Because of a previous report that under certain conditions acetone can react with primary amines (16), we checked this possibility by including in the assay mixture dextroamphetamine, a typical primary amine drug and the enantiomer of amphetamine. Only one peak, dextroamphetamine acetate, was present upon GC/MS analysis after extraction. Thus, no side reactions appear to occur under these conditions of analysis.

The aqueous acetylation procedure used in this method has been described in detail in our urine extraction method (11). A significant advantage in using acetyl derivatives is that one of the most widely available, comprehensive, drug databases (17, available also on disk from Hewlett-Packard, makes extensive use of acetates. This database greatly facilitates identification of drug metabolites, many of which do not exist in many in-house data bases and for which there are no easily available standards.

The procedure described here was developed in a tertiary care hospital setting, with a clinical purpose in mind. However, on two occasions, our Pathology Department requested analysis of postmortem blood samples, from which we extracted and identified a variety of drugs. Thus this method may also have forensic applications.

References


Lactate Analyzer with Continuous Blood Sampling for Monitoring Blood Lactate during Physical Exercise

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To monitor changes in the concentration of blood lactate during physical exercise, we used an automated lactate analyzer based on an electro-enzymatic method with continuous blood sampling through a catheter. The lactate concentration was measured every 2 min; between measurements, the instrument was calibrated with a lactate standard. Ascorbic acid, bilirubin, hemoglobin, creatinine, uric acid, and glucose did not interfere with the measurements. The lactate concentrations in blood samples from apparently healthy subjects before and after exercise correlated well ($r = 0.993$) with results by the conventional enzymatic method. We measured the blood lactate concentrations in nine apparently healthy volunteers during exercise on a treadmill with an increasing workload. The point at which lactate concentrations started to increase was detected easily. Thus, the lactate analyzer is suitable for monitoring changes in blood lactate concentrations during exercise.

Additional Keyphrases: enzyme electrode technique · anaerobic threshold

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The concentration of lactate circulating in blood is a biochemical indicator of anaerobic metabolism, which occurs when oxygen delivery to the tissues is insufficient. Increased concentrations of blood lactate are seen during physical exercise, and the changes in these concentrations are useful for estimating the intensity of the exercise (1). The anaerobic threshold, the point at which anaerobic glycolysis begins, as denoted by an increase in blood lactate, has been used to assess physical fitness and the effects of exercise training in patients with cardiopulmonary or metabolic disease (2, 3). To monitor lactate concentrations in blood, we developed a lactate analyzer with a continuous blood-sampling system and an electrochemical sensor. This instrument consists of an analyzer pump that controls the withdrawal and dilution of blood, a lactate analyzer, a microcomputer that calculates lactate concentrations, and a printer. Here, we present the results of an evaluation of this lactate analyzer.

**Materials and Methods**

We analyzed for blood lactate with this apparatus by use of an electrochemical sensor covered by a membrane on which lactate oxidase was immobilized (Figure 1). A sensor for a glucose analyzer was provided by Kyoto Daiichi Kagaku Co., Ltd. (Kyoto, Japan) and modified for lactate measurements. The diluted blood was drawn into a probe in which the catalytic action of lactate oxidase on L-lactate and oxygen produced hydrogen peroxide and pyruvate. The hydrogen peroxide was polarographically oxidized at the platinum anode and the current produced corresponded to the lactate concentration. A flow diagram of the analyzer is shown in Figure 2. To establish the dilution ratio of blood with saline containing heparin (200 USP units/mL) in a double-lumen catheter, we aspirated a sterilized lactate solution (10.0 mmol/L) from the catheter as an external standard before inserting the catheter into a vein. The blood sample was drawn continuously from the subject at the rate of 3 mL/h through the double-lumen catheter and conducted to the flow-cell of the analyzer, where the lactate concentrations were measured every 2 min. A single-point calibration with an internal lactate standard (4.4 mmol/L) was performed during each interval between measurements. The line to the lactate sensor was switched as follows:

- Blood sample (20 s)
- Buffer (30 s)
- Lactate standard (20 s)
- Buffer (30 s).

We examined interference with the results by ascorbic acid, bilirubin, hemoglobin, creatinine, uric acid, and glucose. We added concentrated solutions of each of these substances to different blood samples and compared the lactate values with those of a blank blood sample without this addition.

For the correlation study, we used blood samples from nine healthy volunteers before and after exercise. Before beginning the study, we obtained informed consent from all participants. Immediately after sampling the blood with a syringe, we transferred the blood to a test tube, placed the catheter in the tube, and measured the lactate concentration. Another portion of the blood sample was treated with perchloric acid and assayed by a comparison method. The comparison method, a Determiner LA kit (Kyowa Medix, Tokyo, Japan), is based on the same reaction principle as that of the analyzer, except that the color develops in the presence of N-ethyl-N-(3-methylphenyl)-N'-acetylmethylendiamine.

During physical exercise, a catheter was inserted into a vein in the forearm of each volunteer and fixed in place. The subjects then exercised on a treadmill and the workload was increased until the subjects reached exhaustion. The speed and slope of the treadmill started at 2.4 km/h and 0%, accelerating every 3 min by 0.8 km/h with a 4% increase in the slope.

**Results**

The time for a single lactate measurement from sampling to printing of the final result was 3 min. The lower limit of detection by the system was 0.1 mmol/L, and flushing the sensor with buffer for 30 s prevented
carryover completely. The standard curve was linear for lactate concentrations as great as 23 mmol/L. Table 1 shows precision of the lactate analyzer and the comparison (kit) method in the assay of blood samples with three different concentrations; the precision of the lactate analyzer seems acceptable compared with that of the kit. Ascorbic acid at a final concentration of 5.7 mmol/L, bilirubin at 0.29 mmol/L, hemoglobin at 0.22 mmol/L, creatinine at 4.4 mmol/L, uric acid at 3.0 mmol/L, or glucose at 56 mmol/L had a negligible effect on the results of blood lactate measurement. The correlation between lactate concentrations in 58 blood samples from the subjects before and after exercise, measured by the lactate analyzer (y) and by the comparison method (x, which is the usual method in Japan) was \( r = 0.993 \) (\( y = 1.036x + 0.068 \)). The effectiveness of the enzyme membrane, checked electrically during measurements of an internal lactate standard, lasted for six weeks when the analyzer was run for 2 h a day, five days a week.

The changes in blood lactate concentrations in the nine healthy volunteers during exercise on a treadmill until they reached exhaustion are shown in Figure 3. The mean (SD) lactate concentration in these subjects at the start of exercise was 0.90 (0.27) mmol/L, increasing to 9.41 (3.27) mmol/L at the end of exercise, and at a maximum 2 to 6 min after exercise ended (12.16 ± 4.49 mmol/L).

**Discussion**

Measurement of lactate concentrations by conventional methods requires some treatment of blood samples, e.g., deproteinization by perchloric acid, because of the instability of lactate after blood is collected. An electrode analyzer with an enzymatic sensor can measure lactate concentrations directly in whole blood, overcoming certain limitations of conventional enzymatic assays of lactate, which are time-consuming and technically complex (4, 5). The test-strip method for measuring lactate in whole blood also provides rapid and precise measurements of lactate concentrations (6). The importance of lactate in energy metabolism during physical exercise is now known and the anaerobic threshold, shown as either an increase in blood concentrations of lactate or a disproportionate increase in ventilation, is used to estimate the intensity of exercise (1). The lactate analyzer is designed to monitor lactate concentrations in blood, and because it is portable (it weighs about 10 kg), it can be used during exercise on a treadmill or an ergometer.

The standard curve was linear up to 23 mmol/L, which exceeded the maximum concentrations of blood lactate during exercise in our subjects. Each subject had a low concentration of blood lactate for 8 to 10 min after exercise started, after which the blood lactate concentration began to increase. This point, which indicated the anaerobic threshold, could be clearly identified because the lactate concentrations were stable before increasing. A more useful role of lactate measurements might be in quantifying regional ischemia, in which continuous monitoring of lactate, if coupled with blood-flow measurements, would yield lactate production/extraction data. We conclude that the lactate analyzer, with its continuous blood sampling, can measure lactate concentrations precisely in blood and can detect small changes in lactate concentrations during exercise.

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**References**