Cerebrospinal fluid concentrations of hypoxanthine, xanthine, uridine and inosine: high concentrations of the ATP metabolite, hypoxanthine, after hypoxia

RA HARKNESS, RJ LUND*

*From the Division of Perinatal Medicine, MRC Clinical Research Centre, and *Department of Paediatrics Northwick Park Hospital, Watford Road, Harrow*

SUMMARY CSF obtained for clinical purposes from newborn, children and adults has been analysed by high pressure liquid chromatography for hypoxanthine, xanthine, inosine, uridine and urate. Large rises in hypoxanthine and to a lesser extent xanthine occur for about 24 h after hypoxia. High concentrations were associated with later evidence of brain damage or subsequent death. Changes in CSF could be independent of those in plasma. Small or negligible rises were associated with localised and generalised infections including bacterial meningitis, fits, or both. Marked and rapid rises were found after death. These estimations may "predict" the extent of brain damage or brain death.

Damage in many diseases is due to hypoxia. Intrapartum hypoxia is recognised as a major cause of morbidity and mortality,[1][2] yet objective methods for confirming the diagnosis lack specificity and comparative measurements are not available.[3][4] The CNS is the most vulnerable system to damage by hypoxia.[5] The central processes in metabolic damage due to hypoxia involve ATP,[6] the universal energy currency of cells. A reduction in intracellular ATP can be a measure of the metabolic damage due to hypoxia. Means of estimating ATP breakdown by non-invasive methods are needed in clinical practice because serial tissue samples are not available. Since some of the uncharged products of ATP breakdown, hypoxanthine, xanthine, and inosine, can escape from cells, concentrations of these oxypurines in extracelluar fluids like CSF can reflect ATP breakdown. The compounds hypoxanthine, xanthine, and inosine can now be estimated specifically by methods of sufficient sensitivity.[7] Since metabolic events in brain will be reflected in its extracellular fluid,[8] we have used CSF samples to study ATP breakdown in the CNS after hypoxia.

Results obtained from 1978 to 1982 show raised concentrations of hypoxanthine and xanthine in CSF at least 24 h after serious hypoxia in newborn. These

rises could be separated from the slight or negligible changes associated with infection or fits. Brain damage or brain death proven by clinical, electrophysiological and ultrasonic methods was associated with high concentrations.

Methods

CLINICAL

Northwick Park is a large general hospital with a perinatal department delivering about 3400 infants per year. The CSF samples analysed were all aliquots of samples taken for conventional clinical indications from 1978–1982. In the last two years of the study these aliquots were immediately centrifuged to remove blood cells and then stored at −20°C. This was advisable because blood staining of samples was frequent in newborn CSF in contrast to that from older children and adults. Contamination of over about 10⁹ erythrocytes per litre or fluid which was obviously blood stained, resulted in high oxypurine concentrations and such specimens were not analysed or the results were discarded. Clinical records were surveyed to determine the final diagnoses, the timing of clinical abnormalities such as an asphyxial episode, and the time of the CSF sample. The clinical criteria for intrapartum asphyxia and some aspects of the neurological assessment have already been described.[2] Neurological status at the time of the CSF

sample was specifically noted by JL in 1981. We are indebted to Dr M O'Connor for post-mortem samples of vitreous humour and CSF.

For a set of results to be accepted for a normal range no abnormality apart from mild fever or poor feeding had to exist at the time of the sample and subsequent investigation and progress had to be essentially negative. In newborn, the majority of such specimens were taken during a search for evidence of infection. The adult normal range was determined from a series of CSF samples from patients without marked evidence of metabolic or other disease, stored at −20°C by the Clinical Chemistry Department at Northwick Park Hospital. However, both sets of "normal" ranges are probably falsely high because some abnormality existed to prompt the taking of the CSF sample.

BIOCHEMICAL
Trichloracetic acid extracts of CSF were analysed by high pressure liquid chromatography by the methods of Simmonds and Harkness.[7] The results of bacteriological examinations, cell counts, protein and glucose concentrations by conventional methods were obtained from the case notes.

Nucleosides, inosine and adenosine, were specifically measured using boronate affinity column chromatography followed by high performance liquid chromatography (HPLC) using a gradient of methanol in phosphate buffer on a C18 Hypersil column. A similar method has been used to measure adenosine and inosine in plasma.[9] The nucleoside, uridine, the central compound in pyrimidine metabolism, was measured by the simple and by the selective method and this provided a measure of the overall recovery of nucleosides which was used to correct the final analytical results.

Results

NORMAL INFANTS AND ADULTS
In 18 "normal" CSF samples from 16 newborn infants the concentrations of hypoxanthine, xanthine, uridine, and inosine are shown in Fig. 1; the distribution of values are symmetrical about a mean. An arithmetic mean and range of ±2 SD were therefore used. Values (μmol/l) were for hypoxanthine 3·6 (1·8–5·5), xanthine 5·0 (0·9–9·1), uridine 3·3 (0·6–6·3) inosine 0·7 (0–2·0). Urate, the catabolic product of hypoxanthine and xanthine, was also estimated in nine samples which had a mean (±SD) concentration of 30 (±25) μmol/l.

In a "normal control" series of 29 adults the concentrations in CSF of hypoxanthine, xanthine, uridine, inosine are shown in Fig. 2. The results showed an approximately log-normal distribution.
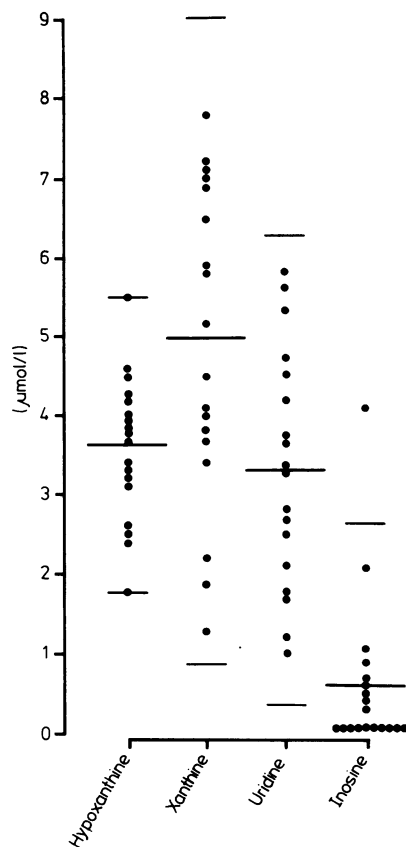

Fig. 1 *Normal concentrations in CSF from newborn of hypoxanthine, xanthine, uridine and inosine. The longer bars indicate arithmetic means with shorter bars to indicate ±2 SD limits except for inosine in which only an upper limit is shown.*

Geometric mean and range of ±2 SD of the logarithmically transformed data are therefore shown in Fig. 2. The mean and ranges (μmol/l) were hypoxanthine 1·8 (0·6–5·1), xanthine 1·7 (0·6–4·7), uridine 1·6 (0·3–8·2), and inosine 0·2 (0–1·0).

CONCENTRATIONS IN PATHOLOGICAL CONDITIONS: HYPOXIA
In cases of hypoxia CSF concentrations of hypoxanthine and xanthine were raised most with lesser rises in uridine and inosine. Results for uridine and inosine are not therefore shown with the pattern of results in Fig. 3 but are given in Table 1. The upper limits of the normal ranges for hypoxanthine and xanthine are shown as a line in Fig. 3. In the first two days after an episode of hypoxia there was a marked rise in the concentration of hypoxanthine. We have little evidence after this but the trend in the data suggests no abnormalities are to be expected on day 3
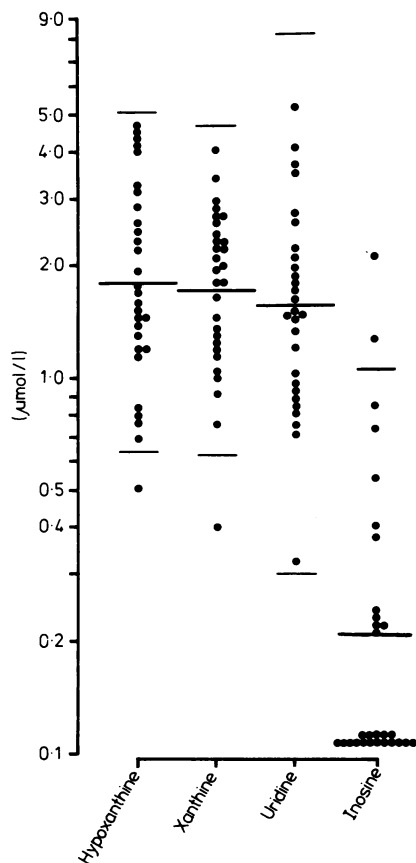
Fig. 2 *Normal concentrations in CSF from adults of hypoxanthine, xanthine, uridine and inosine. The longer bars indicate geometric means with shorter bars to indicate ±2 SD of logarithmetically transformed data except for inosine in which only an upper limit is shown.*

Fig. 3 *Concentrations of hypoxanthine and xanthine in CSF after hypoxia. Symbols show subsequent major clinical findings: ■ = died, ● = brain damaged, ○ = normal, □ = congenital hypothyroidism, △ = chloramphenical toxicity. The horizontal lines are the +2 SD limit of concentration.*

after an episode. The three children who survived after showing high concentrations of hypoxanthine and xanthine have cerebral damage as shown in one by electrophysiological evidence of cerebral cortical blindness and in another by ultrasonographic evidence of severe cerebral atrophy. The third is an athetoid spastic. Five infants died with clinical evidence of asphyxia and had high concentrations of hypoxanthine in their CSF. Two infants died with raised concentrations of hypoxanthine which were less than 10 μmol/l; both died within 24 h of birth. In both these cases with concentrations between 5 and 10 μmol/l there is the problem of accurately diagnosing intrapartum asphyxia from fetal heart rates and Apgar scores alone. In one at least there is some evidence for an alternative cause of death, however, both are included because they fell within
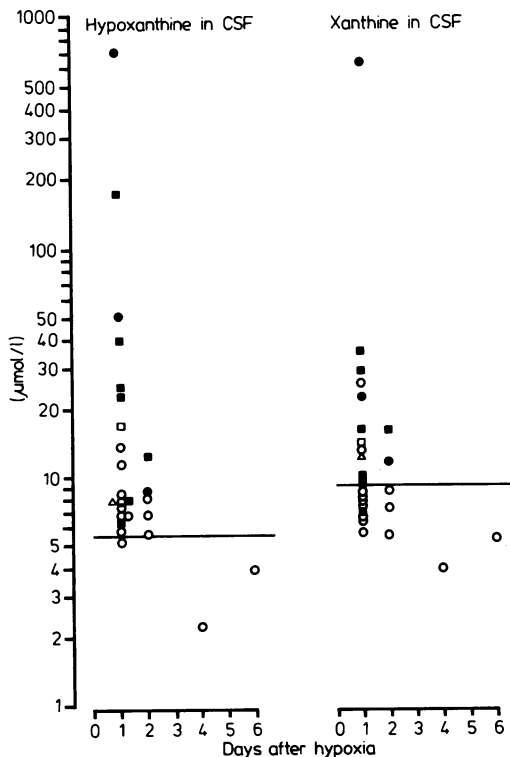
the predetermined criteria. Two of three of our survivors with cerebral damage and high hypoxanthine concentrations were observed to be markedly cyanosed and apnoeic in postnatal episodes, one had subsequent renal tubular necrosis. The third had sustained low Apgar scores, one at 1 min, four at 5 min and subsequent fits. One infant was shown to have brain death by conventional criteria. This infant had a cardiac arrest at the age of 12 min; the heart then continued after a later respiratory arrest sustaining normal plasma concentrations of ATP metabolites, hypoxanthine 1·5, xanthine 1·2, inosine 1·4 μmol/l presumably largely by anaerobic glycolysis[10] since the blood pH was 6·6 with Po_2 2·4 kPa (normal 12–15 kPa). CSF concentrations of hypoxanthine and xanthine were 25·3 and 35·8 μmol/l respectively consistent with the need for O_2 to sustain ATP concentrations in the brain. At necropsy there was no cerebral haemorrhage.

The high concentrations in the asphyxiated newborn later shown to have congenital hypothyroidism

Table 1   *Major clinical features in infants studied after a hypoxic episode with uridine, inosine and urate concentrations in CSF*

Infant	Age	Days after hypoxia	Major clinical features	Outcome	Concentrations in μmol/l CSF		
					Uridine	Inosine	Urate
A	4 days	4	Post-term, severe fetal distress, cardiac arrest at delivery	Good	4·4	0·1	23
B	3 months	1	Missed cot death, renal tubular necrosis	Cerebral cortical blindness	18·6	77	91
C	1 day	1	Fetal distress, emergency caesarean section, focal fit	Good	3·3	1·1	20
D	1 day	1	Confirmed brain death	Death	11·3	2·1	—
E	2 days	1	Two episodes apnoea	Cerebral atrophy	24·6	11·5	49
F	1 day	1	Cardiac arrest at birth, fits	Good	7·6	0·1	240
G	2 days	2	Low Apgar scores, hypothermia	Good	6·4	0·1	34
H	4 wk	1	Preterm, apnoea—ventilated	Good	6·8	0·1	33
I	6 days	6	Severe birth asphyxia, intubated 40 min, fit	Good	—	0·1	26
J	1 day	1	Severe birth asphyxia meconium inhalation	Good	2·3	0·9	56
K	—	1	Preterm—recurrent apnoea ventilated	Good	9·5	1·4	27
L	2 days	2	Intrapartum asphyxia, fits	Death	7·5	0·1	42
M	2 days	2	Intrapartum asphyxia, fits	Athetoid spastic	5·4	0·1	35
N	9 wk	1	Apnoea in car	Died	3·9	14·3	14
O	2 days	2	Fetal distress, post term	Good	3·4	0·6	14
P	1 day	1	Preterm, cyanotic episodes	Good	27	1·9	47
Q	1 day	1	Undiagnosed second twin, intrapartum asphyxia	Died	25·9	20	90
R	2 days	1	Preterm, difficult mechanical ventilation	Good	9·2	3·8	—
S	1 day	1	Preterm, fetal distress	Good	4·9	0·1	—
T	1 day	1	Preterm, RDS difficult mechanical ventilation	Died	26·9	7·9	—
U	2 days	2	Intrapartum hypoxia, post-term	Good	8·4	0·7	—
V	1 day	1	Intrapartum hypoxia, hypothyroid	Good	2·4	1·5	—
W	1 day	1	Intrapartum hypoxia, fits	Good	6·4	1·2	—
X	1 day	1	Preterm, intrapartum hypoxia, ruptured uterus	Died	3·0	1·5	—
Y	1 day	1	Intrapartum hypoxia	Died	3·4	0·6	—
Z	5 days	1	Preterm, chloramphenicol tox, ventilated	Good	5·1	1·1	40

contrast with the normal concentrations but probably abnormal fall in urinary excretion of hypoxanthine and xanthine in this infant.[2] Both may be consistent with diminished ATP turnover in hypothyroidism. In Fig. 3 the results shown four and six days after hypoxia are derived in one case from an infant after a cardiac arrest and from another with sustained low Apgar scores, 2 at 1 min, 4 at 5 and 10 min. The other result meriting detailed description was obtained during ventilation for the gray syndrome due to chloramphenicol toxicity. The acute generalised toxicity of chloramphenicol in some preterm newborn may be due to its action in inhibiting synthesis of mitochondrial cytochromes and if concentrations are high enough, a direct inhibition of cellular respiration.[11] Our patient's hypoxanthine and xanthine concentrations were raised despite effective mechanical ventilation at the time of sampling.

Overall there is a marked rise in concentrations of hypoxanthine and to some extent xanthine for 24–48 h after an hypoxic episode. Mortality is high in those with high concentrations and both our survivors with concentrations above 50 μmol/l on day one showed residual cerebral damage. The majority of infants who survived showed no neurological evidence of damage (Table 1). Our clinical results are therefore similar to those of others.[12]

CHANGES AFTER DEATH

After death there was a rapid rise in the first hour in hypoxanthine, xanthine, uridine and inosine; these concentrations rose more slowly thereafter (Table 2). In two cases samples were taken from upper and lower portions of the vertebral canal. The results were similar in the samples from the same individual from the two different sites. Corresponding concentrations in vitreous humour were lower in five simultaneous comparisons for hypoxanthine and xanthine ($p = 0.03$) and in four of five for uridine. In general urate concentrations were higher in vitreous humour than in CSF which may be a reflection of differences in membrane transport mechanisms. Death therefore causes a rapid and marked rise especially in hypoxanthine concentrations in CSF.

CONCENTRATIONS IN PATHOLOGICAL CONDITIONS OTHER THAN HYPOXIA

The specificity of the rises in ATP metabolites after hypoxic damage was assessed from CSF concentrations in other conditions (Fig. 4). No increases over 10 μmol/l for the compounds measured was obtained in conditions causing fever or fits. CSF hypoxanthine concentrations were increased in newborn infants with bacteriological evidence of infection or a fever (Mann-Whitney U test $p < 0.05$). Surprisingly two children with meningitis had

Table 2 *Hypoxanthine, xanthine, uridine, inosine and urate concentrations in post mortem samples from newborn of cerebrospinal fluid and of vitreous humour*

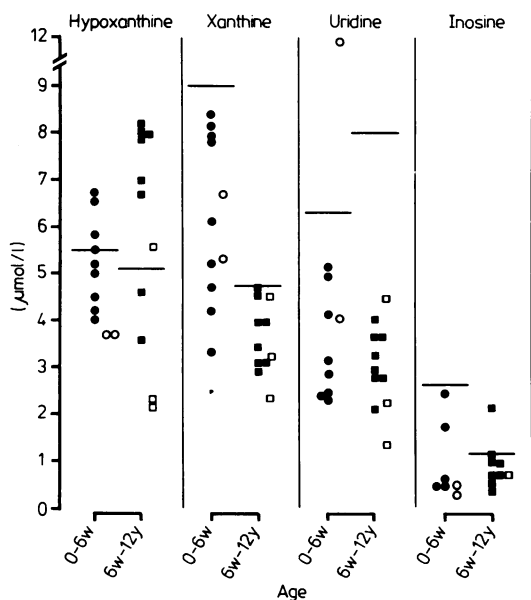|		Concentrations (μmol/l)				
		Hypoxanthine	Xanthine	Uridine	Inosine	Urate
CSF 0–1 h	Mean	102	53	39	25	70
(n = 7)	SEM	27	29	14	10	20
CSF 1–24 h	Mean	312	73	42	55	136
(n = 5)	SEM	103	17	11	29	97
Vitreous 0–1 h	Mean	326*	20	10	0·1	213
(n = 3)	SEM	294	9	2		125
Vitreous 1–24 h	Mean	111	13	19	5	75
(n = 3)	SEM	31	3	5	3	5

* One value 904.

Fig. 4 *Concentrations of hypoxanthine, xanthine, uridine and inosine in CSF from newborn and children aged 6 wk–12 yr. Symbols show the major clinical findings: ● = infection and/or fever including bacterial meningitis in newborn, ○ = fits in newborn, ■ = convulsions with fever (6 wk–12 yr), □ = infection (6 wk–12 yr).*

concentrations little different from those with fever or one with a urinary tract infection, one with pneumonia or two with β-haemolytic streptococcal infection. Results for older children (6 wk–12 yr) with convulsions with fever may be raised; results are shown with an upper limit from adults. Since no entirely adequate corresponding control data are yet available no precise statistical analysis is justified but the adult normal range used is probably applicable. Fits in newborn not associated with any obvious cause were not associated with high concentrations in the

two newborn infants studied. Infections in three older children including one with viral meningitis were associated with lower concentrations than in convulsions with fever.

CSF concentrations were stable in one individual infant with hydrocephalus treated by serial CSF drainage over a period of six weeks. Mean (SEM) CSF concentrations (μmol/l) were hypoxanthine 5·7 (0·5), xanthine 9·8 (0·8), uridine 1·9 (0·2) and inosine 0·5 (0·2) in eight samples. The neurological function of this child was assessed as only mildly impaired and her subsequent progress has been good. This is consistent with the very slight rise in the ATP metabolites, hypoxanthine and xanthine, in CSF. It may be relevant to these findings that increases in cAMP concentration are found in CSF after cerebral damage[13 14] and even migraine;[15] findings consistent with increased leakage of intracellular compounds.

The concentrations of inosine in CSF should be accepted with caution because the usual HPLC method was not sensitive enough to detect this nucleoside in about half the samples of CSF. The immediate metabolic precursor of inosine, adenosine, is also difficult to measure. Using the specific method described above with a pool of CSF, inosine concentration was 0·06 and adenosine 0·034 μmol/l suggesting that the simple method may overestimate inosine concentrations.

Discussion

The prediction that large rises of ATP metabolites in CSF would occur as a consequence of damaging hypoxia has been confirmed in our four-year experience. In view of the difficulties of diagnosing and measuring intrapartum asphyxia it would also appear necessary to study older children and adults after proven and measured periods of hypoxia; it may then be possible to relate the period of ventilatory or cardiac arrest to the raised concentrations of ATP metabolites and other evidence of the damage sus-

tained. It would be necessary to use the appropriate normal range because there are slight differences between "normal" values for newborn infants and those for adults (Figs. 1 and 2). Such differences may be related to the increasing maturity of the blood brain barrier in the first year of life.[16] The large changes in the concentrations of hypoxanthine in CSF are consistent with the sensitivity of the brain to damage from failures of energy supply by hypoxia, or by hypoglycaemia.[5] Hypoxanthine was better than xanthine at "detecting" damage which is consistent with the larger increases in hypoxanthine in blood and urine after acute reduction of intracellular ATP concentrations in man.[17] At least for research purposes a method separating hypoxanthine from xanthine is therefore advisable.

Only one other series has been reported; these results were obtained with a xanthine oxidase-based oxygen electrode method, in which hypoxanthine and xanthine were measured together. The sensitivity of this method is not high enough to measure many normal values.[18] However, some patterns of results similar to ours were obtained. Meberg and Saugstad[18] showed a marked rise in asphyxiated children in CSF samples, "taken very shortly after the hypoxic period." Our results show that change is marked for the first 24 h and suggest that changes might be seen for up to about 48 h after the episode but not thereafter.

Brain damage in infants was not obvious before concentrations of hypoxanthine reached 10 μmol/l about twice the upper limit of normal at 5·4 μmol/l. Values ranged up to about 700 μmol/l. Membrane transport mechanisms are probably not rate limiting.[19] Hypoxanthine concentrations are therefore a sensitive method of detecting brain damage. In contrast a standard objective method of assessing hypoxic damage, hydrogen ion concentrations in blood, ranges from pH 7·4 (40 nmol/l) to pH 7·0 (100 nmol/l) but discrimination with good samples and modern equipment is adequate.

In agreement with Saugstad and Olaisen[20] we found rises after death in the concentration of metabolites in vitreous humour. The pattern of a rapid initial rise and little if any change for several hours thereafter seen in CSF vitreous humour oxypurine concentrations is also seen in another major intracellular component, potassium concentrations, in vitreous humour.[21] A similar but inverted pattern is found in concentrations of ATP in rat and guinea pig brain (unpublished observations), suggesting that the extracellular concentrations are reflecting intracellular events. The range of values for all these variables in post-mortem samples is large, rendering their diagnostic use difficult.

An increase in hypoxanthine concentration may be

a specific indicator of hypoxic or ischaemic damage. Rises in newborn infants due to fever are slight although significant and samples from infants with meningitis were not distinguishable from those with infections outside the CNS; similar results were found by Meberg and Saugstad.[18] In contrast, meningitis increases lactate concentrations in CSF.[22 23] Our findings again agreed with Meberg and Saugstad[18] in that older children (6 wk–12 yr) after convulsions with fever probably had raised concentrations; we have shown that this involves hypoxanthine but not xanthine. In three older children (6 wk–12 yr) infection was not associated with similar increases to those found in convulsions with fever. So in older children the fits themselves may be causing rises in hypoxanthine whereas in the children less than six weeks old it is possible that metabolism in brain is altered by fever.

Previous studies of lactate concentrations in CSF may be relevant. CSF lactate increases after asphyxia are only observed for 8 h after the episode.[24] Samples taken in the immediate post asphyxial period have therefore been recommended as a means of assessing the severity of cerebral hypoxia. A similar possibility exists for CSF hypoxanthine which has the advantage of being directly related chemically and biochemically to ATP. CSF lactate is increased by meningitis,[22] intracranial haemorrhage, hydrocephalus and recurrent or prolonged fits.[23–25] Concentrations of hypoxanthine can be normal in these conditions despite rises of the overall mean in infected newborn and probably in convulsions with fever in older children. CSF lactate reflecting anaerobic glycolysis[10] may therefore be increased and maintaining ATP concentrations in the brain as reflected by normal or slightly increased CSF hypoxanthine. Overall the results of CSF lactate and hypoxanthine show similar trends. However, major increases in CSF lactate are caused by bacterial meningitis whereas practically a CSF hypoxanthine > 10 μmol/l was associated with hypoxic damage and not with meningitis, fever, or fits. The wide variety of abnormalities producing raised CSF lactate concentrations has been said to limit its diagnostic value.[26]

The turnover of CSF is slow compared to blood but it is higher than generally realised. Although we have been unable to locate figures for human newborn in eight older children (4–13 yr) CSF production was sufficient to renew the total CSF volume 5·5 times per day and was independent of short-term alterations in pressure over a range of 0–220 mm of CSF.[27] No age or sex dependence has been shown from 15–85 yr.[28] Secretion of CSF by the choroid plexus accounts for about 70–40% of the production; this leaves a substantial fraction which is derived from the extracellular fluid of the brain; "the active processes

in brain are secondarily enacted upon this extracellular fluid."[8][23] In CSF the very high concentration of hypoxanthine in our child with brain death were associated with normal concentrations of hypoxanthine in plasma. The independent behaviour of many components, for example hydrogen ion, in CSF is well documented.[29][30] Therefore if information on brain is required then CSF is the clinically available relevant material. However, renal damage may be a useful marker; the "missed cot death" showing the highest oxypurine concentrations in Fig. 3 also had acute tubular necrosis. Our data showing an association between urinary excretion of ATP metabolites and neurological signs in newborn also suggest that damage to the kidney can act as a model for damage to the brain.[2]

The relation between concentrations in CSF and those in brain cells probably differs with the compound. Hypoxanthine concentrations although possibly higher in actively metabolising cells are probably similar inside and outside the cell.[17] The concentrations of inosine could be different.[31] Adenosine is probably higher in the cells than in CSF. Rat brain frozen in situ had a concentration of 0·9 μmol/kg,[32] whereas our estimate of adenosine concentration in human CSF was 34 nmol/l. Although human brain cannot be obtained rapidly enough for comparable data to that in the rat, adenosine concentrations of about 10 μmol/kg are available from freeze clamped samples of a metabolically stable human organ, placenta.[33]

From this evidence it seems probable that the physiological concentrations of hypoxanthine, inosine, and adenosine are low relative to the binding constants for these compounds in brain and other tissues but sufficient for some interaction to occur.[34] Inside cells high concentrations of ATP, ADP and AMP could ensure occupancy of the purinergic receptors demonstrable at concentrations of 5 μmol/l–1 mmol/l.[35] Possible physiological roles for adenosine and the adenine nucleotides have been reviewed, for the CNS[36] and other tissues.[37]

Speculatively, changes in adenosine, inosine and the quantitatively more important hypoxanthine could be signals which cross cell membranes from damaged areas. This signal by interacting with "diazepam" receptors[38] could produce a more generalised "inhibition" than that produced by failure of the more active and more sensitive components. This mechanism could, in part, explain the generalised loss of consciousness during hypoxia, hypoglycaemia or after the excessive cerebral activity of epileptic fits, even during the induction of anaesthesia.

Although our methods are well suited to research and development a simpler method would be needed for clinical use. More data is needed to provide a good basis for more widespread use in prognosis. At present, normal CSF hypoxanthine concentrations would be a sensitive indicator for the absence of damage from hypoxia available immediately after the episode. In the most extreme case, brain death, it appears that it may prove possible to provide the quantitative objective data apparently necessary to reassure some of the accuracy of the diagnosis.[39]

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