CONCERNING THE NATURALLY OCCURRING PORPHYRINS. V.
PORPHYRINS OF THE FECES 1, 2

BY CECIL JAMES WATSON

(From the Department of Medicine, University of Minnesota Hospital, Minneapolis)

The complexity of the porphyrin problem is due in large part to the occurrence of spectroscopically identical isomers, those corresponding in configuration to hemoglobin or aetioporphyrin III, and those of the aetioporphyrin I type, whose formation is to be regarded as an independent synthesis. Recent work has demonstrated that porphyrins of the latter group are much more frequently encountered in the excreta. Coproporphyrin I was isolated from normal urine (1, 2, 3), and from the urine in increased amount in a variety of pathological conditions other than the idiopathic porphyrinurias (3, 4, 5). In earlier communications the writer described its isolation from the feces in hemolytic jaundice and pernicious anemia (6, 7). Coproporphyrin III has been encountered less frequently; as yet only in the urine in the following diseases: 1, Lead poisoning (3, 8); 2, exceptional instances of chronic porphyrinuria (9, 10); 3, the majority of instances of acute porphyrinuria (11, 12, 13, 14); 4, salvarsan treated individuals (15); 5, instances of hemochromatosis and atrophic cirrhosis (4). Schreus (16) has recently maintained that excretion of coproporphyrin III would be found to accompany increased blood destruction. This view is not supported by the fact that coproporphyrin I is excreted in urine and feces in hemolytic jaundice (3, 4, 6). As will be noted below, this finding is confirmed in the present investigation.

Since the reports of Snapper (17), Papendieck (18), Schumm (19, 20, 21), and Boas (22, 23, 24, 25), interest in the porphyrins of the feces has centered upon their significance in the detection of occult bleeding. The exact nature of the porphyrins derived from blood in the gastrointestinal tract was first demonstrated by Kämmerer (26), and Fischer and Lindner (27). These are protoporphyrin, deuterohemin, and deuteroporphyrin. The last two are undoubtedly identical with the substances which Schumm named copratin and copratoporphyrin (19). The existing evidence concerning the derivation of coproporphyrin from blood in the intestinal tract is conflicting; Schumm (28) believed it to be the source of the coproporphyrin of the urine. However, Fischer and Schneller (29) demonstrated coproporphyrin in the urine and feces of vegetarians. The writer (30) isolated crystalline deuteroporphyrin IX, corresponding to hemoglobin, from the feces of a normal individual receiving meat in the diet. Coproporphyrin was not increased in this sample of feces although an increase might have been expected if one assumes that hemoglobin can give rise to coproporphyrin in the intestine. As will be noted below, this is borne out in the present investigation.

As yet the isomeric type of the coproporphyrin of normal feces has not been determined. If hemoglobin or meat were the source, coproporphyrin III would be expected. Kämmerer and Gürsching (31) found that many of the common foods, particularly those of plant origin, contain traces of coproporphyrin, which, by analogy to that formed in yeast cells, is probably coproporphyrin I. Fischer and Schneller (29) obtained crystals which were probably those of coproporphyrin I, from the feces of a vegetarian. Correlation of these findings suggests that the coproporphyrin of the feces is exogenous; it should be emphasized, however, that a great disproportion in amounts of porphyrin undoubtedly existed in these two studies, since it is certain that infinitely less porphyrin is necessary for the production of considerable fluorescence (the method of detection used by Kämmerer and Gürsching), than for the isolation of crystalline material. For this reason it is entirely possible that most of the porphyrin obtained by Fischer and Schneller was endogenous. Garrod (32) concluded that the bulk of the normal urinary and fecal porphyrin was endogenous. He pointed to the constant presence of porphyrin in the meconium. This

---

1 Aided by a grant from the research fund of the Graduate School, University of Minnesota.
2 Presented in part before the American Society for Clinical Investigation, Atlantic City, May 4, 1936.
was later identified by Schumm (28) as a coproporphyrin, and Waldenström (33) has recently made the important observation that it is coproporphyrin I. Fischer and Zerweck (34) likewise held the view that coproporphyrin is endogenous, and spoke of it as a normal product of metabolism. The occurrence of protoporphyrin in the erythroblasts of the marrow (35) and in the circulating erythrocytes (36) further supports the endogenous theory.

The porphyrin of human bile was identified by Schumm (28) as a coproporphyrin. Whether this is endogenous or whether absorbed from the bowel and re-excreted is not known. Borst and Königsdörffer (37) were unable to obtain experimental evidence for the direct reabsorption of porphyrins from the bowel. On the contrary, H. Fischer and Hilmer (38), and recently Brugsch (39) noted definite increases in urinary porphyrin after feeding small amounts of coproporphyrin. Although quantitative studies might solve the problem of the origin of coproporphyrins in the excreta, those so far reported (39, 40, 41) have dealt with only the total porphyrin excretion without attempt to separate proto-, deuter-, and coproporphyrin. In addition to the necessity of determining them separately, it is evident that knowledge of the isomeric types excreted normally and in disease must be obtained prior to quantitative studies, since the latter cannot distinguish isomers.

The present investigation continues previous studies of the porphyrins in feces and urine; the scope of the investigation is as follows: (1) Isolation of porphyrins from \(a\) normal feces, \(b\) bile, \(c\), feces in lead poisoning, \(d\), urine in pernicious anemia, \(e\), feces in further instances of hemolytic jaundice and pernicious anemia. (2) Comparison of amounts of porphyrins in the feces in \(a\) pernicious anemia before, during, and after liver induced reticulocyte response, \(b\), normal individuals, patients with hemolytic jaundice and patients with jaundice due to complete common duct obstruction.

**MATERIAL AND METHODS**

**Group I**

*Case 1.* Normal. Male, 18 years of age. Eight day collection of feces. From the urine of this individual, collected for a longer, but contemporary period, coproporphyrin I was isolated, as described in Study IV (3). *Case 2.* Normal. Male, 24 years of age. Four day collection of feces. Urobilinogen was 89.8 mgm. per day.

*Case 3.* Lead poisoning. Male, 46 years of age. Coproporphyrin III was isolated from the urine of this patient, as described in Study IV (3). The present collection of feces did not take place until four weeks after this isolation. In the interval there had been considerable improvement, and it was therefore necessary to re-examine the patient's urine as to porphyrin content. For this purpose the entire amount was collected during an eight day period of collection of feces. Urobilinogen in the feces was 173.4 mgm. per day.

*Case 4.* Hemolytic jaundice, familial. Male, 38 years of age. Eight day collection of feces. Patient had recurrent jaundice since infancy, and has known of enlarged spleen for many years. Father and one brother have jaundice and splenic enlargement. Hemoglobin was 56 per cent (Sahli; 17 grams per 100 cc. = 100 per cent), and average diameter of erythrocytes 6.6 μ. There were many hyperchromatic microcytes in stained preparation of blood. The resistance of erythrocytes to hypotonic saline was H₀ 0.7 per cent, H₀ 0.46 per cent; control H 0.44 per cent, H 0.36 per cent. The icteric index was 18 and the Van den Bergh reaction on blood serum indirect. No bilirubin was demonstrated in the urine. Urobilinogen in feces was 2475 mgm. per day, in urine 2.6 mgm. per day. The normal range with the method (42) used is 40 to 280 mgm. and 0 to 4 mgm. per day respectively.

*Case 5.* Hemolytic jaundice, acquired. Female, 18 years of age. Eight day collection of feces. This case was described in Part IV ((3) Case 2), where the isolation of coproporphyrin I from the patient's urine was reported. The present study was made prior to the first operation. At this time the hemoglobin was 32 per cent, icteric index 42, feces and urine urobilinogen 1106 and 9.8 mgm. per day, respectively.

*Case 6.* Hemolytic jaundice, acquired. Female, 31 years of age. The clinical features in this instance are described elsewhere (43); for the present, it is sufficient to note that a hemolytic, macrocytic anemia appeared during the course of a long-enduring painless jaundice. Correlation with other manifestations of the disease, such as marked enlargement of the liver and spleen, marked bilirubinuria, and prompt Van den Bergh reaction of the blood serum indicated a diffuse affection of the liver. During the period of increased blood destruction and consequent regenerative anemia (the reticulocyte level attaining 15 per cent), urobilinogen excretion was greatly increased; the amounts in the feces ranged from 460 to 1250 mgm. per day during the two weeks in which the anemia developed. The urine urobilinogen at this time varied between 57 and 224 mgm. per day. During the first half of the present eight day period of collection of feces, the feces urobilinogen was 990 mgm. per day; in the second half 894 mgm. per day. The icteric index was 104, and, since bilirubinuria was prominent, it is
clear that normal bile flow had not yet returned. During the ensuing weeks, the jaundice gradually disappeared; five months from the time of onset the patient appeared to have recovered completely, although the spleen and liver were still palpable; at present she has remained well for nearly two years.

Case 7. Complete common duct obstruction. Male, 26 years of age. The patient had epigastric distress, hematemesis and melena of 3 years' duration. There was progressively deepening jaundice during the last two months of life. The urobilinogen excretion was of neoplastic obstructive type (43); feces: 0.3 mgm. per day; urine—trace. Occult blood was present in the feces.

In each instance, the entire amount of urine was subjected to the procedure described in Studies I and IV (3, 5). The coproporphyrin obtained was in such small amount that it was clearly necessary to combine the final solutions from the two cases in order to isolate sufficient crystalline material to determine the melting point. This combination was less objectionable in view of the close clinical similarity of the two patients, in both of whom the findings were typical of pernicious anemia in relapse. Feces in the above instances were subjected to the isolation procedure described in Study II (6).

---

**Fig. 1. Modified Fractionation of Porphyrins of Feces as Employed in Cases of Group 2.**

Necropsy on October 28, 1935, revealed a diffuse scirrhous carcinoma of the stomach of limitis plastica type, with marked diffuse involvement of the duodenum and common bile duct. Eight day collection of feces, October 11 to October 19.

Case 8a. Pernicious anemia. Urine. Male, 55 years of age. Progressive weakness of legs had been noted for 3 years, paralysis for 1 year. There was definite evidence of combined degeneration of the spinal cord. Slight icterus and smooth tongue were observed. The hemoglobin was 38 per cent, erythrocytes were 1,400,000 per cu. mm. Marked macro-anisocytosis and poikilocyctosis were seen. The reticulocytes were 1.0 per cent. Achlorhydria gastrica was present. The feces urobilinogen ranged from 457 to 640 mgm. per day during the eight day period of urine collection prior to liver therapy.

Case 8b. Male 72 years of age. The patient complained of progressive weakness, pallor with slightly yellow skin, for 4 months. There was a smooth tongue. The hemoglobin was 28 per cent, erythrocytes 1,000,000 per cu. mm. Marked macro-anisocytosis and poikilocyctosis were noted in the blood smear. The reticulocytes were 2.0 per cent. The feces urobilinogen was 803.6 mgm. per day during a six day period of collection of urine prior to liver therapy.
Material from the second group was subjected to the following method (Figure 1) of purification modified from that previously described (6), with the intention of minimizing loss of porphyrin.

The protoporphyrin fraction was returned to ether, thence to 10 per cent sodium hydroxide; the insoluble sodium salt was redissolved in 10 per cent HCl, and the protoporphyrin further purified by taking it back and forth repeatedly between 5 per cent HCl and ether; after removal of the final ether the dry porphyrin was either esterified with HCl-methyl alcohol, or converted to mesoporphyrin by the micro method of H. Fischer and Kögl (44). The latter method was applied as follows. The ester or free porphyrin was dissolved in 1 cc. of a mixture of glacial acetic acid (six parts) and hydriodic acid of specific gravity 1.50 (two parts). This was stirred in a boiling water bath for 2 minutes, then cooled under the tap, and diluted with equal parts of distilled water. Ten per cent NaOH was now added, drop by drop, until the solution became Congo negative. The precipitated porphyrin was collected on a small filter paper and washed three times with small amounts of distilled water. It was then dissolved in 1 to 2 cc. of 1 per cent NH₄OH. A sufficient amount of 10 per cent NaOH was next added to make 0.8 per cent. If no precipitation occurred on standing overnight, enough NaOH was added to make 2 per cent, and the solution was again allowed to stand. H. Fischer and Kirrman (45) have shown that the sodium salt of mesoporphyrin IX (corresponding to aetoporphyrin III) is insoluble in 0.8 per cent NaOH, while the salts of meso-I and II, corresponding to aetio-I, are first precipitated at approximately 2 per cent NaOH. This different behavior enabled them to separate mixtures of mesoporphyrin I and IX rather completely.

Porphyrins in the deuteroporphyrin fraction, after precipitation of the sodium salt as noted in the above table, were dissolved in 10 per cent HCl. Upon addition of enough NaOH to make the solution Congo negative, the porphyrin was taken into ether. Further purification was effected by taking back and forth repeatedly between 1 per cent HCl and ether. The dried porphyrin residue from the final ether fraction was then esterified in the usual way, and an attempt made to crystallize the ester from CHCl₃-methyl alcohol.

The above coproporphyrin fractions were returned to ether, thence to 10 per cent NaOH in order to eliminate any porphyrin exhibiting an insoluble sodium salt (although as yet never encountered). From this point, further purification was carried out in the manner previously described (6). The methyl ester was repeatedly recrystallized from CHCl₃-methyl alcohol.

**Group II**

The following material comprises the second group, in each of which the fraction shown in Figure 1 was employed.

**Case 1.** Normal male 19 years of age. (The same individual as Case 1 in Group I, after an interval of eight months.) Eight day collection of feces.

**Case 2.** Pernicious anemia (For clinical summary, see Case 8a, Group I).

a. Period prior to institution of liver therapy. May 20 to May 24, May 27 to May 31. Eight day collection of feces (Involuntary defecation from May 24 to May 27 with result that feces were lost during this interval).

<table>
<thead>
<tr>
<th>Date</th>
<th>June 21</th>
<th>23</th>
<th>25</th>
<th>27</th>
<th>29</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, per cent</td>
<td>38</td>
<td>38</td>
<td>37</td>
<td>39</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>Reticulocytes, per cent</td>
<td>1.0</td>
<td>0.9</td>
<td>0.75</td>
<td>1.3</td>
<td>1.4</td>
<td>1.7</td>
</tr>
</tbody>
</table>

b. Period immediately following intramuscular administration of 20 cc. of liver extract (3 cc. derived from 100 grams of raw liver) June 1 to June 8. Eight day collection of feces.

<table>
<thead>
<tr>
<th>Date</th>
<th>June 2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, per cent</td>
<td>38</td>
<td>40</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes, per cent</td>
<td>1.6</td>
<td>18</td>
<td>25</td>
<td>16</td>
</tr>
</tbody>
</table>

c. Period following reticulocyte response June 8 to June 16. Eight day collection of feces.

<table>
<thead>
<tr>
<th>Date</th>
<th>June 8</th>
<th>10</th>
<th>12</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, per cent</td>
<td>48</td>
<td>50</td>
<td>51</td>
<td>56</td>
</tr>
<tr>
<td>Reticulocytes, per cent</td>
<td>16</td>
<td>8</td>
<td>4.8</td>
<td>4.2</td>
</tr>
</tbody>
</table>

**Case 3.** Pernicious anemia (For clinical summary, see Case 8b, Group I).

a. Period before and including first two days after institution of liver therapy April 13 to April 19. Six day collection of feces.

<table>
<thead>
<tr>
<th>Date</th>
<th>April 14</th>
<th>17</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, per cent</td>
<td>28</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Reticulocytes, per cent</td>
<td>2.0</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

b. Period of reticulocyte response to intramuscular injection of 20 cc. liver concentrate. April 19 to April 23. Four day collection of feces.

<table>
<thead>
<tr>
<th>Date</th>
<th>April 20</th>
<th>22</th>
<th>23</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, per cent</td>
<td>30</td>
<td>32</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>Reticulocytes, per cent</td>
<td>31</td>
<td>28</td>
<td>16</td>
<td>8</td>
</tr>
</tbody>
</table>
c. Period following reticulocyte response April 23 to May 1. Eight day collection of feaces.

<table>
<thead>
<tr>
<th>Date</th>
<th>Hemoglobin, per cent</th>
<th>Reticulocytes, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 26</td>
<td>44</td>
<td>6</td>
</tr>
<tr>
<td>April 27</td>
<td>47</td>
<td>3</td>
</tr>
<tr>
<td>April 28</td>
<td>48</td>
<td>3</td>
</tr>
<tr>
<td>April 29</td>
<td>48</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Case 4. Hemolytic jaundice. Male, 64 years of age. The patient complained of chronic mild icterus and enlarged spleen. The patient's father was similarly affected; his son and grandson exhibited definite evidence of hemolytic jaundice. The hemoglobin was 56 per cent, erythrocytes 3,000,000 per cu. mm., average diameter 7.1 μ. There were frequent deeply staining microcytes. The icteric index was 34. The resistance of erythrocytes to hypotonic saline was: H, 0.66 per cent, H₂, 0.44 per cent; control H, 0.44 per cent, H₂, 0.36 per cent. The reticulocytes were 6 to 9.9 per cent. The feces urobilinogen was 1236 to 1267.0 mgm. per day during the period of collection. Eight day collection of feaces.

Case 5. Paroxysmal hemoglobinuria. Female, 6 years of age. The patient was entirely well prior to March 28, 1936, when marked hemoglobinuria was noted. On March 29 the hemoglobin was 22 per cent, erythrocytes 1,270,000, leukocytes 27,100. The urine and blood serum contained large amounts of hemoglobin. A relatively small amount of methemoglobin was demonstrated in the serum. The latter also contained an obvious excess of indirect reacting bilirubin. Correspondingly, the feces contained relatively large amounts of urobilinogen, 569 mgm. per day from March 29 to April 2. Four day collection of feaces, March 29 to April 2.

Case 6. Complete common duct obstruction. Female 47 years of age. The patient was found to have a carcinoma of the descending colon with metastases to the liver. The feces urobilinogen was: 0 mgm. per day. The urine urobilinogen was 0 mgm. per day. The feces contained blood, often macroscopic. Eight day collection of feaces.

Case 7. Fistula bile.

a. Male, 28 years of age. Painless jaundice beginning about December 1933. Eight weeks later the patient had had an exploratory operation: the liver was found moderately enlarged, but no other abnormality was noted. A catheter was tied with catgut into the main hepatic duct. The jaundice gradually diminished and on February 20, 1934, had entirely disappeared; the icteric index was 10. The bile was replaced by stomach tube daily. From March 21 to March 23 (48 hours) the patient collected the entire amount of bile, measuring 1150 cc., for the present investigation. Ten per cent of this collection (115 cc.) was employed for another purpose; the remainder was diluted with ten volumes of distilled water. Twenty cc. of glacial acetic acid were added to each 1000 cc. of the diluted bile. The material was then extracted with ether (200 cc. for each 1000 cc.). The acetic and ether extract was further subjected to the fractionation described above for feces (Figure 1).

b. Male, 62 years of age. The patient complained of mild jaundice, without pain, of 5 months' duration. There were associated symptoms of diabetes with progressive weight loss. Glycosuria was present. The blood glucose varied from 0.37 to 0.39 per cent. The insulin requirement was 150 to 200 units daily. The feces urobilinogen was 76.6 mgm. per day. The icteric index was 51. Operation on May 24th revealed diffuse chronic inflammatory disease of pancreas, gallbladder, cystic duct, and lower end of the common bile duct. Multiple gallstones were found in the gallbladder. A T-tube was left in the common duct. Following operation the jaundice rapidly disappeared, icteric index on August 13th was 13. (Severe diabetes has persisted to date, January 26, 1937, although patient has gained weight and is considerably improved. Diagnosis: Chronic cholecystitis, choleodochitis, and pancreatitis.)

Bile was collected for a period of 11 days (August 7 to August 18). At this time, the feces urobilinogen ranged from 8.6 to 30 mgm. per day. After dilution with five volumes of water and acidification with glacial acetic acid, the material was extracted with ether in a continuous extraction apparatus. The duration of extraction was two hours for each portion. The extract was combined and further subjected to the above described fractionation.

The feces from each of the above instances in Groups I and II were examined for the presence of occult blood by the benzidine method. Positive tests were encountered in but two cases, in both of which bleeding was incident to gastrointestinal neoplasms. (Case 7 in Group I, Case 6 in Group II.)

In several of the instances of Group II, spectrometric estimations of the porphyrin content of some of the final fractions were made. For this purpose a double spectrocoloroscope attachment for a colorimeter was employed. This was entirely comparable to the apparatus described by Weiss (46), except that it did not have a wavelength scale, which was unnecessary in the present study. In Cases 1, 2 (a, b, and c), 3 (b and c), 4, and 6 the amount of coproporphyrin in 1 per cent

---

2 The writer is indebted to Dr. J. B. Carey of Minneapolis, for information concerning this patient, also for the collections of bile.
HCl, after the above fractionation, was estimated by spectrocolorimetric comparison of the HCl spectrum with that of a 1 mgm. per cent coproporphyrin solution in 1 per cent HCl. The data and calculations for the first instance will serve as an example of such a comparison. Forty cc. of final 1 per cent HCl-coproporphyrin solution (from 8 days feces). Ten cc. of this was further diluted to 30 cc. This dilution was compared in the spectrocolorimeter with the standard 1 mgm. per cent coproporphyrin solution. With the standard set at 15, the readings were: 15, 15.0, 16.1, 15.6, 16.1, average 15.6. \[
\frac{15}{15.6} \times 0.4 \times 3 = 1.46 \text{ mgm. or 0.18 mgm. per day.}
\]

In addition, by employing the above apparatus, a direct comparison was made of the amounts of porphyrin in the proto- and copro-fractions from normal feces (Case I, Group I) with the amounts in the corresponding fractions from hemolytic jaundice feces (Case 4, Group II).

Whenever possible, in both of the above groups of material, the porphyrin ester was crystallized and recrystallized repeatedly from chloroform-methyl alcohol; melting point and mixed melting point determinations were then made. Spectroscopic studies were carried out with a Zeiss comparison spectrometer of grating type (Löwe-Schumm), as previously described (3).

**RESULTS**

The yields in Group I are comparable, insofar as concerns the method of isolation, with those of previous communications. Because of the modified procedure employed in the instances of Group II the yields of this group are not quantitatively comparable with those of Group I. It appears that the amount of coproporphyrin obtained with the present method is considerably less than with that used previously; the final protoporphyrin fractions, however, were obviously richer in porphyrin.

In all of the cases in Table I, the absorption spectra were identical with that of a known coproporphyrin, both by measurement and by superimposition of the spectra (3, 5, 6, 7). With one exception, the crystal form was that characteristic of copro-I methyl ester, i.e.; fine curving and branching needles. The exception was Case 3a

<table>
<thead>
<tr>
<th>Case number</th>
<th>Source</th>
<th>Number of days collection</th>
<th>Crystal- line ester after 3 crystallizations</th>
<th>Melting point</th>
<th>Melting point of mixture with coproporphyrin I ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Normal feces</td>
<td>8</td>
<td>1.3</td>
<td>243-245</td>
<td>243-246</td>
</tr>
<tr>
<td>2.</td>
<td>Normal feces</td>
<td>4</td>
<td>0.4</td>
<td>243-245</td>
<td>243-246</td>
</tr>
<tr>
<td>3.</td>
<td>Lead poisoning</td>
<td>a. Urine</td>
<td>8</td>
<td>0.6</td>
<td>143-156</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. Feces</td>
<td>8</td>
<td>1.0</td>
<td>240-242</td>
</tr>
<tr>
<td>4.</td>
<td>Hemolytic jaundice-feces</td>
<td>8</td>
<td>3.6</td>
<td>247-250</td>
<td>248-248</td>
</tr>
<tr>
<td>5.</td>
<td>Hemolytic jaundice-feces</td>
<td>8</td>
<td>3.4</td>
<td>244-247</td>
<td>245-247</td>
</tr>
<tr>
<td>6.</td>
<td>Hemolytic jaundice-feces</td>
<td>8</td>
<td>1.5*</td>
<td>235-238</td>
<td>249-245</td>
</tr>
<tr>
<td>7.</td>
<td>Common duct obstruction-feces</td>
<td>8</td>
<td>None obtained</td>
<td>None obtained</td>
<td>None obtained</td>
</tr>
<tr>
<td>8a and b.</td>
<td>Pernicious anemia urine</td>
<td>4</td>
<td>0.19</td>
<td>243-246</td>
<td>243-246</td>
</tr>
</tbody>
</table>

| Group II    |        |                           |                                          |              |                                                  |
| 1.          | Normal feces | 8 | 0.16 | 241-243 | 244-247 |
| 2.          | Pernicious anemia-feces | a. | 1.0 | 243-247 | 243-247 |
|             |          | b. | 1.1 | 243-247 | 243-247 |
|             |          | c. | 0.5 | 243-245 | 244-244 |
| 3.          | Pernicious anemia-feces | a. | 6 | 0.5 | 242-245 |
|             |          | b. | 0.5 | 242-245 | 243-246 |
|             |          | c. | 0.39 | 242-245 | 243-246 |
| 4.          | Hemolytic jaundice-feces | 8 | 2.38 | 245-249 | 246-249 |
| 5.          | Paroxysmal hemoglobinuria | a. | 4 | 0.01 | 240-244 |
|             |          | b. | 0.10 | 240-244 | 240-244 |
| 6.          | Common duct obstruction-feces | 8 | None obtained | None obtained | None obtained |
| 7.          | Fistula bile | a. | 2 | None obtained | None obtained | None obtained |
|             |          | b. | 10 | 0.17 | 244-247 |

* It should be observed that this patient still had partial biliary obstruction.

† As noted previously, this fraction was lost before the yield could be determined. It was evident, however, that the intensity of color was not greater than that of fraction (b) in the same dilution; in view of the fact that fraction (a) represented 6 days, and (b) only 4 days it may be assumed that the per diem excretion of coproporphyrin was somewhat less in the first period.
**TABLE II**

Spectrocolorimetric comparisons

a. Relative per diem excretion of coproporphyrin in feces of several cases of Group II

<table>
<thead>
<tr>
<th>Case number</th>
<th>Source</th>
<th>Excretion of coproporphyrin mgm. per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal</td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td>2a. Pernicious anemia (relapse)</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>b. Pernicious anemia (during reticulocyte response)</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>c. Pernicious anemia (after response)</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>3a. Pernicious anemia (relapse) *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Pernicious anemia (during reticulocyte response)</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>c. Pernicious anemia (after response)</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>4. Hemolytic jaundice</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>6. Common duct obstruction</td>
<td>0.0†</td>
<td></td>
</tr>
</tbody>
</table>

b. Direct comparisons of copro- and protoporphyrin in normal and hemolytic jaundice feces (instances 1 and 4, respectively, in Group II)

**Coproporphyrins:**

Hemolytic jaundice: 200 cc. final 1 per cent HCl solution. Set at 15.

Normal: 30 cc. final 1 per cent HCl solution

Average 22.2.

22.2 \( \times \) 200 = 9.8 (hemolytic jaundice)

Average 26.2.

26.2 \( \times \) 200 = 13.1 (hemolytic jaundice)

**Protoporphyrins:**

Hemolytic jaundice: 200 cc. final 5 per cent HCl solution. Set at 20.

Normal: 20 cc. final 5 per cent HCl solution

Average 22.2.

22.2 \( \times \) 200 = 9.8 (hemolytic jaundice)

Average 26.2.

26.2 \( \times \) 200 = 13.1 (hemolytic jaundice)

* See footnotes 5 and 6.

† The actual value in this instance was 0.019; after taking the porphyrin into ether, however, the absorption was that of deuteroporphyrin, not coproporphyrin; the maximum of Band I was at 622.8 μ. It is evident that this represented a small fraction of the deuteroporphyrin initially present, most of which had been extracted by chloroform from 0.2 per cent HCl.

**Other porphyrins**

Protoporphyrins were encountered in considerable amount in the following cases of Group II: 1, 2a, 3a, b, and c, 4, 5, 6, and 7a. In Cases 1 (normal) and 7a (fistula bile), the amounts were only sufficient for spectroscopic identification. In each of these, as well as in the other instances mentioned, the absorption spectrum was that of protoporphyrin, and superimposition with the spectrum of known protoporphyrin, revealed identity. The protoporphyrin obtained from the feces of Case 6 was almost certainly derived from hemoglobin, since this patient had blood in the feces, and in addition had complete exclusion of bile from the duodenum, due to metastatic carcinoma. With this in mind, it is of considerable interest that the protoporphyrin in the feces of Cases 2a, 3, 4, and 5, patients who had no occult blood in the feces, appeared to be present in just as large or even larger amounts than in Case 6. The protoporphyrin methyl ester from Case 6 crystallized readily in long thick prisms; that from Cases 2a, and 5, obviously did not behave similarly. In these two instances the ester came out of methyl alcohol partly crystalline (very minute prisms), and partly in the form of small round amorphous balls. The ester from Case 5 (paroxysmal hemoglobinuria) was recrystallized six times from chloroform methyl alcohol without improving in appearance; the amount was too small for a melting point determination. The esters from Cases 2a (pernicious anemia) and 6 (common duct obstruction, cancer of colon) were converted to mesoporphyrin in the way described previously; the sodium salt of the mesoporphyrin from Case 6 was quite insoluble in 0.8 per cent NaOH, while that from Case 2a remained entirely in solution even after standing for 48 hours. This difference in behavior was not referable to a greater dilution, since the final solution of Case 2a (1.62 cc.) had a greater concentration of porphyrin than that of Case 6 (4.32 cc.). It should be noted, however, that no precipitation occurred from the solution of 2a even after the concentration of NaOH had been brought to 2 per cent.

In Case 3, the amounts of protoporphyrin from the three periods investigated were in an approximate proportion of 4 (a) to 5 (b) to 1 (c). This was determined by simple comparison of color intensity after equal dilution of each fraction. The combined protoporphyrin from these fractions was submitted to Fischer and Kögl's procedure (44); the sodium salt of the resultant mesoporphyrin did not precipitate from 0.8 per cent NaOH, but came out readily when the solution was brought to a concentration of 3 per cent. The precipitated substance was esterified after drying in the air; a few very small crystals, in the form of short needles, were obtained from chloroform-methyl alcohol, but the amount was too small to permit of recrystallization and melting point determination. Spectroscopic identification was carried out; the ester dissolved in chloroform was spectroscopically identical with
superimposed mesoporphyrin IX ester: I 621.7, II 574.2, III 533.0, IV 500.0. (The mesoporphyrin dimethyl ester employed for this comparison was obtained from hemin by Fischer and Kögl's method (44) and subsequent esterification by the usual HCl-methyl alcohol procedure; melting point 212° C.)

As already noted, a relatively considerable amount of protoporphyrin was obtained in Case 4 of Group II (hemolytic jaundice). Two weeks elapsed, from the time of the above mentioned spectrometric comparisons with the protoporphyrin of normal feces, until further study could be carried out. In this interval, although the 5 per cent HCl solution was most of the time in the refrigerator, it was evident that a change had occurred. Most of the porphyrin now present was no longer a protoporphyrin but had characteristics more nearly those of a deuteroporphyrin. The majority was now extractible from ether with 1 per cent HCl (absorption: I 592.7, II 548.9) while a small proportion, having a more bluish color in dilute HCl, still behaved like a protoporphyrin in that it was not extracted from ether with 1 per cent HCl, and in 5 per cent HCl exhibited a protoporphyrin absorption spectrum, i.e., I 594.8, II 556.0. The porphyrin extractible with 1 per cent HCl had the following absorption in ether and acetic: I 624.0, II 577.4, III 531.6, IV 498.5. In 10 per cent NaOH the sodium salt was almost entirely insoluble. Further mention of this porphyrin will be deferred until a description has been made of a similar deuteroporphyrin-like substance which occurred in several instances.

Particular attention was given to the following deuteroporphyrin fractions. Group I, Cases 1, 4, and 7; Group II, Cases 2a and b, 3a, b, and c, 4, 5, and 6. In the other cases of both groups, the amount present was too small to permit spectroscopic identification. Deuteroporphyrin was encountered in Case 7 of Group I, and in Cases 2b and 6 in Group II. In the first and last of these three instances its presence was accounted for by bleeding; the relatively small amount present in the feces of Case 2b, Group II, may have been due to meat in the diet. It is of considerable interest that the same fraction in the other cases contained a porphyrin with an absorption spectrum differing from deuteroporphyrin, but quite identical with that of the porphyrin described in Study III (7) whose ester crystallized in long prisms melting at 189 to 191° C. For the time being at least this will be designated as pseudo-deuteroporphyrin A. In the present investigation it has been encountered in six instances, i.e., in all of the above except Cases 2a and b of Group II. In two of these, crystals of the methyl ester were obtained; these were noted as having the same form illustrated in Study III (p. 118 (7)); in neither was the amount sufficient for melting point determinations. In each of the six instances the absorption spectrum was identical (by superimposition) with that of the porphyrin described in Study III: in ether I 623.8, II 575.5 (midpoint of absorption band; maximum intensity at 568.0), III 528.7, IV 496.3. In 1 per cent HCl, I 590.4, II 547.6.

In each instance superimposition of absorption spectra was carried out with solutions of known copro-, hemato-, and deuteroporphyrin. The absorption differed only slightly from that of copro-, and more sharply from hemato- and deuteroporphyrin. On the other hand, the sodium salt of this porphyrin is insoluble, its HCl number is 0.3 to 0.4, and it is extractible from 0.2 per cent HCl with chloroform, characteristics which distinguish it sharply from coproporphyrin and which are responsible for its designation as a pseudo-deuteroporphyrin. In Case 3 of Group II, the amounts in the three periods, a, b, and c, were in an approximate proportion of 4 to 6 to 1, respectively. It has already been noted that the ratio of amounts of copro- and protoporphyrins for the three periods was essentially the same as this.

In the above it was noted that the protoporphyrin fraction of Case 4 (Group II) after standing for two weeks, contained only an inconsiderable amount of protoporphyrin; most of the porphyrin present now had the characteristics of pseudo-deuteroporphyrin A. The substance was readily extracted from ether with 1 per cent HCl, and after dilution to 0.2 per cent HCl it was in turn extracted by chloroform. In 10 per cent NaOH the sodium salt quickly precipitated. The absorption spectrum was as follows: In ether and acetic acid, I 623.3, II 596.8 (faint), III 575.3, IV 528.8, V 496.0; in 1 per cent HCl, I 588.8, II
547.3. This was entirely identical with pseudodeuteroporphyrin A, when the absorption spectra were superimposed. A very slight difference was noted when superimposed with copro- and a greater difference when with deuteroporphyrin.

In Study II, a new porphyrin, also encountered in the deuteroporphyrin fraction, was found in the feces of a patient with hemolytic jaundice. The methyl ester of this porphyrin crystallized in flower-like aggregates and melted at 202° C.; spectroscopically it was characterized by absorption bands about midway between those of proto- and copro- or deuteroporphyrin. Thus the maximum of the redward band was at 627 to 629 m\(\text{\AA}\). In view of the exceptional broadness of this band, Professor H. Fischer considered the possibility of a molecular compound between two porphyrins, such as proto- and deuteroporphyrin. Because of the limited amount of material this question could not be decided. Later, a porphyrin with the same solubilities and absorption spectrum, but in an amount insufficient for isolation, was noted in the feces of another patient with hemolytic jaundice. In the present investigation a porphyrin of the same type was encountered in Case 3a (Group II), an instance of pernicious anemia in relapse. When the absorption spectrum of this porphyrin was superimposed with that of the porphyrin from the earlier investigation (ester melting point 202° C.), complete identity was noted. In view of the fact that this also behaves like a deuteroporphyrin in many respects, it may be designated as pseudodeuteroporphyrin B (pending more exact information as to its chemical structure).

**DISCUSSION**

In the present investigation coproporphyrin I has been isolated repeatedly from normal feces and in one instance from fistula bile. It was absent from the feces of two patients having complete biliary obstruction; this lends support to the belief that the substance is chiefly endogenous, especially when correlated with the marked increase again observed in hemolytic jaundice.

Since coproporphyrin I is not a derivative of hemoglobin, but is rather the product of an independent synthesis, and since the amounts excreted are greatest in instances where regeneration and bone marrow activity are most marked, it has become more and more evident that the excretion of coproporphyrin I in the feces, at least in patients with normal liver function and without biliary obstruction, is related to erythropoietic activity. There is much reason to believe that the coproporphyrin I of bile and feces is derived from the protoporphyrin which Van den Bergh and Hyman (36) found to occur in a very small amount in the circulating erythrocytes, and which Borst and Königsdörffer (35) had earlier observed in marrow erythroblasts. Van den Bergh et al. (47) first suggested this relationship after demonstrating that the surviving liver is capable of converting proto- into coproporphyrin. The amount of protoporphyrin in the normal erythrocytes is obviously very small; it is unlikely that even a sudden destruction of a majority of the erythrocytes would provide the amounts of porphyrin encountered in hemolytic jaundice feces. This is probably illustrated by the present case of paroxysmal hemoglobinuria in which intravascular hemolysis was sufficient to reduce the hemoglobin rapidly to 22 per cent; in spite of this, relatively little coproporphyrin was found in the feces, and although considerable protoporphyrin was also present, the total porphyrin did not compare in amount with that seen in hemolytic jaundice. The difference seemed too great to be explained by the disparity of ages. In this patient, however, the period\(^7\) of collection of feces followed immediately upon the first hemolysis, and probably preceded any appreciable increase of hemopoietic activity particularly when the time required for traversal of the intestinal tract is taken into account. By contrast, hemopoietic activity is quite constantly increased in hemolytic jaundice, so that the feces of any interval are certain to represent a period of heightened erythrocyte metabolism.

The above considerations focused the writer's attention upon the possibility of relationship between the erythrocyte-protoporphyrin and the reticulated cells. It has in fact been shown (48) that most if not all of this protoporphyrin resides in the reticulocytes, not in the mature erythrocytes. It is quite probable, therefore, that much larger amounts of protoporphyrin are available in blood containing a large number of maturing reticulocytes.

\(^7\) Opportunity for investigating feces of subsequent periods was not afforded in this instance.
If the protoporphyrin of the reticulocytes is the parent substance of the coproporphyrin I found in bile and feces, then it is highly probable that it likewise has the configuration of aetoporphyrin I. Otherwise one would have to assume that the protoporphyrin underwent destruction to its component pyrrol nuclei with subsequent re-synthesis of coproporphyrin I. H. Fischer (14) believes a transformation of this type to be extremely unlikely.

In the present investigation it was usually noted that protoporphyrin was increased in the same instance where the feces contained definite increases of coproporphyrin I. The only exceptions were the two patients with total biliary obstruction, whose feces contained no copro- but considerable proto- and deuteroporphyrin, the occurrence of which was readily explained by the presence of blood. Of greater interest was the protoporphyrin found in feces which did not contain demonstrable occult blood, i.e., in instances of hemolytic jaundice, pernicious anemia, paroxysmal hemoglobinuria. Attempts to identify this protoporphyrin with certainty were unsuccessful; nevertheless its behavior, as well as the behavior of the mesoporphyrin obtained from it, suggested that it differed from protoporphyrin as obtained from hemoglobin. In the case of paroxysmal hemoglobinuria, the amount of protoporphyrin was relatively large, unquestionably exceeding that of the coproporphyrin. In this instance, as in the others where an attempt was made to isolate the substance, it was evident that constant deterioration occurred during the various fractions.

In the cases of pernicious anemia who were followed through the period of reticulocyte response, it was noted that the excretion of coproporphyrin I in the feces increased slightly over that observed during relapse; after the reticulocyte response, a considerable diminution occurred. This again points to a relationship between erythropoietic activity and excretion of coproporphyrin I. The increases were not as great as one would have expected if the circulating reticulocytes were assumed to be the sole source of porphyrin. However, there is no reason to doubt that porphyrin may be furnished directly from the megaloblasts in the pernicious anemia marrow (37); whether this is dependent upon release occurring with maturation, or due to the phagocytic destruction which Peabody and Broun (49) described, remains to be determined.

The significance of the two "pseudodeuteroporphyrins" is not clear. Certain evidence, already mentioned, suggests that they are likewise derivatives of protoporphyrin. The spectroscopic character, chloroform solubility, HCl-number, and insoluble sodium salt of pseudodeuteroporphyrin A suggest similarity with the porphyrin which Schumm (50) described under the name "saproporphyrin."

It is probable that a porphyrin which the writer noted (30) in increased amount in the feces of a patient with hemolytic jaundice, which was believed then to represent deuteroporphyrin, was in reality pseudodeuteroporphyrin A.

Borst and Königsdörffer (37) observed a porphyrin in fetal liver, and in the liver of the famous case Petry (congenital porphyrinuria, pernicious anemia) which they designated as 627 (maximum absorption of redward band at that wavelength). Their studies indicated that this was derived from protoporphyrin, and they evidently considered the possibility that it might be a transition between proto- and coproporphyrin. The similarity of absorption between this porphyrin 627, and the above mentioned pseudodeuteroporphyrin B suggests a close relationship or possible identity.

The isolation of coproporphyrin I from the feces of the patient with lead poisoning, during a period in which the urine contained coproporphyrin III, emphasizes that one may not draw conclusions about the type of urinary porphyrin on the basis of the isomer present in the feces. Coproporphyrin I had been isolated repeatedly from the feces of patients with pernicious anemia, but the type in the urine had not been identified. The porphyrin isolated in the present study from the urine of two typical cases during relapse, proved to be coproporphyrin I.

The findings in the present instance of lead poisoning suggest that coproporphyrin III is either eliminated less easily by the liver, or is re-absorbed to a greater degree from the bowel than is coproporphyrin I. It is quite possible that coproporphyrin III will be found in the feces of
a more severe or more acute case than was represented in this study.

The results of the present investigation support the contention of Boas (22) that protoporphyrin in the feces does not originate solely from ingested hemoglobin nor from occult bleeding, but is also "physiological." Boas noted that it was still demonstrable in feces of a normal individual even after a long period on a milk and vegetable diet. The present study reveals that it is often increased in association with increases of coproporphyrin I in the feces of patients with heightened hemoglobin metabolism. Corresponding increases of deuteroporphyrin were not observed; in agreement with Boas (23) it is believed that this porphyrin owes its formation solely to the putrefaction of hemoglobin in the bowel. It is important to emphasize, however, that deuteroporphyrin may be confused readily with pseudodeuteroporphyrin A except when considerable purification and an exact spectrometric study have been resorted to. Because of this, the usefulness of the deuteroporphyrin test for occult bleeding is distinctly reduced. In some instances of persistent occult bleeding due to gastric carcinoma, Boas (25) noted an increased amount of coproporphyrin. The isomer type was not identified, but it is unlikely that this was other than coproporphyrin I. Since many individuals with persistent occult bleeding exhibit elevation of reticulocytes, the increased coproporphyrin observed by Boas may have been due to increased hemopoietic activity.

SUMMARY AND CONCLUSIONS

1. Coproporphyrin I has been isolated from normal human feces, and from human fistula bile. It was not found in the blood-containing feces of two patients having gastro-intestinal neoplasms, who also suffered from complete biliary obstruction. Considerable increases of coproporphyrin I were again noted in feces from patients with hemolytic jaundice. Correlation of these findings indicates that coproporphyrin I is chiefly endogenous. That it may be derived from the protoporphyrin of the circulating reticulocytes or from that of immature erythrocytes in the bone marrow, is a possibility which must be considered.

2. Increases of protoporphyrin were frequently encountered in association with increased coproporphyrin I in feces not containing demonstrable occult blood. Certain differences in behavior suggest that the isomer type of this protoporphyrin is not the same as that derived from hemoglobin.

3. Coproporphyrin III was isolated from the urine of a patient recovering from lead poisoning; the feces for the same period contained coproporphyrin I. Thus it is clear that conclusions about the isomer type of a coproporphyrin occurring in increased amount in the urine may not be based upon the type found in the feces.

4. Coproporphyrin I was isolated from the mixed extracts of urine of two patients with pernicious anemia, in relapse.

5. Tests for occult blood in the feces which are based upon the presence of porphyrin are of doubtful value unless a careful fractionation is carried out, with subsequent spectrometric identification. In particular, the pseudodeuteroporphyrins described here must be distinguished; their behavior is very similar to that of deuteroporphyrin, but their origin has not been established.

BIBLIOGRAPHY


