References

1. Ascheim, S., and Zondek, B., Hypophysenvorderlappenhormon and Ovarialhormon im Harn von Schwangeren. *Klin. Wochenschr.* **6**, 1322 (1927).

2. Mishell, D. R., Wide, L., and Gemzell, C., Immunologic determination of human chorionic gonadotropin in serum. *J. Clin Endocrinol. Metab.* **23**, 125–131 (1963).

3. Braunstein, G. D., Vaitukaitis, J. L., Carbone, P. P., and Ross, G. T., Ectopic production of human chorionic gonadotropin by neoplasms. *Ann. Intern. Med.* **78**, 39–45 (1973).

4. Wide, L., Early diagnosis of pregnancy. *Lancet* **ii**, 863–864 (1969).

5. Midgley, A. R., Radioimmunoassay: A method for human chorionic gonadotropin and human luteinizing hormone. *Endocrinology* **79**, 10–18 (1966).

6. Halpern, B., Eckman, T. R., and Dolkart, R. E., Seven hour radioimmunoassay of human chorionic gonadotropin. *Am. J. Obstet. Gynecol.* **110**, 412–414 (1971).

7. Odell, W. D., Ross, G. T., and Rayford, P. L., Radioimmunoassay for human luteinizing hormone. *Metabolism* **15**, 287–289 (1966).

8. Wide, L., Roos, P., and Gemzell, C., Immunological determination of human pituitary hormone (L.H.). *Acta Endocrinol.* **37**, 445–449 (1961).

9. Closset, J., Hennen, G., and Lequin, R. M., Human luteinizing hormone. The amino acid sequence of the beta subunit. *FEBS Lett.* **29**, 97–100 (1973).

10. Goldstein, D. P., and Kosasa, T. S., The beta subunit radioimmunoassay for human chorionic gonadotropin—clinical applications. In *Progress in Gynecology*, **6**, M. L. Taymor and T. C. Green, Eds., Grune and Stratton, New York, NY, 1975, pp 145–184.

11. Amir, S. M., Dissociation of glycoprotein hormones. *Acta Endocrinol.* **70**, 21–34 (1972).

12. Morgan, F. T., and Canfield, R. E., Nature of the subunits of human chorionic gonadotropin. *Endocrinology* **88**, 1045–1053 (1971).

13. Chen, H. C., Ayala, A. R., Hodgen, G. D., et al., First specific assay for chorionic gonadotropin in human urinary extracts. *Clin. Res.* **24**, 375A (1976).

14. Ayala, A. R., Nisula, B. C., Chen, H. C., et al., Highly sensitive radioimmunoassay for chorionic gonadotropin in human urine. *J. Clin. Endocrinol. Metab.* **47**, 767–773 (1978).

15. Bahl, O. P., Pandian, M. R., and Ghai, R. D., Immunological properties of the beta subunit of human chorionic gonadotropin. *Biochem. Biophys. Res. Commun.* **70**, 525–532 (1976).

16. Vaitukaitis, J. L., Braunstein, G. D., and Ross, G. T., A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. *Am. J. Obstet. Gynecol.* **113**, 751–758 (1972).

17. Jones, W. B., Lewis, J. L., and Lehr, M., Monitor of chemotherapy in gestational trophoblastic neoplasm by radioimmunoassay of the beta subunit of human chorionic gonadotropin. *Am. J. Obstet. Gynecol.* **121**, 669–673 (1975).

18. Kardana, A., and Bagshawe, K. D., A rapid sensitive and specific radioimmunoassay for human chorionic gonadotropin. *J. Immunol. Methods* **9**, 297–305 (1976).

19. Soules, M. R., Tyrey, L., and Hammond, C. B., The utility of a rapid assay for human chorionic gonadotropin in the management of trophoblastic disease. *Am. J. Obstet. Gynecol.* **135**, 384–392 (1979).

20. Pastofide, G. B., Goldstein, D. P., and Kosasa, R. S., The use of a radioimmunoassay specific for human chorionic gonadotropin in patients with molar pregnancy and gestational trophoblastic disease. *Am. J. Obstet. Gynecol.* **120**, 1025–1028 (1974).

21. Boyko, W. L., and Barrett, B., Detection and quantitation of the β-subunit of human chorionic gonadotropin in serum by radioimmunoassay. *Fertil. Steril.* **33**, 141–150 (1980).

22. Hunter, W. M., and Bennie, J. G., Reduction of non-specific serum responses in human pituitary gonadotropin radioimmunoassays. *J. Endocrinol.* **80**, 59–68 (1979).

CLIN. CHEM. 26/13, 1895–1897 (1980)

Conductometric Titration of Hydrochloric Acid in Gastric Juice

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We describe a procedure for the determination of hydrochloric acid concentration in gastric juice by means of a conductometric titration of the 50-fold diluted sample with an aqueous ammonia solution. The conductometric method of endpoint indication leads to a definite location of the equivalence point in the titration of hydrochloric acid. The proposed method is simple and accurate and shows a good correlation with an accepted method for the measurement of gastric acidity.

The determination of hydrochloric acid in gastric juice by titration to a fixed potentiometric endpoint is incorrect on a theoretical basis, because of the presence of weak acids and their salts in gastric juice (*1*). Moore and Scarlata (*2, 3*) proposed a method by which the hydrogen-ion concentration is calculated from pH measurements with the aid of tabulated activity coefficients. In this paper we propose an alternative solution to the analytical problem of titrating a mixture of a strong acid in the presence of a weak acid, namely, conductometric titration of the 50-fold diluted sample.

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Materials and Methods

Gastric juice was obtained by a standard procedure (*4*) after an overnight fast and after stimulation with pentagastrin, 6 μg/kg of body weight. After being centrifuged, the clear samples were analyzed on the day of collection. Samples grossly contaminated with blood or bile were discarded.

Potentiometric measurements were performed at 25 °C with an IL 305 pH meter (Instrumentation Laboratory S.p.A., 3-20037 Paderno Dugnano, Italy) and an Elkay OHP-1433-U combined pH reference electrode (Elkay Products Inc., Worcester, MA 01613). The pH meter was calibrated with a phosphate buffer (25 mmol/L; pH 6.865 at 25 °C) and with a tetroxalate buffer (50 mmol/L; pH 1.679 at 25 °C).

Conductometric Titrations

Pipette a 2-mL volume of gastric juice into a suitable titration vessel and dilute to 1000 mL with distilled water. Standardize the titration reagent, 0.1 mol/L NH_4OH, against

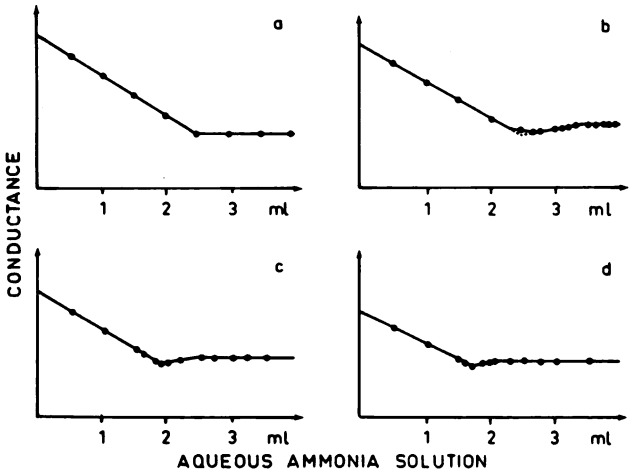

Fig. 1. Typical conductometric titration curves obtained after titration of: *a*, 0.1 mol/L hydrochloric acid; *b*, 0.1 mol/L hydrochloric acid and 50 mmol/L acetic acid; *c*, 0.1 mol/L hydrochloric acid and 20 mmol/L trisodium phosphate; *d*, gastric juice

Solutions titrated with aqueous ammonia

0.1000 mol/L HCl, and place it in a 5-mL microburette. Add the NH_4OH reagent in small portions, stirring the solution after each addition.

The conductivity may be measured with any available set-up. In our laboratory we use a Seybold LTB conductometer equipped with a conductivity cell of cell constant 1.45 cm^{-1} (Seybold, A-1010 Vienna, Austria). The conductivity is measured after the well-mixed solution has been allowed to stand for a minute or two.

Continue adding the 0.1 mmol/L NH_4OH until at least five readings beyond the equivalence point have been made. The exact equivalence point is derived from the titration curve. A review of the theory and practice of conductometric titration is given in references *5* and *6*.

Results

Conductometric titration curves were determined by using the proposed method in "model systems" of (*a*) a strong acid, (*b*) a strong acid/weak acid mixture, and (*c*) a strong acid in the presence of the salt of a weak acid. Figure 1 shows the resulting conductometric titration curves for these systems and for a single sample of gastric juice. Using the proposed method, we determined the hydrochloric acid concentrations of 49 samples of gastric juice. Hydrogen-ion concentrations were also determined in these samples as described by Moore and Scarlata. The results, expressed as hydrogen ion concentration vs hydrochloric acid concentration or as solution pH were correlated with each other in Figures 2 and 3. Figure 3 also shows the conductometric results as transformed by use of the hydrogen-ion activity data of Moore and Scarlata.

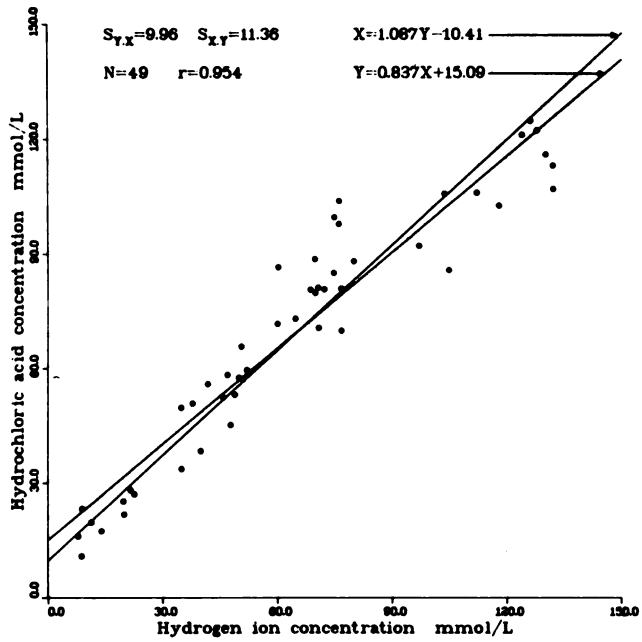

Fig. 2. Measurement of gastric acidity by comparison of hydrochloric acid concentration vs hydrogen ion concentration (determined according to Moore and Scarlata)

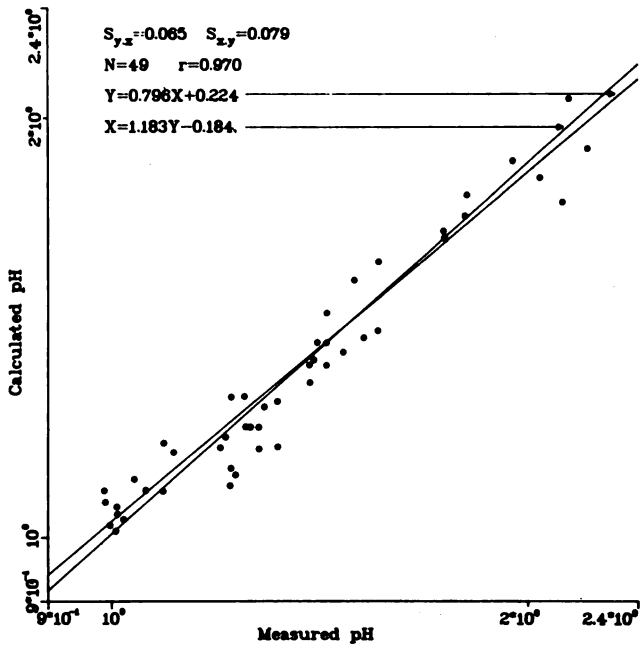

Fig. 3. Measurement of pH in gastric juice: comparison of experimentally determined pH vs pH values as calculated from titrimetrically determined hydrochloric acid concentrations and the tabulated activity coefficients from Moore and Scarlata

Discussion

The direct titration of gastric juice acidity presents a number of analytical problems, because the pH at the stoichiometrical equivalence point cannot be predicted in advance; moreover, the potentiometric titrations are difficult to perform on undiluted and sometimes viscous material.

Hydrochloric acid is the only strong acid of clinical significance in gastric juice. Thus the proposed method, although not specific for hydrochloric acid, may be used in gastric juice analysis.

A reasonable correlation (r = 0.95) was found between the hydrochloric acid concentration determined with the proposed method and the hydrogen ion concentration determined by the method of Moore and Scarlata. These results, expressed as solution pHs, showed a somewhat better correlation (r = 0.97) after transformation by use of the activity coefficients quoted by these authors.

In each scattergram the standard errors of estimate were determined for both methods ($S_{x.y}$, $S_{y.x}$). The differences between these standard errors were not significant.

We suggest that problems associated with electrode standardization and the measurement of pHs in the low pH region (e.g., >100 mmol/L hydrochloric acid concentration) contribute to the relatively larger scatter in these correlations.

The proposed method has an advantage over that of Moore and Scarlata, in that pHs of gastric juice do not have to be measured in a region of low pH, where small variations of pH produce relatively large variations in hydrogen-ion concentrations after antilog transformation. Conversely, where acid concentration is less than 25 mmol/L the proposed method is somewhat less accurate than that of Moore and Scarlata, at least with the titrimetric equipment specified in *Materials and Methods*.

The conductometric titration of hydrochloric acid in gastric secretions is simple and accurate and deserves consideration as the method of choice in those cases that demand an accurate determination of hydrochloric acid secretion, e.g., determination of hydrochloric acid concentration in gastric secretion of patients suffering from Zollinger–Ellison syndrome and in gastric secretions obtained during the insulin stimulation test.

References

1. Lubran, M., Measurement of gastric acidity. *Lancet* **ii**, 1070–1071 (1966).
2. Moore, E. W., and Scarlata, R. W., The determination of gastric acidity by the glass electrode. *Gastroenterology* **49**, 178–188 (1965).
3. Moore, E. W., Determination of pH by the glass electrode: pH meter calibration for gastric analysis. *Gastroenterology* **54**, 501–507 (1968).
4. Sun, D. C. H., and Roth, J. L. A., Test employed in analysis of the stomach contents and their clinical application. In *Gastroenterology*, **1**, H.L. Bockus, Ed., W. B. Saunders, Philadelphia, 1963, pp 419–453.
5. West Loveland, J., Conductometry and oscillometry. In *Treatise on Analytical Chemistry I*, **4**, I. M. Kolthoff, and P. J. Elving, Eds. Interscience Publishers, New York, NY, 1963, pp 2569–2630.
6. Kraft, G., and Fisher, J., *Indikation von Titrationen.* Walter de Gruyter, Berlin, 1972, pp 218–251.

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Multivariate Analysis of an Enzymic Profile for the Differential Diagnosis of Viral Hepatitis

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Differential diagnosis of acute viral hepatitis, persistent chronic hepatitis, aggressive chronic hepatitis, and post-necrotic cirrhosis can reasonably be achieved on the basis of three well-known liver-function tests: aspartate aminotransferase, alanine aminotransferase, and glutamate dehydrogenase. With use of principal-component analysis, these four liver diseases can be characterized by two criteria: a "cytolytic" criterion, correlated particularly with a membrane-permeability test—namely, alanine aminotransferase activity—and a "mitochondrial damage" criterion, which is associated with above-normal ornithine carbamyltransferase and glutamate dehydrogenase activities.

Assessing the permeability of the hepatocyte membrane by assay of a single enzyme is difficult. To avoid superfluous information from multiple assays, however, a selection must be made. On the basis of cytolytic and mitochondrial damage criteria, four enzymes seem to be of interest: AST, ALT, OCT, and GlDH.[1]

To characterize diseases by use of results for several biochemical parameters, various investigators have used multivariate statistical techniques (*1–4*). We applied stepwise discriminant analysis to evaluate the utility of these four enzymes in the differential diagnosis of liver diseases. We used principal-component analysis, which expresses the total variability of all the groups of patients, to establish the interrelationships among these four tests.

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[1] Nonstandard abbreviations used: AST, aspartate aminotransferase, EC 2.6.1.1; ALT, alanine aminotransferase, EC 2.6.1.2; OCT, ornithine carbamoyltransferase, EC 2.1.3.3; and GlDH, glutamate dehydrogenase, EC 1.4.1.3.

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Study Population

Our study population consisted of 57 cases of acute viral hepatitis, 44 cases of persistent chronic hepatitis, 40 cases of aggressive chronic hepatitis, and 77 cases of post-necrotic cirrhosis.

The diagnosis of acute viral hepatitis was based on classical clinico-biological signs. All other patients were diagnosed on the basis of laparoscopy and biopsy data. The cases of chronic hepatitis were subdivided in accordance with the classification proposed in 1968 by Degroote et al. (*5*).

Methods

Enzyme Activities

Plasma AST, ALT, OCT, and GlDH were determined in each subject.

"Optimized" methods (reagents from E. Merck, D-1600 Darmstadt, F.R.G.) were used to measure the activities of AST (cat. no. 3397) (*6*), ALT (cat. no. 3398) (*6*), and GlDH (cat. no. 3373) (*7*). OCT was determined with a continuous-flow analyzer (Technicon Corp., Tarrytown, NY 10591) according to the method of Strandjord and Clayson (*8*).

Data Analysis

Canonical analysis. The principles of this statistical technique are described in ref. *9*.

Stepwise discriminant analysis. Stepwise discriminant analysis was performed by using the program BMDP 7M (*10*). The percentage of patients correctly classified is called the "Diagnostic Effectiveness" (*1*).

Principal-component analysis. This descriptive technique