Primary biliary cirrhosis (PBC) is a chronic cholestatic disease in which there is a crucial need for quantitative liver-function tests. We have developed a mixed sulfobromophthalein (BSP)–indocyanine green (ICG) test and have applied it to 15 healthy subjects and 50 patients with PBC to determine its relevance to the histological severity of the disease. The two dyes were administered intravenously and sequentially as boluses. Plasma concentrations were measured over 60 min. Pharmacokinetic analysis of the plasma elimination curve permitted the calculation of clearance, constants $k_1$ and $k_2$, and the retention percentage at 45 min. In PBC patients, ICG kinetics were within the normal range except for those with stage IV disease (cirrhosis). BSP clearance and the $k_2$ constant were reduced in all the patients, whereas the $k_1$ constant was reduced only in stage III and IV disease. The BSP retention percentage at 45 min was highly correlated with histological stage ($r = 0.89, P < 0.001$). The BSP–ICG mixed test may thus prove useful in the diagnosis and follow-up of patients with PBC.

**Additional Keyphrases:** liver function • pharmacokinetics • cholestasis

Primary biliary cirrhosis (PBC) is a chronic cholestatic disease most often diagnosed in middle-aged women. Morphologically, PBC is characterized by a chronic nonsuppurative cholangitis affecting small and medium-sized bile ducts, leading to the destruction and loss of intrahepatic bile ducts. As in all long-term obstructive cholestasis, lobular lesions and cirrhosis may occur. The terminal phase is characterized by hyperbilirubinemia (>100 μmol/L), a greatly decreased number of patent intrahepatic bile ducts, and extensive fibrosis or cirrhosis. Orthotopic liver transplantation is currently the treatment of choice for end-stage patients, with a two-year survival rate as great as 80%.

Among the drugs evaluated in controlled trials for the medical therapy of PBC, cyclosporine and ursodeoxycholic acid are very promising.

To assess the effects of medical treatment on the progression of the characteristic lesions of PBC, i.e., destruction of bile ducts and hepatocytes and the development of fibrosis and cirrhosis, there is a crucial need for quantitative liver-function tests. The concentration of serum bilirubin is a specific marker of entry into the terminal phase of the disease but is not sufficiently sensitive to detect changes in liver function in the early stages. Other routine liver tests are useful for the diagnosis of PBC but do not provide reliable information concerning the histological progression of the disease. Morphologically, PBC is characterized by a heterogeneous distribution of lesions throughout the liver, making liver histology somewhat unreliable for the follow-up of the disease in individual patients.

Clearance tests of sulfobromophthalein (BSP) and indocyanine green (ICG) have been widely used in hepatology. ICG clearance depends on liver blood flow and uptake and is rather insensitive to changes in biliary excretion (5, 6). In contrast, BSP clearance is critically dependent on biliary excretion (7). We therefore postulated that BSP and ICG pharmacokinetic parameters might provide an accurate assessment of the overall histological severity of PBC by evaluating the degree of bile duct paucity and cholestasis on the one hand, and the degree of hepatocyte necrosis, fibrosis, or cirrhosis on the other hand.

To test this hypothesis, we devised a mixed BSP–ICG test and applied it to 50 patients with PBC to assess the usefulness of BSP–ICG pharmacokinetics in the diagnosis of PBC and in the quantification of histological progression of the disease.

**Patients and Methods**

**Patients**

Fifty PBC patients were included in the study. The diagnosis was based on accepted criteria (1). Histological liver specimens were all examined by the same pathologist and classified as follows: stage I, chronic nonsuppurative cholangitis; stage II, portal and periporal inflammation and fibrosis with ductular proliferation; stage III, lobular fibrosis with bridging; stage IV, cirrhosis.

We also performed the BSP–ICG test with a control group of 15 apparently healthy subjects.

**BSP–ICG Test**

ICG (0.25 mg/kg of body weight) and BSP (5.0 mg/kg) were administered intravenously and sequentially as boluses 2 min apart after an overnight fast. The elimination of these doses follows a first-order process (8, 9). Venous blood was collected into heparinized (ICG) or siliconized tubes (BSP) at 4, 8, 12, 16, 20, 24, 30, 40, 50, and 60 min after the injection of each dye. The plasma concentration of ICG was measured by the spectrophotometric method of Nielsen (10), with correction for blank absorbance at 900 nm. A calibration curve was...
constructed for each patient. BSP concentrations in serum were determined by measuring the absorbance of alkalized serum at 580 nm relative to an acidified sample blank. The data derived from the plasma and serum disappearance curves were fitted to a biexponential function \( (C(t) = A e^{-\alpha t} + B e^{-\beta t}) \) by using the graphic method of residuals (11). Briefly, in the terminal part of the curve (after 30 min), the first distribution phase \( (C(t) = A e^{-\alpha t}) \) was considered to be negligible. Thus, the slope of the terminal regression line is equal to \( \beta \) and the extrapolated zero time concentration is equal to \( B \). Then, the extrapolated values of this line were subtracted from experimental data in the first part of the curve (before 30 min), which represents the residual function \( (C(t) = B e^{-\beta t}) \), and the parameters \( A \) and \( \alpha \) were calculated by linear regression as above. The regression fit the data well in every case (\( P < 0.01 \)). The hepatic clearances (Cl) of ICG and BSP were then calculated from the macroconstants \( A, \alpha, B, \beta \), and the dose \( q_0 \) as Cl = \( q_0/[ (A/\alpha) + (B/\beta) ] \).

In addition, "fractional clearance," \( k_1 \), was computed from the slope of the least-squares regression line for the experimental data and determined in the initial phase of the curve (from 4 to 20 min after the injection). The BSP terminal elimination rate \( (k_\text{t}) \) was considered equal to the value of \( \beta \). Finally, the BSP retention percentage at 45 min was deduced from the extrapolated BSP concentration at time zero: % retention (BSP) at 45 min = 100 \( \times \) C(BSP) at 45 min/(A + B).

Statistical Analysis

Data (mean \( \pm \) SD) were compared by using Kruskall–Wallis nonparametric analysis of variance and the Mann–Whitney U-test. Differences were considered significant at \( P < 0.01 \).

Results

The data are summarized in Table 1. Mean ICG fractional clearance \( (k_1) \) and hepatic clearance \( (\text{Cl}) \) were within the normal range in the PBC patients, except for those with stage IV disease.

BSP hepatic clearance was significantly reduced, mainly because \( k_2 \) values were very low. In stage III and IV disease, decreased BSP clearance also resulted from reduced \( k_2 \) values. The BSP retention percentage at 45 min was significantly increased in stage II, III, and IV disease and was correlated with histological stage \((r = 0.89, P < 0.001; \) Figure 1). There was a significant correlation \((r = 0.64; P < 0.01) \) between the BSP retention percentage at 45 min and the area under the second part of the biexponential curve \( (\text{AUC} = B/\beta) \).

Typical patterns of BSP–ICG disappearance from the serum and plasma, respectively, of a PBC patient (stage II) and a healthy subject are presented in Figure 2.

Discussion

We have shown the feasibility of measuring ICG and BSP pharmacokinetics simultaneously. Regarding possible BSP–ICG interaction, studies in rats (12, 13) and dogs (12) have shown a competitive inhibition of hepatocyte BSP uptake by ICG (13). Meijer et al. (14) also observed enhanced ICG clearance in the presence of dibromosulphthalein, probably attributable to an increase in the plasma protein-unbound ICG fraction. However, they used a molar BSP/ICG ratio in the range of 1 to 10, whereas our conditions \((\text{BSP/ICG} = 19.7) \) make it unlikely that the decreased BSP clearance was related to ICG-induced inhibition of uptake processes. Furthermore, the values in the mixed BSP–ICG test observed with healthy subjects did not differ from those obtained with separate BSP and ICG tests (data not shown).

The two dyes showed distinct plasma disappearance patterns, whatever the severity of PBC. ICG elimination was monophasic, with a clearance value not significantly different from that of the controls, except in the stage IV (cirrhotic) disease. These findings are in agreement with previous studies that showed that cholestasis has little or no influence on ICG clearance in humans (5). This is supported by the physiology of ICG hepatic extraction: the decreased clearance of ICG in patients with stage IV disease is consistent with functional flow-limited uptake of the dye (5).

BSP showed a biphasic elimination profile, whatever the stage, unlike that found in healthy subjects. The greater the histological severity, the lower the biliary elimination, as reflected by either the \( k_2 \) constant or the 45-min retention percentage. In PBC, cholestasis is caused by the destruction of interlobular and septal bile ducts, with a progressive loss of bile ducts as the disease progresses towards cirrhosis (15). This is entirely consistent with the progressive decrease in the \( k_2 \) constant and the 45-min retention percentage we found. Thus,

### Table 1. Pharmacokinetic Parameters (Mean \( \pm \) SD) of ICG and BSP Elimination in PBC Patients by Disease Stage

<table>
<thead>
<tr>
<th>Patients</th>
<th>ICG Cl, mL min(^{-1})</th>
<th>BSP Cl, mL min(^{-1})</th>
<th>BSP % retention at 45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBC stage I</td>
<td>7</td>
<td>0.166 ± 0.047</td>
<td>556 ± 187</td>
</tr>
<tr>
<td>PBC stage II</td>
<td>21</td>
<td>0.172 ± 0.047</td>
<td>518 ± 195</td>
</tr>
<tr>
<td>PBC stage III</td>
<td>10</td>
<td>0.132 ± 0.039</td>
<td>429 ± 194</td>
</tr>
<tr>
<td>PBC stage IV</td>
<td>12</td>
<td>0.064 ± 0.053*a</td>
<td>201 ± 187</td>
</tr>
<tr>
<td>PBC all stages</td>
<td>50</td>
<td>0.130 ± 0.061</td>
<td>437 ± 226</td>
</tr>
<tr>
<td>Control group</td>
<td>15</td>
<td>0.172 ± 0.030</td>
<td>544 ± 86</td>
</tr>
</tbody>
</table>

*a P < 0.01 vs control group. P < 0.01 vs stage I. P < 0.01 vs stage II. NA: a second slope is not present in subjects without liver disease.
the values of these two parameters might represent an index of bile duct paucity. However, the 45-min retention percentage was more closely related to histological severity, probably because the percentage of retention depends essentially on biliary elimination (as indicated by the correlation between the AUC/β and the 45-min retention percentage: $r = 0.64, P < 0.01$).

The BSP elimination constant, $k_1$, was markedly reduced in stage IV disease, suggesting that cholestasis is associated with decreased uptake in these patients. In this case, BSP parallels the elimination profile of ICG, a pattern that is also observed in cirrhotic patients of alcoholic or posthepatic origin (data not shown).

Data concerning the metabolism of BSP and ICG might be useful for the interpretation of these results. Both anions are initially removed from the plasma by a common carrier-mediated transport process (13). Hepatic storage of ICG is important because of low liver to plasma transport (14). In humans, the recovery of unchanged ICG in bile $> 18$ h after intravenous injection is $80\%$ of the dose (14). In cholestatic disease, impairment of canalicular transport processes would lead to an accumulation of the dyes in the hepatic compartment. The fact that the plasma elimination of BSP, but not of ICG, is reduced in PBC might be explained by the hepatic BSP conjugation process (15), which could be saturated when canalicular transport is altered.

In conclusion, this study shows the potential value of measuring dye clearances in PBC patients, for the following reasons: (a) BSP elimination showed a characteristic biphasic pattern, which is not shared by ICG; (b) BSP pharmacokinetic parameters correlated strongly with the histological stage, whereas ICG remained within the normal limit except in patients with cirrhosis. These data suggest that the proposed BSP-ICG test may be useful in the diagnosis and follow-up of PBC patients. Prospective controlled evaluation is now required to determine the prognostic value of this test.

References