Hypoxanthine as a Test of Perinatal Hypoxia as Compared to Lactate, Base Deficit, and pH

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Summary

The possibility of detecting past hypoxia during the first 2 h after birth by means of blood analyses of hypoxanthine, lactate, base deficit, and pH was investigated in six infants with a 1 min Apgar score of ≤ 4 . Reference values for the four biochemical variables were obtained in 16 healthy infants with a normal 1 min Apgar score of ≥ 8 . In the asphyxiated infants, elevated values for hypoxanthine were found in 48%, for lactate in 54%, and for base deficit in 46% while 21% of the pH values were lower than the reference. In the group of asphyxiated infants, significantly elevated hypoxanthine values were found during the first 20 min after birth, base deficit during 30 min, and lactate values during 120 min while lower pH values than the reference were found during the first 30 min. Significant correlations were found between hypoxanthine and lactate concentrations and also between hypoxanthine and base deficit but not between hypoxanthine and pH. It is concluded that the optimal time for detecting past intrauterine hypoxia in the newborn using the hypoxanthine test is between 10 and 20 min after birth, but in individual cases, significantly increased hypoxanthine concentrations may be found at any time in the 10-120 min period. We propose an equation that can be used to calculate an upper normal limit for hypoxanthine concentration in any sampling time during this period.

Speculation

The addition of the plasma hypoxanthine concentration to established biochemical tests of perinatal asphyxia such as lactate, base deficit and pH analyses may improve the possibility of detecting past hypoxia accurately. Differences in appearance time, duration and degree of significant changes in the biochemical tests indicates that the optimal sampling time for the disclosure of past asphyxia differs for different tests.

When the cell lacks oxygen, the production of ATP is dramatically reduced, and ATP is broken down to ADP and AMP and further as far as to hypoxanthine. This metabolite can be used as an indicator of hypoxia in the perinatal period (7, 8). A previous paper described the pattern of changes in the hypoxanthine concentration in arterial plasma during the first 2 h after birth (2). Rapid changes in this concentration occurred in both normal and asphyxiated infants in the early neonatal period and it was noted that marked differences in the plasma hypoxanthine level between these two groups of infants are best revealed by repeated blood samples.

The present study was undertaken to examine the reliability of the hypoxanthine test and to ascertain how long after birth it could be used to discriminate asphyxiated infants from a control group presented earlier (2). A comparison was also made between the discriminating capacity of hypoxanthine in detecting past hypoxia and that of base deficit, lactate, and pH.

MATERIALS AND METHODS

Hypoxanthine. Hypoxanthine analyses were performed by the method of Saugstad, with his original equipment as described in an earlier paper (2). The error of a single measurement was 1.8 mmole/liter, in accordance with Saugstad's findings (7).

Arterial blood gases and acid base. Blood gases were determined in IL 313 Instrumentation Laboratory equipment. Analyses were made in duplicate and the analytical error for pH was $\pm 1\%$, for $\text{PaO}_2 \pm 2.2\%$, and for $\text{PaCO}_2 \pm 3.2\%$. Base deficit values were obtained from the Siggaard-Andersen Alignment nomogram (9) from PCO_2 and pH and were calculated for extracellular fluid, that is at Hb 50 g/liter as advised by Rooth and Jacobson (6), thus avoiding the influences of PCO_2 and haemoglobin values on the estimations of metabolic acidosis.

Lactate. Immediately after sampling, blood was mixed with ice-cold metaphosphoric acid. These assays were performed by the enzymatic method of Marbach and Weil (5). The analytical error for lactate was $\pm 2.3\%$.

Subjects. The subjects comprising the reference group and the clinical procedures have been presented earlier (2). The subjects were investigated as part of another study (10). All infants had a normal Apgar score (≥ 8) and were full-term with weight and length appropriate for their gestational age. Blood samples were obtained at 10, 20, 30, 60, 80, and 120 min of age through an umbilical arterial catheter. Blood gases in the first 2 h after birth did not indicate any delayed respiratory adaptation, the PaO_2 and PaCO_2 being in accordance with reference values from the literature (11).

To be assigned to the asphyxia group, the infants should have a 1 min Apgar score ≤ 6 . As it turned out, all six infants in this group were clinically severely asphyxiated, with 1 min Apgar scores ≤ 4 and 5 min scores ≤ 7 . The case histories of cases 1-3, 5, 6, and case A have been described earlier (2). Case 4 is described below. Clinical data for the asphyxiated infants are summarized in Table 1.

Case A did not comply with the criteria used for selecting infants for the reference group, as this child was born in the breech position, nor did it fit the criterion for the asphyxia group, as the 1 min Apgar score was 9. He was included in the study because of biochemical signs of asphyxia. In the presentation of results, however, values from this infant are used only for the correlations between different variables presented in Table 2.

In case 4 the Apgar score was 3 at 1 min and 7 at 5 min. The birth weight was 3700 g and gestational age 41 wk. This infant was delivered by Caesarean section because of protracted labour with a face presentation. It was observed that the placenta constituted a mechanical obstruction.

Immediate resuscitative measures were taken in the six clinically asphyxiated infants and all blood gases indicated that the asphyxia was successfully treated with no relapsing episode of hypoxia ($\text{PaO}_2 \leq 6.7$ kPa), or carbon dioxide retention ($\text{PaCO}_2 \geq 6.7$ kPa). The blood gases in case A were also within these limits. For practical and clinical reasons lactate determinations were not

Table 1. *Clinical data in the asphyxia group*

Case	Asphyxiated infants	Birth weight (g)	Gestational age (wk)	Apgar score	
				1 min	5 min
1	Fetal bradycardia, emergency Caesarean section	3160	40	2	2
2	Breech delivery	3220	43	1	4
3	Fetal bradycardia, passage of meconium	3200	43	3	6
4	Fetal bradycardia, anomalous presentation	3700	41	3	7
5	Prolapsed extremity, emergency Caesarean section	2830	36	4	7
6	Fetal bradycardia, meconium passage	3000	42	4	7
A	Breech delivery	3170	38	9	9

Table 2. *Correlation between different variables indicating hypoxia in asphyxiated and in nonasphyxiated infants. BD = base deficit*

Correlation	Sampling time, min after birth					
	10	20	30	60	90	120
Hypoxanthine/lactate						
<i>r</i>	0.68	0.75	0.48	0.56	0.56	0.22
<i>n</i>	15	11	17	14	16	12
<i>P</i> <	0.01	0.01	0.05	0.05	0.02	n.s. ¹
Hypoxanthine/BD						
<i>r</i>	0.72	0.63	0.54	0.48	0.40	0.39
<i>n</i>	15	13	18	14	16	12
<i>P</i> <	0.001	0.02	0.05	n.s.	n.s.	n.s.
Hypoxanthine/pH						
<i>r</i>	-0.43	0.13	-0.43	0.04	-0.13	0.24
<i>n</i>	15	13	19	14	16	12
<i>P</i> <	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Lactate/BD						
<i>r</i>	0.86	0.88	0.77	0.66	0.70	0.56
<i>n</i>	17	16	20	20	20	20
<i>P</i> <	0.001	0.001	0.001	0.001	0.001	0.01
Lactate/pH						
<i>r</i>	-0.60	-0.39	-0.41	-0.30	-0.29	-0.10
<i>n</i>	17	16	20	20	20	20
<i>P</i> <	0.01	n.s.	n.s.	n.s.	n.s.	n.s.
BD/pH						
<i>r</i>	-0.74	-0.55	-0.63	-0.68	-0.73	-0.34
<i>n</i>	17	20	21	20	20	20
<i>P</i> <	0.001	0.01	0.01	0.001	0.001	n.s.

¹ n.s., not significant.

made in Cases 1 and 5 and some sampling times differed from those of the reference group (Table 3).

RESULTS

Mean values for hypoxanthine concentration, base deficit, lactate, and pH $\pm$ 1 S.D. at different sampling times in the reference group are presented in Table 4. In Table 3 the individual values of these variables are given for the six asphyxiated infants (cases 1-6) and case A.

As compared with the reference group (Student's unpaired *t* test), significantly higher mean values were found for hypoxanthine concentration, base deficit, and blood lactate and significantly lower mean values for blood pH in the asphyxia group. The levels of significance for the differences at various sampling times are given in Figure 1. Whereas the mean hypoxanthine

values differed significantly from those of the reference group only at 10 and 20 min of age, base deficit and pH values were still significantly different 30 min, and lactate values 120 min after birth.

When the asphyxia group and the reference group were combined, a significant correlation ($r = 0.86$, $n = 17$, $P < 0.01$) was found between the sum of the Apgar scores at 1 and 5 min of age and the hypoxanthine concentrations at 10 min of age. At 20 min of age there was only a tendency to such a relationship ($r = 0.57$, $n = 17$, $P < 0.05$).

In order to examine the possibility of disclosing past hypoxia from single blood samples in the asphyxiated infants, all values noted in this group were related to the corresponding mean values of the reference group at equivalent sampling times. Any value of hypoxanthine, base deficit, or lactate above the reference mean + 2 S.D. or any pH value below the reference mean - 2 S.D. was regarded as indicating past hypoxia. Over the whole sampling period the fraction of such discriminating samples in the asphyxia group was 14/29 for hypoxanthine, 13/28 for base deficit, 12/22 for lactate and 6/28 for pH (Table 3). In the reference group the corresponding fractions were 4/81 for hypoxanthine, 2/88 for base

Table 3. *Base deficit, pH and blood concentrations of hypoxanthine and lactate in the asphyxiated infants at different sampling times*

Case	Sampling time (min after birth)	Hypoxanthine (μ mole/liter)	Lactate (mmole/liter)	Base deficit (mmole/liter)	pH
1	14	48.3			
	17	39.9		16	7.27
	30	17.5			
	45			16	7.30
2	11	37.8	11.5	17	7.11
	20	16.1	11.2	18	7.23
	30	20.3	10.8	16	7.27
	60	11.9	9.4	15	7.32
	90	9.8	9.1	14	7.34
	120	4.9	7.0	10	7.36
	17	18.2	6.5	10	7.30
3	20	10.5	6.6	11	7.31
	30	5.6	6.5	11	7.37
	60	16.6	4.4	10	7.45
	90	9.1	3.6	9	7.42
	120	18.9	3.5	10	7.43
	20	1.4	5.3	6	7.31
	30	0	4.2	5	7.37
4	60	0	2.3	0	7.40
	90	0	1.6	1	7.40
	120	1.4	1.5	1	7.38
	11	36.0		13	7.23
	17	23.8		11	7.17
	22	33.6		3	7.37
	27	19.6		9	7.35
6	47				
	28	7	6.5	10	7.33
	30	4.9	6.4	11	7.37
	60	1.4	4.8	10	7.39
	90	0.7	3.2	8	7.40
	120	0	2.6	6	7.38
	120	0			
Case A	9	18.9	8.8	16	7.09
	10	22.4	10.5	16	7.13
	20	28.0	7.5	17	7.24
	30	5.6	7.0	12	7.39
	60	0	3.5	15	7.29
	90	9.8	3.6	16	7.24
	120	7.0	7.0	14	7.33

Table 4. Base deficit, pH and blood concentrations of hypoxanthine and lactate in the reference group at different sampling times

	Sampling time, min after birth					
	10	20	30	60	90	120
Hypoxanthine, $\mu\text{mole/liter}$						
Mean	12.2	7.7	7.8	3.8	3.4	1.3
± 1 S.D.	7.2	5.9	5.1	3.7	2.5	1.2
n	14	12	15	14	15	12
Lactate, mmole/liter						
Mean	4.6	4.2	3.6	2.5	2.3	2.1
± 1 S.D.	1.6	1.5	1.3	0.8	0.8	0.7
n	15	13	15	15	15	15
Base deficit _{BCF} , mmole/liter						
Mean	7.4	8.3	7.0	6.0	5.9	5.9
± 1 S.D.	2.3	2.0	2.5	1.7	2.3	2.0
n	15	14	15	15	15	15
pH						
Mean	7.28	7.33	7.38	7.41	7.40	7.40
± 1 S.D.	0.05	0.04	0.03	0.04	0.04	0.03
n	15	14	15	15	15	15

Fig. 1. Level of significant increase in hypoxanthine (O--O), lactate (■—■), base deficit (▲—▲) and decrease in pH (▼—▼) in the asphyxia group as compared with the reference group at different sampling times. Hypoxanthine values differed for 20 min, pH and base deficit for 30 min and lactate for 120 min.

deficit, 3/86 for lactate and 4/88 for pH. The result in the latter group is of course due to the statistical limit used.

It was noted empirically that in the reference group the mean hypoxanthine values at the various sampling times best fitted a regression line $Y = 107.8 X + 2.1$ ($r = 0.95$, $P < 0.001$), where Y = the mean reference values of hypoxanthine and X = the sampling times⁻¹ (Equation 1), Figure 2. In order to obtain values that could be used as an upper normal hypoxanthine limit at any sampling time between 10 and 120 min of age, a new regression line was drawn, based on the mean values + 2 S.D., namely $Y = 222.9 X + 6.4$ (Equation 2). The corresponding hyperbolas are presented in Figure 3 together with all hypoxanthine values noted in the asphyxia group and in case A.

The extent to which the mean values for the four different variables in every sample obtained in the asphyxiated infants deviated from the mean reference values is shown in Figures 4a and 4b. The deviation from the reference mean is expressed as the standard deviation from this mean. Hypoxanthine was the strongest discriminator in cases 1, 3 and 5, with values increased 6.0, 4.8, and 5.5 S.D. above the reference mean values at 14, 120, and 22 min of age, respectively. Lactate was the strongest discriminator in infants 2 and 6, with increases by 9.0 and 2.8 S.D. at 90 and 60 min, respectively. It is worthy of note that in infant No. 4, all variables indicated less hypoxia than in the reference group at most sampling times.

When all infants in the reference group, cases 1-6 of the asphyxia group, and case A were combined, and all variables were correlated with each other at the different sampling times (Table

Fig. 2. Correlation between mean hypoxanthine concentration and sampling time⁻¹ after birth.

Fig. 3. Plasma concentrations of hypoxanthine in clinically asphyxiated infants, cases 1-6 (O), and in an infant, case A (■), with metabolic acidosis indicating past hypoxia. Reference mean values are represented by the hyperbola I ($Y = 107.8 \times \text{sampling time}^{-1} + 2.1$). Reference mean values + 2 S.D. are represented by the hyperbola II ($Y = 222.9 \times \text{sampling time}^{-1} + 6.4$).

4) hypoxanthine showed a significant correlation with lactate and base deficit at 10 and 20 min of age, but at no sampling time with pH. A tendency to a positive correlation with base deficit up to 30 min and with lactate up to 90 min of age was observed.

DISCUSSION

If past hypoxia is to be detected in newborn infants by means of hypoxanthine analyses, it is of obvious importance to have appropriate reference values. The difficulty in defining such standard values lies in the fact that even uncomplicated deliveries are accompanied to a certain extent by some degree of hypoxia, as indicated by an increased metabolic acidosis early after birth even though the Apgar score is normal (14). Consequently, hypoxanthine should also be expected to show some increase after birth in all infants. When excessive hypoxia occurs, the hypoxanthine values noted must be compared with "normally" increased levels of hypoxanthine, just as in the case when lactate or base deficit is used for detection of past hypoxia (1).

In the reference group all infants were judged to have sustained no more and no less hypoxia during their deliveries than could normally be expected. Their 1 min Apgar scores were ≥ 8 and their base deficit, lactate, and pH values after birth (Table 4) corresponded to reference values reported in the literature (1, 11). Furthermore, analyses of PaO_2 and PaCO_2 from 10 to 120 min after birth indicated a normal gas exchange during the sampling period. These findings supported our concept that the postnatal hypoxanthine concentrations noted in our reference group were representative for healthy full-term infants with uncomplicated deliveries.

The asphyxia group was selected only on the basis of low Apgar scores, which is an established clinical way of identifying asphyxiated infants. It should be remembered, however, that the

Fig. 4 a and b. Base deficit (B), hydrogen ion activity (P) and blood concentrations of hypoxanthine (H) and lactate (L) in the asphyxiated infants (cases 1-6) and in an infant with metabolic acidosis (case A) related to the corresponding mean values of the reference group. The deviation from the reference mean is expressed as standard deviation from this mean.

Apgar score is not specific for hypoxia. If the hypoxic event occurred sometime before birth, it is quite possible for an asphyxiated infant to be born with a normal Apgar score (1). Infants with a low Apgar score may also be depressed for other reasons

than hypoxia. From the biochemical point of view, it may be suspected that case 4 of the asphyxia group, if any, sustained less hypoxia than the infants in the reference group, in spite of a low Apgar score. The metabolic acidosis in this infant was significantly less pronounced than that in the reference group from 60 to 120 min of age (Fig. 4a).

If, for this reason, case 4 is removed from the asphyxia group, the fractions of values indicating hypoxia, i.e., values increased by more than 2 S.D. above the reference mean, will increase for all the biochemical tests of hypoxia used. For hypoxanthine it will be 14/25 (58%), for lactate 12/17 (71%), for base deficit 16/23 (52%) and for pH 6/25 (24%). When the mean hypoxanthine values in the remaining five asphyxiated infants with low Apgar scores are then compared with those in the reference group, significant differences ($P < 0.001$) are found both at 10 and 20 min of age, but not at subsequent sampling time while base deficit and lactate discriminate between the 2 groups significantly from 10 to 120 min.

Obviously hypoxanthine values, just as pH values, are best suited for disclosing past hypoxia in the immediate neonatal period, while lactate and base deficit are more lasting discriminating variables. An explanation for this is that hypoxanthine, being an uncharged metabolite, quickly crosses the membranes along its diffusion gradient and then, when oxygenation is restored after resuscitation, reaches a peak and is eliminated fairly rapidly (2). The pH values are quickly normalized after the onset of respiration with the elimination of carbon dioxide. Lactate and base deficit, on the other hand, are closely interrelated. Lactate is a charged metabolite that does not pass through membranes so easily, which may explain its prolonged discriminating power.

The optimal time for disclosing past hypoxia with the hypoxanthine test thus seems to be in the 10 to 20 min period, when the concentrations are best related both to biochemical signs such as base deficit or lactate (Table 4) and to clinical signs such as Apgar scores. In individual cases, however, it may also be of use after this period, as indicated by the fact that nine out of 14 hypoxanthine values in the asphyxia group were increased by more than 2 S.D. above the reference mean after 20 min of age. The sampling times closest to the asphyxial event were not necessarily best for disclosing past hypoxia. Thus, in case 3, only lactate was increased by more than 2 S.D. above the reference mean in the first 30 min. Hypoxanthine, lactate, and base deficit all showed such increases at 60 min of age (Fig. 3a). A progressively increasing deviation from reference values with time can be seen for lactate in case 2. The time when asphyxia is best disclosed in any individual case depends upon several factors, as discussed in a previous paper (2).

Considering the rapid changes observed in the hypoxanthine concentration both in the asphyxiated infants and in the reference group during the first 20 min of life, it seems important that any value obtained in asphyxiated infants during this period be related to a reference value valid for an equivalent sampling time. In clinical work it is seldom possible to wait for any specific sampling time for which known reference values are available. By means of the two equations presented in the results, reference values for any point of time between 10 and 120 min of age, based on all samples analysed for hypoxanthine in the reference group, can be obtained (Fig. 3). All values in the asphyxia group were equally well discriminated by equation 2 as by the 2 S.D. limit calculated from samples obtained at one specific sampling time. It should be noted that no reference values can be calculated from this equation for the time period earlier than 10 min after birth.

In a recent paper, Tuchschild *et al.* (13) found no elevation in plasma hypoxanthine concentration in high altitude induced hypoxia in healthy adults while the blood lactate was slightly increased and a ST-T depression was found in the ECG. These authors speculated that the increased plasma hypoxanthine concentration in newborns after delivery may result from other factors, additional to hypoxia. Most probably, however, their own hypoxic subjects exposed to simulated high altitude were not hypoxic and one might assume that the indicators of hypoxia as used by these authors were unspecifically positive due to increased sympathetic tone and/or hyperventilation induced by the test

situation in the low pressure chamber. The ST-T changes as described by Tuchschnid *et al.* (13) are not specific to myocardial hypoxia, but are most often interpreted as sympatheticotonic or functional (3). At a simulated altitude of 6000 m the PaO_2 of the subjects was 35–30 mm Hg. They hyperventilated which may, in turn, lead to lactic acidosis without the presence of hypoxia. When, in such a situation, the respiratory alkalosis is prevented by carbon dioxide breathing in animals or human volunteers the blood lactate will not increase until arterial oxygen pressure falls below 30 torr (4) indicating the presence of nonhypoxia above this level of PaO_2 .

In an experimental study on lamb fetuses, Thieringer *et al.* (12) recently found increased plasma hypoxanthine concentrations during periods of oxygen debt and highly significant correlations between hypoxanthine and other hypoxia variables as pH, base excess, lactate, PaO_2 and PaCO_2 which appears to corroborate the conclusion that plasma hypoxanthine concentration reflects tissue hypoxia in the fetal lamb.

To detect and quantify past hypoxia clinically in the newborn infant is much more complicated than doing so in an animal experiment. Based on earlier knowledge and experience from the present study we may conclude that there is no ideal measure of neonatal hypoxia available for clinical practice. In the present study the hypoxanthine test was as valid as lactate and base deficit in disclosing past hypoxia. The optimal time after birth in doing so was different for the different biochemical variables. One main conclusion is that the hypoxanthine test may improve the possibilities of disclosing past hypoxia accurately in the newborn infant and that the most likely time period in which hypoxanthine will reveal past hypoxia is the first 10–20 min after birth.

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