 8.2	19.9 $\pm$ 4.1	6.2 $\pm$ 1.4
II	6.8 $\pm$ 1.7	4.7 $\pm$ 1.5	23.2 $\pm$ 5.1	15.0 $\pm$ 2.8	3.9 $\pm$ 1.0
III	6.4 $\pm$ 1.5	5.8 $\pm$ 0.9	32.7 $\pm$ 6.3	20.9 $\pm$ 4.8	5.3 $\pm$ 1.5
IV	6.3 $\pm$ 1.8	6.6 $\pm$ 1.8	23.2 $\pm$ 9.4	14.6 $\pm$ 5.5	4.4 $\pm$ 1.3
V	5.3 $\pm$ 1.4	8.5 $\pm$ 2.5	27.9 $\pm$ 9.9	14.3 $\pm$ 4.0	5.0 $\pm$ 0.9
VI	29.2-63.5	16.5-31.3	3.8-11.2	10.8-30.7	2.5-15.2
	(r)	(r)	(r)	(r)	(r)

	LEU	TYR	PHE	LYS	ORN
I	14.8 $\pm$ 3.9	9.5 $\pm$ 2.6	9.9 $\pm$ 2.0	20.8 $\pm$ 4.0	3.8 $\pm$ 0.9
II	10.1 $\pm$ 2.1	6.4 $\pm$ 1.5	6.5 $\pm$ 1.2	21.7 $\pm$ 3.7	3.7 $\pm$ 1.0
III	14.9 $\pm$ 3.3	9.0 $\pm$ 2.4	9.5 $\pm$ 2.1	29.1 $\pm$ 4.8	4.9 $\pm$ 1.8
IV	10.9 $\pm$ 3.6	9.1 $\pm$ 5.0	9.2 $\pm$ 5.8	18.7 $\pm$ 6.6	5.7 $\pm$ 1.8
V	11.6 $\pm$ 2.4	7.9 $\pm$ 2.3	7.5 $\pm$ 2.2	18.6 $\pm$ 6.4	5.7 $\pm$ 1.8
VI	26.1 $\pm$ 18.3	24.1 $\pm$ 14.5	17.0 $\pm$ 8.2	41.8 $\pm$ 18.7	5.1-29.3
					(r)

I = McGale et al [18]  
 II = Gjessing et al [12]  
 III = Perry et al [24]  
 IV = Dickinson et al [9]  
 V = van Sande et al [27]  
 VI = Present study  
 r = range

of taurine, glycine, leucine, tyrosine, phenylalanine and lysine was much higher than in control subjects. Figure 3 demonstrates the remarkable variations of CSF/plasma amino acid ratios, concerning each amino acid measured. It should be noted that the greater the number of calculated ratios, the wider was the range of values.

Statistical analysis revealed no significant correlation between CSF/plasma amino acid ratio and gestational age, birth weight, postnatal age,

furthermore the CSF/plasma ratio of glucose and protein. At the same time, a highly significant ( $r = 0.5414$ ,  $p < 0.001$ ) direct relation was observed between the plasma and CSF concentration of all individual amino acids measured.

## DISCUSSION

The composition of CSF is kept more stable than that of plasma (2-5, 8, 15, 20, 26). Pathological distur-

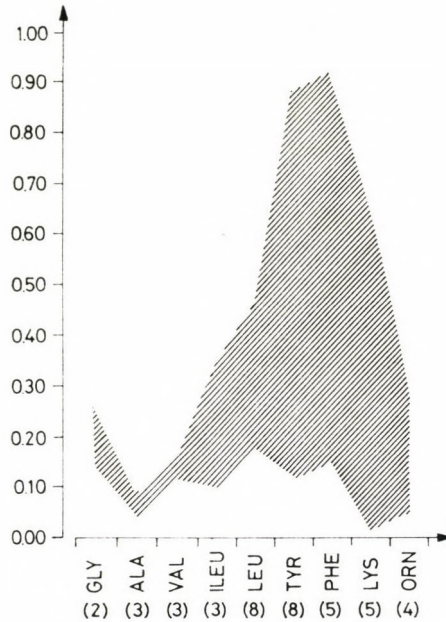


Fig. 3. CSF/plasma amino acid ratios in newborn infants with perinatal hypoxia (ranges). Figures in parentheses indicate the number of values

tions in the composition of CSF may result from changes in the permeability of the blood-brain-CSF barrier, or the metabolism of brain tissue and the clearance of various substances from CSF. All three mechanisms are certainly maturity-dependent, but may be easily damaged in newborn infants suffering from perinatal hypoxia. Consequently, studies on the composition of CSF of pathological newborn infants may provide useful information for the clinician in the diagnosis, treatment and prognostication of ill newborn babies.

Despite the relatively small number of studies on cerebrospinal fluid of normal and pathological newborn infants, it seems to be firmly established that CSF protein concentration is remarkably higher in preterm and term newborn infants than in later life

(1, 10, 14, 20, 23). The high protein content of CSF may result from intracranial haemorrhage or from the increased permeability of the blood-brain-CSF barrier, but the real cause is still disputed. We found a significant negative correlation between gestational age, birth weight and CSF protein level and CSF/plasma protein ratio. The rise in CSF protein level in the newborn infants must be independent of the serum protein concentration, since a strong reverse correlation was observed between the two parameters. The finding that plasma protein concentration correlated directly with gestational age suggests that both plasma and CSF protein concentration is the function of maturity, but in an inverse way. We conclude therefore that the higher protein concentration in

the CSF of preterm infants is probably due to the increased permeability of the barrier and has nothing to do with plasma protein concentration. Since the correlations could be found after perinatal hypoxia of various severity, the CSF protein concentration is probably much more dependent on the functional maturity of the newborn infant than on the hypoxic insult suffered perinatally.

CSF and plasma concentration of glucose has been known to be lower in full term and even more so in preterm newborn infants than in older infants or children (10, 21, 23, 28). In view of the uncertainty in the diagnostic evaluation of a low CSF glucose level in newborn infants, simultaneous measurement of blood glucose and calculation of CSF/plasma glucose ratio has been proposed (10, 21). This is especially important in pathological newborn infants since bidirectional blood glucose and secondary CSF glucose alterations may occur due to the pathologic changes but also to the therapeutic procedures. The CSF and plasma glucose levels of our patients corresponded well to the normal values reported by others. In newborn infants with perinatal hypoxia we found the CSF/plasma glucose ratio to be similar to that observed by Sarff et al. (28) and Monaco et al. (21). No relation could be detected between gestational age, birth weight, postnatal age and CSF glucose level or the CSF/plasma glucose ratio. Still, no conclusion can be drawn as to the spontaneous connections and interrelationships be-

tween the two parameters as all patients received glucose drip infusion before and during the time of sample collection. Nevertheless, the fact that no significant correlation was found between gestational age and actual CSF/plasma glucose ratio, furthermore that a highly significant positive correlation was observed between plasma and CSF glucose concentration, deserves some consideration. It is not unrealistic to suggest on the basis of these observations that the blood-brain-CSF barrier for glucose is either not maturity-dependent, or that the permeability for glucose increases remarkably due to the hypoxic insult and the CSF glucose level becomes dependent on the blood glucose concentration. If this conclusion is correct, then apart from other reasons, special care must be taken in order to avoid iatrogenic hyperglycaemia in newborn infants with perinatal hypoxia, since the effects of a secondary rise of the CSF glucose concentration and CSF hyperosmolality are unknown at that time but may well be dangerous.

An increasing body of knowledge has been accumulated on the amino acid concentration of CSF (6, 7, 9, 11-13, 16-18, 24, 25, 27, 31) in children and adults with various pathological conditions, but data are lacking for the human newborn. It is known that the concentration of most amino acids in the CSF is usually lower than their equivalent levels in plasma, but remarkable inter-individual variations character-

ize the CSF/plasma concentration ratios of different individual amino acids. It has also been pointed out that a raised CSF amino acid concentration or CSF/plasma amino acid ratio may reflect a disturbed cerebral metabolism, but may also be due to damage to the blood-brain-CSF barrier or the amino acid clearance mechanism. The concentration of the 10 amino acids measured in the CSF was raised in the 11 newborn infants with perinatal hypoxia (Fig. 2 and Table III) in comparison with the controls. In evaluating this observation, care must be taken because of the small number of samples and the wide range of individual values. Furthermore, the question remains unanswered whether or not the increased CSF amino acid concentration was related with gestational or post-natal age, or with the perinatal hypoxia suffered by the infants.

Similarly to other authors (18) we observed a wide range of CSF/plasma amino acid ratios and of each individual amino acid (Fig. 3). If the postulation is accepted that the CSF/plasma ratio reflects amino acid transport into and out of CSF directly or indirectly from or to plasma and brain tissue, then this transport is not related to gestational age in hypoxic newborn infants.

Settergren et al. (30) studied 12 infants and 7 children aged 5 months and 12 years, respectively, and found a significant correlation between the arterial concentration and the cerebral exchange of 7 individual amino acids. McGale et al. (18) observed

a direct relationship between venous plasma and CSF concentration of 13 individual amino acids in normal adult humans. Our finding of a significant correlation between the plasma and CSF concentration of amino acids in newborn infants with perinatal hypoxia might have some practical clinical implications. The close relationship of plasma and CSF amino acid level indicates an unrestricted transport, and so a non-physiological rise in plasma amino acid concentration may well result in abnormal amino acid accumulation in the brain and CSF. Considering the fact that birth asphyxia (29) and uncontrolled total or partial parenteral nutrition both affect the plasma amino acid pool, secondary adverse effects on the CNS might be anticipated; the more so as many amino acids are known to have toxic effects on the brain if given in excess, while others are transmitter substances and have neuroexcitatory or inhibitory effects on synaptic transmission (31).

#### REFERENCES

1. Bauer, C, New, M, Miller, J: Cerebrospinal fluid protein values of premature infants. *J. Pediatr* 66:1017, 1965
2. Bering, EA, Jr: The cerebrospinal fluid and the extracellular fluid of the brain. *Fed Proc* 33:2061, 1974
3. Bering, EA: Problems of the dynamics of the cerebrospinal fluid with particular reference to the formation of cerebrospinal fluid and its relationship to cerebral metabolism. *Clin Neurosurg* 5:77, 1958
4. Betz, AL, Goldstein, GW: Polarity of the blood-brain barrier: neutral amino acid transport into isolated brain capillaries. *Science* 202:225, 1978
5. Bradbury, MWB, Crowder, J, Desai, S, Reynolds, JM, Reynolds, M, Saunders,

- NR: Electrolytes and water in the brain and cerebrospinal fluid of the foetal sheep and guinea-pig. *J Physiol (Lond)* 277:591, 1972
6. Buryakova, AV, Sytinsky, JA: Amino acid composition of cerebrospinal fluid in acute neuroinfections in children. *Arch Neurol* 32:28, 1975
  7. Corston, RN, McGale, EHF, Stonier, C, Hutchinson, EC, Aber, GM: Abnormalities of cerebrospinal fluid amino acids in purulent meningitis. *J Neurol Neurosurg Psychiatr* 42:881, 1979
  8. Cserr, HF: Relationship between cerebrospinal fluid and interstitial fluid of brain. *Fed Proc* 33:2075, 1974
  9. Dickinson, JC, Hamilton, PB: The free amino acids of human spinal fluid determined by ion exchange chromatography. *J Neurochem* 13:1179, 1966
  10. Escobedo, M, Barton, LL, Volpe, J: Cerebrospinal fluid studies in an intensive care nursery. *J Perinat Med* 3: 204, 1975
  11. Franklin, GM, Dudzinski, DS, Cutler, RWP: Amino acid transport into the cerebrospinal fluid of the rat. *J Neurochem* 24:367, 1975
  12. Gjessing, LR, Gjesdahl, P, Sjaastad, O: The free amino acids in human cerebrospinal fluid. *J Neurochem* 19:1807, 1972
  13. Goodnick, PJ, Evans, HE, Dunner, DL, Fieve, RR: Amino acid concentrations in cerebrospinal fluid: effects of aging, depression and probenicid. *Biol Psychiatry* 15:4, 1980
  14. Gyllensward, A, Malmstrom, S: The cerebrospinal fluid protein values in immature infants. *Acta Paediatr Scand Suppl.* 135:54, 1962.
  15. Himwich, WA: Physiology and pharmacology of the central nervous system. In: *Perinatal Physiology*, ed. Stawe, U, Plenum Medical Book Company, New York 1978
  16. Korobkin, RK, Cutler, RWP: Maturation changes of amino acid concentration in cerebrospinal fluid of the rat. *Brain Res* 119:181, 1977
  17. Lorenzo, AV: Amino acid transport mechanisms of the cerebrospinal fluid. *Fed Proc* 33:2079, 1974
  18. McGale, EHF, Pye, IF, Stonier, C, Hutchinson, EC, Aber, GM: Studies of the inter-relationship between cerebrospinal fluid and plasma amino acid concentrations in normal individuals. *J Neurochem* 29:291, 1977
  19. Mestyán, J, Soltész, Gy, Schultz, K, Horváth, M: Hyperaminoacidemia due to the accumulation of glyconeogenic amino acid precursors in hypoglycemic small-for-gestational age infants. *J Pediatr* 87:409 1975
  20. Milhorat, TH: Choroid plexus and cerebrospinal fluid production. *Science* 166:1514, 1969
  21. Monaco, M, Kopen, P, Bloom, E, Carter, A, Vanucci, R: Cerebrospinal fluid/blood glucose ratios in premature and full term infants. *Pediatr Res* 12: 554, 1978
  22. Naidoo, B: The cerebrospinal fluid in the healthy newborn infant. *S. Afr. Med. J.* 42:204, 1975
  23. Otila, E: Studies on the cerebrospinal fluid in premature infants. *Acta Paediatr Scand Suppl.* 8:5, 1948
  24. Perry, TL, Hansen, S, Diamond, S, Stedman, D: Plasma amino-acid levels in Huntington's chorea. *Lancet* 1:806, 1969
  25. Pye, IF, McGale, EHF, Stonier, C, Hutchinson, EC, Aber, GM: Studies of cerebrospinal fluid and plasma amino acids in patients with steady-state chronic renal failure. *Clin Chim Acta* 92:65, 1979
  26. Rapaport, SI: Blood-brain barrier. In: *Physiology and Medicine*, New York, Raven Press 1976
  27. Sande, van M, Mardens, Y, Adriaenssens, K, Lowenthal, K: The free amino acids in human cerebrospinal fluid. *J Neurochem* 17:125, 1970
  28. Sarff, LD, Platt, LA, McCracken, GH: Cerebrospinal fluid evaluation in neonates: comparison of high risk infants with and without meningitis. *J Pediatr* 88:473, 1976
  29. Schultz, K, Mestyán, J, Soltész, Gy: The effect of birth asphyxia on plasma free amino acids in preterm newborn infants. *Acta Paediatr Acad Sci Hung* 18:123, 1977
  30. Settergren, C, Lindblad, BS, Persson, B: Cerebral blood flow and exchange of oxygen, glucose, ketone bodies, lactate, pyruvate and amino acids in infants. *Acta Paediatr Scand* 65:334, 1976
  31. Snyder, SH, Young, AB, Bennett, JP, Mulder, AH: Synaptic biochemistry of amino acids. *Fed Proc* 32: 2039, 1973
  32. Ujsághy, P: Eiweissfraktionen des normalen und pathologischen Liquors im Kindesalter. *Monatschr Kinderheilkd* 66:137, 1936

Received 7 June 1981

M. FEKETE, M. D.

József A. u. 7, H-7623 Pécs, Hungary