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The effect of birth asphyxia on plasma free amino acids in preterm newborn infants

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The postnatal plasma amino acid pattern was compared in 16 asphyxiated and 13 non-asphyxiated preterm newborn infants. The lactic acidosis induced by asphyxia was associated with a marked rise in the total amino acid content of the plasma. Among the 17 individual amino acids determined the concentrations of alanine, proline, taurine, glutamate, valine, methionine and lysine were significantly elevated. The accumulation of alanine was particularly marked and its concentration showed a significant linear correlation with that of lactate ($p<0.001$). A similar relationship was observed between other potentially glucogenic amino acids and lactate. It is suggested that an impaired gluconeogenesis may be responsible for the accumulation of glucogenic amino acids. The response of the plasma aminogram to asphyxia resembles that associated with hypoglycaemia in the small-for-gestational-age infant, where a delay in the maturation of key gluconeogenic enzymes seems to account for the reduced hepatic disposal of glucose precursors.

The postnatal accumulation of gluconeogenic amino acids [7, 12], lactate and pyruvate [7] in the plasma of hypoglycaemic SGA newborn infants strongly suggests a decreased capacity of hepatic glucose synthesis, leading to a reduced uptake of the glucose precursors by the liver. In addition to reduced disposal, an increased production or release of amino acids can also contribute to the hyperaminoacidaemia associated with hypoglycaemia. This particularly applies to alanine, the most prominent glucose precursor among the amino acids.

It is well-known that besides conversion to lactate, transamination of pyruvate to alanine is the alternate pathway of pyruvate disposal and is the key metabolic process of the glucose-alanine cycle [5]. It seems therefore reasonable to suppose that under conditions associated with pyruvate accumulation in the muscles, e.g. hypoxia, an increased production of alanine can be a major mechanism in the development of hyperaminoacidaemia. In view of this possibility and the frequent occurrence of birth asphyxia in severe intrauterine malnutrition, the plasma amino acid pattern has been studied in neonates suffering from moderate or severe birth asphyxia. It appeared interesting to establish whether or not a correlation existed between the asphyxia-induced

lactic acidosis and the postnatal changes of plasma amino acids. To exclude the effect of intrauterine undernutrition on the plasma aminogram, only appropriate for gestational age preterm infants have been included in the study.

Material and Methods

Two groups of preterm newborn infants appropriate for gestational age (AGA) were studied: 13 non-asphyxiated and 16 asphyxiated infants. Mean birth weight, gestational age, postnatal age and ranges are shown in Table I. All infants had a birth weight between the tenth and ninetieth percentile on our local intrauterine growth chart. All of the non-asphyxiated infants were delivered vaginally after uncomplicated pregnancy and had an Apgar score higher than 7 at one minute.

Two of the asphyxiated infants were delivered by Caesarean section and five after difficult breech presentation. Infants included in this group had an Apgar score lower than 7 at birth, and were, after being resuscitated in the delivery room, admitted to the neonatal referral centre for further observation and treatment. Pertinent data regarding pregnancies, deliveries and infants in the asphyxiated group are shown in Table II. Eleven severely asphyxiated infants died between 1 to 3 days of age; necropsy revealed massive intracranial haemorrhage in all of them.

Blood was drawn by puncture of the antecubital or cephalic vein at the time of admission, 1—12 hours after birth, before the first feeding. Glucose and lactate concentrations were determined by the orthotoluidine method of Price [15] and by the method reported by Huckabee [9]. Specimens of heparinized venous blood were promptly centrifuged and the plasma was deproteinized by addition of four volumes of 5% sulphosalicylic acid. The protein-free supernatant was immediately frozen and stored at —20°C until assayed.

Amino acid analysis was performed by an automatic Beckman Multichrom Liquid Column Chromatograph using norleucine as an internal reference standard. The levels of 17 amino acids were quantitated.

Regression equations were calculated by the method of least squares. Differences between group averages were compared by the standard t test.

Table I

Mean gestational age, postnatal age and birth weight in the two groups of infants*

	Non-asphyxiated preterm infants	Asphyxiated preterm infants
Gestational age (wk)	32.7 (29—35)	31.1 (28—34)
Birth weight (g)	1772 (1250—2450)	1501 (800—1970)
Postnatal age (hr)	8.3 (3—12)	3.6 (1—7)
Number of infants	13	16

* Ranges for age and weight are in parentheses.

TABLE II

Clinical data of 16 preterm infants with birth asphyxia

Infant No	Infant Sex	Pregnancy, labor and delivery	Apgar score at 1 min	Apgar score at 5 min	pH (on admission)	Gestational age (wk)	Birth weight (g)	Outcome	Necropsy
1	M	Cholestasis during pregnancy, breech delivery	1	3	6.97	30	1680	Died	Meningeal haemorrhage. HMD
2	M	Toxemic pregnancy, meconium stained amn. fluid	4	8	7.27	32	1880	Died	Ventricular haemorrhage
3	M	Uneventful pregnancy, spontaneous delivery	6	10	7.27	33	1970	Survived	—
4	F	Twin pregnancy, spontaneous delivery	1	3	7.14	30	1250	Died	Periventricular haemorrhage. HMD
5	F	Twin pregnancy, spontaneous delivery	5	3	7.12	30	1350	Died	Meningeal haemorrhage. HMD
6	F	Transverse presentation, difficult breech delivery	6	10	7.36	32	1840	Survived	—
7	M	Uneventful pregnancy, spontaneous delivery	5	9	7.37	29	1240	Died	Meningeal haemorrhage.
8	M	Twin pregnancy, breech delivery, traction	2	9	7.12	30	1280	Died	Meningeal haemorrhage. HMD
9	M	Uneventful pregnancy, spontaneous delivery	4	6	7.09	31	1320	Died	Ventricular haemorrhage. Pulmonary haemorrhage. Epidural haemorrhage. HMD
10	F	Uneventful pregnancy, spontaneous delivery	6	8	7.20	31	1630	Died	Ventricular haemorrhage. HMD
11	M	Uneventful pregnancy, breech delivery	6	8	7.04	28	1080	Died	Meningeal haemorrhage.
12	F	Uneventful pregnancy, breech delivery	5	8	7.34	31	1500	Died	Pulmonary atelectasis. HMD
13	M	Breech presentation, cesarean section	2	4	7.25	—	1550	Survived	—
14	M	Early separation of the placenta, cesarean section	1	7	7.33	34	1850	Survived	—
15	F	Uneventful pregnancy, spontaneous delivery	6	10	7.30	34	1800	Survived	—
16	M	Uneventful pregnancy, spontaneous delivery	5	7	7.18	—	800	Died	Meningeal haemorrhage

Results

As Table III shows, the mean blood lactate concentration and the combined concentration of 17 amino acids were significantly higher in the asphyxiated AGA preterm newborn infants (3.38 ± 0.28 vs. 1.92 ± 0.09 mmoles/l and 2713 ± 82 vs. 2081 ± 91 μmoles/l; $p < 0.001$). Mean blood glucose concentration was higher in the asphyxiated than in the non-asphyxiated group, but the difference was not significant statistically. It should be noted that 9 of the asphyxiated infants received 10 % glucose and bicarbonate infusion before admission to the neonatal unit.

The plasma concentrations of 17 individual amino acids in the two groups of infants are shown in Table IV. In newborns suffering from birth asphyxia the plasma concentrations of alanine, proline, taurine, glutamate, valine, lysine and methionine were significantly elevated. If the concentrations of the glucogenic proline, valine, lysine obtained in both groups, were related to alanine (Fig. 1), their elevation in the plasma turned out to be closely related.

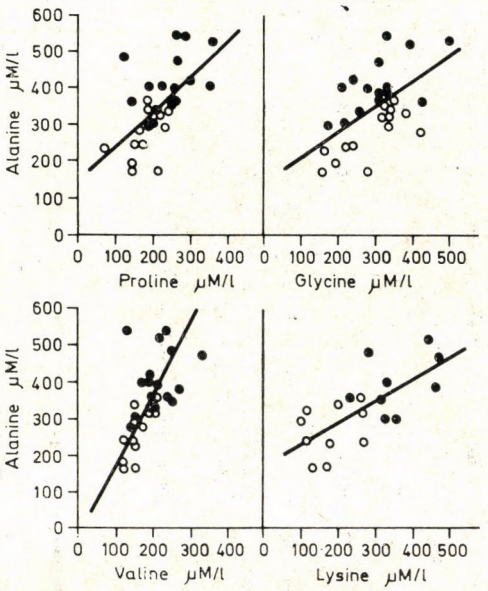

Fig. 1. Plasma glycine, proline, valine and lysine* concentrations in 16 asphyxiated (closed circles) and 13 non-asphyxiated (open circles) preterm newborn infants, related to plasma alanine concentrations.

Alanine vs. glycine, $y = 140.1 + 0.69\times$, $r = 0.564$ $p<0.01$
Alanine vs. proline, $y = 150.4 + 0.93\times$, $r = 0.594$ $p<0.001$
Alanine vs. valine, $y = 14.3 + 1.94\times$, $r = 0.949$ $p<0.001$
Alanine vs. lysine, $y = 177.6 + 0.57\times$, $r = 0.687$ $p<0.01$

*10 measurements neglected in statistical analysis because of poor resolution of peaks.

TABLE III

Mean total plasma amino acid, blood glucose and blood lactate concentration

	Mean (±SE) total plasma amino acid concentration μM	Mean (±SE) blood lactate concentration mM	Mean (±SE) blood glucose concentration* (mg/100 ml)
Non-asphyxiated preterm infants (n = 13)	2081 ± 91	1.95 ± 0.09	52.8 ± 5.7
Asphyxiated preterm infants (n = 16)	2713 ± 82	3.38 ± 0.29	72.4 ± 12.6
p value	$p < 0.001$	$p < 0.001$	—

* Nine infants received glucose infusion prior to admission.

TABLE IV

Plasma concentration (μM±SE) of 17 amino acids in the two groups of newborn infants

Amino acid	Non-asphyxiated preterm infants	Asphyxiated preterm infants
Taurine	252±13	315±18++
Aspartate	46±5	40±3
Glutamate	48±3	68±7+
Citrulline	25±2	26±2
Proline	175±14	237±17++
Glycine	285±24	308±21
Alanine	267±19	409±20+++
Cystine	122±24	140±13
Valine	156±8	210±13++
Methionine	22±3	31±3+
Isoleucine	47±4	56±6
Leucine	98±8	122±12
Tyrosine	141±17	117±8
Phenylalanine	107±9	128±10
Lysine	179±21	358±27++
Histidine	52±10	74±8
Arginine	57±8	76±6

\+ $p<0.05$
++ $p<0.01$
+++ $p<0.001$

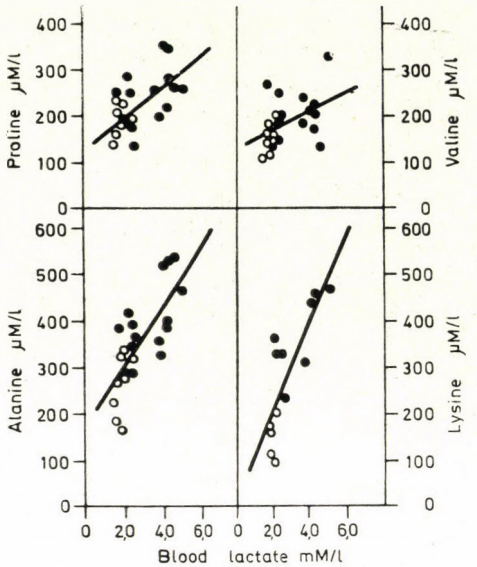

FIG. 2. Regression analysis of alanine vs. lactate, proline vs. lactate, valine vs. lactate and lysine* vs. lactate concentrations in the first twelve hours of life in 15 asphyxiated (closed circles) and 8 non-asphyxiated (open circles) preterm newborn infants.

Alanine vs. lactate, $y = 167.4 + 65.29\times$, $r = 0.752$ $p<0.001$
Proline vs. lactate, $y = 122.6 + 34.25\times$, $r = 0.593$ $p<0.01$
Valine vs. lactate, $y = 131.6 + 19.59\times$, $r = 0.437$ $p<0.05$
Lysine vs. lactate, $y = 20.3 + 94.32\times$, $r = 0.798$ $p<0.01$

*10 measurements neglected in statistical analysis because of poor resolution of peaks.

In Fig. 2, plasma alanine, proline, valine and lysine values are plotted against blood lactate concentrations. A significant positive linear correlation was observed between blood lactate and the four glucogenic amino acids (alanine vs. lactate, $r = 0.752$, $p < 0.001$; proline vs. lactate, $r.= 0.593$, $p < 0.01$; valine vs. lactate, $r = 0.437$, $p < 0.05$; lysine vs. lactate, $r = 0.798$, $p < 0.01$). Even individual observations point towards a close relationship between alanine and lactate levels. The relevant data of a pair of twins, with different degrees of birth asphyxia are shown in Table V. As it can be seen, plasma alanine and blood lactate concentrations were much higher in the severely asphyxiated twin A.

TABLE V

Blood lactate and plasma alanine levels in asphyxiated twins

	Birth weight (g)	Gestational age (wk)	Postnatal age (hr)	Apgar score at 1 min	Lactate mM	Alanine µM
Twin A	1250	30	3	1	4.37	392
Twin B	1350	30	3	5	2.08	296

Discussion

The results allowed to conclude that lactic acidosis due to birth asphyxia was associated with hyperaminoacidaemia. This is in agreement with the observation made in human adults suffering from circulatory failure or idiopathic lactic acidosis without evidence of poor tissue oxygenation [11]. The changes in plasma amino acid pattern were also similar as those obtained in adults. Among the 17 amino acids determined, the increase of alanine concentration was particularly marked and accounted for a portion of the total increment in the plasma amino acid content.

It appears reasonable to assume that an increasing amount of alanine is produced in lactic acidosis caused by asphyxia, which leads to an imbalance between release from the muscle and uptake by the liver. The close correlation between pyruvate and alanine concentration found under different conditions [6, 11] on the one hand, and the significant correlation between lactate and alanine level observed in the present study, on the other hand, support the contention that an increased availability of pyruvate may result not only in increased lactate production but also in increased conversion of pyruvate to alanine catalyzed by alanine aminotransferase.

Since the plasma concentrations of other amino acids were also found to be elevated, and the elevation of certain glucogenic amino acids were closely related to that of alanine, mechanisms other than increased production and release must also be considered in the mechanism of hyperaminoacidaemia associated with birth asphyxia. On the basis of some experimental studies [2, 3, 10, 11, 13, 17, 18] it may be assumed that a failure of amino acid removal, due to the impaired hepatic gluconeogenesis can be an additional mechanism which contributes to amino acid accumulation in the plasma. This assumption is supported by studies in adult rats, according to which hypoxia causes a marked inhibition of gluconeogenesis [1]. Observations in newborn rats [1, 8, 14] also point toward the importance of oxygenation in the establishment of hepatic glucose synthesis after birth. Even the increased lactate and pyruvate concentration can be partly responsible for the reduced hepatic utilization of glucogenic amino acids. In the isolated perfused rat liver Marliss et al. [11] have shown that when lactate and pyruvate concentrations were increased in the perfusate, uptake of alanine and its conversion to glucose decreased.

Another aspect of the possible impairment of gluconeogenesis associated with hyperlactataemia, induced by asphyxia, is the inhibitory effect of the low cellular pH. According to rat liver perfusion studies [3, 4, 10], lactate consumption decreases when intracellular H^+ concentration increases. This might also apply to the hepatic utilization of amino acids. Furthermore, examinations in vitro have shown that the activity of pyruvate carboxylase, an important enzyme in

hepatic glucose synthesis, is highly pH dependent [16, 17].

Finally, the quantitative and qualitative plasma amino acid profile observed in birth asphyxia resembles that associated with hypoglycaemia in the small-for-gestational-age newborn infant [7, 12]. In such babies a delay in the maturation of key enzymes appears to be an important factor in the reduced removal rate of glucose precursors. In view of the similar responses in the circulating free amino acid pool in hypoxia and hypoglycaemia and the frequent occurrence of perinatal asphyxia in severely malnourished newborn infants, it is conceivable that a transient or lasting tissue hypoxia might aggravate the metabolic consequences of hypoglycaemia.

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