immunoglobulin antibodies in the patients are either monoclonal or oligoclonal in origin with a very narrow specificity (recognizing sites only on the Fc portion), whereas the animal anti-immunoglobulin antibodies are surely polyclonal (raised by hyperimmunization) and, not surprisingly, react with a number of antigenic sites along the heavy chain, including those on the F(ab')2 or Fab fragment and Fc fragment.

We have observed (unpublished) a patient with anti-mouse IgG activity that caused falsely increased TSH concentrations in spite of the presence of added mouse IgG in the assay kit. Thus, we are concerned that addition of mouse immunoglobulin does not completely solve the problem of interference. We think that use of antibody-binding immunoglobulin fragments such as F(ab')2 is an alternative worth investigating.

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References

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Determination of Plasma Bicarbonate of Neonates in Intensive Care

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Most modern blood-gas analyzers are programmed to use the Henderson–Hasselbalch equation to calculate a value for plasma bicarbonate. It has been suggested, however, that among acutely ill patients, including newborns, these calculated values may be at variance with measured total CO2. To assess the clinical significance of such errors, we compared calculated bicarbonate with measured total CO2 in 79 blood samples from 40 babies in intensive care. The calculated bicarbonate values consistently exceeded the measured values by about 1.5 mmol/L. Of the errors, 94% were within the range −10% to +20%. When the systematic bias was removed, calculated and measured bicarbonate values agreed within ±3.3 mmol/L in 95% of cases. Because calculated values can be obtained much more quickly and frequently than laboratory measurements, we believe that these limits are clinically acceptable.

Additional Keyphrases: blood gases · analytical error · emergency (urgent) procedures

Disturbances of acid–base status occur frequently and rapidly during neonatal intensive care and, because of the influence of hydrogen ion concentration on cerebral and pulmonary blood flow, they must be corrected as quickly as possible. Routinely, pH and blood gases are measured every 2 h but, in situations where acute changes are expected, measurements may be made as often as every 15 to 30 min. On each occasion, the relative importance of the metabolic and respiratory components of a pH disturbance must be established so that appropriate clinical action can be taken. At this rate of sampling it is impracticable to rely on measurements of total CO2 content, which are made in the central biochemistry laboratory; thus, changes in pH are usually characterized by the value for plasma bicarbonate [HCO3−], as calculated by the neonatal unit blood-gas analyzer from the Henderson–Hasselbalch equation:

\[ \text{pH} = \frac{\text{pK}' + \ln[HCO_3^-]}{\text{S}} + \ln[PCO_2] \]

where S is the solubility coefficient of CO2 in plasma, and pK' is the apparent first dissociation constant of carbonic acid. Both are assumed to be constant, but there is considerable controversy concerning the validity of calculated bicarbonate, in the light of evidence that pK’ may be altered in some disease states (1, 2). Indeed, pK’ not only varies both within and between healthy individuals (3, 4), it also is affected by the ionic strength and bicarbonate concentration of the plasma (4). On theoretical grounds alone the Henderson–Hasselbalch equation, being applicable only to very dilute aqueous solutions (5), may be unreliable when applied to biological fluids. Even relatively small departures from the accepted value of 6.10 for pK’ may produce large errors in calculated bicarbonate. Natelson and Nobel (2) calculated that pK’ values ranging from 5.80 to 6.30 would cause errors in excess of 50%.

Against this body of evidence stand studies in which calculated bicarbonate has been found to be reliable, even in

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very sick patients (6, 7). The neonatal intensive-care situation typifies the polarization of views. Rosan et al. (8) claimed to find bicarbonate errors of -55% to +170% in their study of 22 neonates and stated that "pK" cannot any longer be considered a constant in pediatrics or neonatology." In another study on critically ill newborns, however, no large variations in pK' were found (9) and the authors concluded by supporting the use of calculated bicarbonate.

The aim of the present study was to see how often reliance on the value for plasma bicarbonate derived by the blood-gas analyzer would lead to clinically significant errors in the assessment of acid-base status of sick newborns.

Materials and Methods

Arterial blood was sampled from 40 babies in the Neonatal Unit of St James's University Hospital between March and October 1987. All samples were necessary for the clinical management of the babies, who were all being treated with intermittent positive-pressure ventilation for either respiratory distress syndrome, pneumonia, meconium aspiration, or bronchopulmonary dysplasia. Six of the 40 babies studied were born at term. The rest were preterm, 17 of less than 30 weeks gestation.

Each sample was drawn into a heparinized syringe, and 200 μL was immediately injected into the ABL 300 (Radiometer, Copenhagen, Denmark) blood-gas analyzer in the neonatal unit. Actual bicarbonate in plasma was calculated according to the Henderson–Hasselbalch equation. The rest of the sample was taken by messenger to the Department of Chemical Pathology, where plasma electrolytes and total CO₂ content were measured with a Beckman "Astra 8" automated analyzer as part of the routine service, both inside and outside normal laboratory hours. Both machines were used within their usual operating ranges and were calibrated according to manufacturers' instructions. All samples were measured within 30 min of collection.

In all, we analyzed 79 samples and calculated a value for pK' for each by substituting measured CO₂ for bicarbonate in the Henderson–Hasselbalch equation. We assumed that total CO₂ = HCO₃⁻, because the samples were not collected anaerobically and were subsequently left in an open cup for at least 5 min before analysis. This means that both dissolved CO₂ and CO₂ derived from carbonic acid will have been lost to the atmosphere, and further losses of bicarbonate will not occur without further generation of H⁺ ion.

Coefficients of variation for both methods were calculated from assays with quality-control samples.

For statistical analysis of the data we used a "Compaq" computer with the "Oxstat" package (Medstat Ltd., Nottingham, U.K.)

Results

The coefficients of variation for the Radiometer and Beckman machines were 2.5% and 5.8%, respectively.

The scattergram of calculated bicarbonate values vs measured total CO₂ (Figure 1) shows a good agreement between the two methods, with a systematic bias such that approximately 90% of the points lie to the left of the line of equality. We calculated the correlation, taking into account the imprecision of the two methods, to give a Deming slope of 0.91 (P < 0.0001) and a Deming intercept of 3.46.

A histogram of the frequency distribution of pK' (Figure 2) shows a gaussian distribution. The largest bicarbonate errors found were -13% and +45%. However, 94% of the errors were within the range -10% to +20%. The largest single percentage error was a calculated bicarbonate value of 6.7 mmol/L with a measured total CO₂ of 4.6 mmol/L; hence the difference, although large in percentage terms, was numerically small.

Discussion

The data presented in Figure 1 show both systematic bias and random scatter. Both are expected and acceptable. We would be surprised if there were no bias, because fundamentally different measurements are made by the Radiometer and Beckman analyzers. When the data are analyzed in the way suggested by Bland and Altman (11) for the statistical comparison of methods of clinical measurement, the mean bias for calculated bicarbonate is -1.54 (SD 1.65) mmol/L. The limits of agreement (11), taken as the mean difference between the methods ±2 SD, are -4.84 and 1.76 mmol/L—limits wide enough to be of some clinical significance, although they are unlikely to lead to seriously inappropriate
management. Because the bias is consistent it can be allowed for by subtracting the value of 1.54 mmol/L from each derived bicarbonate value; and because the differences between the methods are normally distributed we can be confident that in 95% of cases there will be agreement to within ±2 SD, i.e., ±3.3 mmol/L. We regard these limits as clinically acceptable.

Our findings are clearly at variance with those of Rosan et al. (8), who found errors in the calculation of bicarbonate of between −55% and +170% in sick newborns. Still, we believe that our population was similar to that studied by Rosan et al., both in terms of diagnosis and severity of illness. They attributed their large errors to a state of disequilibrium not accounted for in the Henderson–Hassebalch equation. Karlowicz et al. (9), however, could find no alteration in pK' when the acid–base status of newborn lambs was acutely disturbed. Re-analysis of the data presented by Rosan et al. shows that, although errors ranged from −55% to +72% (not 170% as stated by the authors), almost all could be explained on the basis of variance between the two machines used to measure total CO2 content. Far from the negligible inter-instrument variance claimed, we calculated the standard deviation for the paired measurements to be 2.17 mmol/L, enough to account for all but three of the reported errors.

We also are unconvinced by the data presented by Natelson and Nobel (2), whose dissatisfaction with the calculated bicarbonate value was the foundation for the Rosan study. This often-cited Letter presented a frequency distribution curve for pK' in which the cumulative frequency exceeded 140%! The scattergram on which this was based did not appear to have enough discrepant points to account for the range of pK' values stated.

We conclude that the values for bicarbonate calculated by a ward-based blood-gas analyzer agree sufficiently closely with the total CO2 measured in the chemical pathology laboratory for the former to be a clinically useful (and safe) method of characterizing acid–base disturbances in sick newborns. Also, the speed with which a result can be obtained from the ward-based instrument is a major advantage in a situation where derangements of acid–base status are so common and develop so rapidly.

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References

Influence of Hypertension and Antihypertensive Drugs on the Biological Intra-Individual Variation of Electrolytes and Lipids in Serum

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Biological intra-individual CVs for Na+, Cl−, K+, calcium, cholesterol, high-density lipoprotein cholesterol, triglycerides in serum, and hemoglobin were estimated in men with essential hypertension (EH) treated with beta-blockers and diuretics, and compared with those of normotensive men. Although in EH the mean concentrations of Na+, Cl−, K+, hemoglobin, and triglycerides were increased and that of HDL cholesterol was decreased, the average intra-individual CVs did not significantly differ between the two groups. The mean concentration of cholesterol, as well as the average intra-individual CV for it, was significantly higher in EH. There was no correlation between the intra-individual CVs for the analytes and the mean blood pressure of the individuals. Individual values were normally distributed for all analytes. There was no increase of the intra-individual CV with the lapse of time between consecutively measured values. The estimated average biological intra-individual CV was used to derive decision-making criteria for interpretation of test results observed in monitoring EH.

Judging the clinical significance of changes between consecutively observed values in monitoring diseases requires decision-making criteria that are derived from the random intra-individual variation of the analytes. This random variation has two components, biological and analytical. The biological component must be specifically studied for each disease, because the disease, or the drugs used in its treatment, may influence the magnitude of variation.