The Porphyrins of Acute Porphyria. The Detection of hitherto Unrecognized Porphyrins

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Waldenström (1934) reported the natural occurrence in acute porphyria of a uroporphyrin III, but Watson, Schwartz & Hawkinson (1945) and Prunty (1946) found evidence for the belief that this Waldenström porphyrin was a mixture of a large proportion of uroporphyrin I with a smaller amount of a type III porphyrin. The observations of Watson *et al.* (1945) also indicated the presence in the urine of porphyrin other than coproporphyrin and the uroporphyrin 'mixture'. The mechanism involved in the porphyrin metabolism of acute porphyria must be regarded as being of great importance, and a necessary step in the elucidation of this mechanism is the fullest possible characterization of the porphyrins present in this disease. Material from two new cases of acute porphyria observed in St Thomas's Hospital forms the basis of the present study on the nature of these porphyrins. The electrolyte metabolism in case 1 is the subject of a separate report (Prunty, 1949).

EXPERIMENTAL

Quantitative methods. The methods previously used for the determination of urinary and faecal coproporphyrin and faecal uroporphyrin (Prunty, 1946) were employed. The

urinary coproporphyrin extracts in 0.2% (w/v) HCl were given a final washing with CHCl_3 before estimation in a

Fig. 1. Paper partition chromatography of known porphyrins. Relation of R_f values to number of carboxyl groups in porphyrins.

modified Hilger fluorimeter. Urinary total porphyrin was estimated by the calcium phosphate adsorption technique

Fig. 2. Isolation of faecal porphyrins.

Fig. 3. Isolation of liver porphyrins.

of Sveinsson, Rimington & Barnes (1949), the final assays being carried out fluorimetrically. This alteration from the method previously used was necessitated by inability to obtain suitable samples of kieselguhr for porphyrin adsorption. Differential estimation of coproporphyrin I and III isomers in mixtures was carried out by a modification (McSwiney & Prunty, unpublished) of the method of Schwartz, Hawkinson, Cohen & Watson (1947). Porphobilinogen was estimated with *p*-dimethylaminobenzaldehyde (Prunty, 1946) and urobilinogen by the method of Watson (1936).

Chromatography of porphyrin esters. Adsorbents employed were 'alumina for adsorption' (Savory & Moore, 'after Brockmann'); Talc B.P. or CaCO_3 A.R. Solvents used were redistilled CHCl_3 A.R., benzene A.R. and redistilled light petroleum A.R. (b.p. 40–60°).

Partition chromatography of porphyrin mixtures. The technique used was that described by Nicholas & Rimington (1949), employing a lutidine-water system. Preliminary purification was performed as follows: Crude ester fractions were hydrolysed by contact with 7N-HCl for 48 hr. at room temperature, the porphyrins and accompanying brown pigments were then adsorbed directly on to a column (0.5 × 3 cm.) packed with talc suspended in distilled water. The non-porphyrin pigment was adsorbed on the top of the column with the porphyrins in a band beneath. The column was washed with distilled water until free from inorganic salts and then with N-NH_3 which eluted the non-fluorescent pigments. The porphyrin was eluted with 0.1 ml. 10N- NH_3 and this solution applied to the paper for partition chromatography. Ester fractions from previous chromatography were dissolved in CHCl_3 and a fraction containing approx. 50 μg . ester was evaporated to dryness under reduced pressure and the residue saponified as above. The HCl was removed in a vacuum desiccator over KOH, and the porphyrin hydrochloride dissolved in 0.1 ml. 2N- NH_3 . In order to avoid misinterpretation through slight changes in R_F values owing to variation in conditions, known markers were run on the strips in every case alongside the material under investigation. The results obtained with such markers are depicted in Fig. 1, the R_F values bearing a linear relation to the number of carboxyl groups in the porphyrin.

Conversion to mesoporphyrin. Protoporphyrin was reduced with HI according to Grinstein & Watson (1943) and isolated as mesoporphyrin ester.

Decarboxylation of uroporphyrin. This was carried out as previously described (Prunty, 1946).

Absorption spectra. These were measured with a Hartridge reversion spectroscope.

Melting points. These were observed microscopically on an electrically heated block.

Extraction of urinary porphyrins. Urine (12 l.) from case 1 collected from the 15th to 21st hospital days inclusive was brought to pH 3.4 with 2N-HCl and 100 g. talc added. After shaking thoroughly the mixture was allowed to stand at 4° for 5 days, when nearly the total porphyrin was adsorbed on the talc. The latter was filtered off, air-dried and treated overnight with 100 ml. methanol saturated with HCl. After filtration and washing with this HCl-methanol mixture and then with CHCl_3 , the filtrate and washings were poured into excess water. The CHCl_3 solution of porphyrin esters was washed successively with water, 10% (w/v) NH_3 and with water, and then evaporated to dryness. The esters were chromatographed on alumina after dissolving in CHCl_3 and

adding 7 vol. of light petroleum, the addition of which caused some precipitation. The precipitated porphyrin was filtered off and the filtrate put on the column. The precipitate was then dissolved in CHCl_3 and 5 vol. of light petroleum added. The process was repeated with 1:4, 1:3, 1:2.5 and 1:1 CHCl_3 -light petroleum mixtures. By this means all the esters were introduced on to the column, the chromatogram developed and the successive bands eluted and collected.

Isolation of faecal porphyrins. The flow sheet of this process is illustrated in Fig. 2. Faeces (582 g.) collected from case 1 from 13th to 23rd hospital days inclusive were partially dried on the water bath and treated overnight with methanol saturated with HCl. The methanolic solution contained the porphyrin esters and much fatty material, and these substances were transferred to CHCl_3 . After washing the latter it was evaporated to dryness. The residue was shaken mechanically with successive portions of 10% (w/v) HCl (total vol. 260 ml.) for 1 hr., and then allowed to stand 2 days with the last portion. The combined HCl solutions were extracted with ether, a black precipitate appearing in the ether layer. The precipitate was filtered off, and repeatedly extracted with 10% (w/v) HCl, the latter being returned to the main portion. The ether was also extracted with a small volume of 10% (w/v) HCl and the latter also added to the main bulk. Sodium acetate was added to the HCl solution of porphyrin esters until the solution was no longer acid to congo red and the ether-soluble porphyrin removed with ether. Ether-insoluble porphyrin precipitated at the interface and after filtration was dissolved in a small vol. of 10% (w/v) NH_3 and returned to the aqueous fraction which was then adjusted to pH 3.4 and treated with talc. The further procedure was identical with that used in the extraction of urine.

Isolation of porphyrins from liver. The liver freshly obtained post mortem from case 2 weighed 1162 g. and was partially dried *in vacuo* over P_2O_5 after mincing. It was moistened with acetic acid and repeatedly extracted with ether in a mechanical shaker and the porphyrins fractionated as described in the flow sheet (Fig. 3). The ether extracts were fractionated into HCl. The residual liver tissue was treated with HCl-methanol. The porphyrin esters thus extracted were chromatographed in the manner described above.

RESULTS

Quantity of porphyrin excreted

Fig. 4 indicates the results obtained for the coproporphyrin and uroporphyrin excretion in case 1. Urinary coproporphyrin reached levels as high as

2.78 mg./day and uroporphyrin 35.2 mg./day. In the faeces there were up to 4.3 mg./day coproporphyrin and 2.0 mg./day of uroporphyrin.

Urinary porphyrins

A sample of the crude porphyrin ester fraction, from case 1, prior to initial chromatography on alumina was subjected to paper chromatography. The free porphyrin mixture was found to contain porphyrins with 4, 5, 6, 7 and 8 carboxyl groups (Table 1). The porphyrins with 6 and 7 carboxyl

Table 1. Partition chromatograms of crude esters from urine

R_F value	Amount	No. of carboxyl groups	Nature of porphyrin present
0.07	Large	8	Uroporphyrin
0.12*	Trace	—	—
0.18	Trace	7.0	?
0.27	Trace	6.2	?
0.37	Moderately large	5.3	?
0.5	Large	4.0	Coproporphyrin

* Pure samples of uroporphyrin consistently give two spots. It is thought both are due to uroporphyrin (Nicholas & Comfort, 1949). To avoid confusion in subsequent tables the 0.12 spot is omitted.

groups occurred only in traces and the material containing 6 carboxyl groups was lost on subsequent purification (see below).

The primary chromatogram from the porphyrin extraction yielded four main fractions. The first was eluted with CHCl_3 -light petroleum 1:3 and after re-esterification rechromatographed on alumina from CHCl_3 -light petroleum 1:9 in which it was soluble. This yielded mainly fraction U_1 eluted with CHCl_3 -light petroleum 1:5 and traces of fraction U_2 eluted with 1:4 solvent mixture. The second fraction from the primary chromatogram, eluted with 1:2.5 solvent mixture was likewise rechromatographed, after re-esterification, from 1:8 solvent mixture in which it was soluble. This yielded mainly fraction U_2 and some U_1 . Fraction U_3 was eluted from the primary chromatogram with 1:1 solvent mixture. Fraction U_4 on the primary chromatogram was 'red brown' in appearance and after elution with CHCl_3 -acetic acid 5:1 and re-esterification was rechromatographed on alumina and yielded mostly material corresponding to fraction U_3 , with small amounts of U_2 and U_1 which were

Table 2. Characteristics of urinary porphyrins

Fraction no.	Porphyrin	Ester			Proportion of CHCl_3 to light petroleum used in the eluent from Al_2O_3	Absorption band I (λ_{max} ; m μ .)	Results from partition chromatogram	
		Amount (mg.)	No. of times crystallized	M.p. ($^{\circ}$)			R_F value	No. of carboxyl groups
U_3	Uroporphyrin	55	2	271–274	1:1	625.6	0.07 + trace 0.16	8 7
U_2	?	1	5	222–227	1:4	622.6	0.4 + trace 0.5	5 4
U_1	Coproporphyrin I	4.8	5	250–251	1:5	621.4	0.5	4

again rechromatographed on alumina from 1:7 solvent mixture. The respective moities of fractions U_1 , U_2 and U_3 were combined and crystallized from CHCl_3 -methanol (Table 2).

Fraction U_2 . 1.0 mg. ester, m.p. 222–227° after five crystallizations, containing mainly a porphyrin with five ester groups, and traces of porphyrin with four (the latter probably coproporphyrin) (Fig. 7).

Fig. 4. Daily porphyrin excretion, case 1.

Fraction U_1 . This yielded 4.8 mg. ester m.p. 250–251° after five crystallizations and by partition chromatography the material contained only four ester groups. This fraction is coproporphyrin I tetramethyl ester.

Fraction U_3 . 55 mg. ester, m.p. 271–274°, after two crystallizations. This was composed mainly of material with eight ester groups plus a trace with seven. A portion of this material was hydrolysed with 10% (w/v) HCl overnight;

the porphyrin could be extracted with ethyl acetate after the pH of the solution had been adjusted for 3.4 with sodium acetate. This fraction corresponds to 'uroporphyrin III'. Attempts to separate this fraction into components on calcium carbonate columns from benzene-light petroleum mixtures, after solution in a minimal amount of CHCl_3 , were unsuccessful.

Decarboxylation and esterification of 9.1 mg. yielded an ester which was chromatographed on calcium carbonate from benzene-light petroleum 1:1. Benzene- CHCl_3 20:1 eluted the major part of the porphyrin, which was rechromatographed on alumina. A microscopic amount of porphyrin remaining as a second band on the calcium carbonate was eluted with CHCl_3 , and after three crystallizations had m.p. 224–228°. By partition chromatography it contained four carboxyl groups, but was not further examined. The main bulk of the porphyrin was chromatographed on alumina from CHCl_3 -light petroleum 1:8 and was eluted with 1:4 mixture. Partition chromatography of the eluted porphyrin showed the presence of only four ester groups (Table 3). After two recrystallizations from CHCl_3 -methanol this fraction yielded 3.0 mg. coproporphyrin I ester, m.p. 250–252° (Fig. 5). On recrystallization from ether the mother liquor yielded a microscopic quantity of ester of m.p. 140°. The substance did not remelt above 144°, but the form of the crystals strongly resembled that of authentic coproporphyrin III methyl ester (Fig. 6).

An additional 2.7 mg. of the uroporphyrin ester was decarboxylated and the ester obtained chromatographed on alumina from CHCl_3 -light petroleum. The porphyrin, which appeared homogeneous, was assayed for the relative proportions of coproporphyrins I and III. This assay showed that the mixture contained approximately 75% coproporphyrin I and 25% coproporphyrin III.

Faecal porphyrins

Ether-soluble porphyrins. The ether-soluble porphyrins, from case 1, were fractionated into fractions soluble in 0.2, 1 and 5% (w/v) HCl respectively.

Fraction F_1 . The porphyrin soluble in 5% (w/v) HCl was esterified and treated with HI. After esterification and chromatography on alumina it yielded 5.8 mg. mesoporphyrin IX ester, m.p. 216–218°, containing two ester groups (Table 4).

Fraction F_2 . The porphyrin soluble in 1% (w/v) HCl yielded after esterification, chromatography on alumina and crystallization from CHCl_3 -methanol 3.1 mg. deuteroporphyrin IX ester, m.p. 223°, containing two ester groups.

The porphyrin soluble in 0.2% (w/v) HCl was returned to ether, the latter removed and the residue esterified with HCl-methanol. Four chromatograms were run on alumina in the manner described above. Table 5 represents a composite chromatogram of this series.

Fraction F_3 . In running the secondary chromatogram there was a marked tendency for this fraction to separate into two bands on the column (Band Ia, b, Table 5). Component *a* weighed 5.5 mg. and after five crystallizations had m.p. 248–250°. Component *b* after five crystallizations yielded 14 mg. ester of m.p. 250–252°. The mother liquor of this yielded from ether after three crystallizations 0.8 mg. ester of m.p. 149°, remelting at 168–171°. Equal parts of ester crystals *a* and the portion of *b* melting at 250–252° were mixed and gave crystals from CHCl_3 -methanol of m.p. 248–252°. The mother liquor contained only a minute amount of porphyrin. The mixed crystals on chromatography on alumina behaved as an entity. Each of the two batches of ester used for the mixed crystallizations contained four ester groups as shown by partition chromatography.

Table 3. *Properties of decarboxylation products of urinary uroporphyrin*

Porphyrin	M.p. of ester (°)	Results from partition chromatogram		
		Absorption band I ($\lambda_{\text{max.}}$; $m\mu$.)	R_F value	No. of carboxyl groups
Coproporphyrin I	250–252	621.1	0.5	4
Coproporphyrin III?	140–144	—		

Table 4. *Properties of faecal porphyrins*

Fraction no.	Nature of porphyrin	Ester			Proportion of CHCl_3 to light petroleum in eluent from Al_2O_3	Results from partition chromatogram		
		Amount (mg.)	No. of times crystallized	M.p. (°)		Absorption band I ($\lambda_{\text{max.}}$; $m\mu$.)	R_F value	No. of carboxyl groups
F_5	Uroporphyrin	4.2	2	272–275	1:1	625.5	{ + trace	8
								7
F_4	?	0.4	4	227–232	1:4	622.2	{ + trace	5
								4
F_3	{ Coproporphyrin I Coproporphyrin III	19.5 0.8	5 3	248–252 149/168–171	1:4.5–1:5	621.1	0.5	4
F_2	Deuteroporphyrin	3.1	4	223				
F_1	Mesoporphyrin (from protoporphyrin)	5.8	4	216–218	1:5	622.5*	0.79	2

* A sample of Fischer's mesoporphyrin IX methyl ester gave band I $\lambda_{\text{max.}}$ 623.7 $m\mu$.

Table 5. *Composite chromatograms of faecal porphyrin esters on alumina*

(Free porphyrin soluble in 0.2% (w/v) HCl.)

Fraction no.	Band	Amount	Proportion of CHCl ₃ to light petroleum in eluent	No. of carboxyl groups from partition chromatogram
	III	Trace	1:1	—
F ₄	II	Small	1:4	5 + trace 4
F ₅	Ib	Much	1:4.5	4
	Ia	Moderate	1:5	4

Table 6. *Properties of liver porphyrins*

Fraction no.	Porphyrin	Ester			Proportion of CHCl ₃ to light petroleum in eluent from Al ₂ O ₃	Absorption band I (λ _{max.} , mμ.)	Results from partition chromatogram	
		Amount (mg.)	No. of times crystallized	M.p. (°)			R _F value	No. of carboxyl groups
L ₄	Uroporphyrin	2.8	3	270	1:1	625.2	0.07	8
L ₃	?	2.8	3	246–250	1:6	622.8	{ Mainly 0.49 Also 0.63	{ 4 3
L ₂	?	—	3	—	1:5	622.1	{ Equal 0.5 Amounts 0.64	{ 4 3
L ₁	Mesoporphyrin (from protoporphyrin)	—	3	215–218	—	622.1*	0.8	2

* See footnote to Table 4.

Fraction F₄. This yielded 0.4 mg. ester of m.p. 227–232° after four crystallizations from CHCl₃-methanol and contained primarily material with five ester groups (Table 4), plus a trace with four ester groups. The crystals were very similar in appearance to those from the urine fraction U₂ (Fig. 7).

The ether-insoluble porphyrin. The primary chromatogram of this material yielded mainly two bands. The first was eluted with CHCl₃-light petroleum ether 1:1, and the second with CHCl₃-acetic acid 5:1. The latter after re-esterification rechromatography and elution with CHCl₃-light petroleum 1:1 was combined with the ether (F₅). The primary chromatogram also yielded traces of ether-soluble porphyrins which had escaped complete extraction.

Fraction F₅. This, after two crystallizations from CHCl₃-methanol, yielded 4.2 mg. of ester of m.p. 272–275°. The free porphyrin obtained by hydrolysis with 10% (w/v) HCl was extracted into ethyl acetate from an aqueous solution of pH 3.4. Partition chromatography showed it to consist almost entirely of material containing eight carboxyl groups and traces of porphyrin with seven carboxyl groups. Its behaviour suggests a 'uroporphyrin III'.

The fatty residue from which the porphyrins had been extracted with 10% (w/v) HCl was exhaustively examined for the presence of further porphyrins by solution in ether and extraction with 10% (w/v) NH₃. The ammoniacal extract was adjusted to pH 3.4 and treated with talc in the manner described above. On chromatography on alumina, traces of coproporphyrin and uroporphyrin esters were obtained, and a quantity of chlorophyll degradation products was eluted with CHCl₃. This fraction has not been further studied. No other porphyrin esters were found.

Liver porphyrins (case 2)

Ether-soluble porphyrin. The extraction of ether-soluble porphyrins proved to be only partially complete. The porphyrin soluble in 0.2% (w/v) HCl and not extracted from this solvent with CHCl₃ was esterified and combined with a small quantity of similar porphyrin from liver tissue (Fig. 3, Table 6, fraction L₃). A small amount of porphyrin soluble in CHCl₃ from 0.2% (w/v) HCl was esterified and chromatographed on alumina from which it was eluted with CHCl₃-light petroleum 1:5. This constituted fraction L₂, and as shown by partition chromatography contained equal amounts of porphyrins with three and four ester groups. It melted over a wide range and no precise m.p. could be obtained.

Ether-insoluble porphyrin. The primary chromatogram on alumina yielded four zones: fraction A was eluted with CHCl₃-light petroleum 1:6–1:4. After hydrolysis with 5% (w/v) HCl for 24 hr. it was extracted into ether. Fraction L₁ consisted of material extractable from ether by 5% (w/v) HCl and from the latter it could be extracted into CHCl₃. This material was combined with the corresponding fraction from the ether-soluble porphyrins. Treatment of the ester with HI yielded a small amount of mesoporphyrin IX ester of m.p. 215–218°. Fraction L₃ consisted of material extractable by 0.2% (w/v) HCl from ether. It was not extracted by CHCl₃ from 0.2% (w/v) HCl. This material was combined with the corresponding fraction from ether-soluble porphyrins. It was taken into ether, the ether removed and the residue esterified with HCl-methanol. After twice chromatographing on alumina it gave 2.8 mg. ester of m.p. 246–250° after softening at 225°. Partition chromatography showed the main fraction to contain four ester groups, but a large

Fig. 6. Coproporphyrin III methyl ester crystals from decarboxylation products (fraction U_3) ($\times 350$).

Fig. 8. Crystals of mixed coproporphyrin I methyl ester and tri-carboxylic porphyrin methyl ester from liver (fraction L_3) ($\times 120$).

Fig. 5. Coproporphyrin I methyl ester crystals from decarboxylation of urinary uroporphyrin (fraction U_3) ($\times 550$).

Fig. 7. Pentacarboxylic porphyrin methyl ester crystals from urine (fraction U_3) ($\times 550$).

amount of material with three ester groups was also present. The two components could not be separated on alumina columns, although there was a strong tendency for the mixture to give two bands in a similar manner to the faecal coproporphyrin fractions. The ester crystals (Fig. 8) strongly resemble those of coproporphyrin I methyl ester, except that the needles formed tend to be straighter.

Fractions *B* and *C* from the primary chromatogram were eluted respectively with CHCl_3 -light petroleum 1:2 and 1:1 and then further fractionated on five secondary alumina chromatograms. A porphyrin ester eluted with CHCl_3 -light petroleum 1:1 was combined with the product from fraction *D*. There were also recovered traces of porphyrin ester too small for identification. Fraction *D* formed a 'red-brown' zone at the top of the chromatogram and was eluted with CHCl_3 -acetic acid 5:1. It was re-esterified and after two further chromatograms were run the ester eluted with CHCl_3 -light petroleum 1:1 was combined with the product from fractions *B* and *C* mentioned above.

Fraction *L*₄ constituted this combined product and consisted of a total of 2.8 mg. ester of m.p. 270°. After hydrolysis with 10% (w/v) HCl it was also extracted into ethyl acetate on increasing the pH of the aqueous phase to 3.4. Partition chromatography showed that it contained only material with eight carboxyl groups. The behaviour of this porphyrin suggests that it is a 'uroporphyrin III'.

DISCUSSION

The porphyrins of the urine and the faeces of case 1 presented a similar pattern. These porphyrins were easily separable into coproporphyrin I (and a small amount of III from faeces), a pentacarboxyl porphyrin and a uroporphyrin extractable into ethyl acetate from aqueous solution of pH 3.4. Re-examination of the material obtained from a previous case (Prunty, 1946) showed that in each instance uroporphyrin from liver, faeces and urine had a similar solubility in ethyl acetate. The uroporphyrin from the liver of case 2 also shows this behaviour. Decarboxylation of the uroporphyrin obtained from the urine of case 1 showed clearly that it contained in the main uroporphyrin I, but the data from both differential crystallization and the quantitative estimation of the isomers present, suggest the decarboxylation products also contained a small proportion of coproporphyrin III. This would indicate the presence of a small amount of type III porphyrin in the original uroporphyrin ester. This ester therefore, like the ester previously obtained (Prunty, 1946), falls into the category of a Waldenström type *B* porphyrin as defined by Watson *et al.* (1945). The esters from the urine and faeces of case 1 and liver of case 2 are similar, their melting points ranging from 270 to 275°. This is rather high for so-called 'uroporphyrin III', but a figure of 275° has been reported by Watson *et al.* (1945).

The quantity of pentacarboxyl porphyrin obtained from the urine and faeces of case 1 was unfortunately insufficient for elementary analysis. It would appear to correspond with a porphyrin with a band I

maximum of 622.7 m μ . mentioned by Grinstein, Schwartz & Watson (1945). The crude porphyrin ester mixture from urine also contained traces of porphyrins with six and seven carboxyl groups, but these were present in quantities too small for isolation. There was, however, neither in urine nor faeces any suggestion of the presence of a tricarboxylic porphyrin present in the liver of case 2. On the other hand, the liver did not contain detectable amounts of the porphyrins with five, six and seven carboxyl groups. Separation of the tricarboxylic porphyrin from coproporphyrin could not be achieved except by paper chromatography. There is a striking resemblance between these ester crystals (Fig. 8) and those of coproporphyrin I methyl ester illustrated by Rimington (1936).

The mesoporphyrin obtained from the liver may be derived in part from the protoporphyrin present in the blood contained in the organ. Uroporphyrin has previously been isolated from the liver by Grinstein *et al.* (1945) and Prunty (1946), and the latter author has correlated this with the presence of porphyrin granules in the liver cells as shown by ultraviolet microscopy. It must be acknowledged that the possibility remains that the coproporphyrin and tricarboxylic porphyrin were present in the blood in the organ. Notwithstanding these questions the presence of porphyrins containing 2, 3, 4, 5, 6, 7 and 8 carboxyl groups has been demonstrated, although the evidence for the presence of the members with 3, 5, 6 and 7 carboxyl groups rests for the present on their behaviour on paper partition chromatograms. Of these porphyrins those with 8, 4, 5 and 3 carboxyl groups were present in that order of decreasing quantities. It may be that these porphyrins represent intermediate steps in ascending carboxylation of the porphyrin system.

The peculiar tendency of the faecal coproporphyrin esters to separate into two bands on alumina chromatograms is worthy of note. In general increase in the proportion of chloroform in the eluent of the chromatograms of the methyl esters of these porphyrins results in an increase of the number of ester radicles in the porphyrin contained in the eluted fraction (see Tables 2, 4 and 6). The separation of the coproporphyrin esters into two bands might be due to the formation of a molecular mixture of coproporphyrin I and III esters giving rise to one of the observed bands, whereas the remainder or excess of the coproporphyrin I ester appeared as a second band below this. Watson *et al.* (1945) demonstrated experimentally that molecular combinations of esters could occur, and that the ratio of the components tended to be strictly limited. Possibly, also, similar mixtures are formed between coproporphyrin ester and tricarboxylic porphyrin ester.

It should be noted that in case 1, although during the acute attack there was an excessive excretion of

urobilinogen (Fig. 4) and both urine and faecal porphyrin excretions were high, on clinical improvement the fall in the excretion of urine and faecal porphyrin took place simultaneously. This sequence is similar to that reported before (Prunty, 1946) but is in sharp contrast to the findings of Gray, Rimington & Thomson (1948) in an unusual case of porphyria. In their case the faecal porphyrin excretion rose as the urine excretion fell, suggesting that the liver had temporarily been unable to excrete porphyrin via the biliary tract.

SUMMARY

1. The porphyrins of two cases of acute porphyria have been studied. Both uroporphyrin and coproporphyrin excretion in urine and faeces reached high values during an acute attack in the first of these cases. With clinical improvement there was a concurrent fall in these amounts and a decrease of porphobilinogen excretion in the urine.

2. Fractionation of the porphyrins present in the urine of the first case showed the presence of coproporphyrin I, a pentacarboxylic porphyrin and traces of porphyrins with six and seven carboxyl groups.

The uroporphyrin was similar to 'uroporphyrin III' of Waldenström, but proved to consist mainly of uroporphyrin I.

3. The porphyrins of the faeces in case 1, in addition to protoporphyrin IX and deuteroporphyrin IX, consisted of coproporphyrin I and a small amount of the type III isomer, a pentacarboxylic porphyrin similar to that in the urine, and traces of a porphyrin with 7 carboxyl groups. There was also isolated a uroporphyrin of similar properties to that in the urine.

4. The liver of the second case contained protoporphyrin IX, and fractions consisting of mixtures of coproporphyrin and a tricarboxylic porphyrin were obtained. A uroporphyrin with similar properties to that from the urine and faeces of the first case was isolated.

5. The importance of these findings in relation to porphyrin metabolism has been indicated.

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A Note on the Urinary Uroporphyrin in Acute Porphyria

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It is well known that the urine in acute porphyria contains a substance porphobilinogen which, on standing or heating with weak acid, is converted into a brown pigment, porphobilin. There is evidence (Waldenström & Vahlquist, 1939; Prunty, 1945) that porphobilinogen may be a precursor of porphyrin also. Accordingly, methods of pretreatment of the urine in this disease to bring about maximal formation of porphyrin have been recommended by various workers: e.g. Rimington (1943b) states that the same full porphyrin production may be obtained

by heating the weakly acidic urine on the water bath or by standing overnight with 1% (w/v) hydrochloric acid. It is, however, by no means clear to what extent such methods of pretreatment have been adopted by workers in this field or, in general, if the urine has been used fresh or after spontaneous darkening has been allowed to take place. If it could be shown that the porphyrin obtained after these treatments was identical with that present in the fresh urine, it would clearly be advantageous to apply such methods before beginning the isolation.