Fig. 1. Effect of creatinine (□), uric acid (▲), urea (×), and NaCl (○) on determination of albumin with bromphenol blue

All points are mean values of fivefold determinations. Concentrations indicated are final concentrations in the reaction mixture

munonephelometry) have been recommended for this purpose.

In order to use the simple and inexpensive dye-binding method with bromphenol blue (1), we tried to improve the analytical sensitivity by increasing the ratio of urinary sample to reagent volume. To detect microalbuminuria (20 mg/L) with sufficient sensitivity, we found, in model experiments with solutions of human albumin, that this ratio had to be about 1:2. Then we added known amounts of human albumin (Impfstoffwerke, Dessau, G.D.R.) to various urine specimens and made recovery experiments. Large differences were observed between measured and calculated values as compared with values obtained by immunodiffusion (VLC-Partigen-Albumin; Behring AG, Marburg/Lahn, F.R.G.). These differences depended both on the sample:reagent volume ratio and on the individual urine investigated. To evaluate interfering reactions of nonprotein components of urine with bromphenol blue, we added 0.5 mL of a 100 mg/L albumin solution to 0.5 mL of urea, uric acid, creatinine, or NaCl solutions of different concentrations. Then we mixed in 0.1 mL of bromphenol blue reagent (2 mmol of bromphenol blue and 8.25 g of Brij 35 per liter of glycine buffer, 1.1 mol/L, pH 3.0) and, 30 s later, measured the absorbance at 610 nm in a spectrophotometer with a 10-mm lightpath (Model DU-8; Beckman Instruments, Fullerton, CA). Interference by creatinine, uric acid, and NaCl with bromphenol blue is clearly demonstrated in Figure 1.

When we consider that creatinine and uric acid, for example, are excreted in concentrations of about 15 and 5 mmol/L, we can already observe positive biases caused by these interferences at a sample:reagent volume ratio of 1:5 (1). However, this ratio does not yet permit measurement of microalbuminuria. Thus, the interfering reactions of urinary components with bromphenol blue preclude use of this dye-binding method for determination of microalbuminuria in native urine samples. These interferences by nonprotein constituents of urine would explain why the Microalbutest (Miles Labs., Ames Div.) showed falsely positive results (2).

References

1. Schosinsky KH, Vargas M, Esquivel AL, Chavarria MA. Simple spectrophotometric determination of urinary albumin by dye-binding with bromphenol blue. Clin Chem 1987;33:223–6.

2. Schmitz A. Microalbutest®: a new screening method for detecting microalbuminuria in diabetes mellitus. Uremia Invest 1986;9:79–84.

Klaus Jung
Erika Nickel

Dept. of Exptl. Organ Transplantation
Univ. Hosp. Charité
Humboldt University Berlin
Leninallee 49
DDR-1017 Berlin, G.D.R.

More on Oligoclonal Bands and Diagnosis of Multiple Sclerosis

To the Editor:

We would like to report our contribution regarding the "dilemma" raised by Stanley et al. (1) and recently discussed by Tate and Campbell (2), concerning the relationship between the diagnosis of multiple sclerosis (MS) and the presence of oligoclonal bands that are unique to the cerebrospinal fluid (CSF).

We considered isoelectric focusing (IEF) patterns for CSF and serum from 94 patients, who met the criteria of McDonald and Halliday (3) for a clinical diagnosis of definite MS. The specimens, collected between January 1985 and July 1988, were all treated in the same way. Essentially, we standardized the concentration of IgG in CSF (by using Amicon CS 15 concentrators) and in serum (by diluting the sera with distilled water) so we could apply a constant amount, 20 μg, of IgG to the

gel surface by use of pieces of filter paper. For the IEF we used thin-layer polyacrylamide gel (Ampholine PAG-plate, pH range 3.5–9.5; LKB), staining with Coomassie Brilliant Blue.

On the basis of the IEF findings, the 94 patients could be divided into three groups. Group I, comprising 66 patients (70%), had oligoclonal bands, numbering three or more, only in their CSF. Group II, 20 patients (21%), had some bands common to both CSF and serum but also some unique to CSF. Group III, eight patients (9%), had no oligoclonal bands in either CSF or in serum. Thus Groups I and II, with at least three or more bands in CSF only, represented 91% of all these patients.

One patient affected by both MS and, in association, Sjögren's syndrome (specific antibodies to SSA/Ro present in his serum) had multiple oligoclonal in his CSF and also three that appeared in IEF patterns for both CSF and serum. This might result from the combination of two different immunological mechanisms, functioning in the systemic and intrathecal compartments, respectively.

We also found bands unique to CSF, together with some others paired in CSF and in serum, in 78% (18 of 23) cases of AIDS with central nervous system involvement.

In a group of other neurological diseases (e.g., extrapyramidal degenerative disease, neurosyphilis, chronic myelopathy of unknown cause, polyneuropathy, Guillain–Barré syndrome, and dementia) we found some cases that showed, on IEF, patterns of bands identical in CSF and serum and, occasionally (<5%), bands unique to CSF. Moreover, our preliminary data concerning patients with acute unilateral optic neuritis indicate that those patients who have oligoclonal banding in CSF have an increased risk for future development of MS. This underlines the importance of detecting oligoclonal fractions in CSF from cases of suspected MS, as an aid in early diagnosis of the disease.

In any case, in agreement with Tate and Campbell (2), we never found any patient with clinically definite MS who had an identical banding pattern in both CSF and serum.

References

1. Stanley MJ, Schmierer DM, Millingen KS, Yeo PT. Oligoclonal bands are found in electrophoretograms of serum of patients with multiple sclerosis [Letter]. Clin Chem 1986;32:709.

2. Tate J, Campbell B. Must CSF oligoclonal bands be unique to cerebrospinal fluid and absent from serum to support the

diagnosis of multiple sclerosis? [Letter]. Clin Chem 1988;34:1521–2.

3. McDonald WL, Halliday AM. Diagnosis and classification of multiple sclerosis. Br Med Bull 1977;33:4–8.

Diego Michele Franciotta
Gianvico Melzi D'Eril

Serv. Anal. Chim. Clin. e Neurochim.
Fondazione "C. Mondino"
via Palestro 3
27100 Pavia, Italy

Assessing Metabolic Acidosis

To the Editor:

Kost et al. (*1*) recently reported the case of a 10-year-old boy with diabetic ketoacidosis in whom there was a discrepancy between the value for plasma bicarbonate calculated by the blood-gas analyzer (with use of the Henderson–Hasselbalch equation) and the value for TCO_2 measured in the laboratory. They were able to calculate that at the height of the acidosis the value for pK_1' was 5.49, differing markedly from the 6.1 used by the blood-gas analyzer. We are entirely in agreement with them that the most nearly accurate estimation of the bicarbonate concentration in plasma is obtained by directly measuring the TCO_2 in arterial blood. However, we can not agree with their statement that the assumption of constant pK_1' is no longer tenable when therapeutic decisions are necessary for acid–base management. We maintain that the characterization of an acid–base disturbance provided by the calculated value for plasma bicarbonate, as measured in a blood-gas analyzer, is quite accurate enough for clinical purposes. We have shown this to be the case in the neonate (*2*), and we believe that in the case reported by Kost et al. the value for calculated bicarbonate served its purpose in demonstrating that there was a profound metabolic acidosis. The use of intravenous bicarbonate in the treatment of diabetic ketoacidosis remains controversial (*3*), but we think that most clinicians who use intravenous bicarbonate in these circumstances would prefer to titrate the bicarbonate infusion against a series of arterial blood-gas analyses, rather than expect to calculate a "once-and-for-all" dose aimed at a particular desired plasma bicarbonate concentration. The clinical situation is by its very nature unpredictable because of the interplay of the disease state and the therapy with insulin. Because the greatest deviations in pK_1' are likely to coincide with the extremes of disordered metabolism, the value for plasma bicarbonate calculated by the blood-gas analyzer should become more nearly accurate as the patient improves—and this was indeed what happened in the case reported by Kost et al.

References

1. Kost GJ, Trent JKT, Saeed D. Indications for measurement of total carbon dioxide in arterial blood [Case Report]. Clin Chem 1988;34:1650–2.
2. Masters P, Blackburn MEC, Henderson MJ, Barrett JFR, Dear PRF. Determination of plasma bicarbonate of neonates in intensive care. Clin Chem 1988;34:1483–5.
3. Hale PJ, Crase J, Nattrass M. Metabolic effects of bicarbonate in the treatment of diabetic ketoacidosis. Br Med J 1984;289:1035–7.

P. R. F. Dear
M. J. Henderson

St. James's University Hosp.
Beckett St.
Leeds LS9 7TF, U.K.

Follow-up of Monoclonal Gammopathies in Asymptomatic HIV-Infected Subjects

To the Editor:

Monoclonal gammopathies (MG) have been described in AIDS patients (*1, 2*) and, more recently, in asymptomatic subjects infected with human immunodeficiency virus (HIV) (*3–5*). Because the prognostic value of finding MG in these subjects is not known, we followed up these asymptomatic subjects, to determine whether their outcome differed from that of seropositive subjects without MG. Both groups of subjects were under observation in our unit.

Eight HIV-seropositive subjects (positive by ELISA, with confirmation by Western blot) with MG (six IgG kappa, two IgG lambda) were seen semiannually over a mean period of 25.5 months (range 18–30). The subjects, ages 23–45 y (mean 33) were four homosexual men, two transfusion recipients, one female partner of a high-risk subject, and one subject without known risk factors. All subjects were asymptomatic for AIDS during the study period (stage II or III according to the criteria of the U.S. Centers for Disease Control). MG was identified by immunofixation and quantified by electrophoresis on cellulose acetate. Semestrial observations included physical examination (lymph nodes) and laboratory tests for CD4 and CD8 lymphocyte count; p24 antigen; anti-core (p24 and p17) antibodies; erythrocyte sedimentation rate; concentrations of IgG, IgA, and IgM in serum (quantified by laser nephelometry); and serum protein electrophoresis to detect monoclonal bands. No subject received antiviral therapy before or during the study period. Table 1 gives, for each visit, the concentration of MG and the CD4 lymphocyte count of the eight subjects over the study period. We observed the appearance of MG in five subjects (they did not have MG at the start of the study and were retro-

Table 1. Monoclonal Gammopathies during the Follow-up of Eight Asymptomatic HIV-Seropositive Subjects

	Patients							
	A (II)[a]	B (II)	C (II)	D (II)	E (III)	F (II)	G (II)	H (III)
Semiannual test no.								
1	— (204)[b]	— (622)	9 (400)	2 (350)	— (213)	— (440)	5 (464)	— (656)
2	5 (298)	ND	9 (510)	3 (370)	— (350)	— (420)	5 (467)	ND
3	ND	6 (311)	ND	3.5 (580)	7 (332)	7 (443)	ND	5.4 (640)
4	5 (210)	6 (412)	11 (300)	3.7 (530)	15 + 7[c] (240)	— (310)	ND	ND
5	— (220)	4 (240)	ND	3 + 10[c] (680)	—	— (340)	7 (420)	5 (600)
6		— (410)	11 (380)			— (340)		

ND = not done.

[a] CDC stage at start of and throughout study is listed in parentheses. [b] Results shown are concentration (mg/L) or absence (—) of MG, with number of CD4 lymphocytes per microliter listed parenthetically. [c] A second MG (IgG kappa) was detected at this testing.