and 2.0% for patients’ samples having mean total CO$_2$ of 7 and 35 mmol/L, respectively (n = 27). Least-squares linear-regression analysis of the total CO$_2$ values obtained from the potentiometric ($x$) and new enzymatic slides (y) for 113 clinical samples (range 15–41 mmol/L) showed the following relationship: $y = 0.92x + 0.35$ (r = 0.976, P < 0.01) (4). Only samples assayed within 5 min of each other by the two methods were included in the comparison.

Post-production evaluation confirmed the above results: an analytical range of 5–40 mmol/L, and a relationship to the potentiometric method represented by the equation $y = 1.06x + 0.01$ (n = 59).

Importantly, nitrate/nitrite did not interfere in the new enzymatic slide, no effect on total CO$_2$ values being noted with as much as 15 mmol/L cerosus nitrate added to patients’ sera. Furthermore, we observed no interference from icteric (bilirubin ≥270 mg/L), lipemic (triglyceride ≥8850 mg/L), or hemolyzed (hemoglobin ≥5000 mg/L) samples. The latter finding is notable because some enzymatic total CO$_2$ methods are prone to interference from hemolyzed samples (5). The new enzymatic total CO$_2$ slides were stable for at least 36 days when stored on the Ektachem instrument.

Overall, the enzymatic CO$_2$ slide appears to be a viable alternative to the potentiometric method and is not subject to significant nitrate ion interference. It also eliminates the maintenance requirements inherent to potentiometry (and recommended by the manufacturer) such as regular cleaning of the electrolyte reference fluid (ERF) reservoir and warming of the ERF reservoir temperature before use.

References

Emad W. Daoud1
Adrian McClellan2
Mitchell G. Scott1

1 Washington Univ. Sch. of Med.
Depts. of Pathol. and Med., Box 8118
660 S. Euclid Ave.
St. Louis, MO 63110
2 Barnes Hospital
Dept. of Labs.
St. Louis, MO 63110

Oligoclonal Banding in Sera of Hospitalized Patients

To the Editor:

The paper by Myara et al. (1) on monoclonal and oligoclonal gammopathies in heart-transplant recipients is one of several in recent years describing oligoclonal immunoglobulin abnormalities in specific clinical situations such as transplantation (1–3) or HIV infection (4, 5). These papers, including the study of Myara et al., generally admit that the oligoclonal-banding phenomenon is not specific for the disease being studied, but nevertheless imply that there is some specificity by providing a list of several other conditions in which the phenomenon has been noted.

I showed some years ago that the presence of one or several monoclonal bands is common in the serum of hospitalized patients when examined by a high-resolution electrophoresis technique (6). The phenomenon is not specific for any group of clinical disorders. However, it is seen more often when there is evidence of immune system activation, e.g., a high gamma globulin concentration or an acute-phase pattern on electrophoresis. The oligoclonal banding phenomenon may be related to the presence of circulating immune complexes (7, 8).

Although the presence of an oligoclonal pattern in association with a specific disease may help provide information on the pathophysiologial process involved, I wish to re-emphasize that the significance of this phenomenon in any given disease must be evaluated in the context of its common occurrence in patients with evidence of immune system activation.

References

Albert A. Keshegian
Dept. of Pathol.
Bryn Mawr Hospital
130 S. Bryn Mawr Ave.
Bryn Mawr, PA 19010

Negative Interference by Ethamsylate in Enzymatic Assay of Serum Creatinine Involving Peroxidase-Coupled Reaction

To the Editor:

The quantitative determination of creatinine in serum and urine has long been performed by the Jaffé method (1). Recent enzymatic methods for serum creatinine have been established. Of the coupled-enzyme systems used to determine creatinine, the peroxidase (POD; EC 1.11.1.7)-coupled method is superior because of its sensitivity and easy application to the automated analyzer (2).

The serum creatinine value in a patient with renal dysfunction who was taking ethamsylate (3)[(diethylammonium 2,5-dihydroxybenzenesulfonate (a drug for stabilizing capillaries)] was 22 mg/L by an automated POD method (One Pharmaceutical Co., Osaka, Japan), 83 mg/L by the Jaffé reaction-rate method (performed with an Astra 8; Beckman Instrument Inc., Fullerton, CA 92634), 79 mg/L by a deproteinized Jaffé
method (Wako Pure Chemical Co., Osaka, Japan), and 85 mg/L by a coupled-enzyme ultraviolet absorbance method (Boehringer Mannheim Co., Mannheim, F.R.G.). To compare the POD and Jaffé reaction-rate methods, we analyzed 37 serum samples obtained from different patients and observed good correlation except for this patient’s sample. Analytical recoveries of standard solution added to this patient’s serum were 77–89% by the POD method and 101–102% by the Jaffé reaction-rate method.

To confirm the molecular nature of inhibitory factor, we fractionated the patient’s serum with an FPLC system (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). The substance that was eluted in the fraction of very low molecular mass had an inhibitory effect in the POD method. Pure diethylammonium 2,5-dihydroxybenzenesulfonate also eluted in the same position, indicating that the inhibitory factor was ethamsylate itself.

The direct inhibitory effect of ethamsylate on coloration in the POD method was confirmed in vitro. To clarify the in vivo effect, a healthy subject ingested three tablets of ethamsylate and supplied blood samples every 30 or 60 min for 5 h. The serum samples obtained were analyzed with an identical volume of creatinine standard solution (100 mg/L) and analyzed for creatinine (Figure 1). Fixed creatinine values, 49–51 mg/L, were obtained by the Jaffé reaction-rate method during the 5 h, but the creatinine concentration measured by the POD method was reduced to 40 mg/L after 3 h and to 30 mg/L after 4 h.

In this study, we found that ethamsylate interfered negatively with the color development in the creatinine assay by POD-coupled reaction. In the reaction with POD, the rate constant (K) for hydrogen peroxide as hydrogen acceptor is 9 × 10⁹ L mol⁻¹ s⁻¹ (4), so that hydrogen peroxide reacts with POD very fast as hydrogen acceptor. Moreover, many compounds act as hydrogen donors with the POD reaction, e.g., uric acid, ascorbic acid (K = 2 × 10⁹), and pyrogallol (K = 3 × 10⁹). The fundamental structure of ethamsylate is a hydroquinone ring; the rate constant for hydroquinone as hydrogen donor is K = 3 × 10⁸ (4). Thus, hydrogen peroxide will be consumed by the ethamsylate in the presence of POD to form colorless products. Consequently, ethamsylate causes negative interference in assays based on the POD reaction.

We should carefully evaluate clinical laboratory data obtained by the methods involving POD reactions in patients taking ethamsylate.

![Graph](image)

**Fig. 1.** Effect of ethamsylate, administered to a healthy subject, on creatinine assayed in the subject’s serum.

Methods obtained by the Jaffé reaction-rate method; ○, results of the POD method.

**References**


**Development of an Immunoradiometric Assay Kit for Ventricular Myosin Light Chain I with Monoclonal Antibodies**

**To the Editor:**

Trahern et al. (1) demonstrated by radioimmunoassay the presence of cardiac myosin light chains (MLC) in the serum of patients with acute myocardial infarction. Nagai et al. (2) demonstrated both a unique time course curve for serum myosin light chain II (MLCII), after acute myocardial infarction in dogs, and a good correlation between the serum MLCII and histologically determined myocardial infarct size. Isobe et al. (3) showed that serum cardiac myosin light chain I (MLCI), unlike serum creatine kinase (CK), reflected infarct size, as determined by left ventriculography, four weeks after attack even when the patient received coronary reperfusion treatment. We describe a simple one-step assay for human ventricular MLCI and clinical findings with this assay.

Myosin from human ventricular myocardium, atrial myocardium, skeletal muscle, and smooth muscle was extracted by the dilution technique (4), then denatured with guanidine to dissociate the light chains. Light chains I and II were separated by preparative disc gel electrophoresis (5). Protein concentrations of these materials were determined by the method of Lowry et al. (6), with bovine serum albumin as a standard. BALB/c mice were immunized by five or six biweekly intraperitoneal injections of 10 μg of human ventricular MLCI in 50 μL of complete Freund's adjuvant, then received a booster injection of 10 μg of human ventricular MLCI intravenously two weeks after the last immunization. Three days later, the spleen cells were fused with P3-X63-Ag8-U1 by the method of Köhler and Milstein (7). Using an enzyme-linked immunosassay (8), we screened for hybridomas producing anti-human ventricular MLCI antibody. Expansion of positive clones, cloning, and propagation of the cloned hybridomas in ascitic fluid were performed by a standard method (9). Monoclonal antibodies were purified from the ascites by adding an equal volume of saturated ammonium sulfate, dissolving the precipitate in 0.1 mol/L Tri·HCl, pH 7.6, and dialyzing this solution against the same buffer. The dialyzed solution was passed through a DE52 column (Whatman, Maidstone, U.K.) column equilibrated in the same buffer. The flow-through fractions were collected and concentrated with an XM50 ultrafiltration membrane (Amicon, Danvers, MA). Gel filtration of the concentrated solution was performed with ACA44 (LKB, Bromma, Sweden) equilibrated with phosphate-buffered saline (PBS). Antibody MLM508 was iodinated with 125I (Amer sham, Bucks, U.K.) by the Chloramine-T method (10).