BEDSIDE ESTIMATION OF WHOLE BLOOD LACTATE

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SUMMARY

A new instrument for the measurement of lactate in biological fluids, the Lactate Analyzer 640, has been evaluated. The method of use recommended by the manufacturer for blood samples was found to be inadequate. A new method of sample preparation for the instrument, based upon immediate haemolysis and fluoridation of blood, has been developed, allowing measurement of whole blood lactate concentration to be performed on samples as small as 150 μl within 5 min of withdrawal. The instrument is designed to be operated by a medical practitioner. Excellent correlation with a conventional enzymatic assay was found. These features make this new method particularly applicable to rapidly changing clinical situations such as shock in adult patients, asphyxia neonatorum and intrapartum foetal hypoxia.

The value of blood lactic acid determination is well known but is seldom exploited in routine clinical management because of the length of time required for analysis and the rapid increase in lactate concentrations in blood samples after withdrawal. The Lactate Analyzer 640 (Racine et al., 1975; Racine, Klénk and Kochsieck, 1975) seemed to have overcome these problems, providing rapid, simple measurement of extracellular lactate within 2–3 min using diluted samples of whole blood.

During a preliminary evaluation of the instrument the recommended method of preparing blood samples for analysis was found to be inadequate. We report the nature of the problems associated with the recommended method and describe a superior method.

METHODS

Lactate Analyzer 640

The instrument uses an electrochemical enzymatic sensor (fig. 1). L-Lactate is specifically and irreversibly oxidized to pyruvate by the enzyme, cytochrome b₅, in the presence of an electron acceptor, hexacyanoferrate III (fig. 2). Hexacyanoferrate II is reoxidized at a platinum electrode biased at 0.25–0.40 V against a silver/silver chloride electrode. Thus a current is developed which is related linearly to the concentration of L-lactate. The measurement cell contains the platinum electrode sensor covered with a thin layer of enzyme solution which is retained on the electrode by a semipermeable cellophane membrane (fig. 1). This membrane is permeable to low molecular weight compounds (lactate, pyruvate and hexacyanoferrate III), but is impermeable to the enzyme. The counter electrode is separated from the test solution by a glass frit and a semipermeable membrane. Because these membranes are highly permeable to lactate, it is essential to dilute the sample 1:10 for assay in order to conserve the enzyme, which requires to be changed only once in 3 weeks. The enzyme layer is kept at 18°C by a Peltier element and a reference thermistor in the platinum electrode sensor. Emptying and rinsing of the cell are automatic. Within 60 s of injecting the sample, the value is read from a digital display where the final result, which is calculated automatically from the slope of the increments in current, is shown and retained. Calibration is recommended twice daily with standard solutions of lactate provided. The response of the sensor is linear from 0 to 10 mmol litre⁻¹.

“Roche” method of sample preparation

The blood is placed in a chilled test-tube on ice, 100 μl is added rapidly to 900 μl of chilled diluent buffer on ice (100 mmol phosphate buffer at pH 7.3 with sodium fluoride 2 g litre⁻¹ and sodium azide 1 g litre⁻¹); the lactate concentration is not stabilized until the blood is in the ice-cold diluent. Thereafter a 100-μl aliquot of the diluted sample is injected into the measuring cell (fig. 3).

The volume injected is not critical, but the manufacturer stresses the importance of standardizing the speed of injection. Using this recommended method, only the extracellular or plasma lactate concentration is measured, as the intracellular lactate will not leak out sufficiently rapidly to take part in the enzymatic reaction. Since this method measures the plasma lactate on a diluted sample of whole blood the
FIG. 1. The measuring cuvette, showing the sample chamber with the lactate sensor on the left and counter electrode on the right. (Diagrammatic.)

FIG. 2. The oxidation of L-lactate to pyruvate in the presence of hexacyanoferrate which carries the electrons to the platinum electrode.

packed cell volume (PCV) must be known in order to calculate how much the plasma has been diluted and to correct for this.

For example, consider a specimen of blood PCV = 50 (fig. 4). Using the “Roche” method of sample preparation, 50 μl of plasma would be diluted in 900 μl of diluent plus 50 μl of red cells, a dilution of 1:20—not 1:10, for which the instrument is calibrated. This dilution error will vary with PCV. This problem was not mentioned by Racine and his co-workers (1975) and is only alluded to in the handbook supplied with the instrument.

To confirm the need to measure PCV using the “Roche” method of sample preparation, two specimens of blood were taken on different occasions from the same subject and allowed to stand at room temperature for 30 min. The extracellular lactate was measured in duplicate in one aliquot from each specimen using diluted whole blood as recommended.

Another aliquot was centrifuged at room temperature to obtain plasma which was diluted 1:10 and the lactate concentration measured in duplicate. The PCV of the subject’s blood was determined also.

The authors’ method of sample preparation

Long (1944, 1946) and Powell (1974) demonstrated the value of a mixture of sodium fluoride, citric acid and cetyltrimethylammonium bromide (Cetrimide) in haemolysing red cells and in stabilizing both pyruvate and lactate in blood. In the preparation of samples for the Lactate Analyzer such an approach would offer the advantages of very rapid “fixation” of the lactate concentration so that accurate dilution could be performed at leisure, and avoid the need to measure PCV (fig. 5). This would enable measurement of whole blood lactate.

As a result of initial trials, Long’s method of haemolysing the blood and stabilizing the lactate concentrations was modified to meet the requirements of small blood samples. A mixture of citric acid 16.8 g, cetyltrimethylammonium bromide 8.0 g and sodium fluoride 4.2 g was dissolved in about 70 ml of distilled water. The pH was adjusted to 4.0 with 40% solution of sodium hydroxide in water and the volume made up to 100 ml with distilled water. This resulted in a suspension rather than a solution. One hundred microlitre aliquots of this suspension were added to 2-ml plastic tubes and evaporated to
Fig. 3. A diluted sample of blood being injected into the measuring cuvette of the Lactate Analyzer 640.

**Fig. 4.** If 100 µl of whole blood is added to 900 µl of diluent, a 1:10 dilution of blood is obtained. However, the Lactate Analyzer measures only the extracellular or plasma lactate in a whole blood sample. If the PCV of the blood sample is 50, then 100 µl of whole blood will contain 50 µl of plasma, which is therefore diluted 1:20, not 1:10 as expected.

**Fig. 5.** In the authors' method, the lactate value is stable before dilution. Furthermore, since all of the lactate in a haemolysed blood sample will be measured by the Lactate Analyzer, the dilution is exactly as expected and is unaffected by PCV.

dryness. The dried residue was separated from the walls of the tube to ensure rapid and complete dissolution of the powder in added blood. This is suitable for approximately 1 ml of blood but the amount of the suspension added to each tube may be scaled down for samples as small as 150 µl. As soon as the blood is added to the powder, the tube is shaken vigorously for at least 15 s. This haemolysed sample is now ready for dilution (1 : 10) in a buffer modified from that provided by Roche so that the final concentration of sodium fluoride does not exceed 2 g litre⁻¹ (100 mmol potassium phosphate buffer, pH 7.3, sodium fluoride 1.58 g litre⁻¹, sodium azide 1 g litre⁻¹) and for injection into the Lactate Analyzer.

**Stability of lactate at room temperature.** One-millilitre samples of venous blood were collected from healthy adults into tubes containing the powder. The whole blood lactate was assayed in duplicate in the Lactate Analyzer within 24 h and after 3 and 7 days of storage at room temperature.

**Recovery of lactate.** Lactic acid was added to both haemolysed samples and to diluted haemolysed samples of whole blood whose lactate concentration had already been measured using the author's modification. The resultant lactate concentrations were measured and expressed as a percentage of the expected values.

**Spectrophotometric lactate assay**

As a control for the authors' method a standard enzymatic spectrophotometric assay was used (Sigma Chemical Co., London). Approximately 1 ml whole blood was added to a pre-weighed test-tube containing 8% w/v perchloric acid (PCA) on ice. This was reweighed to measure the volume of blood added assuming an average specific gravity of 1.055
(Documenta Geigy). The protein-free supernatant was separated by centrifugation and the lactate concentration estimated by the method of Marbach and Weil (1967) using a Gilford 250 spectrophotometer. Each sample was assayed in duplicate and at least one lactate standard was included in every batch assay. Usually the assay was performed on the day on which the sample was obtained, but when an assay was delayed until the following day the samples were stored at 4 °C. The recovery of added lactate was measured four times by adding lactic acid to precipitated specimens from which aliquots of the supernatant had already been taken for assay. The specimens were mixed, recentrifuged and the supernatants reassayed.

Comparison of the authors' method of sample preparation with the spectrophotometric assay

Two-millilitre samples of blood were collected and, as rapidly as possible, 1 ml was added to 2 ml PCA on ice and 1 ml to a tube at room temperature containing the powder. The tube was stoppered, mixed vigorously for 15 s and stored at room temperature until assay in the Lactate Analyzer. The PCA tube, in which the blood proteins were already precipitated, was inverted and stored at 4 °C until assay using the Sigma method. All assays were performed within 24 h of collecting the samples.

RESULTS

Effect of PCV on the “Roche” method of sample preparation

The results of this experiment are shown in table I. The “calculated” PCV is derived from the following equation: 100\[1-(A/B)\], where \(A\) is the extracellular lactate as measured from a diluted sample of whole blood (“Roche” method) and \(B\) the lactate value obtained from the specimen of diluted plasma prepared by centrifugation. The good agreement between this “calculated” PCV and the measured PCV confirms that the large difference between \(A\) and \(B\), both of which allow measurement of plasma lactate, results from the effect of the red cells on the dilution of whole blood necessary in the “Roche” method.

Stability of lactate at room temperature using the authors’ method

The change in lactate values in 10 samples of blood haemolysed by the powder and stored at room temperature was minimal at 3 days (mean ± SD = −0.025 ± 0.079 mmol litre\(^{-1}\)) and at 7 days (mean ± SD = −0.027 ± 0.104 mmol litre\(^{-1}\)).

Recovery of lactate by the authors’ method

There was no difference in the recovery if lactic acid was added to the haemolysed blood before or after dilution. The average recovery measured was 101.8% (SD = ± 2.1%).

Reproducibility of the authors’ method

This was estimated by calculating the average variation about the mean of duplicate lactate measurements on diluted samples of haemolysed blood. This calculation does not take into account possible errors in diluting the samples. The average variation and SD for 53 duplicate assays was 0.009 mmol litre\(^{-1}\) ± 0.008 mmol litre\(^{-1}\). The 95% confidence limits of the mean of duplicates were therefore ± 0.025 mmol litre\(^{-1}\). Ten samples were diluted in duplicate and analysed in duplicate. The average variation of these duplicates about their means was 0.043 mmol litre\(^{-1}\) (SD ± 0.072 mmol litre\(^{-1}\)), with 95% confidence limits of ± 0.188 mmol litre\(^{-1}\). This demonstrates that careless dilution is a major potential source of error.

Enzymatic spectrophotometric assay

The recovery of added lactate using the Sigma assay was measured four times and was between 100.5 and 106.5%. The average variation of duplicate measurements about their means in 53 assays was 0.030 mmol litre\(^{-1}\) (SD ± 0.019 mmol litre\(^{-1}\)). The 95% confidence limits of the mean of duplicates were therefore ± 0.069 mmol litre\(^{-1}\).

<table>
<thead>
<tr>
<th>(A) Diluted wholeblood</th>
<th>(B) Diluted plasma</th>
<th>Calculated PCV (%)</th>
<th>Actual PCV of subject (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.55</td>
<td>0.93</td>
<td>41</td>
<td>43</td>
</tr>
<tr>
<td>0.90</td>
<td>1.58</td>
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</table>
ESTIMATION OF WHOLE BLOOD LACTATE

Authors' lactate (mmol litre\(^{-1}\))

Sigma lactate (mmol litre\(^{-1}\))

\[ r = 0.9813 \]
\[ b = 1.0073 \]
\[ a = + 0.024 \]

**Fig. 6.** A comparison of the authors' whole blood lactate assay using the Lactate Analyzer with the conventional Sigma whole blood lactate assay on 53 samples. This shows, not only excellent correlation, but also a 1 : 1 relationship between values obtained by the different methods.

The range of values measured was from 0.45 to 5.39 mmol litre\(^{-1}\). The average difference in the values obtained with the different methods was 0.035 mmol litre\(^{-1}\) (SD ± 0.198 mmol litre\(^{-1}\)). The correlation coefficient was 0.9813 and the slope of the regression line fitted by least squares was 1.007 (fig. 6).

**DISCUSSION**

The Lactate Analyzer 640 makes it possible for the clinician to measure blood lactate simply and quickly. The major problem encountered with the instrument was the unsatisfactory method of sample preparation recommended by the manufacturer for samples of whole blood. Our experience in this regard is at variance with that of Racine, Klenk and Kochsiek (1975) who described excellent correlation of this method with a conventional assay. They made no mention of measuring PCV. However, Bossart and Janecek (1976) found that, using this method on whole blood, they obtained good correlation with, but values which were on average 73% of, those obtained by conventional assay. On the other hand, when they diluted plasma the values agreed well. They were not able to explain this difference, but it is now clear that most of this error was a result of the effect of PCV on the dilution step. A further disadvantage of this method is the need to dilute the blood in ice-cold buffer rapidly and accurately in order to prevent an increase in lactate concentration. This is obviously not very practical in a busy clinical situation.

The authors' modification of the method of sample preparation appears to overcome these problems with a rapid, convenient method of stabilizing the lactate concentration for up to 7 days and haemolysing the cells, so avoiding the effect of PCV on dilution. This method correlates very well indeed with a conventional assay but is both simpler and faster. Of particular interest to obstetricians and neonatologists is the fact that this modified method can be readily adapted for samples as small as 150 μlitre.

The instrument is easy to use. The volume of sample injected into the chamber does not need to be accurate, but large differences in the speed of injection will give poor replication. However, in practice this is not a problem, as good results are obtained when every sample is injected rapidly. Subsequent experience of clinical colleagues using the machine has confirmed this. It is preferable that calibration and sample measurement are carried out by the same operator. Full daily calibration and maintenance take 10–15 min and thereafter only the 10-mmol litre\(^{-1}\) standard needs to be checked from time to time during the day. The sensor and counter electrode must be refurbished every 3–4 weeks. This process takes about half-an-hour and thereafter the instrument is not ready for use until after 12 h. A result can be obtained within 5 min of taking the blood sample. The reading is usually stable about 60 s after injecting a sample and another 2 min are required to complete the cycle before the next sample can be introduced.

This instrument, using the authors' method of sample preparation, offers a simple, reliable and rapid method of measuring whole blood lactate. It should prove of great value in the management of shock, lactic acidosis, neonatal asphyxia and intrapartum hypoxia.

**ACKNOWLEDGEMENTS**

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REFERENCES


MESSUNG VON VOLLLUT-LAKTAT AM KRANKENBETT
ZUSAMMENFASSUNG


EVALUACION INMEDIATA DEL LACTATO EN LA SANGRE COMPLETA
SUMARIO

Se ha evaluado un instrumento nuevo para la medición de lactato en fluidos biológicos, el "Lactate Analyzer 640". Se descubrió que el método de empleo recomendado por el fabricante para muestras de sangre es inadecuado. Se ha desarrollado un nuevo método de preparación de muestras para el instrumento basado en la hemólisis y fluoridación inmediatas de la sangre, permitiendo la medición de la concentración del lactato de sangre completa con muestras de un volumen mínimo de 150 μl dentro de 5 min de extraídas. El instrumento ha sido diseñado para ser manejado por un médico. Se encontró una excelente correlación con una evaluación enzimática convencional. Estas características permiten que este método sea especialmente aplicable a las situaciones clínicas de cambio rápido tales como "shock" en pacientes adultos, asfixia del neonato y hipoxia fetal intrapartum.

ESTIMATION DU LACTATE DU SANG TOTAL FAITE AU CHEVET D'UN MALADE
RESUME

On a évalué un nouvel instrument permettant de mesurer le lactate contenu dans les fluides biologiques: le Lactate Analyzer 640. Il a été trouvé que le mode d'emploi recommandé par le fabricant était inadéquat en ce qui concerne les échantillons de sang. On a mis au point une nouvelle méthode de préparation des échantillons destinés à cet instrument, méthode basée sur l'hémolyse immédiate et la fluoridation du sang permettant de mesurer la concentration de lactate dans le sang total sur des échantillons de l'ordre de 150 μl, dans les 5 minutes qui suivent le prélèvement. Cet instrument est conçu pour être utilisé par un médecin. On a trouvé une excellente corrélation avec un essai de conformité enzymatique conventionnel. Ces caractéristiques font que cette nouvelle méthode est tout particulièrement applicable dans les situations cliniques variant rapidement, comme par exemple l'état de choc chez les malades adultes, l'asphyxie des nouveau-nés et l'hypoxie fœtale au cours de la délivrance.