Effects of Various Medications on Values from the HABA and BCG Methods for Determining Albumin

Solomon Notrica, Don S. Miyada, Vern Baysinger, and Robert M. Nakamura

We investigated the effects of acetylsalicylic acid, salicylic acid, sulfisoxazole, and phenobarbital—at concentrations at or just above those in serum after therapy—on results of the 2-(4′-hydroxyazobenzene) benzoic acid (HABA) and bromcresol green (BCG) methods for measuring serum albumin. Although the HABA method was more prone to such interference, only interference by salicylic acid with the HABA method is significant. Salicylic acid at a concentration of 400 mg/liter decreases the apparent values for HABA-albumin by about 10%.

Serum albumin is commonly measured by dye-absorption methods. In a previous report in which the dye-binding reagents—2-(4′-hydroxyazobenzene)-benzoic acid (HABA) and bromcresol green (BCG)—were compared, results for icteric, lipemic, and hemolytic serum samples were less reliable by the HABA procedure (1).

Alcohol combines with various compounds that may interfere with quantitation by the dye-binding methods. Here, we report studies of possible interference caused by therapeutic (or somewhat greater) concentrations of acetylsalicylic acid, salicylic acid, phenobarbital, or sulfisoxazole with serum albumin determinations by the HABA and BCG methods. We selected salicylic acid, acetylsalicylic acid, and sulfisoxazole for study because they reportedly displace the binding of bilirubin to albumin (2, 3). Phenobarbital was selected because it has been used therapeutically to decrease serum bilirubin concentrations in neonatal jaundice (4).

Materials and Methods

**HABA dye method.** The procedure used was the modification of Ness et al. (5) adapted to the “AutoAnalyzer” SMA-12/30 (Technicon Instruments Corp., Tarrytown, N.Y. 10591). In this procedure the HABA dye is used at a buffered pH of 6.2. The instrument was standardized with reference serum supplied by Technicon (lot No. B8C110).

In this procedure a serum blank is not used.

**BCG method.** The procedure used is a modification of that of Babson (6). The stock reagent consisted of 26 ml of sodium hydroxide (100 g/liter), 30 ml of L-lactic acid, 500 mg of BCG, and 10 ml of “Tween-20,” all diluted to 1 liter with distilled water, mixed, and the pH adjusted to 4.0 at 25°C.

The stock reagent was diluted one part plus four parts of distilled water before use. Ten microliters of serum was added to 5 ml of diluted reagents, mixed, and read at 630 nm vs. the reagent blank. Measurements were made on a Coleman spectrophotometer, Model D, using a 12-mm flow-through cuvet.

The BCG procedure was standardized with commercial reference sera assayed at 32, 42, and 44 g/liter (“Calibrate”; General Diagnostics Division, Warner-Chilcott Labs., Morris Plains, N.J. 07950).

Salicylic acid, acetylsalicylic acid, and phenobarbital were all U.S.P. grade.

Sulfisoxazole diolamine (“Gantrisin”; Roche Labs., Nutley, N.J. 07100) was used.

Lyophilized sera (“Versatol”; Warner-Chilcott), containing, per liter, 44 g of albumin and less than 10 mg of bilirubin, were reconstituted with aqueous solutions of various concentrations of the above compounds. The reconstituted sera were analyzed for serum albumin by both dye methods.

Results and Discussion

The greatest effect was observed with salicylic acid, on the HABA method. Salicylic acid at a concentration of 1 g/liter decreased the HABA-albumin results by 8 g/liter, or about 18% (Table 1). Acetylsalicylic acid at the same concentration had virtually no effect (Table 1). The converse was true of the BCG method: salicylic acid had no effect on the BCG method, whereas acetylsalicylic acid at a concentration of 1 g/liter decreased serum albumin values by about 7%. The latter effect, however, was not significant at acetylsalicylate concentrations below 800 mg/liter. Therapeutic plasma salicylate concentrations are generally less than 400 mg/liter (6). Our findings are in agreement with previous reports (7, 8). Arvan and Ritz (7), in their investigation of the HABA method, found sodium salicylate at a concentration of 450 mg/liter to decrease serum albumin values by about 15%. Doumas et al. (8) reported that sodium salicylate at a concentration of 600 mg/liter did not interfere with the BCG method.

The above data are consistent with the hypothesis that salicylates, bilirubin, and HABA all compete for similar binding sites on the albumin molecule. Previous investigators have shown that sodium salicylate decreases bilirubin—albumin binding (2, 3). Salicylic acid and acetylsalicylic acid both bind albumin, as shown by gel filtration as well as by equilibrium dialysis methods (9, 10). The binding characteristics of acetylsalicylic acid are complicated further by covalent bond formation. Pinckard et al. (11) found acetylsalicylic acid to acetylate albumin both in vivo and in vitro at a concentration of 180 mg/liter.

Sulfisoxazole at concentrations to 500 mg/liter had no significant effect on the dye binding methods (Table 1). Therapeutic concentrations are generally considered to be from 70–170 mg/liter (12). Previous investigators have reported that sulfisoxazole at concentrations above 250 mg/liter partially displaced bilirubin from albumin in vitro (2, 3).

Phenobarbital at a concentration of 250 mg/liter, well above the concentration producing coma, had no effect on
Table 1. Effects of Salicylic Acid, Acetylsalicylic Acid, Sulfisoxazole, and Phenobarbital on the HABA and the BCG Methods for the Determination of Albumin

<table>
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<th>Salicylic acid, mg/liter</th>
<th>Albumin, g/liter&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BCG</th>
<th>HABA</th>
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<sup>a</sup>Av. of duplicate determinations.

either of the methods (Table 1). Phenobarbital was observed by Doxiadis (13) to decrease serum bilirubin concentrations in neonatal jaundice, whether administered to the mother antenatally or to the infant neonatally. Khan- na et al. (3) were not able to detect any differences in the spectral absorbance curves of icteric sera with and without phenobarbital, and concluded that displacement of bilirubin from albumin was not responsible for the bilirubin lowering effect of phenobarbital in the neonate.

References