ABNORMAL EXCRETION OF THE ISOMERS OF URINARY COPROPORPHYRIN BY PATIENTS WITH DUBIN–JOHNSON SYNDROME IN ISRAEL

JUDITH BEN-EZZER, C. RIMINGTON, M. SHANI, U. SELIGSOHN, CH. SHEBA AND A. SZEINBERG

Departments of Chemical Pathology, Internal Medicine and Haematology, Government Hospital, Tel-Hashomer and Tel-Aviv University Medical School

(Received 26 May 1970)

SUMMARY

1. The urinary excretion of isomers I and III of coproporphyrin by fifty-nine patients with Dubin–Johnson syndrome has been examined, and compared with the results obtained for normal control subjects and patients with various types of jaundice.

2. The control subjects (with one exception) excreted less than 45% of the coproporphyrin as isomer I. Fifty-six patients with the Dubin–Johnson syndrome excreted more than 65% isomer I (mode, 87%). In three cases the relative content of isomer I was normal. These exceptional patients differed also in some other characteristics from typical cases of the Dubin–Johnson syndrome.

3. Patients with obstructive jaundice or infectious hepatitis showed an intermediate pattern of the isomer distribution. In Gilbert's disease the isomer pattern was normal (six cases). In Rotor syndrome the relative content of isomer I was increased (three cases).

4. The abnormal urinary excretion of coproporphyrins in the various types of jaundice is probably caused by different mechanisms. In obstructive jaundice and infectious hepatitis the absolute excretion of isomer I is raised and isomer III is normal or elevated. This pattern may be explained by a shift from the biliary to the urinary route of excretion affecting mainly isomer I. On the other hand, in Dubin–Johnson syndrome an increased excretion of isomer I was accompanied by a significant decrease of isomer III excretion in the urine. It is suggested that this abnormal pattern might be a result of a deficiency or inhibition of uroporphyrinogen isomerase (uroporphyrinogen III co-synthetase) in the liver of patients with the Dubin–Johnson syndrome.

The Dubin–Johnson syndrome is characterized by chronic or intermittent hyperbilirubinaemia, usually with predominance of conjugated bilirubin in the serum and deposition of a dark
pigment in the liver cells. In the majority of patients there is also retention of injected bromsulphthalein and no visualization of the gall-bladder on cholecystography. The basic abnormality in this syndrome is thought to consist of a selective hepatic excretory defect. Most investigations suggest that the disease is hereditary; however the mode of inheritance is uncertain. Most of the previous studies suggest autosomal dominant inheritance with slight penetrance (Dubin, 1958; Dubin & Johnson, 1954; Arias, 1961; Mandema, De Fraiture, Nieweg & Arends, 1960; Schillinger, 1967). Our own material indicates recessive transmission (Shani, Adam, Seligsohn, Gilon & Sheba, 1970).

In Israel the Dubin–Johnson syndrome is relatively frequent, particularly among the Iranian Jews; until now 101 cases have been detected, sixty-four of them among immigrants from Iran (Shani et al., 1970). Koskelo, Toivonen & Adlercreutz (1967) studied thirteen patients with Dubin–Johnson syndrome and demonstrated an abnormal distribution of urinary coproporphyrin isomers I and III. In normal subjects, the isomer I constituted 5–20% of the total urinary coproporphyrin, whereas patients with the Dubin–Johnson syndrome excreted 80–95% of the coproporphyrin as isomer I. The aim of the present investigation has been to examine the distribution of urinary coproporphyrin isomers in a large sample of Dubin–Johnson patients in Israel in order to establish the validity of the above observation in our case material and its usefulness for the differential diagnosis of this type of jaundice.

Investigations were also conducted on unaffected family members of the Dubin–Johnson patients in order to study the mode of inheritance of this disorder. The results of this part of the investigation will be reported separately.

MATERIALS AND METHODS

Total urinary coproporphyrin. These were estimated by a slight modification of the method of Rimington (1961). Random urine specimens were preserved at 4°C with sodium carbonate (0.3–1% w/v of urine) and tested usually between 1 and 10 days after collection. Urine (40 ml) was placed in a separating funnel and 10 ml buffer, consisting of one part of saturated sodium acetate and four parts of glacial acetic acid (pH = 2.8), were added. The final pH of the mixture should be approximately 4.5 (checked with indicator paper) and if necessary more of the buffer was added to achieve the desired pH. Ether (B.P. grade, 80–90 ml) was added and the mixture shaken vigorously for 1 min. The aqueous layer was removed, 25 ml of 3% (w/v) sodium acetate (hydrated, 'Analar') and 25 ml of 0.005% aqueous iodine solution (prepared fresh each day from a stock solution of 1% (w/v) iodine in absolute ethanol) were added and the mixture shaken for 1 min. The aqueous phase was removed and the ether extract washed once with 30 ml of water. The coproporphyrin was extracted from the ethereal solution with successive 1:5 ml portions of 1:37 M HCl until the extract no longer showed fluorescence under ultraviolet light (usually three extractions were sufficient). The acid extracts were collected in a graduated cylinder, mixed and their total volume recorded. The concentration of coproporphyrin was determined spectrophotometrically and calculated according to the original method. The results were expressed as µg/g creatinine. After the spectrophotometric determination the acid extracts were retained and examined for isomer I and III content. Urinary creatinine content was determined by an alkaline picrate method (Owen, Iggo, Scandrett & Stewart, 1954).
Porphyrin isomers in Dubin–Johnson syndrome

Examination of coproporphyrin isomers

Materials. Chloroform—'analar'; acetone—'analar'; ammonia solution 33% (w/v) s.g. 0.885; 2,6-lutidine. Talc should be purified to remove contaminating iron and possibly other metals. This was accomplished by boiling with about 5 volumes of approximately 5 M HCl for 5–10 min. After the talc had settled the acid was removed by decantation, the powder transferred to a Buchner funnel with water and washed with water until the washings were neutral. The product was finally dried overnight in an oven at 100° and preserved in a closed container. Thin layer chromatography plates of 0.25 mm thickness were prepared from Kieselgel G (Nach Stahl, Merck, for thin layer chromatography) according to Jensen (1963) on glass plates 50 × 200 mm.

Procedure. The HCl extracts of urinary coproporphyrin prepared as described above were mixed with 2 ml of chloroform and centrifuged. The aqueous layer was transferred to a clean tube, about 50 mg of talc powder added, mixed well on a Vortex mixer for 30 s and the talc separated by centrifugation. The tube was examined under u.v. light and if the supernatant showed fluorescence more talc was added, mixed and the centrifugation repeated. The supernatant was discarded and the talc washed twice with about 10 ml of water. After centrifugation the water was discarded and the coproporphyrin eluted from the talc with 1–1.5 ml of a solution of acetone : ammonia : water (7:1:2 v/v). After thorough mixing and centrifugation the supernatant was placed in a small, wide-mouthed vial. The talc remaining in the test tube was examined under u.v. light and if some fluorescence was seen, the elution was repeated with an additional portion of the eluting solution. The porphyrin extracts were evaporated to dryness in vacuo over sodium hydroxide and concentrated sulphuric acid.

The coproporphyrins were separated from one another as follows: 2,6-lutidine (10 ml) and water (3.2 ml) were mixed and poured into a cylindrical chromatography tank (53 × 230 mm). A small glass vial containing about 14 ml of 33% (w/v) ammonia solution was placed on the bottom of the tank which was then closed tightly and equilibrated for 30 min. The dry porphyrin extracts were dissolved in 1–2 drops of 33% (w/v) ammonia and 25 μl portions of these solutions were placed on the thin layer plate (1.5 cm from the bottom, 1.5 cm apart) by application of small drops from a micropipette and drying with a hairdryer. The plates were placed in the tanks, developed in the dark for 90 min, dried for 10 min at 60° and examined under u.v. light. The fluorescent areas were marked with a fine stylus, the Rf values of coproporphyrins I and III being usually 0.42 and 0.45 respectively. The plates were then replaced in the oven at 60° for 30 min and subsequently the silica was removed from the previously marked fluorescent areas with a spatulate piece of photographic film, placed in centrifuge tubes and the porphyrin eluted into 1 ml of 1.37 M HCl. After centrifugation spectrophotometric determinations were performed on the supernatants in 1 ml capacity (10 mm light path) cells. The relative content of the two isomers in the extracts was calculated by the formula:

\[
\text{Corrected } E = 2E_{\text{max}} - (E_{380} + E_{430})
\]

\[
E_{\text{max}} = \text{extinction at peak of Soret band}
\]

\[
E_{380} = \text{extinction at 380 nm}
\]

\[
E_{430} = \text{extinction at 430 nm}
\]

The sum of the values of corrected \(E\) representing isomers I and III respectively was taken as 100% and the proportion of each isomer calculated accordingly.
The absolute values for isomers I and III excretion were calculated from the total coproporphyrin values and the percentage of each isomer.

**Patient material**

Fifty-nine cases of the Dubin–Johnson syndrome have been examined. The diagnosis was based on the finding of chronic or intermittent hyperbilirubinaemia in all cases and the presence of brown-yellow pigmentation in liver parenchymal cells on histologic examination in forty-five cases. In the remaining fourteen cases no liver biopsy was performed, but they were hyperbilirubinaemic siblings of histologically positive cases. Bromsulphthalein tests were performed in thirty-nine cases and prolonged retention was observed in thirty-five of them. Oral cholecystography was carried out in thirty-seven cases and the gall-bladder was not visualized in thirty among them. A detailed description of these cases is being published separately (Shani et al., 1970).

In addition to the cases of Dubin–Johnson syndrome the following subjects were studied. A control group of thirty-one healthy subjects, selected at random (mainly hospital employees); a group of thirty-one Iranian Jews (mainly hospital patients without liver disease or haemolytic anaemia); twenty cases of obstructive jaundice; eighteen cases of infectious hepatitis; thirteen cases of haemolytic anaemia; three cases of Rotor syndrome and six cases of Gilbert’s disease.

The criteria for diagnosis of Gilbert’s disease were the existence of chronic unconjugated hyperbilirubinaemia with normal results for reticulocyte count, haemoglobin, red blood cell count, osmotic fragility, serum glutamate oxaloacetate transaminase, alkaline phosphatase and bromsulphthalein (BSP) retention test. None of the patients had a history of hepatitis. In a majority of cases there was clinical and biochemical proof of the existence of familial hyperbilirubinaemia.

The diagnosis of Rotor syndrome was established by the existence of chronic conjugated hyperbilirubinaemia, high retention of BSP at 45 min, visualization of the gall-bladder on oral cholecystography and absence of pigmentation in liver biopsy material with normal histological appearance.

The majority of the subjects studied were in the age range of 17–76 years with the exception of four children with the Dubin–Johnson syndrome (ages 5–13), and four children in the control group of Iranian Jews (ages 6–9).

**RESULTS**

**Evaluation of the methods for porphyrin examination**

**Reproducibility of the method for quantitative estimation of total urinary coproporphyrin.** Thirty-five urine samples from normal control subjects were examined in duplicate on the same day for total coproporphyrin content. The mean value was 54.8 μg/g creatinine (SD 42.6, n = 35). The SD of the duplicate examinations calculated according to Mikkelsen, Dodge & Valkenburg (1965) was 6.9 μg/g creatinine.

Total coproporphyrin was determined twice on ten urine samples at an interval of 1–14 days. The mean value was 51.2 μg/g creatinine (SD 21.9, n = 20). The SD of replicate examinations was 6.9 μg/g creatinine. These results demonstrated that the reproducibility was satisfactory and that storage of urine for at least 14 days did not alter the results.

**Efficiency of separation of coproporphyrin isomers by thin layer chromatography.** A solution
Porphyrin isomers in Dubin–Johnson syndrome

containing pure coproporphyrin I and III in 2 M ammonia in exactly equal concentrations (by spectrophotometric assay) was examined by thin layer chromatography. In four replicate determinations the proportion of isomer I was found to be 48·3%, 45·9%, 49·2% and 52·9% (mean value = 48·5%). Subsequent examinations of the pure coproporphyrin I sample by thin layer chromatography showed a trace contamination with coproporphyrin III isomer.

Reproducibility of the chromatographic stage of the method for separation of coproporphyrin isomers in the urinary extracts. Dried extracts of urinary coproporphyrin, prepared as described under Methods, were examined by thin layer chromatography in duplicate at intervals of 1–2 days between the examinations. The SD of duplicate examinations for the relative content of isomer I were within 2–3% calculated in terms of total coproporphyrin (Table 1).

Table 1. Reproducibility of the method for separation of coproporphyrin isomers I and III in urinary extracts

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. subjects</th>
<th>Mean content of coproporphyrin I*</th>
<th>SD of mean*</th>
<th>SD of duplicate* examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproducibility of the chromatographic stage of the method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>17</td>
<td>28·4</td>
<td>5·6</td>
<td>3·2</td>
</tr>
<tr>
<td>Dubin–Johnson patients</td>
<td>6</td>
<td>78·3</td>
<td>9·2</td>
<td>2·1</td>
</tr>
<tr>
<td>Patients with jaundice due to various causes</td>
<td>12</td>
<td>50·1</td>
<td>19·3</td>
<td>2·0</td>
</tr>
<tr>
<td>Reproducibility of the complete procedure performed in duplicates on the same day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>27</td>
<td>29·8</td>
<td>6·9</td>
<td>3·3</td>
</tr>
<tr>
<td>Reproducibility of the complete procedure performed in replicates on different days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>11</td>
<td>27·5</td>
<td>6·8</td>
<td>2·7</td>
</tr>
<tr>
<td>Dubin–Johnson patients</td>
<td>10</td>
<td>83·8</td>
<td>5·4</td>
<td>2·7</td>
</tr>
</tbody>
</table>

* Expressed as a percentage of the total coproporphyrin content.

Reproducibility of the complete procedure for the relative content of isomers. The complete procedure for extraction and separation of coproporphyrin was carried out in duplicate simultaneously or in replicate on the same urine samples at an interval of 1–14 days (Table 1). The SDs of these duplicate or replicate determinations were within 2·7–3·3% calculated in terms of total coproporphyrin content, and thus the method was considered to be satisfactory.

Results on clinical material

Total urinary coproporphyrin excretion (Fig. 1 and Table 2). The range of total urinary coproporphyrin in the normal control group was 8·4–117·9 µg per creatinine (mean 58·1 µg/g creatinine, SD 24·0, n = 31). The mean coproporphyrin excretion of the Iranian control group was slightly higher (68·1 µg/g creatinine, SD 40·0, n = 31) but the difference was not statistically significant, therefore for further statistical evaluations the two groups were combined, totalling sixty-two control subjects with mean total coproporphyrin excretion of 61·6 µg/g creatinine (SD 33·8, n = 62).

The range of coproporphyrin excretion by patients with the Dubin–Johnson syndrome was
39.6–211.0 μg/g creatinine (mean 97.0 μg/g creatinine, SD 34.2, n = 59). These values were significantly higher than those found in the control group (P < 0.001), though the values in individual cases showed a very marked overlapping between the two groups. The mean values in patients with infectious hepatitis and obstructive jaundice were markedly higher than those observed in the control group or Dubin–Johnson syndrome patients, but a very large spread, ranging from normal values to 626 μg/g creatinine in a case of obstructive jaundice was noted.

Urinary coproporphyrin isomers distribution in control subjects and Dubin–Johnson patients (Fig. 2 and Table 2). Isomer I constituted less than 45% of the total coproporphyrin in all the control subjects with one exception (an Iranian with 59% isomer I). The mean values were 24% for the control group of random subjects and 29% for the group of Iranian controls. As the difference between these values was not significant they were combined for further statistical evaluation (mean isomer I content 27.2%, SD 9.0, n = 62).

The mean excretion of isomer I by the Dubin–Johnson syndrome patients was 84%. The frequency distribution curve of values observed in this group was not normal and therefore no SD has been calculated. The mode value was represented by the group of patients excreting 85–90% isomer I. The statistical analysis demonstrated a highly significant difference between the Dubin–Johnson and control group.

Fifty-six out of fifty-nine Dubin–Johnson cases excreted more than 65% of isomer I. However, three cases showed a normal pattern of isomer excretion (15, 23 and 36% isomer I). In
Porphyrin isomers in Dubin–Johnson syndrome

order to evaluate the significance of this finding we have compared the results of the isomers distribution with the results of other tests performed on the same patients. About 70% of the patients have been examined for BSP retention and visualization of gall-bladder on cholecystography. Among those with increased excretion of isomer I (above 65% of total coproporphyrin) only one case showed a normal BSP test, four showed a normal gall-bladder visualization and one case showed both normal BSP and cholecystography. On the other hand two of the three cases with normal isomer I excretion presented atypical results on the other tests, namely normal BSP retention and normal cholecystography (the third case has not yet been examined.

Table 2. Statistical evaluation of the differences between the urinary coproporphyrin excretion by the control subjects and the patients

<table>
<thead>
<tr>
<th>Parameter evaluated</th>
<th>Groups compared</th>
<th>Statistical significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>t or Z values</td>
</tr>
<tr>
<td>Total</td>
<td>Control subjects versus Dubin–Johnson patients</td>
<td>5.73</td>
</tr>
<tr>
<td>coproporphyrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>excretion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isomer I excretion</td>
<td>Control subjects versus Dubin–Johnson, infectious hepatitis or obstructive jaundice groups</td>
<td>6.13−8.95</td>
</tr>
<tr>
<td>(% of total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>coproporphyrin)</td>
<td>Dubin–Johnson patients versus infectious hepatitis or obstructive jaundice groups</td>
<td>5.73−5.82</td>
</tr>
<tr>
<td>Isomer III excretion</td>
<td>Control subjects versus Dubin–Johnson, infectious hepatitis groups</td>
<td>6.25−8.95</td>
</tr>
<tr>
<td>(absolute values)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isomer I excretion</td>
<td>Control subjects versus Dubin–Johnson or infectious hepatitis groups</td>
<td>4.27−7.76</td>
</tr>
<tr>
<td>(absolute values)</td>
<td></td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>Control subjects versus obstructive jaundice group</td>
<td>6.17−6.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The statistical analysis relating to total coproporphyrin excretion was performed by the t-test for samples with equal variances (Snedecor, 1967). All the other analyses were performed by the Mann–Whitney U-test (Siegel, 1956) since some of the parameters relating to Dubin–Johnson patients did not show a normal distribution curve.

by these procedures). Two of these cases had elevated serum bilirubin levels with preponderance of the unconjugated pigment while the third had normal serum bilirubin levels at the time when urinary coproporphyrins were examined.

These three cases will be provisionally designated as atypical Dubin–Johnson cases. The clinical data on these cases are given in the appendix.

Urinary coproporphyrin isomers distribution in other types of jaundice (Fig. 2 and Table 2). The pattern of isomer I excretion in urine of patients with infectious hepatitis and obstructive jaundice was found to be intermediate between that observed in the control groups and Dubin–Johnson syndrome patients, there being some overlap with both groups. The mean content of isomer I was 54% for the infectious hepatitis group and 59% for the obstructive jaundice
FIG. 2. Urinary coproporphyrin isomer I content expressed as percentage of total coproporphyrin. The printed values denote the range ± 1 SD about the mean (m). The values in the patients with the Dubin–Johnson syndrome did not have a normal frequency distribution, and a SD was therefore not calculated.
Porphyrin isomers in Dubin–Johnson syndrome

Absolute values of urinary isomers I and III excretion. In the previous sections we have dealt with the relative proportions of the two isomers excreted in the urine. The absolute values of excretion of isomer I and III are presented in Figs. 3 and 4 and statistically evaluated in Table 2. Most of the Dubin–Johnson syndrome patients excreted significantly increased amounts of isomer I. The three atypical Dubin–Johnson syndrome cases, which had normal proportional distribution of the isomers in the urine, were exceptional also in this respect, as the absolute amounts of isomer I excreted by them were very similar to the mean values observed in the control group.

Increased excretion of isomer I was also observed in patients with infectious hepatitis and obstructive jaundice and they did not differ significantly in this respect from Dubin–Johnson patients.

The comparison of the amounts of isomer III excreted by the various groups of patients produced very interesting results. The mean excretion of isomer III by control subjects was 44 µg/g creatinine. Patients with infectious hepatitis and obstructive jaundice excreted on the average significantly increased amounts of this isomer (mean values of 97 and 83 µg/g creatinine). On the other hand the Dubin–Johnson group demonstrated a significantly decreased excretion of the type III isomer, with a mean value of 14 µg/g creatinine. The three atypical
cases were included in the Dubin–Johnson syndrome group for the purpose of statistical evaluation, but it should be pointed out that their isomer III excretion was at the upper limit of or above that observed in all other patients with the Dubin–Johnson syndrome.

**Patterns of urinary coproporphyrin excretion by patients with Rotor syndrome and Gilbert's disease.** Three cases of Rotor syndrome were investigated. The proportion of isomer I in their urine (57–73 %) was above that observed in the normal controls and at the lower limit of that observed in the Dubin–Johnson syndrome patients. The absolute amounts of coproporphyrin I excreted by them were increased while isomer III excretion was within the normal range.

Six cases of Gilbert's disease were examined and they showed normal total coproporphyrin excretion as well as normal distribution and absolute amounts of the two isomers.

**DISCUSSION**

The present results, based upon examination of a large sample of patients with the Dubin–Johnson syndrome, confirm the original observation of Koskelo et al. (1967) concerning the abnormal distribution of coproporphyrin isomers in the urine. However in three of our patients a normal pattern of excretion was observed, and these patients differed from the majority of
Porphyrin isomers in Dubin–Johnson syndrome

typical cases of the Dubin–Johnson syndrome also in some other aspects. It has been suggested that the Dubin–Johnson syndrome does not constitute a separate entity, but might possibly be considered as one type of a group of disorders including Gilbert and Rotor syndromes with much overlap between the different types (Sherlock, 1967). The overlap between Dubin–Johnson and Rotor syndromes was shown by finding patients from the same families with conjugated hyperbilirubinaemia but with or without pigment in the liver cells (Arias, 1961; Wolf, Pizette, Richman, Dreiling, Jacobs, Fernandez & Popper, 1960). The overlap with Gilbert's disease might be represented by cases with unconjugated hyperbilirubinaemia in whose liver a pigment, similar to that observed in the Dubin–Johnson syndrome was found (Butt, Anderson, Fouk, Baggenstoss, Schoenfield & Dickson, 1966; Sagild, Dalgaard & Tygstrup, 1962; Herman, Cooper, Takeuchi & Sprin, 1964). Abnormal handling of unconjugated bilirubin has been observed as the only abnormality in some members of families of two Dubin–Johnson syndrome patients (Billing, Williams & Richards, 1964). Furthermore, in our recent studies, hereditary deficiency of factor VII was frequently observed in Dubin–Johnson, Rotor and Gilbert syndromes (Seligsohn, Shani, Ramot, Adam & Sheba, 1969, 1970; Seligsohn, Shani & Ramot, unpublished) suggesting an interrelationship between these three entities. It is possible therefore that our three cases, which showed a normal distribution of urinary coproporphyrin isomers should be regarded as atypical variants, possibly illustrating the overlap between the Dubin–Johnson syndrome and Gilbert's disease.

Our results suggest that the estimation of the relative content of the two isomers of coproporphyrin can be helpful in the differentiation of most cases of infectious hepatitis, obstructive jaundice and haemolytic anaemia from cases of the Dubin–Johnson syndrome. However, this procedure cannot be considered an absolutely specific tool for differential diagnosis. It is possible that an increased specificity can be obtained by combining the results representing the relative content of the two isomers and the absolute values of isomer III excretion. Thus, in four out of five patients with obstructive jaundice, in whose urine 70–80% isomer I was found (i.e. values observed also in a few cases of the Dubin–Johnson syndrome) the absolute excretion of isomer III was 38–51 µg/g creatinine, namely above the range observed in the typical Dubin–Johnson syndrome cases.

It has been known for a long time that hepatic excretory dysfunction leads to an increased excretion of porphyrin in the urine and decreased excretion in the faeces via the bile (Localio, Schwartz & Gannon, 1941; Nesbitt & Snell, 1942a, b; Gray, Rimington & Thomson, 1948; Rimington, 1952a, b; MacGregor, Nicholas & Rimington, 1952; Hoffbauer, Watson & Schwartz, 1953). According to Aziz, Schwartz & Watson (1964) this shift from the biliary and faecal to the urinary route of excretion mainly affects isomer I, while isomer III is affected less or not at all. Relative increase of urinary coproporphyrin I was observed in hepatic cirrhosis of non-alcoholic origin, infectious hepatitis, obstructive jaundice, cardiac jaundice (Aziz et al., 1964; Koskelo et al., 1967) as well as in mild liver functional impairment observed in the last trimester of normal pregnancy, and in women taking oral contraceptives containing oestrogen (Koskelo & Toivonen, 1968). In these conditions, the absolute amount of coproporphyrin III in the urine remains within the normal limits or is elevated to a lesser degree than isomer I (Koskelo & Toivonen, 1968). The results obtained by us in the present investigation suggest that in cases of the Dubin–Johnson syndrome, the change in the relative proportion of the two isomers in the urine is caused by a different mechanism, the increase in the isomer I excretion in these patients being accompanied by a decrease in the amount of
Judith Ben-Ezzer et al.

isomer III excreted. The formation of uroporphyrinogens from porphobilinogen is accomplished by the 'porphobilinogenase' enzyme system (Lockwood & Rimington, 1957). In this system two enzymic activities are associated, porphobilinogen deaminase, which acting alone transforms porphobilinogen into uroporphyrinogen I, and uroporphyrinogen isomerase (uroporphyrinogen III co-synthetase) which directs the synthesis to the type III isomer although it has no action upon porphobilinogen in the absence of the first enzyme. If the isomerase is removed or selectively destroyed or inhibited only uroporphyrinogen I results (Sancovich, Battle & Grinstein, 1969; Bogorad, 1958). It is possible therefore that the finding of a significant increase of isomer I and decrease of isomer III in the urinary coproporphyrin excreted by Dubin-Johnson patients, reflects a diminished activity of the isomerase. Such a decreased enzyme activity could stem from a primary effect of the gene responsible for the Dubin-Johnson disease or be a result of inhibition by some metabolites accumulating in the liver of these patients.

In a large family studied by Butt et al. (1966) the propositi had a classic picture of the Dubin-Johnson syndrome, but many family members had significant pigmentation of the liver without hyperbilirubinaemia or abnormality in the BSP and cholecystography tests, suggesting that at least in some cases the pigment accumulation might be the earliest or the only manifestation of the genetic abnormality. Investigations conducted by us on asymptomatic children and parents of patients with the Dubin-Johnson syndrome have demonstrated a relative increase of urinary coproporphyrin I, usually in the range intermediate between normal controls and the patients (unpublished data). It is possible therefore that the abnormal porphyrin isomer distribution in the urine may provide a sensitive indicator of the disorder present in the carriers of the gene (or genes) responsible for the Dubin-Johnson syndrome.

ACKNOWLEDGMENTS

Professor C. Rimington was visiting professor at the Tel-Aviv University Medical School sponsored by the Royal Society of London. This work was supported in part by grants AM-14552 and OG 1272 from the National Institutes of Health, U.S.A.

REFERENCES


Porphyrin isomers in Dubin–Johnson syndrome


APPENDIX

Clinical data on three atypical cases of Dubin-Johnson syndrome

Case 1. A 6-year-old boy, born to a family of Sephardic Jews from Bulgaria, had jaundice with preponderance of conjugated bilirubin from the age of 2 months onwards. Two liver biopsies, performed at the ages of 3 and 4 months respectively, contained black pigment. A laparotomy was performed when he was 5 months old because of suspicion of biliary atresia. The dark liver was observed but operative cholangiography did not confirm this diagnosis and a diagnosis of the Dubin-Johnson syndrome was made. The boy was not seen until the age of 6 years when he was seen at a follow-up study. His serum bilirubin was normal (0.4 mg/100 ml total) and the total urinary coproporphyrin excretion was 109.3 µg/g creatinine with 15% of isomer I.

Case 2. A 40-year-old woman from Turkey, diagnosed 2 years previously as having the Dubin-Johnson syndrome on the basis of the presence of dark pigment with centrilobular localization in liver biopsy material. At that time her conjugated serum bilirubin was 1.1 mg/100 ml and total bilirubin 2 mg/100 ml but the BSP test and cholecystogram were normal. At present she shows slight hyperbilirubinaemia (total bilirubin 1.6 mg/100 ml with 0.2 mg/100 ml conjugated) and her urinary total coproporphyrin excretion is 79.6 µg/g creatinine with 23% of isomer I.

Case 3. A 33-year-old man, born in Afghanistan, in whom the Dubin-Johnson syndrome was diagnosed when he was 30 years old because of a dark pigment in a liver biopsy. At that time his total serum bilirubin was 2.0 mg/100 ml with 1.1 mg/100 ml as conjugated pigment. The results of the BSP test and cholecystography were normal. During the present investigation his serum bilirubin levels showed fluctuations. On one occasion it was 0.4 mg/100 ml while 2 weeks later the total bilirubin was 2.4 mg/100 ml with 0.4 mg/100 ml conjugated bilirubin. On both occasions urinary coproporphyrin examination gave very similar results, total coproporphyrin 42 and 39.6 µg/g creatinine with isomer I content of 35% and 36%.