THE KINETICS OF ORGANIC ANION EXCRETION
BY THE LIVER IN ACUTE INTERMITTENT PORPHYRIA

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SUMMARY

1. The kinetics of disappearance from the blood of three organic anions were studied in patients with acute intermittent porphyria. Indocyanine green (ICG) and \([^{14}\text{C}]{\text{bilirubin}}\) were cleared normally in this disease, whereas bromsulphthalein (BSP) was cleared at less than the normal rate. The first exponential component of the BSP clearance curve was normal in acute intermittent porphyria, but the second exponential component declined less rapidly than in normals. The abnormality was greater in symptomatic than in asymptomatic patients, but both groups were significantly different from normals.

2. The data are discussed in terms of a previously published model of BSP metabolism. The findings suggest normal BSP uptake initially by the liver with increased regurgitation of dye into the blood and decreased excretion of BSP from the liver cell into the bile.

3. In contrast to human acute intermittent porphyria, plasma clearance of BSP was greatly increased in experimental porphyria. This was associated with an increased level of BSP conjugating enzyme in the liver in experimental porphyria.

4. The BSP retention in acute intermittent porphyria, along with certain other findings, raises the question of the existence of an 'oestrogen effect' in this disease, but further studies will be required to determine whether the mechanism of BSP retention is identical to that produced by oestrogen.

Acute intermittent porphyria (AIP) is a type of hepatic porphyria in which overproduction of porphyrin precursors occurs in the liver (Tschudy et al., 1965; Nakao et al., 1966; Dowdle, Mustard & Eales, 1967). Standard tests of hepatic function in this disorder have generally been normal with the exception of the bromsulphthalein (BSP) test (Schmid, Schwartz &
Watson, 1954; Hellman et al., 1963; Taddeini & Watson, 1968). These studies employed a single plasma measurement of dye concentration. The present study examines the kinetics of BSP, indocyanine green (ICG) and \[^{14}C\]bilirubin (BR) disappearance from the plasma in AIP. The enzymatic conjugation rates and plasma disappearance curves of BSP in experimental porphyria are also presented.

**METHODS**

The diagnosis of AIP was established in eleven patients by the demonstration of increased levels of porphobilinogen in the urine. Porphobilinogen was measured by the method of Mauzerall & Granick (1956). The ages of the eight female and three male patients ranged from 26 to 47 years and from 34 to 51 years respectively. Five patients were having exacerbations of AIP when they were studied.

Standard tests of hepatic function were carried out by the routine methods employed in the laboratories of the Clinical Center, National Institutes of Health.

BSP, 5 mg/kg body weight, was injected intravenously over a 30 sec period after an overnight fast. Heparinized blood samples were then obtained from the opposite arm every 3–5 min for at least 60 min. BSP concentration in plasma was determined by the method of Gaebler (1945). Table 1 shows the percentage retention of BSP at 30 min calculated by the standard clinical laboratory method which assumes an initial \( (t_o) \) concentration of 10 mg% in plasma (Gaebler, 1945).

Isotopic bilirubin was prepared biosynthetically in bile fistula dogs from 4-\[^{14}C\]ALA (Barrett, Mullins & Berlin, 1966). \[^{14}C\]bilirubin clearance studies were carried out using the method of Barrett et al. (1968), as modified by Berk et al. (1969). Each patient received 0.4–2.0 \( \mu Ci \) in 0.5 mg \[^{14}C\]bilirubin. The liver dose received was calculated to be 0.12–0.60 mrad.

ICG, 0.5 mg/kg body weight, was injected intravenously to fasting subjects over a 15–30
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sec period, and blood samples were taken 5, 10, 15 and 20 min later. ICG was determined in serum as previously described (Cherrick et al., 1960). Informed consent was obtained from each patient for all the studies performed.

The plasma clearance data for isotopic bilirubin were fitted to a sum of three exponential functions on a Univac 1108 digital computer using the SAAM programme of Berman, Shahn & Weiss (1962) and Berman & Weiss (1967). Typical curves in normals have been presented previously (Berk et al., 1969). Using the plasma curve integral method (Nosslin, 1964), the fraction of the plasma unconjugated bilirubin pool cleared irreversibly per minute by the liver ($k_E$) was determined from the coefficients ($A$'s) and rate constants ($a$'s) of the bilirubin clearance curve, according to the relationship:

$$k_E = \frac{1}{(A_1/a_1) + (A_2/a_2) + (A_3/a_3)}$$

(Berk et al., 1969).

The percentage retention of isotope at 4 hr was calculated as the ratio of specific activity of the plasma at 4 hr to the extrapolated value at time 0.

The plasma clearance data for BSP were fitted to a sum of two exponentials, using the same computer method. The transfer rates presented in Table 2 were calculated by the method of Richards, Tindall & Young (1959).

All studies of experimental porphyria were performed in Sprague-Dawley female rats weighing 120–150 g. The enzyme catalysing the conjugation of BSP and glutathione was measured in pooled liver homogenates from four animals by the method of Goldstein & Combes (1966). Allylisopropylacetamide (AIA) was administered subcutaneously daily at a dosage of 300 mg/kg. Oestradiol was injected daily subcutaneously in a dose of 400 µg/kg. For BSP clearance studies in rats, 75 mg/kg was injected via the tail vein, and blood samples were taken every 5 min thereafter. Each value is the mean value from three animals.

RESULTS

Standard tests of hepatic function including serum glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, lactic dehydrogenase, alkaline phosphatase, prothrombin level and protein electrophoresis were normal. Cholesterol elevation to levels between 250 and 300 mg/100 ml was seen in four patients and has been documented in other patients with AIP previously (Hellman et al., 1963; Taddeini, Nordstrom & Watson, 1964). Levels of direct and indirect-reacting serum bilirubin were within the established range of normal.

The 30 min BSP retention values for the eleven patients studied are presented in Table 1 along with normal controls of similar age. The difference between both groups of porphyria patients and controls is highly significant ($P<0.01$).

A typical plasma disappearance curve for BSP in one patient is plotted semi-logarithmically in Fig. 1. A curve of this type with two exponential components was seen in all patients except one asymptomatic woman whose disappearance curve best fits a single straight line. Such a single component is occasionally seen in normals (Barber-Riley et al., 1961).

The mean values ±SD of the computer generated slopes ($k_1$ and $k_2$) of the two components are presented in Table 2 and are compared to the series of twenty-six normals presented by Barber-Riley et al. (1961) (Fig. 2).
There were no significant differences in the slopes of the first exponential component ($k_1$) in any of the porphyrics (symptomatic or asymptomatic) as compared to normals. However, the differences in the slopes of the second component ($k_2$) are highly significant when normals are compared to all porphyrics ($P < 0.001$), symptomatic porphyrics ($P < 0.001$) and asymptomatic porphyrics ($P < 0.001$). The mean value of $k_2$ in the symptomatic porphyric group is significantly less than that of the asymptomatic porphyrics ($P < 0.01$).

**Fig. 1.** The plasma curve of BSP disappearance in a patient with AIP.

The data have also been analysed in terms of the model of BSP metabolism studied previously by Richards et al. (1959) and Barber-Riley et al. (1961) (Fig. 2). $a$, $b$ and $h$ are the fractional rates (first order rate constants) for transfer of dye from the circulation to liver, from liver back to the circulation, and excretion from liver into the bile, respectively. As shown by Richards et al. (1959), if the experimental data (the plasma BSP curve) are represented by

$$\frac{X}{X_0} = Ae^{-k_1t} + Be^{-k_2t},$$

then $a$, $b$ and $h$ can be calculated when $A$, $B$, $k_1$ and $k_2$ are known. In this equation $X_0$ is the concentration of BSP at time 0 (derived by extrapolation to time 0 of the measured curve), $X$ is the concentration of dye in the circulation at a given time, $A$ and
### Table 2. Kinetic parameters of BSP excretion in acute intermittent porphyria

<table>
<thead>
<tr>
<th>Sex</th>
<th>Patient</th>
<th>Status*</th>
<th>$k_1$ (min)</th>
<th>$T_{1/2}$ (min)</th>
<th>$k_4$ (min)</th>
<th>$T_{1/2}$ (min)</th>
<th>$X_0$</th>
<th>$A$</th>
<th>$B$</th>
<th>Injected dose (mg)</th>
<th>$a$</th>
<th>$b$</th>
<th>$h$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>J.L.</td>
<td>S</td>
<td>0-186 ± 0-020</td>
<td>3.7</td>
<td>0-011 ± 0-006</td>
<td>63.0</td>
<td>17.90</td>
<td>0.79</td>
<td>0.21</td>
<td>254</td>
<td>0.149</td>
<td>0.034</td>
<td>0.014</td>
</tr>
<tr>
<td>M</td>
<td>W.B.</td>
<td>A</td>
<td>0-184 ± 0-029</td>
<td>3.8</td>
<td>0-030 ± 0-001</td>
<td>23.1</td>
<td>15.90</td>
<td>0.82</td>
<td>0.18</td>
<td>395</td>
<td>0.156</td>
<td>0.023</td>
<td>0.035</td>
</tr>
<tr>
<td>M</td>
<td>R.B.</td>
<td>S</td>
<td>0-147 ± 0-008</td>
<td>4.7</td>
<td>0-021 ± 0-0008</td>
<td>33.0</td>
<td>11.66</td>
<td>0.49</td>
<td>0.11</td>
<td>301</td>
<td>0.133</td>
<td>0.012</td>
<td>0.023</td>
</tr>
<tr>
<td>F</td>
<td>D.S.</td>
<td>S</td>
<td>0-168 ± 0-022</td>
<td>4.1</td>
<td>0-008 ± 0-0009</td>
<td>86.6</td>
<td>13.53</td>
<td>0.43</td>
<td>0.17</td>
<td>268</td>
<td>0.141</td>
<td>0.025</td>
<td>0.010</td>
</tr>
<tr>
<td>F</td>
<td>J.C.</td>
<td>S</td>
<td>0-109 ± 0-012</td>
<td>6.4</td>
<td>0-017 ± 0-001</td>
<td>40.8</td>
<td>11.23</td>
<td>0.44</td>
<td>0.26</td>
<td>240</td>
<td>0.085</td>
<td>0.019</td>
<td>0.022</td>
</tr>
<tr>
<td>F</td>
<td>D.B.</td>
<td>S</td>
<td>0-131 ± 0-013</td>
<td>3.3</td>
<td>0-016 ± 0-007</td>
<td>43.3</td>
<td>13.08</td>
<td>0.76</td>
<td>0.24</td>
<td>300</td>
<td>0.103</td>
<td>0.023</td>
<td>0.020</td>
</tr>
<tr>
<td>F</td>
<td>N.F.</td>
<td>A</td>
<td>0-201 ± 0-024</td>
<td>3.4</td>
<td>0-025 ± 0-0009</td>
<td>27.7</td>
<td>18.90</td>
<td>0.80</td>
<td>0.20</td>
<td>260</td>
<td>0.166</td>
<td>0.030</td>
<td>0.030</td>
</tr>
<tr>
<td>F</td>
<td>B.W.</td>
<td>A</td>
<td>0-100 ± 0-001</td>
<td>6.9</td>
<td>—</td>
<td>—</td>
<td>13.46</td>
<td>1.40</td>
<td>—</td>
<td>398</td>
<td>0.100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>F</td>
<td>N.G.</td>
<td>A</td>
<td>0-123 ± 0-023</td>
<td>3.6</td>
<td>0-040 ± 0-008</td>
<td>17.3</td>
<td>11.88</td>
<td>0.79</td>
<td>0.21</td>
<td>217</td>
<td>0.106</td>
<td>0.011</td>
<td>0.046</td>
</tr>
<tr>
<td>M</td>
<td>J.S.</td>
<td>S</td>
<td>0-208 ± 0-044</td>
<td>3.3</td>
<td>0-006 ± 0-0008</td>
<td>115.5</td>
<td>18.15</td>
<td>0.42</td>
<td>0.18</td>
<td>415</td>
<td>0.172</td>
<td>0.035</td>
<td>0.007</td>
</tr>
<tr>
<td>F</td>
<td>A.M.</td>
<td>A</td>
<td>0-205 ± 0-075</td>
<td>3.4</td>
<td>0-022 ± 0-0016</td>
<td>31.5</td>
<td>12.56</td>
<td>0.49</td>
<td>0.31</td>
<td>255</td>
<td>0.148</td>
<td>0.049</td>
<td>0.030</td>
</tr>
</tbody>
</table>

* S: symptomatic, A: asymptomatic. $k_1$ and $k_4$ are the slopes of the two exponential components of the BSP curve and $T_{1/2}$ and $T_{1/2}$ the two half-lives of these components. $X_0$ is the $t_0$ calculated value of plasma BSP concentration. $A$ and $B$ are the normalized coefficients of the two exponential terms of the plasma BSP curve. $a$, $b$ and $h$ are defined in Fig. 2 and in the text. ± = S.D.
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$B$ are the normalized coefficients of the two exponential terms of the plasma BSP curve. The values of $a$, $b$ and $h$ in each of the porphyric patients and in the normal subjects are presented in Table 2.

![Diagram of BSP metabolism](image)

**FIG. 2.** The model of BSP metabolism previously studied by Barber-Riley et al. (1961). $a$: The fractional uptake per unit time of BSP by the liver, $b$: the fractional reflux per unit time of BSP from liver into plasma, $h$: the fraction of BSP in liver which is excreted per unit time into bile.

The mean value of $a$ in the porphyric patients is not significantly different from normals, but $b$ is significantly increased ($P<0.01$) and $h$ significantly decreased ($P<0.01$) in the total group of porphyric patients.

The results of the isotopic bilirubin clearance studies in six patients are presented in Table 3,

<table>
<thead>
<tr>
<th>Sex</th>
<th>Patient</th>
<th>$T_{1/2}$ (min)</th>
<th>$T_{2/4}$ (min)</th>
<th>$T_{3/4}$ (min)</th>
<th>$k_E$ (min$^{-1}$)</th>
<th>% 4 hr retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>N.F.</td>
<td>16.9</td>
<td>53.3</td>
<td>577.5</td>
<td>0.016</td>
<td>3.5</td>
</tr>
<tr>
<td>F</td>
<td>J.L.</td>
<td>15.4</td>
<td>38.5</td>
<td>535.5</td>
<td>0.019</td>
<td>2.3</td>
</tr>
<tr>
<td>M</td>
<td>R.B.</td>
<td>20.0</td>
<td>76.2</td>
<td>626.6</td>
<td>0.019</td>
<td>2.5</td>
</tr>
<tr>
<td>F</td>
<td>B.W.</td>
<td>20.4</td>
<td>97.1</td>
<td>551.8</td>
<td>0.019</td>
<td>2.9</td>
</tr>
<tr>
<td>F</td>
<td>A.M.</td>
<td>8.6</td>
<td>57.1</td>
<td>998.0</td>
<td>0.022</td>
<td>2.2</td>
</tr>
<tr>
<td>F</td>
<td>N.G.</td>
<td>37.7</td>
<td>102.8</td>
<td>1873.0</td>
<td>0.0042</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Normal mean (± SD) (13 subjects) 17.7(± 7.2) 80.5(± 38.6) 578(± 132) 0.015(± 0.004) 5.0(± 1.9)

The three $T_{1/2}$ values are the half-lives of the three exponential components of the bilirubin clearance curve, and $k_E$ represents the fraction of plasma unconjugated bilirubin cleared irreversibly per min by the liver (Berk et al., 1969).
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along with the mean, range and SD for the parameters described by Berk et al. (1969) in thirteen normal adults. In five of the patients the parameters were normal. The sixth patient, N.G., had an abnormal clearance as shown by the increased percentage retention of isotope at 4 hr and the decreased value of $k_E$.

The mean half time for disappearance of indocyanine green from the serum in seven patients

TABLE 4. Effect of experimental porphyria and oestrogen on hepatic BSP conjugating activity

<table>
<thead>
<tr>
<th></th>
<th>BSP conjugating activity/g liver (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>AIA: 5 hr</td>
<td>128</td>
</tr>
<tr>
<td>5 days</td>
<td>360</td>
</tr>
<tr>
<td>10 days</td>
<td>215</td>
</tr>
<tr>
<td>Oestradiol: 5 days</td>
<td>70</td>
</tr>
<tr>
<td>10 days</td>
<td>70</td>
</tr>
</tbody>
</table>

Values are derived from the pooled liver homogenates of four animals as described in the Methods section.

TABLE 5. BSP clearance in experimental porphyria

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (min)</th>
<th>BSP level (mg/100 ml plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>2.2</td>
</tr>
<tr>
<td>AIA treated</td>
<td>5</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.5</td>
</tr>
</tbody>
</table>

BSP (75 mg/kg) was administered via tail vein. Each value is the mean value from three animals.

was 3.1 (SD 0.4) min. In two groups of normals (Cherrick et al., 1960; Cooke et al., 1963) the mean half-times for disappearance were 3.0 and 3.4 min.

The enzyme catalysing the conjugation of BSP and glutathione was measured in liver homogenates of fed rats given the porphyrinogenic compound allylisopropylacetamide (AIA). The values are compared with control litter mates given saline and with litter mates given oestradiol (Table 4).

As seen in Table 5 BSP clearance is greatly increased in experimental porphyria produced by 10 days of injections of AIA.
DISCUSSION

Of the three organic anions studied, BSP, ICG and bilirubin, only the excretion of BSP was defective in AIP. One patient (N.G.) had an abnormal bilirubin clearance curve, but her son and her sister have unconjugated hyperbilirubinaemia and it is thought that this patient has both AIP and Gilbert's syndrome. Other liver function tests were also normal in these patients. It is known that hepatic histology by light microscopy (Goldberg & Rimington, 1962) and electron microscopy (Jean, Lambertenghi & Ranzi, 1968) is usually normal in AIP. It would thus appear that the effect of AIP on BSP excretion is not just a non-specific impairment of liver function. The three anions studied are handled in different ways by the liver. Bilirubin is removed from the plasma and conjugated by glucuronidation. ICG is probably excreted without conjugation (Cherrick et al., 1960), whereas BSP is conjugated with glutathione (Goldstein & Combes, 1966).

The defective BSP clearance in AIP appears to be a slower rate of decline of the second exponential component than is seen in normals. The data also indicate that this defect is greater during activity of the disease.

The exact physiological meaning of the decreased value of $k_2$ in AIP is not clear. When the data are analysed by the model discussed by Barber-Riley et al. (1961) (Fig. 2), the conclusions reached are that uptake of dye by the liver ($a$ in Fig. 2) is normal in AIP, but that increased reflux occurs into the circulation (an increase of $b$) along with decreased excretion of dye from the liver cell into the bile (decrease of $h$). The model is an over-simplification and does not consider problems such as different intracellular pools for unconjugated and conjugated BSP. However, it should be pointed out that the measurements of the dye in blood include both these molecular species. The data are compatible with defective conjugation and/or defective excretion of BSP from the liver cell in AIP. This could result from a decrease of the conjugating enzyme.

As seen in Table 4, the conjugating enzyme is increased and clearance of BSP from the blood is very rapid in experimental porphyria. The increase of BSP conjugating enzyme in animals treated with AIA is similar to the increase of a number of other liver enzymes produced by this compound (Narisawa & Kikuchi, 1966). The differences of blood clearance rates of BSP indicate a definite difference between experimental porphyria and AIP. The increased clearance in experimental porphyria is partly related to the increased amount of hepatic tissue produced by AIA as well as any effect resulting from the increased specific activity of the BSP conjugating enzyme. It is not yet possible to say whether the decreased BSP clearance in AIP results from a decreased activity of the conjugating enzyme or decreased excretion of conjugated dye into the bile.

Natural oestrogens can cause BSP retention in man (Mueller & Kappas, 1964). This is thought to result from a depression of the hepatic secretory maximum for transport of conjugated BSP from the liver to the bile (Mueller & Kappas, 1964). No significant difference in the levels of BSP conjugating enzyme was seen after oestrogen treatment (Gallagher, Mueller & Kappas, 1965), but increased levels of conjugated BSP in the plasma were demonstrable (Mueller & Kappas, 1964). The present studies show a slight decrease of the BSP conjugating enzyme in oestradiol treated animals using a different enzyme assay than that employed by Gallagher et al. (1965). Oestrogens have been implicated on a number of occasions as causing attacks of AIP (Zimmerman, McMillin & Watson, 1966) and can cause an increase of por-
phyrin precursor excretion (Welland et al., 1964). Thus BSP retention is one of the findings, along with the increased thyroid hormone binding globulin levels in the blood (Hollander et al., 1967), which suggests the operation of an 'oestrogen effect' in this disease. Further studies will be required to determine whether the mechanism causing BSP retention in AIP is identical to that produced by oestrogen.

REFERENCES


A modification of the bromsulphthalein liver function test to predict the dye content of the liver and bile. *Clinical Science, 18,* 499–511.


