Carbonic Acid Dissociation Constant (pK_1) in Critically Ill Newborns

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ABSTRACT. In the Henderson-Hasselbalch equation, the apparent first dissociation constant for carbonic acid in plasma, pK_1 , is 6.10 ± 0.01 ($\pm$ SD) in healthy adults. In contrast, values for pK_1 in sick adults and in sick infants and children have been reported to vary widely. Because of the far reaching implications of these findings, we repeated the measurements in 19 newborns in a neonatal intensive care unit. Two measurements were made in each infant, one while the infant was acutely ill and another after recovery. We found that neither the mean value nor the range of pK_1 values was affected by the infants' clinical status. The values during the acute phase of the hospitalization (range, 6.01–6.12; mean $\pm$ SD, 6.08 ± 0.03) did not differ from those after recovery (6.02–6.17; 6.08 ± 0.04). A second study was performed in order to see if the wide range of pK_1 values seen in other studies might be the result of an unstable state accompanying acute changes in acid-base status similar to those that might be encountered in clinical situations. However, data in seven lambs showed no significant difference when pK_1 before an acute alteration in acid-base status (6.10 ± 0.04) was compared with that 10 min after (6.09 ± 0.03). In newborn intensive care units, nomograms are used to calculate total CO_2 from pH and PCO_2 assuming a $pK_1 = 6.10$. Our data support this practice. (*Pediatr Res* 18:1287–1289, 1984)

Assessment of acid-base status requires clinical evaluation and accurate measurement of pH, PCO_2 , and HCO_3 . Although measurement of pH and PCO_2 can be made within minutes, plasma bicarbonate determinations take somewhat longer. As an alternative, the bicarbonate concentration can be quickly derived from the pH and PCO_2 using a nomogram based on the Henderson-Hasselbalch equation:

$$pH = pK_1 + \log [HCO_3 / (S \times PCO_2)] \quad (1)$$

in which S expresses the solubility of carbon dioxide in plasma and pK_1 is the apparent first dissociation constant for carbonic acid in plasma. The calculation of HCO_3 from pH and PCO_2 depends upon the assumption that $S = 0.03$ and $pK_1 = 6.10$.

Hastings *et al.* (4) measured a pK_1 of $6.10 \pm .01$ at $38^\circ C$ in plasma from sick and healthy patients. This value has been remarkably consistent among subsequent studies in man and a variety of other species [see Rispens *et al.* (8)], and the results

have been confirmed using newer electrode technology (8). Further, Austin *et al.* (1) studied 25 adults in intensive care units and found little variation in pK_1 (6.10 ± 0.03) (mean $\pm$ SD).

In contrast, others (7, 9, 10) have measured widely varying pK_1 values in sick adults and children; in one study (10) pK_1 varied from 5.873 to 6.429. Natelson and Nobel (7) suggested that the variability might be due to variation in plasma ionic strength, in the amount of CO_2 held in carbamino compounds, or in the solubility of CO_2 in plasma. However, not all authors agree that these vary widely enough to be of importance (1, 3, 5, 8). Trenchard *et al.* (10) noted that acute changes in the patient's clinical status had the strongest correlation with an abnormal pK_1 .

Hood and Campbell (5) recently reviewed this issue and concluded that the use of the Henderson-Hasselbalch equation to calculate HCO_3 is valid in most clinical situations. Nonetheless, they advised that the total CO_2 content be measured rather than calculated in patients whose clinical condition is changing rapidly.

Sick low birth weight infants have conditions similar to those associated with scatter in values for pK_1 in adults. Indeed, Rosan *et al.* (9) compared measured serum bicarbonate with that calculated assuming constant pK_1 in 22 neonates in an intensive care unit and found errors in calculated bicarbonate ranging from -55 to $+170\%$. They concluded that " pK_1 can no longer be considered a constant in . . . neonatology" (9).

We have examined the hypothesis that pK_1 in sick newborns is too variable to be clinically useful. We then examined the effect of an acute change in acid-base status on pK_1 in the lamb.

MATERIALS AND METHODS

Nineteen newborn infants admitted to the Neonatal Intensive Care Unit of the Johns Hopkins Hospital between December 1981 and October 1982 who were sick enough to require an arterial catheter to monitor their arterial blood gases and blood pressure were selected at random for study. Informed consent was obtained from the parents. The research protocol was approved by the Joint Committee on Clinical Investigation.

The pK_1 was calculated twice in each subject: during the acute illness and after recovery. Infants were considered to have recovered when they were well past their acute illness and were taking feedings and growing consistently. Blood was obtained from the arterial catheter during acute illness. After recovery, arterialized capillary blood was used. One ml of blood was drawn into four Natelson capillary tubes (DADE Division, American Hospital Supply Corporation, Miami, FL). Each tube was closed with a plastic cap and placed immediately on ice.

PCO_2 and pH were measured at $37^\circ C$ with the Radiometer BMS 2 MK 3 (Radiometer, Copenhagen). The PCO_2 electrode was calibrated with 3 and 8% CO_2 in nitrogen (Radiometer)

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before and after each sample. The pH electrode was calibrated before and after each sample with two phosphate buffers pH = 6.841 ± 0.005 (mean $\pm$ SD) and 7.383 ± 0.005 at 37°C (Radiometer). Calibration drift was greater than 1 mm Hg PCO_2 or 0.005 pH unit in only two samples. The measurement was repeated in each case, this time without drift.

Total CO_2 content was measured in plasma after centrifugation (2500 rpm $\times$ 10 min at 5°C) using the Natelson microgasometer (Model 650, Scientific Industries, Inc., Springfield, MA) (6). A three-point calibration line (using 0, 10, and 25 mM sodium carbonate solutions) was determined before each measurement. At least two measurements were made on each sample. The coefficient of variation for repeated measurements was $\pm 1\%$.

pK_1 was calculated from the equation:

$$\text{pK}_1 = \text{pH} - \log \frac{\text{total CO}_2 \text{ content} - (S \times \text{PCO}_2)}{S \times \text{PCO}_2} \quad (2)$$

S was assumed to be 0.0307 at 37°C (2).

Experiments were performed in newborn (ages 3–10 days) lambs to test the hypothesis (10) that pK_1 will change with acute changes in acid-base status. Acute metabolic alkalosis was induced by an intravenous bicarbonate dose of 2 mEq/kg. Acute respiratory acidosis was induced by providing an FiCO_2 of 0.09. Acute metabolic acidosis was induced with acute hypoxia with an FiO_2 of 0.05. Blood samples were drawn from an arterial catheter immediately before and 10 min after the acute perturbation. Samples were handled in the same manner as those of the newborn infants except pH and PCO_2 were measured at 39.5°C (normal temperature for lambs) and appropriate adjustments were made in calibration.

RESULTS

Nineteen newborn infants were studied; 16 had hyaline membrane disease. The other three had primary diagnoses of patent ductus arteriosus, persistent transitional circulation, and Group B streptococcal sepsis. Sixteen of 19 required ventilator management; the remaining three required supplemental inspired oxygen. Each had an umbilical arterial catheter that had been placed to monitor arterial blood gases and blood pressure. Fourteen infants were under 1500 g, and six were under 1000 g; 15 of 19 were studied before 72 h of age.

Table 1 shows that mean value for pK_1 during the acute illness is indistinguishable from that after recovery. There is also no significant difference between the variances in pK_1 during and after the acute illness ($F = 2.41$; critical value $F_{0.05(2)(12,18)} = 2.77$). These two groups of data were therefore pooled to yield 6.08 ± 0.03 (mean $\pm$ SD). For purposes of comparison, Figure 1 displays our values for pK_1 with those of others (1, 4, 9, 10).

Table 2 shows results of the pK_1 studies in newborn lambs. Acute changes in acid-base status did not change pK_1 significantly ($t = 0.700$, $df = 14$, $p > 0.2$). The lamb data can be pooled to give a $\text{pK}_1 = 6.10 \pm 0.03$.

DISCUSSION

The variance of pK_1 in the newborns we studied is different from that in the sick adults studied by Trenchard *et al.* (10) ($F = 5.09$; critical value $F_{0.05(2)(60,32)} = 1.94$). Since the mean pK_1 and frequency distribution for the sick adults investigated by Natelson

and Nobel (7) are nearly identical to those of Trenchard *et al.* (10), their findings are similarly discrepant from ours. In contrast, there was no difference between the variance in newborns as compared to sick adults studied by Austin *et al.* (1) ($F = 1.1$; critical value $F_{0.05(2)(32,24)} = 2.21$).

The reasons for these discrepancies are not clear. It is always possible that conditions which significantly alter pK_1 in the newborn were inadvertently excluded from our study. Trenchard *et al.* (10) hypothesized that acute changes in acid-base status produced the wide variation in pK_1 . We were unable to confirm this hypothesis in newborn lambs in situations that approximate those likely to be encountered on the clinical service. On the other hand, we waited 10 min after the perturbation before blood was sampled; sampling during the change might have yielded different results. Perhaps, too, if we had studied more than seven lambs, we would have found more variation in pK_1 .

The lack of agreement between our results and those of Rosan *et al.* (9) in newborns and children might reside in part on differences in calibration procedures. We calibrated pH and PCO_2 before and after every sample and determined a three-point calibration line for the Natelson method with each sample while Rosan *et al.* (9) "assayed control samples every tenth specimen." It is noteworthy that when Rosan *et al.* performed simultaneous measurement of total CO_2 with Natelson and Beckman analyzers, there were differences as great as 33%. Our coefficient of variation was 1%.

CONCLUSIONS AND RECOMMENDATIONS

We measured the pK_1 in sick newborns and found a value 6.08 ± 0.03 , essentially the same as the value of 6.10 used to calculate bicarbonate from pH and PCO_2 on the clinical service. If our extreme individual values for pK_1 (6.01 and 6.17) were used, there would be errors in calculated bicarbonate as high as 19%; but when exigencies of time require immediate information, derivation of the plasma bicarbonate concentration from the Henderson-Hasselbalch equation with an assumed pK_1 of 6.10 is acceptable until a direct measurement of total CO_2 content

Fig. 1. The range of values for pK_1 in adults and newborns from several studies in the literature (1, 4, 9, 10) compared with those in the present study.

Table 1. pK_1 in infants during acute illness and recovery*

	pK_1	PCO_2	pH	Calculated Tco_2	Measured Tco_2	% Variation†
Acutely ill	$6.08 \pm 0.03^*$	47.0 ± 10	7.28 ± 0.08	23 ± 3	24.7 ± 3	-7
Recovered	6.08 ± 0.04	48.1 ± 14	7.35 ± 0.06	27 ± 7	28.5 ± 7	-5

* Values are mean $\pm$ SD.

† (Calculated total CO_2 - measured total CO_2)/measured CO_2 .

Table 2. pK_1 and acute changes in acid-base status in newborn lambs

	<i>n</i>	Mean pK_1	Range	Mean pH	Range	Mean PCO_2	Range	Mean TCO_2	Range
Control	7	6.10	6.02–6.14	7.43	7.37–7.46	40.3	34.4–48.6	27.1	22.4–33.9
Metabolic alkalosis	5	6.09	6.07–6.11	7.51	7.48–7.53	41.3	32.8–46.8	33.8	27.9–39.2
Respiratory acidosis	2	6.10	6.10–6.11	7.28	7.23–7.34	55.7	52.6–58.8	27.2	22.9–31.5
Metabolic acidosis	2	6.08	6.04–6.13	7.20	7.10–7.30	38.2	36.5–40.0	16.2	15.2–17.1

is available. A 19% error is far less than the 50–170% errors reported by others (7, 9, 10). In addition to differences in calibration procedures, a difference in the timing of samples relative to acute disturbances in acid-base status may have been responsible. Nevertheless, our own data in babies, along with those of Austin *et al.* (1) in critically ill adults, shows that acute illness *per se* need not lead to alterations in pK_1 . Our data in lambs suggest that blood samples drawn as soon as 10 min after a large change in acid-base status may also be assumed to have a pK_1 near 6.10.

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Semiautomated Enzyme Immunoassay of Thyrotropin as a Mass Screening Test for Neonatal Hypothyroidism

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ABSTRACT. A sensitive, simple, and rapid semiautomated sandwich enzyme immunoassay (EIA) was developed for measuring thyrotropin in dried blood samples on filter paper for use in screening for neonatal hypothyroidism. Good correlation was found between values for thyrotropin determined by this method and those determined by radioimmunoassay (RIA) ($r = 0.94$). In pilot tests on 17,160 newborn infants in the general population, five cases of primary hypothyroidism were detected by both EIA and

RIA. The recall rate was slightly higher in EIA than in RIA. (*Pediatr Res* 18:1289–1292, 1984)

Abbreviations

RIA, radioimmunoassay
EIA, enzyme immunoassay
TSH, thyrotropin
IgG, immunoglobulin G

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Because irreversible mental retardation in congenital hypothyroidism can be prevented by early treatment, several mass screening methods have been developed for early diagnosis of this