

Effect of Pyridostigmine Bromide on Serum Bromide Concentration and the Anion Gap¹

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Bromide (Br) causes positive interference with all clinical laboratory methods for the determination of serum Cl (1,2). Interference with Cl measurement is much greater with ion-selective electrode (ISE) technology (1,3) than with coulometry (1-4). We recently described a patient ingesting therapeutic amounts of pyridostigmine Br (Mestinon[®], containing 18.4 mg of Br [30.6%] per 60-mg tablet), the medication of choice for the management of myasthenia gravis (4). His serum Br was sufficiently high to cause artifactual increases in serum Cl and HCO₃, which reduced the apparent anion gap [Na - (Cl + HCO₃)] to -9 mmol/liter. The spuriously high levels of Cl and HCO₃ were measured on a Kodak Ektachem 700 automated analyzer (Eastman Kodak Co., Rochester, NY) and were not apparent when a Beckman Astra-8 analyzer (Beckman Instruments, Inc., Brea, CA), which uses coulometry for Cl determination, was employed (4).

METHODS

We obtained approximately 30 mL of venous blood from 15 patients (one previously reported [4]) already

taking pyridostigmine Br. None had clinical evidence of Br toxicity. Fourteen had myasthenia gravis; one had the Eaton-Lambert syndrome. Thirteen were outpatients; two were hospitalized—both for decompensation of their myasthenic state. The patients' data are compared with those of 18 normal controls not taking any medications whose serum Br levels were within the normal range. All sera were analyzed for Na, Cl, HCO₃, K, and creatinine (Cr) with a Kodak Ektachem 700 automated analyzer (Eastman Kodak Co.) (5). Na, Cl, HCO₃, and K were also determined with a Beckman Synchron CX3 analyzer (Beckman Instruments, Inc.) (6). Serum Br was measured by the trichloroacetic acid method (7,8), which detects Br concentrations as low as 10 µg/mL (0.125 mmol/L). With it, the serum Br level in normal individuals is less than 30 µg/mL (0.375 mmol/L) (8).

The Ektachem 700 measures Cl and HCO₃ (total CO₂ content) by using simultaneous deposition of 10 µL of sample on separate halves of a multilayered "dry slide" (5). The methodology differs for each assay: Cl-ISE (potentiometry) and HCO₃-ISE (membrane sensitive to carbonate ion) (5). The Synchron CX3 measures Cl with a two-phase Ag/AgCl ISE and measures HCO₃ by the differential pH rate of change for CO₂ employing both measuring and reference pH electrodes (6). Statistical analysis was carried out by one-way analysis of variance followed by the Newman-Keuls multiple range test (with *P* set at 0.05) (9). We also used the Bonferroni method (10), wherein dividing the *P* value (*t* test) usually required for significance (0.05) by the number of potential comparisons gives the new *P* value necessary to achieve statistical significance.

RESULTS AND DISCUSSION

The mean age of the 15 patients, 59 ± 3 (SE) years, was greater (*P* < 0.001) than that of the 18 control subjects (30 ± 1 years). The average daily dose of pyridostigmine Br was 362 ± 48 mg (4.4 ± 0.6 mg/kg body wt). According to the manufacturer, the average daily dosage of the medication is 600 mg, with a maximum of 1,500 mg. The period over which the patients had been taking a constant dosage varied widely and averaged 43 ± 9 months.

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TABLE 1. Serum electrolyte and anion gap data in patients receiving pyridostigmine Br and in control subjects^a

	Na	K	Cl (mmol/L)	HCO ₃	AG	Cr (μmol/L)
Patients (N = 15) (serum Br ^b 1.9 ± 0.5 mmol/L)						
Kodak Ektachem 700	141.1 ± 0.7	4.1 ± 0.1	106.1 ± 1.1 ^{c,d}	30.5 ± 0.8	4.4 ± 1.2 ^{c,d}	80.2 ± 5.7
P value	NS	NS	<0.0025	NS	<0.001	
Beckman Synchron CX3						
Control Subjects (N = 18) (serum Br 0.2 ± 0.02 mmol/L)	142.7 ± 0.7	4.2 ± 0.1	101.5 ± 0.8	28.9 ± 0.5	12.3 ± 0.6	
Kodak Ektachem 700	141.8 ± 0.4	4.0 ± 0.1	101.5 ± 0.5	28.4 ± 0.7	11.9 ± 0.7	89.4 ± 4.3
P value	NS	NS	NS	NS	NS	
Beckman Synchron CX3	143.0 ± 0.5	4.2 ± 0.1	101.8 ± 0.5	29.7 ± 0.6	11.4 ± 0.5	

^a Data are means ± SE; NS, not significant ($P \geq 0.05$); AG, anion gap.

^b $P < 0.001$ versus controls.

^c $P < 0.001$ versus Ektachem controls.

^d $P < 0.001$ versus Synchron controls.

Except for serum Br (two comparisons), all P values shown relate to six potential comparisons between four groups.

When measured with the Kodak Ektachem 700, the serum Cl was significantly higher than that obtained with the Beckman Synchron CX3 analyzer (Table 1). The anion gap was strikingly lower with the Ektachem 700 (4.4 ± 1.2 mmol/L) than with the Synchron CX3 analyzer (12.3 ± 0.6 mmol/L). The average serum Br was 1.9 ± 0.5 mmol/L, with individual values ranging from 0.3 to 8.1 mmol/L. Nine values were equal to or exceeded 1.0 mmol/L; five of these had corresponding anion gap levels of 3 mmol/L or less. There was a strong negative correlation between the serum Br and the anion gap ($r = -0.91$; $P < 0.001$), but there was no significant relationship between serum Br and either the dosage or duration of use of pyridostigmine Br.

The average serum Br of the controls (0.2 ± 0.02 mmol/L) was almost 10-fold lower than that of the patients ($P < 0.001$), and there were no significant differences between the serum values for Cl or anion gap determined on the Ektachem 700 versus the Synchron CX3 analyzer. There were no significant differences between patients and controls in serum Cr.

Although the *in vitro* addition of pyridostigmine Br to serum is known to raise serum Cl artifactually (11), interference with Cl measurements by pyridostigmine Br ingestion has, to our knowledge, been reported in only three patients (4,12,13)—two of whom were taking large doses of the medication (in one instance resulting in frank bromism).

In the presence of Br, Cl determination with ISE results in major positive interference. With the Ektachem 700, serum Cl may increase by approximately 3 mmol/L for each 1-mmol/L increase in serum Br (5). In addition, the CO₂ content may increase by approximately 2 mmol/L for each 1-mmol/L increase in serum Br (5). In this present study, the small increase in serum HCO₃ was not statistically significant. On the other hand, Br is not cited as an interfering substance for CO₂ content determination in the Synchron CX3 manual (6).

There were no significant electrolyte or anion gap differences in the control group between the values obtained with the two analyzers, suggesting that, in the patient group, the Ektachem 700 data were faulty. Both of the automated analyzers used in the study presented here employ ISE for the measurement of Cl; nevertheless, the Cl levels differed considerably in the patient group as measured by the two instruments. Of note, Nagamine *et al.* (3) state that the degree of interference by Br with Cl determination may vary among different instruments with the same assay principle—particularly with ISE technology.

Myasthenia gravis is an uncommon but not rare disease whose estimated prevalence in the United States is between 43 and 84 per million (i.e., a total of up to approximately 21,000 patients) (14). Because a majority of the known patients are probably using pyridostigmine Br, the phenomenon described herein

is important. Clinicians should also be aware that a considerable number of other medications available in the United States contain Br (13).

In conclusion, routine therapeutic doses of pyridostigmine bromide may, in the absence of clinical bromism, induce methodology-dependent artifactual increases in serum Cl (and sometimes HCO₃) with a consequent marked reduction of the anion gap. This may be particularly confusing in the diagnostic evaluation of acutely ill patients admitted to an intensive care unit.

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REFERENCES

1. **Elin RJ, Robertson EA, Johnson E:** Bromide interferes with determination of chloride by each of four methods. *Clin Chem* 1981;27:778-779.
2. **Wenk RE, Lustgarten JA, Pappas NJ, Levy RI, Jackson R:** Serum chloride analysis, bromide detection, and the diagnosis of bromism. *Am J Clin Pathol* 1976;65:49-57.
3. **Nagamine Y, Hamai I, Chikamori K, et al.:** Asymptomatic hyperbromidaemia detected as pseudohyperchloridaemia measured with an ion selective electrode meter. *Scand J Clin Lab Invest* 1988;48:177-182.
4. **Wacks I, Oster JR, Perez GO, Kett DH:** Spurious hyperchloremia and hyperbicarbonatemia in a patient receiving pyridostigmine bromide therapy for myasthenia gravis. *Am J Kidney Dis* 1990;16:76-79.
5. **Sodium, carbon dioxide and chloride test methodology for Ektachem 700 automated analyzer.** Eastman Kodak Co, Clinical Products Division. Publications MP-13 (November 1989), MP 2-15 (November 1989), and MP 2-14 (1986).
6. **Carbon dioxide and chloride test methodology for Synchron CX3 automated analyzer.** Beckman Instruments, Inc. System Chemistry Descriptions. Section Three. Publication 015-246770-E. 1987:3-9.
7. **Hepler OE.** Manual of Clinical Laboratory Methods. 4th ed. Springfield: Charles C. Thomas; 1963:325.
8. **Jatlow PI, Bailey DN.** Analytical toxicology. In: Sonnenwirth AC, Jarett L, eds. *Gradwohl's Clinical Laboratory Methods and Diagnosis*. 8th Ed. St Louis: C.V. Mosby Company; 1980:384-434.
9. **Snedecor CW, Cochran WG.** Statistical Methods. Ames: Iowa State University; 1968.
10. **Godfrey K:** Comparing the means of several groups. *N Engl J Med* 1985;313:1450-1456.
11. **Komaiko W, Hussain S, Brecher M, Bissell M:** Positive interference with Ektachem chloride and carbon dioxide methods by bromide-containing drugs. *Clin Chem* 1988;34:429-430.
12. **Ruff RL:** Spuriously elevated serum chloride values caused by pyridostigmine bromide. *Arch Neurol* 1981;38:321.
13. **Rothenberg DM, Berns AS, Barkin R, Glanz RH:** Bromide intoxication secondary to pyridostigmine bromide therapy. *JAMA* 1990;263:1121-1122.
14. **Adams RD, Victor M:** Principles of Neurology. 4th Ed. New York: McGraw-Hill Information Services Co.;1989:1150-1167.