
Francisco Pérez-Jiménez
Antonio Grillo
Juan Jimenez-Alonso
José A. Jiménez Perepérez

Cuidad Sanitaria de la Seguridad
Social Reina Sofia
Univ. of Córdoba Med. School
Cordoba, Spain

Commercially Prepared Bilirubin Standards May Not Be Valid for Use with Direct Spectrophotometric Assay

To the Editor:

Our autopsy service has observed an unusually high incidence of yellow hyaline membrane disease (1). While other institutions have also observed an increase in incidence in recent years, at least one institution with an active neonatal service has yet to find this disease. A brief check of the affected institutions showed that those observing this disease also routinely use the direct spectrophotometric assay for determining bilirubin in their neonates. Of possible significance was the observation that the one institution not seeing this disease has continued to use the Jendrassik-Grof methodology for bilirubin determination.

Pursuing this possibility, we evaluated 70 consecutive samples coming from our neonatal service by both the direct spectrophotometric assay and the Jendrassik-Grof method. For both these analyses we used an American Monitor Corp. (Indianapolis, IN 46268) calibrator for standardization. Results of this survey consistently showed 18% lower bilirubin values when determined by the direct spectrophotometric assay.

We next evaluated the American Monitor standard against a weighed bilirubin standard (Sigma Chemical Co., St. Louis, MO 63178; cat. no. B4126) dissolved in dimethyl sulfoxide and Na$_2$CO$_3$ diluted to a concentration of 200 mg/L in human serum, and the pH adjusted to 7.4 (2). This laboratory-prepared standard showed a peak absorption at 460 nm with a molar absorptivity of between 44 000 and 45 000 L mol$^{-1}$ cm$^{-1}$ at 454 nm on repeat assays (3). The American Monitor standard showed a peak absorbance at 472 nm and a molar absorptivity of 56 100 L mol$^{-1}$ cm$^{-1}$ at 454 nm. A differential spectrum of the American Monitor standard against our prepared standard and a prepared Sigma bilirubin reference (no. 665-10), both adjusted to the approximate listed concentration of the American Monitor standard, showed a positive peak at 490 nm and a negative peak at 410 nm. In collaboration with American Monitor, we were able to ascertain that the interfering peak was produced in the lyophilization procedure and was not present in the material before lyophilization. The lyophilized material apparently is still valid for use as a standard with the Jendrassik-Grof method. The Sigma-prepared standard did not show any interfering material, although on assay it appeared to be approximately 5% lower than the listed value.

It is clear that the American Monitor standard should not be used for the direct spectrophotometric method. Both manufacturers of these commercial standards verify the concentration of their weighed-in bilirubin by using the Jendrassik-Grof methodology. We recommend that before using any commercial standard for the direct spectrophotometric method, the chemist should verify the absorption spectrum and the molar absorptivity. Finally, while it is not yet clear whether this error in bilirubin methodology has contributed to the high apparent incidence of yellow hyaline membrane disease in our institution, we strongly recommend that any institutions observing a high incidence of this disease should verify their analytical method, both against a reference Jendrassik-Grof method and a validated reference material as described above.

References

J. S. Amenta
J. A. Silverman

Dept. of Pathol.
Magee-Women's Hosp.
Forbes Ave. and Halket St.
Pittsburgh, PA 15213

Furosemide and Increased Serum Alkaline Phosphatase (Hepatic Isoenzyme)

To the Editor:

The report by Nanji and Blank is of importance (1). Their possible explanation for increased serum alkaline phosphatase (hepatic isoenzyme) in some of their alcoholic patients with ascites being treated with furosemide needs some clarification.

Erlinger et al. (2) reported that ouabain, ethacrynic acid, and amiloride cause a reduction in canalicular bile flow. Campese and Siro-Brigliani (3) reported that ethacrynic acid stimulates bile flow in rats. Shaw et al. (4) observed that ethacrynic acid increases bile flow in sheep, dogs, rats, guinea pigs, and rabbits. Graf et al. (5) also found canalicular bile flow to be increased by ouabain and ethacrynic acid in the isolated perfused rat liver. Inhibition of active reabsorption of solutes, especially sodium, from bile in the ducts of ductules may account for some of the choleresis caused by ethacrynic acid (4). Thus furosemide, which has a diuretic action similar to ethacrynic acid, may cause an increase in canalicular bile flow. This needs further study, because the metabolism and excretion of furosemide are different from that of ethacrynic acid.

Alkaline phosphatase is an indirect marker of cholestasis (6). Normal serum alkaline phosphatase values have been observed in some patients with cholestasis (7). Brensilver and Kaplan noted increased alkaline phosphatase in a group of patients with no demonstrable liver disease (8).

Mitchell et al. (9) observed hepatic necrosis by furosemide in a dose-dependent pattern in mice. The dose of furosemide they used was 100 to 500 mg/kg, larger than the dose used in clinical practice. Hepatic necrosis was prevented when metabolism of furosemide was inhibited by pretreatment of mice with three different types of cytochrome P450 enzyme inhibitors (piperonyl butoxide, cobaltous chloride, and naphthyl isothiocyanate) (9). Formation of the arylating metabolite of furosemide, which causes hepatocellular necrosis, is mediated by a cytochrome P450-dependent mixed-function oxidase in liver. Human microsomes similar to mouse microsomes can convert furosemide to arylating metabolite (9).

Because other laboratory indexes of liver function did not change in their patients who had an increase in the hepatic isoenzyme of alkaline phosphatase in serum, it is unclear whether furosemide acted as an enzyme inducer for alkaline phosphatase or caused a decrease in degradation or activation of pre-existing enzyme in their patients.

Further studies by Nanji and Blank in their patients may clarify the above problem.

References