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Plasma hypoxanthine and xanthine levels in the early newborn period in problem-free preterm babies and those with idiopathic respiratory distress syndrome

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The use of hypoxanthine measurements for quantitative monitoring of intrauterine asphyxia is generally accepted. A high level in blood or in CSF is a consequence of tissue hypoxia. Hypoxanthine and xanthine were measured by selective high pressure liquid chromatography in mature newborns, in healthy, symptom-free preterm babies, and in preterm babies affected by idiopathic respiratory distress syndrome. The measurements were carried out from peripheral venous blood within three hours after birth and at the age of 48—72 hours. In mature newborns the mean hypoxanthine level was 11.10 μmol/l in the early determinations, and 8.45 μmol/l in the second set of measurements. In unaffected prematures there were significantly higher levels, and the highest values (44.22±15.13 μmol/l) were encountered in premature babies subsequently dying of severe hypoxia. Xanthine showed a similar course. In addition to establishing normal values for prematures we desired to clarify the changes in the levels of purine metabolites during idiopathic respiratory distress and their prognostic value. Hypoxanthine and xanthine levels were found to be informative in postnatal hypoxia, especially together with other parameters.

Catabolism of purine nucleotides is a complex process consisting of several steps. In man, the final metabolite is uric acid arising by oxidation of hypoxanthine and xanthine, a process catalyzed by xanthine oxidase (Fig. 1). Metabolism of nucleic acids, purine containing coenzymes and nucleotides carrying high-energy phosphate bounds is channelled to this final step. The relationship of oxygen and energy carrying purine compounds has long been known. In hypoxia, characterized by anaerobic conditions, their synthesis is impaired and their breakdown is enhanced, ATP is used up [5]. Hypoxanthine, an intermediary product of purine catabolism, is regarded as a good indicator of the severity of hypoxia at all ages [12]. In neonatal hypoxia its level may be increased by two factors. First, ATP is used up at a dramatic speed, this leads to formation of ADP, AMP and hypoxanthine. Second, there is a direct action of the lack of oxygen; xanthine oxidase, converting hypoxanthine to xanthine and xanthine to uric acid, only works in the presence of oxygen. The enzyme ceases to function in hypoxia, thus resulting in an elevation of hypoxanthine and xanthine levels in tissues and body fluids [11].

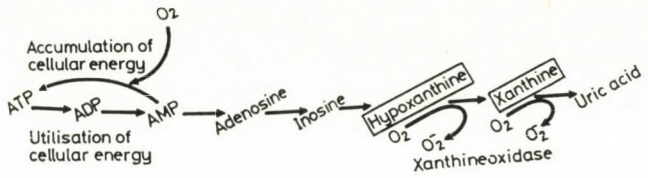

FIG 1. Breakdown of purine derivatives

Improvement inlaboratory techniques added hypoxanthine measurement to the list of indicators of hypoxia; lactate, base deficit and pH measurements.

For exact measurement of purine metabolites, enzymatic, column chromatographic and oxygen electrode methods are available [13]; the method utilizing oxygen electrodes is the most exact one but its drawback is its inability to determine the individual metabolites separately, thus to distinguish between xanthine and hypoxanthine. High pressure liquid chromatography (HPLC) is now the method of choice, being suitable for separate determination of hypoxanthine and xanthine [2, 4, 6, 9, 10, 14].

HPLC was used for measuring the plasma hypoxanthine and xanthine levels in symptom-free premature babies and those affected by idiopathic respiratory distress. Mature healthy babies born by normal delivery after an uneventful pregnancy were chosen for a control group.

MATERIAL

Hypoxanthine and xanthine determinations were performed in all babies admitted ot our intensive care centre, without any selection during the study period. Gestational age was determined on the basis of obstetrical data and the maturity score of Dubowitz. Babies exhibiting intrauterine growth retardation with clinical symptoms of injury were excluded from the study. The distribution of newborns according to gestational age and birthweight is shown in Fig. 2; birthweight

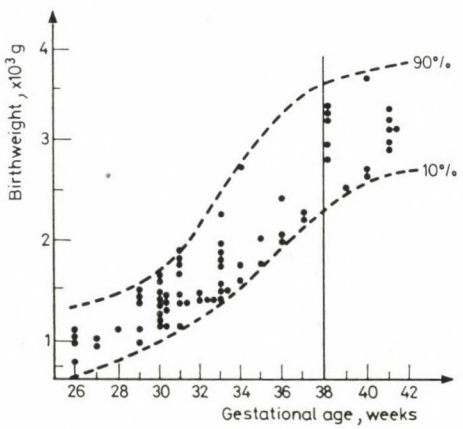

FIG 2. Distribution of newborns according to gestational age and birthweight

TABLE I

Grouping of newborns and distribution of prematures affected by idiopathic respiratory distress syndrome, according to outcome, mean gestational age and birthweight in the various groups

TABLE Ia

Newborns studied

	Age at blood sampling	
	Within 3 hours after birth	48—72 hours
Number of cases	70	60
Mature newborns		
n	15	15
gestational age, weeks	39.3±1.0	39.3±1.0
birthweight, g	2918±406	2918±406
Premature newborns		
Healthy		
n	24	24
gestational age, weeks	32.1±2.8	32.1±2.8
birthweight, g	1444±397	1444±397
IRDS		
n	31	21
gestational age, weeks	30.6±2.7	31.4±2.8
birthweight, g	1498±392	1616±399

n: number of cases
means and standard deviations
IRDS: idiopathic respiratory distress syndrome

TABLE Ib

Prematures affected by IRDS

	n	gestational age, weeks	Birthweight, g
All cases	31	30.6±2.7	1498±392
Survivors	13	31.5±2.2	1641±285
Died during the neonatal period			
within 72 hours	10	29.1±1.4	1252±243
between 4—7 days	8	31.3±3.8	1576±558
together	18	30.0±2.9	1396±432
dying of pulmonary haemorrhage	11	30.1±2.9	1406±459
Sepsis score points			
exceeding 10	14	30.0±2.3	1349±354
died within 72 hours after birth	4	29.8±2.9	1250±334
died within 7 days after birth	8	28.6±2.4	1258±450
		means and standard devations	

n: number of cases
IRDS: idiopathic respiratory distress syndrome

was between the 10th and 90th percentiles in all cases [8].

The symptom-free babies needed only care. The babies affected by idiopathic respiratory distress syndrome exhibited all clinical and radiological signs of the condition and needed respiratory treatment and correction of acid-base imbalance. In all babies who died of IRDS, necropsy revealed the presence of hyaline membrane and alveolar haemorrhages.

Since the idiopathic respiratory distress syndrome can be simulated by sepsis, a score for establishing the probability of severe infection was used; a score value exceeding 10 made sepsis highly probable [18]. Respiration itself may cause infection, therefore the above-mentioned score was applied several times during IRDS and the highest value was taken as the indication for therapy and grouping. Grouping, number of cases, their gestational age and birthweight and the outcome of IRDS are shown in Table I. Autopsy confirmed the primary pulmonary changes resulting from immaturity leading to severe hypoxia in all fatal cases.

The blood samples were drawn from a peripheral vein. Clotting was prevented by addition of one part of 3.8% sodium citrate to nine parts of blood. Hypoxanthine and xanthine were determined in plasma.

Method

Hypoxanthine and xanthine were first extracted from the plasma [14]. A double volume of ice-cold, freshly prepared 10% trichloroacetic acid (TCA) was added to each plasma sample, the precipitate was removed by centrifugation. TCA was removed from the supernatant by 1.5 volume of diethylether saturated with water. The aqueous phase was filtered through a 0.5 μm Millipore filter and the sample was stored at -20°C. This extraction procedure could be carried out from samples of 100 μl.

The measurements were carried out in a Hewlett-Packard 1084/B type HPLC device by the method of Khym and Simmonds modified by ourselves [6, 14]. The column consisted of ODS-Hypersil, length: 10 cm, mesh size: 5 nm, pressure: 86 bar, working temperature: 30°C. The detector with variable wavelength sensitivity was sensitive to ultraviolet light, a lambda_{max} of 255 nm was used for hypoxanthine determination, 270 nm for xanthine. The flow speed was 1 ml/minute. The eluent was 0.01 mol/l potassium hydrogen phosphate pH 5.3, containing 1% methanol. The 20 μl sample was injected by automatic injector. Quantitative determination of xanthine and hypoxanthine was achieved by establishing calibration curves and computer evaluation of the area below the corresponding peak in the chromatogram (Fig. 3).

For calibration, serial dilutions of plasma hypoxanthine and xanthine (Sigma) were used. From all artificial plasma samples the whole procedure including extraction, was carried out and the area below the curve was measured and taken as the relative unit. Figure 3 illustrates, in addition to the calibration curves, a chromatogram as well. The lower limit of sensitivity was 0.1 μmol/l of hypoxanthine and xanthine, respectively.

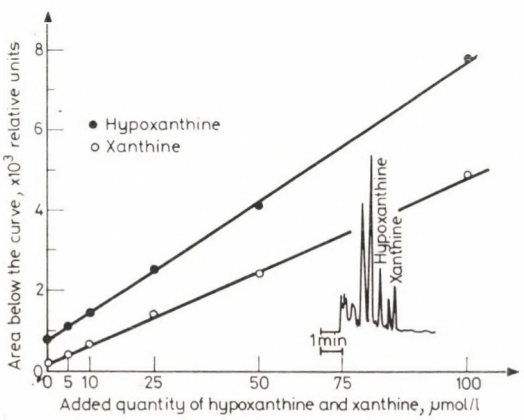

Fig 3. High pressure liquid chromatography curve of a plasma extract and calibration curves of hypoxanthine and xanthine

Since the determinations were carried out in citrated blood, real hypoxanthine and xanthine levels in plasma were calculated as follows: real concentration = measured concentration $\left\langle \frac{1}{9(1-\mathrm{PCV})} + 1 \right\rangle$ where PCV meant packed cell volume.

Results

Figure 4 shows the plasma hypoxanthine level found in the newborns. Within three hours after birth the

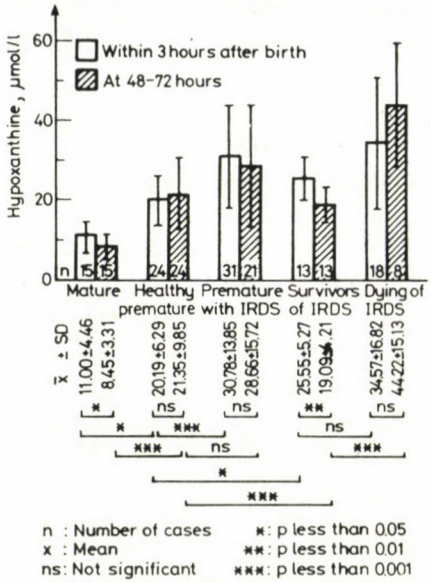

FIG 4. Plasma hypoxanthine levels of newborns within three hours after birth and between 48 and 72 hours of life

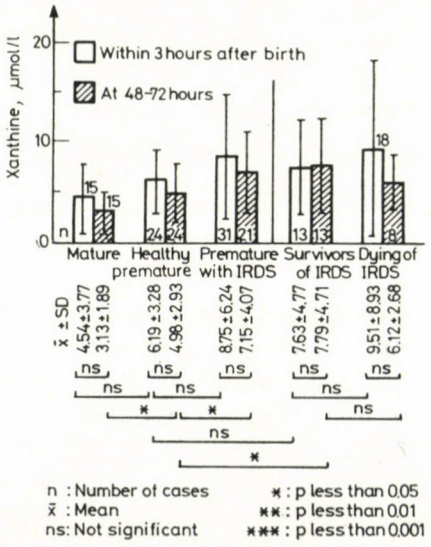

FIG 5. Plasma xanthine levels of newborns within three hours after birth and between 48 and 72 hours of life

lowest values were encountered in mature healthy babies. A mean value nearly twice as high was obtained in premature babies free of symptoms or problems, while in premature neonates affected by idiopathic respiratory distress syndrome the mean value was about three times higher than that of the mature babies. In prematures with IRDS dying subsequently of intractable hypoxia the mean was even higher, 34.57 μmol/1.

In the 15 mature newborns, the mean value observed between 48 and 72 hours after birth was significantly lower than the first mean value. In the premature babies, on the contrary, there was a slight increase instead of a decrease. An especially marked increase was seen in the 8 prematures affected by IRDS in whom the 48—72 hour measurement could still be carried out but of whom two died thereafter: the mean value amounted to 44.22 μmol/1. In some babies dying during the perinatal period, values as high as 100 μmol/1 were measured.

Similarly, both the values obtained within three hours and on the third day of life were higher in the preterm than in the term newborns. The increase was even more pronounced in the babies affected by IRDS. Again, in the newborns dying subsequently, a considerable fall occurred by the third day compared to the three-hour value. In the whole material, the lowest values were encountered in mature newborns on the third day of life while the highest values at three hours after birth in premature neonates dying during the perinatal period. The extreme values were 4.54 and 9.51 μmol/1, respectively (Fig 5).

Discussion

The key to perinatal adaptation is the baby's cardiorespiratory function. Postnatally there are more possibilities for diagnosis and treatment than immediately before birth, but estimation of the severity of hypoxia is still difficult. Measurement of the hypoxanthine level has primarily been used for judging the degree of intrauterine asphyxia; its value was compared to that of the Apgar score, blood lactate, blood pH, base deficit and individual asphyxia scores [1, 15, 17]. It was found that plasma hypoxanthine had a complementary value in this series of tests [16]. The highest hypoxanthine levels in neonates delivered after intrauterine asphyxia were usually measured 20 minutes after birth. The base deficit was highest at 30 minutes, the maximum of lactate during the first three hours. There is a strong correlation between the three parameters but none could be found between the blood pH and plasma hypoxanthine, probably because of the rapid changes in pCO_2 [15]. In intrauterine hypoxia the optimum time for plasma hypoxanthine determination is 10—20 minutes after delivery, but elevated values are still encountered three hours after birth [3]. If a single determination is carried out, the

hypoxanthine level may not be correlated with the clinical features. This is due to the fact that the plasma hypoxanthine concentration is influenced not only by hypoxia but also by other factors. Since hypoxanthine is electrically neutral, it seems very probable that it can escape from the hypoxic cells to the extracellular space by simple diffusion, hence it can easily reach the blood plasma. It appears in the plasma immediately after the hypoxic episode but this may be hindered by poor circulation. After birth there is a marked peripheral vasoconstriction in all newborns, even in mature babies, and this is especially pronounced in asphyxiated neonates. Under pathological conditions the pump function of the heart is impaired and redistribution of the circulating blood occurs: perfusion of all organs but of the brain and the heart deteriorates. Hypoxanthine appears in the circulation of the arteries or veins available for blood sampling only when the peripheral circulation has improved and the bloodstream is able to wash out hypoxanthine from the tissue that had been hypoxic.

The primary determinant of an elevated hypoxanthine level is hypoxia, but its effect may be retarded by impaired heart function and disturbed microcirculation. Furthermore, the plasma hypoxanthine level can be altered by the volume of plasma and the size of the extracellular space. From the practical point of view it is important that no difference should occur between the arterial and venous hypoxanthine levels.

Timing of the first blood sampling within three hours after birth has been chosen on the basis of the dynamics of hypoxanthine levels. In addition to clarify the severity of perinatal and very early postnatal hypoxia, we also wanted to establish the range of basal values for healthy premature babies. To see the course of hypoxanthine and xanthine levels, they were measured in mature newborns, in prematures without problems and in prematures affected by IRDS, 48—72 hours after birth. Determinations in the healthy mature babies had the purpose of obtaining normal values of our own.

The normal values for hypoxanthine and xanthine obtained in this study fell within the upper third of the range described in the literature. In some instances we compared the hypoxanthine and xanthine levels determined by an oxygen electrode or by high pressure liquid chromatography; the results are shown in Table II. It can be seen that the ranges obtained by the two methods were similar but the individual values of the two different determinations did not coincide. We think that the uniformity of the method throughout our present study has contributed to the reliability of our findings.

The lowest early values were found in healthy mature babies. The values then decreased by the third day of life. This indicates that a certain degree of hypoxia occurs even during normal delivery but is soon abolished after birth, consequently the blood level of the purine derivatives is normalized

TABLE II

Normal plasma hypoxanthine and xanthine concentrations obtained by various authors

Author	Method	Site of blood sampling	Compound	Mean ± SD μmol/l	Range
Saugastad [12]	pO_2electrode	cord	hypoxanthine	5.8 ± 3.0	0 – 11
Lipp-Zwahlen [7]	pO_2 electrode	umbilical artery	hypoxanthine	16.1 ± 5.7	≈8 – 26
	pO_2 electrode	umbilical vein	hypoxanthine	14.4 ± 4.7	≈9 – 24
Bratteby [3]	pO_2 electrode	umbilical artery	hypoxanthine	11.9 –	0 – 23.8
	pO_2 electrode	umbilical vein	hypoxanthine	9.0	1.4 – 18.9
Thiringer [16]	pO_2 electrode	cord	hypoxanthine	5.7 ± 5.8	0 – 18.3
O'Connor [11]	pO_2 electrode	cord	hypoxanthine	5.1	1 – 14.0
Swanström [15]	pO_2 electrode	umbilical artery	hypoxanthine	7.7 ± 5.9	–
Simonds [14]	HPLC	adult blood	hypoxanthine	2.04 ± 0.6	–
			xanthine	0.61 ± 0.21	–
Boulieu [2]	HPLC	adult blood	hypoxanthine	3.2	–
			xanthine	2.0	–

when circulation and respiration have become fully adapted. The significantly higher value observed in healthy prematures three hours after birth can be explained by their slightly impaired cardiorespiratory adaptation causing a somewhat protracted transitory hypoxia in the tissues. But in these babies, too, the hypoxanthine level fell by the third day of life, pointing to an improved cellular respiration. Thus, hypoxanthine estimations have proved here a useful laboratory marker of adaptation.

In the case of prematures suffering from IRDS, the relative role of intrauterine asphyxia, immaturity and postnatal respiratory distress in causing high hypoxanthine levels can hardly be established, but the high value itself shows that the persisting respiratory failure maintains a hypoxia. Successful respiratory treatment, restoration of the circulation and correction of the imbalance of acid-base equilibrium result in a fall in the plasma hypoxanthine level, as it has been observed in out survivors of IRDS, and the contrary could be seen in the group of prematures who subsequently have died. If attempts to restore cardiorespiratory functions fail, the hypoxanthine level inexorably increases, showing the persistence of severe energetical disturbances at the cellular level. In this group the xanthine level was comparatively low. It appears that as a consequence of hypoxia, the failure of xanthine oxidase function prevents normal catalysis of the conversion of hypoxanthine to xanthine and thus the xanthine level falls in spite of extremely high hypoxanthine concentrations.

Our results suggest that plasma hypoxanthine and xanthine are good indicators not only of intrauterine asphyxia but also of postnatal adap-

tation and that they even have a prognostic value. These data show that the pathogenesis of IRDS is complex and its therapy must be aimed at improving cardiorespiratory function, microcirculation and cell metabolism.

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