

## MEASUREMENT OF THE URINARY LACTATE:CREATININE RATIO FOR THE EARLY IDENTIFICATION OF NEWBORN INFANTS AT RISK FOR HYPOXIC-ISCHEMIC ENCEPHALOPATHY

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### ABSTRACT

**Background** Newborn infants with perinatal asphyxia are prone to the development of hypoxic-ischemic encephalopathy. There are no reliable methods for identifying infants at risk for this disorder.

**Methods** We measured the ratio of lactate to creatinine in urine by proton nuclear magnetic resonance spectroscopy within 6 hours and again 48 to 72 hours after birth in 58 normal infants and 40 infants with asphyxia. The results were correlated with the subsequent presence or absence of hypoxic-ischemic encephalopathy.

**Results** Hypoxic-ischemic encephalopathy did not develop in any of the normal newborns but did develop in 16 of the 40 newborns with asphyxia. Within six hours after birth, the mean ( $\pm$ SD) ratio of urinary lactate to creatinine was  $16.75 \pm 27.38$  in the infants who subsequently had hypoxic-ischemic encephalopathy, as compared with  $0.09 \pm 0.02$  in the normal infants ( $P < 0.001$ ) and  $0.19 \pm 0.12$  in the infants with asphyxia in whom hypoxic-ischemic encephalopathy did not develop ( $P < 0.001$ ). A ratio of 0.64 or higher within six hours after birth had a sensitivity of 94 percent and a specificity of 100 percent for predicting the development of hypoxic-ischemic encephalopathy. The sensitivity and specificity of measurements obtained 48 to 72 hours after birth were much lower. The mean ratio of urinary lactate to creatinine was significantly higher in the infants who had adverse outcomes at one year ( $25.36 \pm 32.02$ ) than in the infants with favorable outcomes ( $0.63 \pm 1.50$ ) ( $P < 0.001$ ).

**Conclusions** Measurement of the urinary lactate:creatinine ratio soon after birth may help identify infants at high risk for hypoxic-ischemic encephalopathy. (N Engl J Med 1999;341:328-35.)

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**P**ERINATAL asphyxia is an important cause of neonatal mortality and of subsequent neurologic disabilities among the infants who survive.<sup>1-3</sup> Newborn infants who sustain an acute intrapartum hypoxic-ischemic insult of sufficient magnitude to result in long-term neurologic sequelae invariably have recognizable clinical encephalopathy during the first days of life. These infants have evidence of derangements in many organs. Their cerebral function is depressed at birth and remains depressed for days or weeks, and they frequently have seizures soon after birth.<sup>1-3</sup>

It is important to identify infants at high risk for

hypoxic-ischemic encephalopathy soon after birth if neuroprotective therapy is to be given.<sup>2-4</sup> However, most newborns with perinatal asphyxia have uneventful courses and a low likelihood of neurologic sequelae, and the development of hypoxic-ischemic encephalopathy and neurodevelopmental outcome cannot be predicted reliably.<sup>1,3,4</sup> Measurements of neuron-specific enolase, lactate dehydrogenase, hydroxybutyrate dehydrogenase, glial fibrillary acidic protein, brain-specific creatine kinase, glutamate, and interleukin-6 in serum or cerebrospinal fluid are of some value as markers of hypoxic-ischemic encephalopathy,<sup>1,5-10</sup> but samples of cerebrospinal fluid are rarely obtained from infants with no clinical signs of encephalopathy and are dangerous to obtain from infants with evidence of increased intracranial pressure.

Severe tissue hypoxia causes the accumulation of intermediary metabolites excreted by the kidneys, notably lactate,<sup>11-14</sup> which can be measured readily by proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectroscopy.<sup>15,16</sup> We previously reported that increases in urinary lactate excretion could be detected by <sup>1</sup>H NMR spectroscopy in newborn infants with perinatal complications.<sup>16</sup> In the present study, we measured the urinary lactate and creatinine concentrations within the first six hours after birth and determined the sensitivity and specificity of the ratio of urinary lactate to creatinine for the early identification of infants in whom hypoxic-ischemic encephalopathy is likely to develop.

### METHODS

#### Subjects

We studied 40 consecutive newborn infants with perinatal asphyxia who were born in our hospitals between June 1996 and October 1997 after at least 36 weeks' gestation. Perinatal asphyxia was defined as the presence of at least three of the following conditions: intrapartum distress, as indicated by fetal bradycardia with a heart rate of less than 100 beats per minute, late decelerations, or an absence of heart-rate variability; thick, meconium-stained amniotic fluid; an Apgar score of 6 or less at five minutes; a need for resuscitation for more than one minute with positive-pressure ventilation and oxygen immediately after birth; and an arterial blood pH value of 7.20 or less or a base deficit of at least 14 mmol

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**TABLE 1.** CHARACTERISTICS OF 58 NORMAL NEWBORN INFANTS AND 40 INFANTS WITH ASPHYXIA.\*

CHARACTERISTIC	NORMAL INFANTS (N=58)	INFANTS WITH ASPHYXIA		P VALUE†
		NO HIE (N=24)	HIE (N=16)	
Birth weight (g)	3346±408	3078±412	3129±367	0.50
Gestational age (wk)	39.7±1.2	38.7±1.4	39.3±1.1	0.16
Sex (M/F)	38/20	14/10	10/6	1.00
Apgar score				
At 1 min				0.86
Mean	9	2	2	
Range	7-10	0-4	0-4	
At 5 min				0.50
Mean	9	5	5	
Range	9-10	4-6	3-6	
Arterial-blood gases‡				
pH	ND	7.10±0.09	7.09±0.10	0.46
Base deficit (mmol/liter)	ND	17.8±2.3	18.5±2.1	0.32
Blood glucose (mg/dl)§				
At ≤6 hr	ND	106±44	123±42	0.29
At 48-72 hr	ND	83±19	84±18	0.90
Oliguria (no. of infants)¶				
Transient	0	3	4	0.28
Persistent	0	0	1	
Urinary lactate:creatinine ratio				
At ≤6 hr	0.09±0.02	0.19±0.12	16.75±27.38	<0.001
At 48-72 hr	0.09±0.03	0.16±0.17	0.92±1.77	0.008

\*Plus-minus values are means ±SD. HIE denotes hypoxic-ischemic encephalopathy, and ND not determined.

†P values are for comparisons between the infants with asphyxia and no hypoxic-ischemic encephalopathy and those with asphyxia and hypoxic-ischemic encephalopathy.

‡Levels of arterial-blood gas were measured within the first hour after birth.

§To convert the values for blood glucose to millimoles per liter, multiply by 0.056.

¶Transient oliguria was defined as urine output of <1 ml per kilogram of body weight per hour during the first 24 hours of life. Persistent oliguria was defined as urine output of <1 ml per kilogram per hour for at least the first 48 hours.

per liter within the first hour after birth.<sup>1,3,5,6</sup> The exclusion criteria were maternal drug addiction, congenital infections, or perinatal infections, including chorioamnionitis. The control group consisted of 58 normal, full-term newborns who met the following criteria: no maternal illness, normal results of fetal monitoring, an Apgar score of at least 8 at one and five minutes, and a normal course during the first week of life. The infants in both groups were examined daily during the first week after birth by a single examiner who did not know the results of the urinary testing.

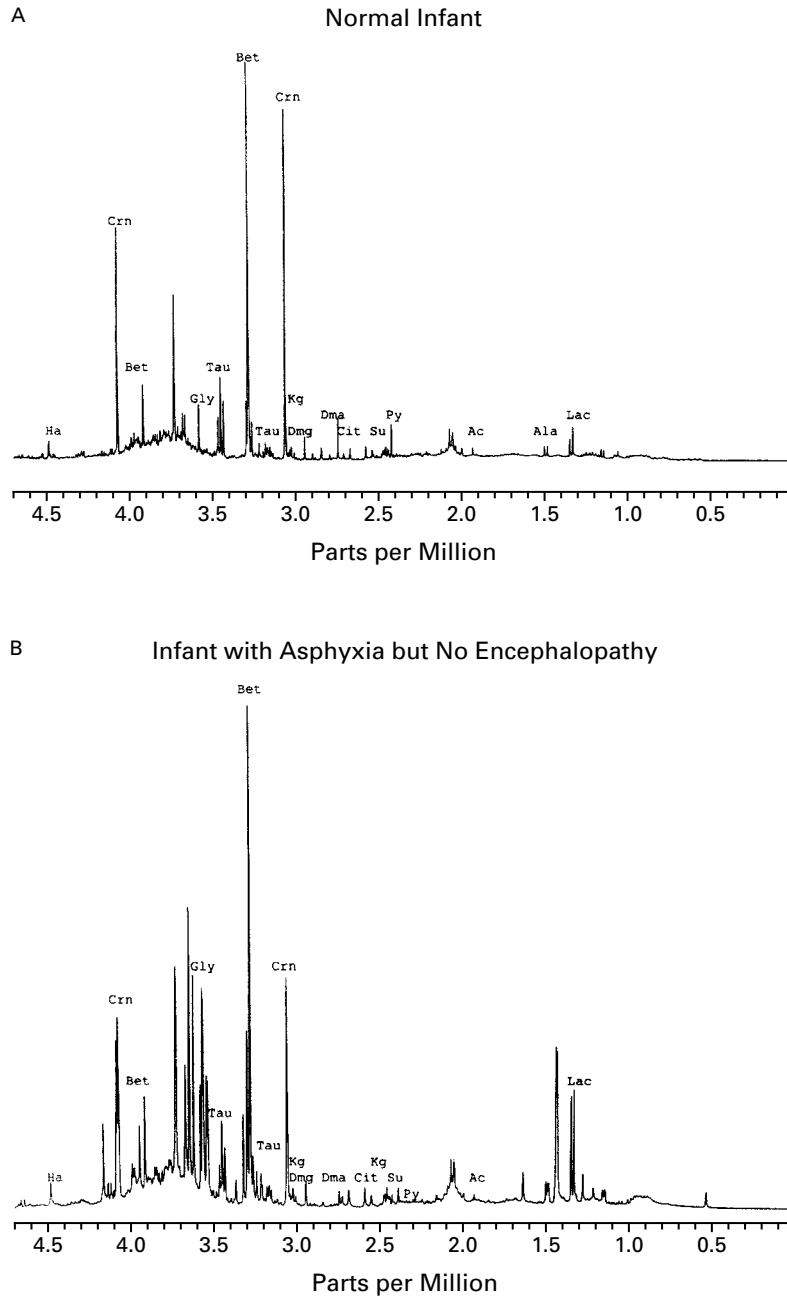
The infants with perinatal asphyxia were divided into two groups, according to whether hypoxic-ischemic encephalopathy developed within the first seven days after birth. Hypoxic-ischemic encephalopathy was classified as mild, moderate, or severe on the basis of the staging system described by Sarnat and Sarnat.<sup>17</sup> This system assesses the infant's level of consciousness, muscle tone, cranial nerves, primitive reflexes, spontaneous motor activity, autonomic function, and seizures. Hypoxic-ischemic encephalopathy was classified as mild if hyperexcitability or hypotonia persisted without seizures for at least 72 hours after birth; as moderate if the infant was lethargic and had hypotonia, weak primitive reflexes, and seizures; and as severe if the infant had frequent seizures, apnea, flaccid weakness, or coma. The study was approved by the institutional review board of National Cheng Kung University Medical Center, and written informed consent was obtained from the parents of the infants.

### Cranial Ultrasonography and Electroencephalography

We performed real-time ultrasonography within 24 hours, 48 to 72 hours, and 10 days after birth in the infants with perinatal asphyxia, using a 5- or 7.5-MHz sector transducer (SSD 630, Aloka, Tokyo, Japan).<sup>18</sup> Ultrasonograms showing increased echogenicity within the cerebral cortical parenchyma, basal ganglia, and thalamus or the presence of encephalomalacia were considered abnormal.<sup>19</sup> Electroencephalography was performed 36 to 52 hours after birth in the infants with perinatal asphyxia and was repeated when necessary. The electroencephalograms were interpreted by a single investigator, according to the criteria of Lombroso.<sup>20</sup>

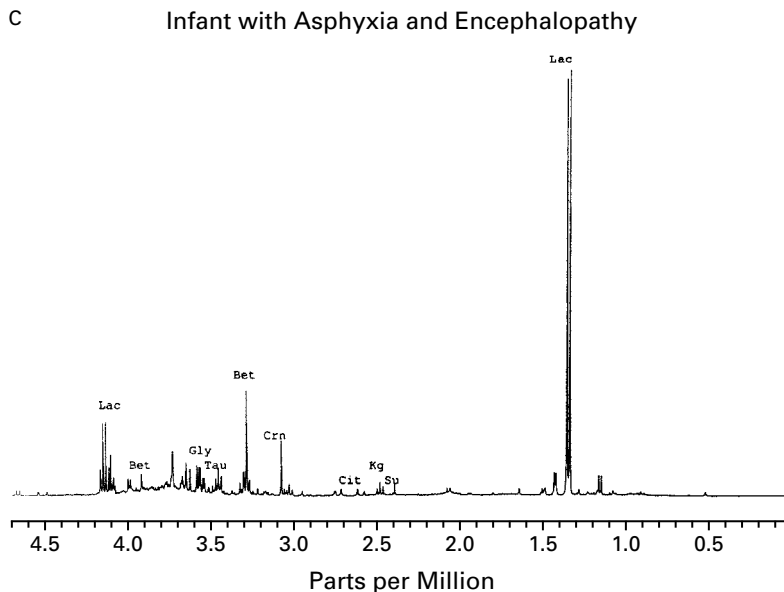
### Neurodevelopmental Outcome

All surviving infants with neonatal asphyxia and 51 normal infants underwent a neurodevelopmental examination at one year of age, which included a neuromotor assessment and the Bayley Scales of Infant Development II (BSID II). The neuromotor assessment was based on Amiel-Tison and Grenier's neurologic examination, which assesses posture and spontaneous motor activity, passive muscle tone, active muscle tone, deep-tendon reflexes, and postural reactions.<sup>21</sup> The neurodevelopmental outcome was classified as favorable or adverse. A favorable outcome was defined as normal neurologic development or only mild impairment (slight abnormalities in muscle tone and reflexes); an adverse outcome



**Figure 1.** Proton Nuclear Magnetic Resonance Spectra of Urine Collected within Six Hours after Birth in a Normal Infant (Panel A), an Infant with Neonatal Asphyxia in Whom Hypoxic–Ischemic Encephalopathy Did Not Develop (Panel B), and an Infant with Neonatal Asphyxia Who Subsequently Had Hypoxic–Ischemic Encephalopathy (Panel C).

We used the peak heights for lactate (a chemical shift at 1.34 ppm) and creatinine (a chemical shift at 3.06 ppm) to calculate the ratio of lactate to creatinine. Ha denotes dihydroxyacetone, Crn creatinine, Lac lactate, Cit citrate, Bet betaine, Gly glycine, Dma dimethylamine, Dmg *N,N*-dimethylglycine, Ala alanine, Ac acetate, Py pyruvate, Su succinate, Kg  $\alpha$ -ketoglutarate, and Tau taurine.



was defined as impairment resulting in death, severe cerebral palsy (hemiplegia, quadriplegia, diplegia, or severely impaired functioning associated with hypertonicity), developmental delay (a BSID II score that was more than 2 SD below the mean score for age), blindness, or deafness.<sup>10,21,22</sup>

#### Urinary <sup>1</sup>H NMR Spectroscopy

Spot urine samples were collected within 6 hours and again 48 to 72 hours after birth and were immediately centrifuged. The supernatants were stored at  $-80^{\circ}\text{C}$  for later assay. Urinary lactate and creatinine concentrations were measured by high-resolution <sup>1</sup>H NMR spectroscopy, as described previously,<sup>16</sup> at a probe temperature of  $25^{\circ}\text{C}$  with an AMX400 spectrometer (Bruker, Karlsruhe, Germany) operating at 400.13 MHz. High-resolution <sup>1</sup>H NMR spectroscopy was used in this study because it is a rapid and highly sensitive method for measuring multiple compounds simultaneously in a single small urine sample. The urine samples were prepared for analysis by adding 0.05 ml of deuterium oxide to 0.45 ml of urine contained in a 5-mm NMR tube. The NOESYPR1D (Bruker) pulse sequence provided efficient water-signal suppression. The 90-degree pulse width for the reverse broad-band probe was 7.6  $\mu\text{sec}$ , and 128 free-induction decays were collected. The methyl proton signal of creatinine with a chemical shift set at 3.06 ppm was selected as an internal standard, and the resonance was assigned for lactate and other metabolites. The peak heights for lactate (1.34 ppm) and creatinine (3.06 ppm) were determined, and the ratio of lactate to creatinine was calculated. The coefficient of variation for the ratio of lactate to creatinine in 26 urine samples was 4 percent. We did not measure urinary lactate by biochemical methods in this study, but a close correlation between <sup>1</sup>H NMR and enzymatic methods has been reported.<sup>23</sup>

#### Statistical Analysis

Unless indicated otherwise, continuous data are expressed as means  $\pm$ SD. We compared the ratio of urinary lactate to creatinine in the different groups using the Kruskal-Wallis test. All statistical tests were two-sided.

### RESULTS

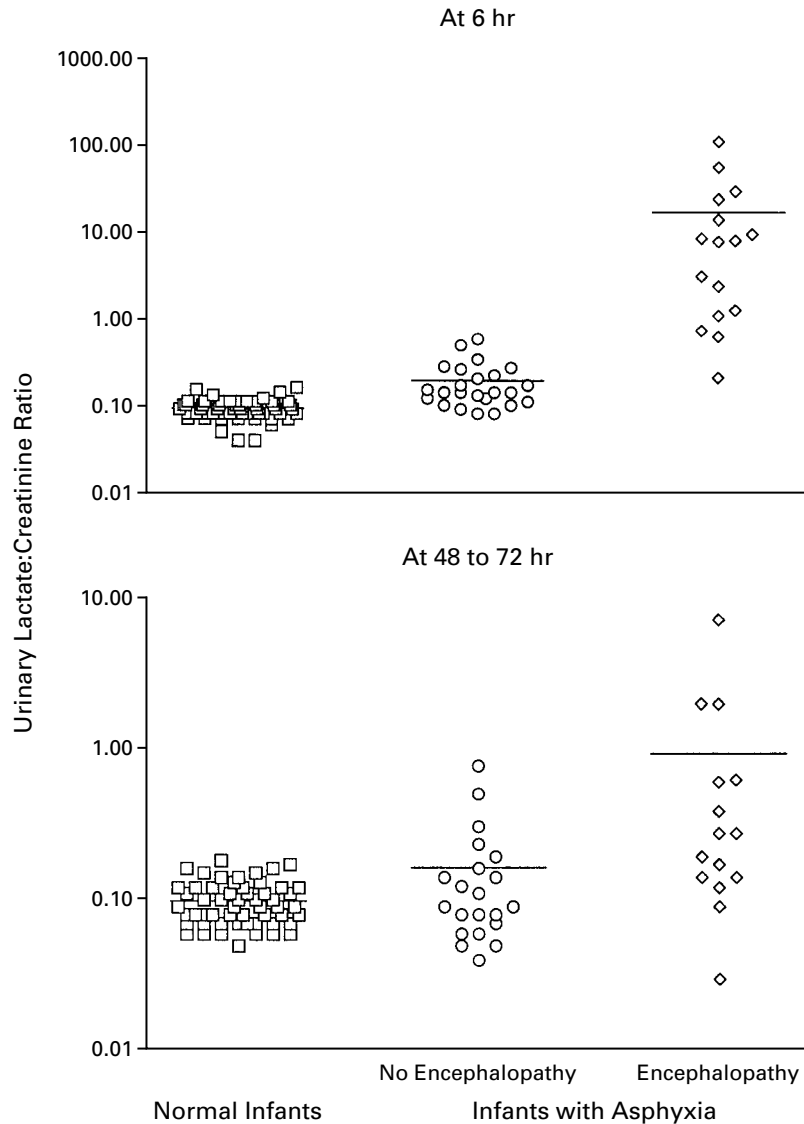
Birth weight, gestational age, and sex were similar among the normal infants, the infants with asphyxia

in whom hypoxic-ischemic encephalopathy developed, and the infants with asphyxia in whom the disease did not develop (Table 1). The first urine sample was obtained at a mean ( $\pm$ SD) of  $4 \pm 1$  hours in all three groups. Of the 40 infants with asphyxia, 24 did not have hypoxic-ischemic encephalopathy and 16 did. The two groups with asphyxia did not differ significantly in mean birth weight, gestational age, Apgar scores, first postnatal arterial pH value, base deficit, blood glucose concentrations, or the incidence of oliguria (Table 1). Among the 16 infants in whom hypoxic-ischemic encephalopathy developed, the disease was judged to be mild in 4, moderate in 5, and severe in 7.

The results of ultrasonography were normal in the infants who did not subsequently have hypoxic-ischemic encephalopathy. Among the infants in whom the disease did develop, five had diffuse brain edema, five had hyperechogenicity in the bilateral basal ganglia and thalamus, one had infarctions in the middle cerebral artery, and one had multicystic encephalomalacia. Eight infants in this group had severe, diffuse abnormalities on the electroencephalogram (a burst-suppression pattern, unreactive traces, or marked voltage suppression), and four had focal epileptiform discharges.

#### Ratio of Lactate to Creatinine in Urine

The <sup>1</sup>H NMR spectra of urine samples collected within six hours after birth in a normal infant, an infant with asphyxia who did not subsequently have hypoxic-ischemic encephalopathy, and an infant with asphyxia in whom hypoxic-ischemic encephalopathy developed are shown in Figure 1. The spectra in the third infant and in the other infants with subsequent hypoxic-ischemic encephalopathy were significantly



**Figure 2.** Urinary Lactate:Creatinine Ratios for the Three Groups of Infants.

The upper panel shows the ratios within six hours after birth. The lower panel shows the ratios 48 to 72 hours after birth. Urine samples from three infants with asphyxia and no encephalopathy and one infant with asphyxia who subsequently had encephalopathy were not available for evaluation 48 to 72 hours after birth. The solid lines represent mean values.

different from those in the other infants. The mean ratio of lactate to creatinine in urine within six hours after birth was  $16.75 \pm 27.38$  in the infants in whom hypoxic-ischemic encephalopathy subsequently developed — a value that was 186 times as high as the ratio in the normal infants ( $0.09 \pm 0.02$ ,  $P < 0.001$ ) and 88 times as high as that in the infants with asphyxia who did not subsequently have hypoxic-ischemic encephalopathy ( $0.19 \pm 0.12$ ,  $P < 0.001$ ) (Fig. 2, upper panel).

A urinary lactate:creatinine ratio of 0.64 or higher had 94 percent sensitivity (15 of 16 infants) and 100

percent specificity in predicting the development of hypoxic-ischemic encephalopathy. This value was chosen post hoc because it discriminated best and gave the maximal efficiency — the sum of the sensitivity and the specificity. Among the infants in whom hypoxic-ischemic encephalopathy developed, there was a nonsignificant trend for the ratio to increase with the severity of the hypoxic-ischemic encephalopathy:  $0.96 \pm 0.32$  in the infants with mild encephalopathy,  $7.48 \pm 9.36$  in those with moderate encephalopathy, and  $32.44 \pm 35.94$  in those with severe encephalopathy.

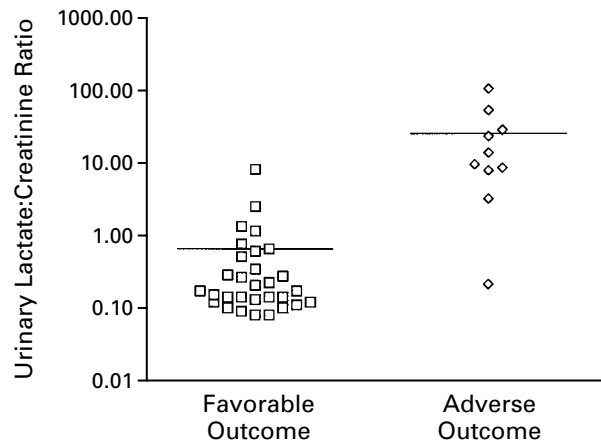
In the normal infants, the urinary lactate:creatinine ratio at 48 to 72 hours did not differ from the earlier value ( $P=0.15$ ), but the ratio decreased in the infants with asphyxia who did not subsequently have hypoxic-ischemic encephalopathy ( $0.16\pm 0.17$ ,  $P=0.02$ ) and in those who did ( $0.92\pm 1.77$ ,  $P=0.002$ ). At 48 to 72 hours after birth, the ratio in the infants who subsequently had hypoxic-ischemic encephalopathy was 10 times as high as that in the normal infants ( $P<0.001$ ) and almost 5 times as high as that in the infants who did not subsequently have hypoxic-ischemic encephalopathy ( $P=0.008$ ) (Fig. 2, lower panel). These values in the two groups of infants with asphyxia overlapped more than did the values within six hours after birth, resulting in lower sensitivity and specificity for predicting hypoxic-ischemic encephalopathy.

#### Urinary Lactate:Creatinine Ratio and Neurodevelopmental Outcome

None of the infants without asphyxia had abnormal neurologic development at one year of age. Among the 24 infants with asphyxia who did not have hypoxic-ischemic encephalopathy, 2 had mild impairment and the rest were normal. Among the 16 infants who had hypoxic-ischemic encephalopathy, 6 infants had favorable outcomes (neurologic development was normal in 5 and mildly impaired in 1), and 10 had adverse outcomes (5 died within the first four months of life, and 5 had severe neurodevelopmental sequelae). Nine of the 10 infants with a urinary lactate:creatinine ratio of at least 3.19 within six hours after birth were either dead or had severe neurodevelopmental sequelae at one year; only 1 of the 10 infants with adverse outcomes had a ratio of less than 1.00. In the group of 40 infants with asphyxia, the urinary lactate:creatinine ratio was higher in those with adverse outcomes than in those with favorable outcomes ( $25.36\pm 32.02$  vs.  $0.63\pm 1.50$ ,  $P<0.001$ ) (Fig. 3).

### DISCUSSION

In our study, 75 percent of newborn infants with perinatal asphyxia (30 of 40 infants) did not have adverse neurodevelopmental outcomes at one year. Conventional indicators (Apgar scores, arterial-blood pH, and base deficits) could not be used to predict the development of hypoxic-ischemic encephalopathy, although a multivariate model that incorporated a combination of these markers was somewhat predictive in other studies.<sup>3,24,25</sup> Most studies of perinatal asphyxia have measured biologic markers (brain-specific creatine kinase, hypoxanthine, erythropoietin, and lactate dehydrogenase in serum or cerebrospinal fluid), but the tests are usually performed several days after birth, when the infants may already have hypoxic-ischemic encephalopathy.<sup>1,5-11,26</sup> These tests may be useful as markers of tissue injury, but they offer little information that can be used to identify



**Figure 3.** Urinary Lactate:Creatinine Ratios within Six Hours after Birth and the Neurodevelopmental Outcomes at One Year in Infants with Perinatal Asphyxia.

The neurodevelopmental outcome was classified as favorable (normal neurologic development or mild impairment) or adverse (impairment resulting in death, severe cerebral palsy, developmental delay, blindness, or deafness). The solid lines represent mean values.

newborn infants at high risk for hypoxic-ischemic encephalopathy.

Our study shows that the urinary lactate:creatinine ratio, determined by  $^1\text{H}$  NMR within six hours after birth in infants with perinatal asphyxia, can be used to identify most of the infants in whom hypoxic-ischemic encephalopathy will develop. Oliguria and increased urinary excretion of beta<sub>2</sub>-microglobulin are also associated with cerebral abnormalities in newborn infants with perinatal asphyxia,<sup>11</sup> but a period of at least 36 hours is required to record the degree of oliguria, an interval not suitable for early diagnosis.

The salient abnormality in our study was a marked increase in the urinary lactate:creatinine ratio within six hours after birth in newborn infants with asphyxia who subsequently had hypoxic-ischemic encephalopathy. Lactate is the main end product of anaerobic glucose metabolism. Urinary lactate may result from systemic tissue hypoxia, skeletal-muscle ischemia, or renal injury during asphyxia.<sup>1,14,27</sup> In fetal sheep, urinary excretion of lactate increases during hypoxia, and it increases further during reoxygenation.<sup>13</sup> Although the serum lactate concentration has been widely used as an indicator of tissue hypoxia, this marker has not been related to the neurologic outcome in newborns with asphyxia.<sup>28</sup> Acute antepartum asphyxia is more likely to be detected by analysis of urine, in which disturbances are cumulative, than by analysis of serum, because lactate is also cleared by the placenta, and hyperlactatemia may be transient if hypoxia is relieved before delivery.<sup>13</sup>

In our study, the newborn infants with asphyxia were examined before hypoxic-ischemic encephalopathy developed. We selected them because they had hypoxia at birth, and we followed them to determine which ones subsequently had hypoxic-ischemic encephalopathy and which had adverse neurodevelopmental outcomes. Within six hours after birth, all the infants in whom hypoxic-ischemic encephalopathy did not develop had urinary lactate:creatinine ratios below 0.64. The values, although variable, were much higher in the infants in whom hypoxic-ischemic encephalopathy developed. The ratio also increased as the hypoxic-ischemic encephalopathy worsened. These results suggest that the urinary lactate:creatinine ratio within six hours after birth is related to the occurrence and degree of hypoxic-ischemic encephalopathy. The clinical value of this ratio decreases by 48 to 72 hours after birth, which suggests that the biochemical derangement detected in the urine after perinatal asphyxia is more pronounced within a few hours after birth than it is later. In infants with asphyxia, the urinary lactate:creatinine ratio within the first six hours after birth was also significantly related to the neurodevelopmental outcome at one year of age. Again, the cutoff value of 0.64 was chosen post hoc, and the sensitivity and specificity of values greater than 0.64 for predicting the risk of hypoxic-ischemic encephalopathy may well be lower in another study.

Whereas the infants who had severe hypoxic-ischemic encephalopathy were hypotonic, inactive, or comatose within six hours after birth, the infants who would later have mild or moderate hypoxic-ischemic encephalopathy were not easily distinguishable from those in whom hypoxic-ischemic encephalopathy did not develop. Some infants with low Apgar scores have signs of progressive neurologic dysfunction, including an initial period of near normality followed by seizures, deterioration of muscle tone, and deepening coma. A moderate brain insult can be difficult to detect when other conditions, such as the meconium aspiration syndrome, are present or when sedative or paralytic drugs have been administered. Electroencephalographic changes can be valuable in identifying infants at high risk for subsequent brain damage, but the interpretation of neonatal electroencephalograms can vary and requires considerable experience.<sup>20,29</sup>

Conditions other than hypoxic-ischemic encephalopathy that may cause high urinary lactate excretion in newborns are acquired diseases (e.g., necrotizing enterocolitis)<sup>30</sup> and congenital metabolic disorders (e.g., pyruvate dehydrogenase deficiency, glucose-6-phosphatase-deficient glycolysis, pyruvate decarboxylase deficiency, propionyl-coenzyme A carboxylase deficiency, and methylmalonic aciduria).<sup>31-33</sup> Although metabolic disorders may masquerade as hypoxic-ischemic brain injury in newborn infants, most

of these conditions are readily distinguishable from asphyxia. In addition, <sup>1</sup>H NMR can also be used to detect these metabolic disorders.<sup>31</sup>

Our study shows that the urinary lactate:creatinine ratio in newborn infants with asphyxia is useful for predicting the development of hypoxic-ischemic encephalopathy. The ratio may therefore be useful in identifying infants most likely to benefit from intervention.

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