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Note

Determination of pyruvate, lactate, acetoacetate, and 3-hydroxybutyrate in serum by capillary isotachophoresis

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In numerous pathological states the concentration of organic acids in the blood increases enormously and the resulting acidosis can lead to acidotic coma and even to death. Prompt determination of organic acids in the blood is therefore of diagnostic and prognostic importance. Pyruvate and lactate concentrations provide information on the state of tissue oxidation [1, 2], acetoacetate and 3-hydroxybutyrate are significant for the monitoring of ketoacidosis, usually as a consequence of diabetes mellitus. The last three anions are the most important for disorders of the acid—base balance [3].

At present, the so-called anion gap ($[Na^+] + [K^+] - [Cl^-] - [HCO_3^-]$) is usually used to evaluate metabolic acidosis; however, it provides only rough information on the content of organic acids.

The photometric determination of these acids in blood is not very sensitive [4] and thus enzymatic [4—8] methods are usually recommended [4] despite the fact that they require pure enzyme preparations, and some other organic acids present in blood (for example, 2-hydroxybutyrate) affect the determination. Chromatographic methods [9] are laborious and time-consuming [4].

In the present paper we show that isotachophoresis may serve as a method suitable for the quantitation of the organic acids mentioned by fast, direct analysis of blood serum.

EXPERIMENTAL

All the chemicals used were of analytical grade, provided by Lachema, Brno, Czechoslovakia, with the exception of sodium 3-hydroxybutyrate (BDH Chemicals, Poole, Great Britain), sodium pyruvate, lithium lactate (both from E. Merck, Darmstadt, G.F.R.), 2-hydroxybutyric acid (Fluka, Buchs, Switzer-

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land), β-alanine (Loba Chemie, Vienna, Austria), and acetoacetic acid (prepared by hydrolysis of the ethyl ester of acetoacetic acid [10]).

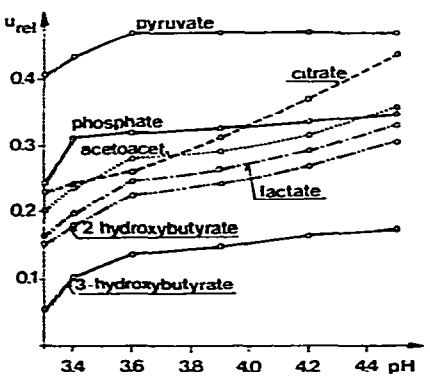
The blood samples were clotted and centrifuged in the cold for 10 min at 1000 *g*. The supernatants were immediately frozen and stored at −20°C until analysis.

Analyses were performed at room temperature in a capillary isotachophoretic column (0.2 × 1 × 200 mm). Zones were detected by a potential gradient detector with two platinum contacts placed in the capillary ca. 0.05 mm apart. A power supply, with a stabilized d.c. current up to 400 μA and with a maximum voltage of 16 kV, was connected to the column. A detailed description of the instrumentation can be found elsewhere [11, 12]. A Perkin-Elmer Model 196 line recorder was used. Leading electrolytes were prepared by adding β-alanine to 10 mmole/l HCl containing 0.3% of polyethyiene glycol until the pH of the solution reached the required value.

Relative mobilities were determined as the ratio of the step height of the leading ion (Cl^-) to the step height of the compound under investigation.

RESULTS AND DISCUSSION

When selecting working conditions for the separation of the acids mentioned above, it is necessary to take into consideration some other blood anions that could interfere with the analysis. Preliminary experiments indicated phosphate, citrate, and 2-hydroxybutyrate. The dependence of the relative mobilities of the respective substances on the pH of the leading electrolyte is presented in Fig. 1. Analyses of model mixtures showed poor separation of lactate and acetoacetate at pH higher than 4. At pH 3.5—4, the pair of lactate and 2-



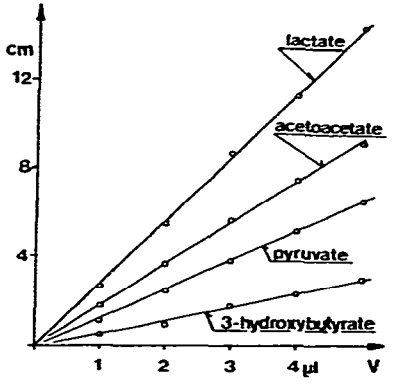


Fig. 1. Dependence of the relative effective mobilities of the investigated acids on the pH of the leading electrolyte. Leading electrolyte: 10 mmole/l HCl, 0.3% polyethylene glycol + β-alanine. Terminating electrolyte: 10 mmole/l propionic acid.

Fig. 2. Dependence of step length (cm) in the trace on the amount analysed. Sample injected: 1—5 μl of the standard mixture containing 5.6, 10, 15 and 19.3 mmole/l 3-hydroxybutyrate, pyruvate, acetoacetate and lactate, respectively. Leading electrolyte: 10 mmole/l HCl, 0.3% polyethylene glycol + β-alanine (pH 3.3). Terminating electrolyte: 10 mmole/l propionic acid. Driving current: 200 μA. Chart speed: 8 cm · min^{-1}.

hydroxybutyrate and that of acetoacetate and citrate were poorly separated from one another. At pH 3.3 all anions shown in Fig. 1 were well separated and the time required for the analysis was acceptable. In the pH range 3.0—3.3 it was possible to separate even some other anions (for example, malate and 2-ketoglutarate); however, the analysis time was longer than 25 min. More detailed experiments showed that these last anions do not interfere with the analysis, and thus pH 3.3 was selected as the optimum.

For quantitation the dependences of the step lengths on the trace upon the amounts injected were measured and are presented in Fig. 2. The dependences were linear, with linear correlation coefficients of 0.9994, 0.9993, 0.9998 and 0.9989 and standard deviations of the regression line of $6.50 \cdot 10^{-10}$, $10.41 \cdot 10^{-10}$, $8.23 \cdot 10^{-10}$, and $4.87 \cdot 10^{-10}$ mole for pyruvate, acetoacetate, lactate and 3-hydroxybutyrate, respectively. The relative standard deviations for the mean of the calibration range were 2.57, 2.77, 1.70, and 3.63%, respectively.

TABLE I

CONCENTRATIONS OF ANALYSED ORGANIC ACIDS IN SELECTED SERA

Results are expressed in mmole/l.

Serum No.	Pyruvate	Acetoacetate	Lactate	3-Hydroxybutyrate
1	0.08	0.09	1.67	<0.03
2	0.11	0.99	3.24	1.18
3	0.06	0.33	2.57	0.73
4	0.05	0.11	3.16	<0.03
5	0.08	0.08	3.76	0.12
6	0.04	0.32	3.53	0.54
7	0.05	0.59	2.75	7.19

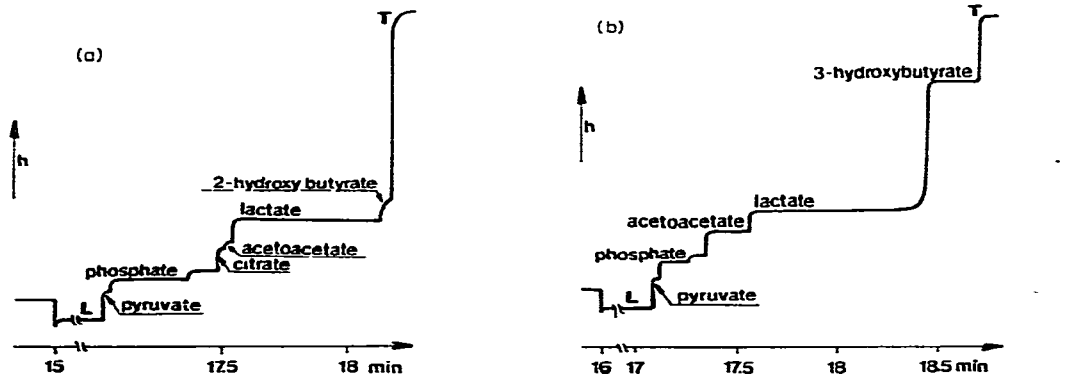


Fig. 3. Analysis of human serum. Sample injected: 15 μl of human serum. (a) Serum of a healthy man (sample No. 1 from Table I). (b) Serum of a patient with diabetes mellitus (sample No. 2 from Table I). For conditions of separation see Fig. 2. The separation proceeded for the first 15 min with a driving current of 300 μA, then it was switched to 200 μA.

For a rough indication of the content of organic acids in normal serum, we analysed sera from three healthy individuals and found values in the range 0.06—0.09 mmole/l, 0.08—0.11 mmole/l, 1.19—1.67 mmole/l, and less than 0.03 mmole/l for pyruvate, acetoacetate, lactate, and 3-hydroxybutyrate, respectively. The concentrations of these acids in selected sera are given in Table I. Serum No. 1 is from a healthy man, the other sera are from acidotic patients. All acidotic sera had elevated lactate levels, and some of them elevated acetoacetate and/or 3-hydroxybutyrate levels. We can thus distinguish between lactic acidosis (serum No. 4) and ketoacidosis (serum No. 7) and their mixed forms. Analysis of sera Nos. 1 and 2 is shown in Figs. 3a and b, respectively.

The isotachophoretic analysis of blood acids is very fast; it needs neither deproteinization, nor derivatization of the sample, and the separation time does not exceed 20 min. Quantitation is easy by simple measurement of the step lengths. Moreover, other pathological blood acids can be analysed simultaneously in one separation run. All these advantages predetermine isotachophoresis to be a useful diagnostic and prognostic tool in intensive medicine.

REFERENCES

1 R.D. Cohen and R. Simpson, Anesthesiology, 43 (1975) 661.
2 A. Kazda, J. Hendl, B. Nejedlý, V. Vacek, S. Dvořáková and D. Miloschevský, Vnitř. Lék., 25 (1979) 685.
3 B. Nejedlý, Milieu Intérieur, Clinical Biochemistry and Use, Avicenum, Prague, 1980 (in Czech).
4 E. Hultman, in H.Ch. Curtius and M. Roth (Editors), Clinical Biochemistry, Principles and Methods, W. de Gruyter, Berlin, 1974, pp. 908—930.
5 C.P. Price, B. Lloyd and K.G.M.M. Alberti, Clin. Chem., 23 (1977) 1893.
6 J.L. Hansen and E.F. Freier, Clin. Chem., 24 (1978) 475.
7 B. Lloyd, J. Burrin, P. Smythe and K.G.M.M. Alberti, Clin. Chem., 24 (1978) 1724.
8 G.A. Noy, A.L.J. Buckle and K.G.M.M. Alberti, Clin. Chim. Acta, 89 (1978) 135.
9 L. Siegel, N.I. Robin and L.J. McDonald, Clin. Chem., 23 (1977) 46.
10 R.C. Krueger, J. Amer. Chem. Soc., 74 (1952) 5536.
11 P. Boček, M. Deml and J. Janák, J. Chromatogr., 106 (1975) 283.
12 M. Deml, P. Boček and J. Janák, J. Chromatogr., 109 (1975) 49.