were degraded chemically after the administration of [*carboxy*-^{14}C] and [*Me*-^{14}C]acetate, [α-^{14}C] and [β-^{14}C]pyruvate and of [1-^{14}C]glucose.

7. The results of degradations showed that the conversion of glucose to fatty acids proceeds by the overall reactions equivalent to glucose → pyruvate → acetate → fatty acid.

We express our gratitude to Dr R. Bentley and to Dr K. Bloch both of whom generously provided us with the samples of [1-^{14}C]glucose. The photosynthetic experiments with tobacco leaves were carried out by Dr Helen Porter at the Botany Department of Imperial College of Science and Technology, whom we thank for her co-operation. The skilful technical assistance of Miss Christin Connolly and Mr F. Gaebler are also gratefully acknowledged.

REFERENCES

Achaya, K. T. & Hilditch, T. P. (1950). *Proc. Roy. Soc.* B, **137**, 187.
Anker, H. S. (1948*a*). *J. biol. Chem.* **176**, 1333.
Anker, H. S. (1948*b*). *J. biol. Chem.* **176**, 1337.
Bernhard, K. (1940). *Hoppe-Seyl. Z.* **267**, 91.
Bloch, K. & Rittenberg, D. (1944). *J. biol. Chem.* **155**, 243.
Bloch, K. & Rittenberg, D. (1945). *J. biol. Chem.* **159**, 45.
Breusch, F. L. (1952). In *The Enzymes; Chemistry and Mechanism of Action.* Ed. by J. B. Sumner and K. Myrbäck, vol. II, part 2, p. 1033. New York: Academic Press Inc.
Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M. & Yankwich, P. E. (1949). *Isotopic Carbon*, p. 248. New York: John Wiley and Sons, Inc.
Folley, S. J. (1949). *Biol. Rev.* **24**, 316.
Folley, S. J. (1952). *Symp. biochem. Soc.* no. 9, p. 52.
Folley, S. J. & French, T. H. (1950). *Biochem. J.* **46**, 465.
French, T. H. & Popják, G. (1951). *Biochem. J.* **49**, iii.
French, T. H., Popják, G. & Malpress, F. H. (1952). *Nature, Lond.*, **169**, 71.
Harrow, B. & Mazur, A. (1932). *J. biol. Chem.* **102**, 35.
Hilditch, T. P. (1952). *Symp. biochem. Soc.* no. 9, p. 63.
Hunter, G. D. & Popják, G. (1951). *Biochem. J.* **50**, 163.
Kuhn, R. & Roth, H. (1933). *Ber. dtsch. chem. Ges.* **66**, 1274.
Popják, G. (1950). *Biochem. J.* **46**, 560.
Popják, G. (1952*a*). *Nutr. Abstr. Rev.* **21**, 535.
Popják, G. (1952*b*). *Symp. biochem. Soc.* no. 9, p. 37.
Popják, G. (1952*c*). *Brit. med. Bull.* **8**, 218.
Popják, G. & Beeckmans, M. L. (1950). *Biochem. J.* **46**, 547.
Popják, G., Folley, S. J. & French, T. H. (1949). *Arch. Biochem.* **23**, 508.
Popják, G., French, T. H. & Folley, S. J. (1951). *Biochem. J.* **48**, 411.
Popják, G., Glascock, R. F. & Folley, S. J. (1952). *Biochem. J.* **52**, 472.
Porter, H. K. & Martin, R. V. (1952). *J. exp. Bot.* **3**, 326.
Rittenberg, D. & Bloch, K. (1945). *J. biol. Chem.* **160**, 417.
Van Slyke, D. D., Plazin, J. & Weisiger, J. R. (1951). *J. biol. Chem.* **191**, 299.
Wood, H. G. (1948). *Cold Spr. Harb. Symp. quant. Biol.* **13**, 201.
Zabin, I. & Bloch, K. (1950). *J. biol. Chem.* **185**, 131.

Urinary Porphyrins in Lead-treated Rabbits

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New chromatographic methods of identifying and separating porphyrins (Nicholas & Rimington, 1949, 1951; Nicholas & Comfort, 1949; Nicholas, 1951; Chu, Green & Chu, 1951) have made possible the detection of previously undescribed porphyrins in biological material. The ether-soluble porphyrins found in the urine of men and of animals exposed to lead have been regarded until recently as consisting mainly, if not entirely, of coproporphyrin III. However, a tricarboxylic porphyrin has recently been found (Nicholas & Rimington, 1951) in the urine of a case of industrial lead poisoning and in a preparation of supposed coproporphyrin from the urine of lead-treated rabbits, as well as from other sources. Di-, penta-, hexa- and hepta-carboxylic porphyrins were found by the same workers in other pathological conditions. Weatherall & Comfort (1952) have also described tri- and tetra-carboxylic porphyrins, and have obtained evidence for the existence of a pentacarboxylic porphyrin in the urine of lead-poisoned rabbits, and have found similar porphyrins in the urine of normal rabbits. Kench, Lane & Varley (1952*a*, *b*) have found coproporphyrin I as well as coproporphyrin III, and an uncharacterized porphyrin in the urine of men suffering from lead poisoning. We have examined the urinary porphyrins of lead-treated rabbits in more detail and the results are described in this paper.

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METHODS

Extraction of ether-soluble porphyrins from urine

Rabbits were maintained on a porphyrin-producing intake of lead as already described by us (Weatherall & Comfort, 1952). Urine was separated from faeces by a grid of suitable mesh in the receiving area of the metabolism cages, collected without preservative and stored at 7°. Volumes of 0·5–2 l. were extracted by one of the two procedures described by Weatherall (1952), involving treatment with ether and acetic acid and subsequent transfer to N-HCl. A few samples were extracted by adsorbing the porphyrins on calcium phosphate (Sveinsson, Rimington & Barnes, 1949) and dissolving in N- or 3N-HCl. Pooled HCl solutions were neutralized with sodium acetate, using bromophenol blue as external indicator, and the porphyrins were taken into ether. The solutions in ether were washed with water and taken to dryness under reduced pressure. The free porphyrins were esterified overnight with methanolic HCl or H_2SO_4, transferred to $CHCl_3$, washed with water, 0·1M-Na_2CO_3, and water, and were dried before chromatography.

Chromatography on aluminium oxide of porphyrin esters

The methyl esters were dissolved in 1–5 ml. of benzene and chromatographed by Nicholas's (1951) method on grade IV aluminium oxide. The chromatogram was developed with increasing concentrations of $CHCl_3$ in benzene and finally with increasing concentrations of methanol in $CHCl_3$. Where ultraviolet fluoroscopy showed red fluorescent material not removed by methanol, the column was extruded, extracted for 36 hr. with 7N-HCl, and the extract filtered free of solid aluminium oxide.

Chromatography on paper of porphyrin esters

The method of Chu *et al.* (1951) was followed in detail, using kerosene b.p. 180°. Fractions obtained from the aluminium oxide column chromatograms were examined in this way, using methyl esters of deuteroporphyrin IX, coproporphyrin I and coproporphyrin III as markers. The modification of Kench *et al.* (1952*b*) was also used, in which the second solvent is run at right angles to the first.

Chromatography on paper of free porphyrins

The method of Nicholas & Rimington (1949, 1951), depending on partition between lutidine and water, was used. Porphyrin esters were saponified in 7N-HCl for 35 hr. at room temperature before chromatography. Details of technique have been described by Weatherall (1952).

RESULTS

Chromatography on aluminium oxide columns

In all, six batches of esterified porphyrins from lead-treated rabbits were examined. Each batch contained between 1 and 5 mg. of porphyrin, and represented the output of about 10–50 rabbit days. Thirty-two rabbits contributed to the various batches; no rabbit contributed to more than two of the batches. One batch of about 0·5 mg. of porphyrin from four normal rabbits has also been examined: in view of the large amount of starting material necessary, detailed studies of the porphyrin of normal rabbit urine are incomplete and will be reported later. The chromatograms of the material from lead-treated rabbits were similar, and usually showed five fluorescent bands (numbered throughout this paper from below upwards) (Fig. 1*a*). Band 1 was a fluorescent colourless trace completely eluted by 10% chloroform in benzene.

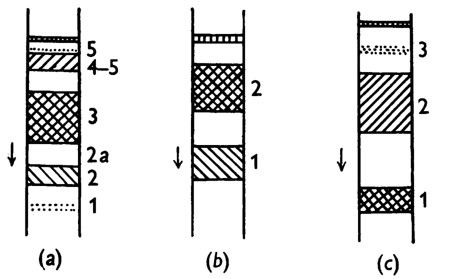

Fig. 1. Chromatograms on aluminium oxide of porphyrin methyl esters from urine of lead-treated rabbits, as developed with 10–20% $CHCl_3$ in benzene. (*a*) Preliminary run; (*b*) pool of predominantly 3-COOH porphyrins; (*c*) pool of high-COOH porphyrins. , tri-carboxylic porphyrins; , tetracarboxylic porphyrins; , penta- and hexa-carboxylic porphyrins; , tri- and tetra-carboxylic porphyrins; , mixed residue at top of column.

Band 2 was a substantial pink zone eluted by 10–15% chloroform. Band 3 was maroon in colour and contained the bulk of the porphyrin. It was also eluted by 10–15% chloroform, though more slowly than band 2, and it was found to consist mainly of coproporphyrin. Bands 4 and 5 were commonly fused. Band 4 ran as a pink zone and 5 as a fluorescent, usually colourless, band close behind it. A pigmented but non-fluorescent zone, pink or violet, probably consisting of bile-pigment esters, occasionally appeared between bands 2 and 3 (2*a*). The concentration of chloroform necessary to elute the various bands differed slightly in different runs, and the figures given are approximate. In one run the coproporphyrin moved exceptionally readily and no separation of antecedent bands was obtained.

The large eluted fractions were taken to dryness, dissolved in a few drops of benzene or chloroform and diluted with an equal volume of light petroleum. They were left at 5° to crystallize. Crystals were obtained on several occasions from band 3, and once from band 5 (sample 465). Some of their properties are shown in Table 1. Most of the crystalline samples behaved as coproporphyrin III on lutidine and on kerosene paper chromatography, though often they were not free from other porphyrins. Sample 465 had a melting point (146°, remelt 168°,

Table 1. *Urinary porphyrins of lead-poisoned rabbits*

(Melting points and paper chromatography of crystalline methyl esters. Sample 420 was an unchromatographed methyl ester. All the other samples were obtained from band 3 of alumina chromatograms, except 465, which came from band 5 of the chromatogram which had already yielded 463. Sample 434*b* was a second crop obtained on concentrating the mother liquor from 434*a*.)

Sample no.	M.p. (uncorr.) (°)	Appearance	Porphyrins detected on paper chromatogram: Lutidine/water system, after hydrolysis	Porphyrins detected on paper chromatogram: Chu method, methyl esters
420	138	—	Tetra- and penta-carboxylic	—
434*a*	146, remelt 164	Needles, typical of copro. III	Tetra-, trace penta- or hexa-carboxylic	Copro. III
434*b*	146	Ill-formed needles	Tri-, tetra-, penta-carboxylic	—
463	142	—	Tetracarboxylic	Copro. III
465	146, remelt 168	—	Tetracarboxylic	Not copro. I or III (see Table 2)
501	120	Rosettes. Not homogeneous	Tetracarboxylic	Three spots—copro. III and two substances with lower R_F values
511	134, remelt 140	Well-formed needles	Tetra-, penta- or hexa-carboxylic	Copro. III

uncorr.) like that of coproporphyrin III, but behaved differently on kerosene paper chromatography: it is further discussed below.

Samples of about 2 μg. were taken from the eluted fractions, and from the mother liquors from which crystals had been obtained. These samples were hydrolysed and chromatographed on lutidine papers to determine the carboxyl number of the porphyrins present. The fractions usually yielded two or more spots. On the whole the early eluates contained porphyrins with high R_F values and the later eluates contained those with lower values. Porphyrins running parallel to standard coproporphyrin were present in nearly all the eluates at this stage. The mother liquors from which porphyrins had been crystallized still contained appreciable amounts of red fluorescent material, and the paper chromatograms suggested that this remaining material was largely composed of tri-, penta- and hexa-carboxyl porphyrins.

The single batch of porphyrins extracted from normal, untreated rabbits' urine showed on aluminium oxide chromatography bands 1, 2, 3 (predominant) and one or more further bands which migrated more slowly than coproporphyrin. The various components appeared to be present in much the same relative proportions as in the extracts of the urine of lead-treated animals, though the quantities were much less.

In the further investigation of the material from lead-treated rabbits, the eluted fractions which were too small to crystallize, or which failed to crystallize, and the mother liquors were combined in groups according to their predominant components and were chromatographed again on aluminium oxide columns. This second series of chromatograms resembled the preliminary runs in giving rise to several fractions, but the proportions differed, as expected, from the composition of the starting material.

Apparently corresponding fractions from these runs were combined as before and a third series of solid chromatograms was run on aluminium oxide columns. Three runs were made, with pools rich in esters of porphyrins with three, four, and five or more carboxyls respectively. The tricarboxylic porphyrin pool (Fig. 1*b*) yielded two bands which separated well. The first band was eluted with 5% chloroform (sample 571) and on hydrolysis yielded only tricarboxylic porphyrins (R_F on lutidine chromatography, 0·77; comparable standard values are given in Table 2), while the second band was eluted with 10% chloroform and yielded tetracarboxylic porphyrins on hydrolysis (lutidine R_F, 0·64). The tetracarboxylic porphyrin pool ran as a single band eluted by 10% chloroform. The polycarboxylic porphyrin pool (Fig. 1*c*) divided into three bands of which the first two appeared well separated. The first fraction was eluted with 10% chloroform and on hydrolysis contained mainly tetracarboxylic porphyrins (lutidine R_F, 0·64) with a trace of pentacarboxylic material (R_F, 0·44). The second band was eluted by 20% chloroform (sample 592) and contained only hexacarboxylic porphyrin (R_F, 0·33). The third band was eluted by 30% chloroform (sample 593), and, rather surprisingly, on lutidine chromatography of a hydrolysed portion appeared to contain tri- and tetra-carboxylic porphyrins (R_F, 0·76 and 0·62).

As the amounts of material available were decreasing and the atypical porphyrins were proving difficult to separate completely, it was

Table 2. *Chromatography of urinary porphyrins from lead-treated rabbits*

(The figures given are the mean of three or less observations, the range of four to ten observations and the mean and standard deviation of more than ten observations.)

		Paper R_F values			
		Free porphyrin (Lutidine/water)	Porphyrin methyl ester (1, kerosene/chloroform and 2, propanol/kerosene)		
			One dimension (Solvents run successively over paper in same direction)	Two dimensions	
Porphyrin	Elution order from Al_2O_3			Solvent 1	Solvent 2
Standards					
Deuteroporphyrin IX	—	0·84±0·04 (S.D.)	0·41±0·02 (S.D.)	0·00	0·53
Mesoporphyrin IX	—	—	0·89*	—	—
Protoporphyrin IX	—	—	0·84*	—	—
Coproporphyrin I	—	0·62±0·04 (S.D.)	0·60±0·03 (S.D.)	0·31–0·64	0·05–0·15
Coproporphyrin III	—	—	0·77±0·03 (S.D.)	0·58–0·64	0·64–0·68
Urophorphyrin I	—	0·03	0·17	0·22	0·01
From Pb urine					
Coproporphyrin III	3	0·56–0·64	0·72–0·82	0·58–0·63	0·57–0·69
Porphyrin *A*	1	0·82–0·91	0·96	—	—
Porphyrin *B*	2	0·70–0·79	0·87	0·74	0·76
Porphyrin *C*			0·67	0·60	0·52
Porphyrin *D*	5	0·55–0·62	0·34–0·45	0·09–0·26	0·34–0·49
Porphyrin *E*	4+5	0·27–0·38	0·67–0·73	0·50–0·60	0·56–0·66
Porphyrin *F*			0·41–0·46	0·0 –0·13	0·24–0·36
Porphyrin *G*	5	0·76	0·51	0·39–0·44	0·46

* Figures given by Chu *et al.* (1951).

decided to get as much further information as possible about the purer fractions already obtained, rather than to embark on extensive further extractions of rabbits' urine in order to obtain additional material.

Elution of the residue at the top of the column with methanol, or its hydrolysis with 7N-hydrochloric acid, always yielded a mixture similar to the substances eluted from the column, and it did not therefore appear that selective loss of any particular fractions was occurring in this way.

Kerosene paper chromatography

Fractions which ran homogeneously on aluminium oxide columns and on lutidine papers were shown by kerosene paper chromatography to be non-homogeneous, and it was possible to recognize several porphyrins which occurred repeatedly in our material and which did not correspond to any of the standards examined. The regions of the two-dimensional chromatograms in which the standard and the unknown porphyrins appeared are shown in Fig. 2, and a provisional interpretation of the findings is discussed below. In order to exclude artifacts, portions of paper containing porphyrin esters separated by this method have been cut out, extracted with acetone and run as before. The fractions have run homogeneously with R_F values comparable to those observed in the first run, and have shown no tendency to divide again.

DISCUSSION

Nicholas (1951) has shown that the elution order of porphyrin esters chromatographed by her technique on grade IV aluminium oxide agrees with the

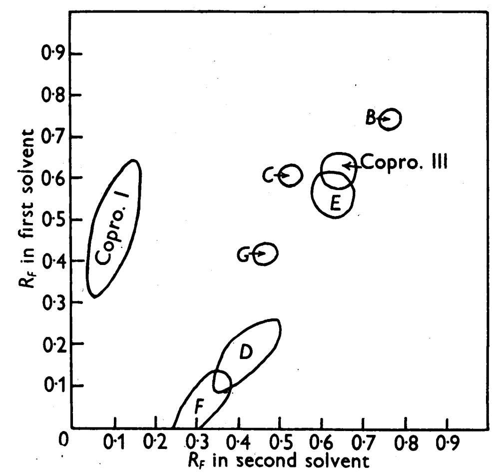

Fig. 2. Paper chromatography of porphyrin methyl esters from urine of lead-treated rabbits, showing range of areas occupied by coproporphyrins I and III and porphyrins *B*, *C*, *D*, *E*, *F* and *G*.

number of esterified carboxyl groups, and Nicholas & Rimington (1949, 1951) have found an inverse linear relation between the number of carboxyl groups in a free porphyrin and its R_F values on chromatography on paper with lutidine and water. Our own results with deuteroporphyrin, coproporphyrin and uroporphyrin (Table 2) support this linear relation, and give us grounds for attributing carboxyl numbers to the various fractions examined. With certain exceptions, all the porphyrins which we have obtained from the urine of lead-treated rabbits are consistently identified by both methods. The lowest band (1), eluted from the aluminium oxide column with 5–10 % chloroform, is a minute trace, sometimes too small even for identification by paper chromatography, but it has been twice identified after hydrolysis as a dicarboxylic porphyrin. Band 2 consists mainly of tricarboxylic porphyrin esters. Band 3, containing the bulk of the material, yields largely coproporphyrin, but in the preliminary chromatograms carried with it several other constituents in small amounts. Bands 4 and 5 contain a mixture of penta- and hexa-carboxylic porphyrin esters in varying amounts. However, lower carboxyl fractions sometimes appear in these final bands. In one preliminary run a tetracarboxylic porphyrin ester (465) was obtained from band 5 in sufficient amounts to crystallize. Also, the final band (593) of the pool of high-carboxyl esters gave both tri- and tetra-carboxylic porphyrins on hydrolysis. These tri- and tetra-carboxylic porphyrin esters did not behave on kerosene chromatography like the tri- and tetra-carboxylic esters which came off the aluminium oxide column at the expected time, and appear to be different entities. They are discussed below.

The majority of samples obtained from bands 4 and 5 gave free porphyrins with R_F values on lutidine papers of about 0·3. If the relation between R_F and number of carboxyls is strictly linear, this indicates a predominance of hexacarboxylic porphyrins. Occasionally spots with R_F about 0·45–0·50 were found. These spots would correspond well with a pentacarboxylic porphyrin and support the hypothesis that the main constituent of the high-carboxyl fraction contains six carboxyl groups. Porphyrins extracted from the urine, adsorbed on talc, eluted and run on lutidine papers have given R_F values about 0·35–0·40. We have previously reported observations indicating that a pentacarboxylic porphyrin is the main constituent of the high-carboxylic fraction (Weatherall & Comfort, 1952), but in the light of the present observations on more highly purified material we regard this interpretation as doubtful.

Kerosene chromatograms of the porphyrin methyl esters show that most of the fractions obtained by alumina chromatography are not homogeneous, even when the corresponding free porphyrins behave as single substances on lutidine chromatography. From the behaviour of the esters we incline to the view that the unknown tricarboxylic and hexacarboxylic porphyrins may exist in two isomeric series. If our chromatographic separations all correspond to structural differences, it is necessary to postulate no less than six or possibly seven porphyrin types other than coproporphyrin III, and perhaps I, in our material. The distinguishable porphyrins which appear to be entities are here cited by letters, and their properties are given in Table 2.

Porphyrin *A* corresponds to band 1 from the aluminium oxide column. It behaves as a dicarboxylic porphyrin on lutidine chromatography. One sample only was examined by the method of Chu *et al.* (1951). It ran with an R_F of 0·96, which is close to the value obtained by Chu *et al.* with mesoporphyrin IX and protoporphyrin IX. It may also correspond with the unknown porphyrin reported in the urine of human lead-poisoned subjects by Kench *et al.* (1952*b*). However, in our material, the possibility that it might arise from faecal contamination of urine specimens, though unlikely, is not wholly excluded.

The fraction eluted by 5 or 10 % chloroform (band 2) contained tricarboxylic porphyrins. The purest samples (e.g. 571) ran as a single spot on lutidine papers, but as two, of roughly equal intensity, in one- and two-dimensional kerosene chromatograms (porphyrins *B* and *C*). Porphyrin *C* had an R_F value by this method very close to that of coproporphyrin III, but no tetracarboxylic material was detectable by Nicholas's method. It seems likely that *B* and *C* are distinct tricarboxylic porphyrins and the possibility exists that they are, in fact, position isomers of series I and III.

The coproporphyrin band (3) almost alone gave crystals. The crystallized material generally contained small traces of tri- and penta- or hexacarboxylic porphyrins on hydrolysis and lutidine chromatography but ran as a single spot corresponding to coproporphyrin III by the method of Chu *et al.* (1951). This behaviour is not unexpected, as the esters of porphyrin *C* and one of the hexacarboxylic porphyrins (*E*, below) have R_F values very close to those of coproporphyrin III.

Evidence of the presence of coproporphyrin I was scanty. Two mother liquors from which coproporphyrin III had crystallized contained a fraction running at $R_F = 0·58–0·63$ on one-dimensional papers by the method of Chu *et al.*, which agreed well with the behaviour of known coproporphyrin I run on the same strip: but the apparently greater solubility of this fraction is not typical of the series I isomer. No unequivocal coproporphyrin I was isolated in the crystalline state. The discrepancy between our finding and that of Kench *et al.* (1952*a*, *b*) might be expected from the far higher proportion of coproporphyrin III normally excreted in rabbit urine as compared with human (Schwartz & Zagaria, 1951).

A third, presumably tetracarboxylic, porphyrin (*D*) was distinguished on kerosene chromatograms (sample no. 465). It was eluted from alumina with unusual difficulty, requiring 100% chloroform for full removal. Crystals obtained from benzene-light petroleum solution had a melting point 146° (remelt 168°, uncorr.) compatible with coproporphyrin III. It ran in lutidine water as a tetracarboxylic porphyrin. On two-dimensional paper chromatography, trace spots corresponding to the positions of coproporphyrins I and III were just detectable, but the bulk of the sample always behaved quite differently (R_F about 0·17/0·41) from coproporphyrin markers, and both on one-dimensional kerosene chromatography and on the column its behaviour was suggestive of a higher number of carboxyl groups than was found in any other substance isolated from our material. No other homogeneous sample of *D* was obtained, but there was evidence of a similar substance in the last fraction obtained by elution with 30% chloroform from an alumina chromatogram of the pooled high-carboxylic fractions. This sample (593) ran on hydrolysis mainly as a tetracarboxylic porphyrin with a trace of a tricarboxylic porphyrin. The ester ran on kerosene papers mainly like *D* with a smaller fraction at R_F 0·51 in one dimension and 0·44/0·46 in two. All these values are lower than those of *B* and *C*. This would suggest that there may be a tricarboxylic porphyrin (*G*) related to these porphyrins much as *D* is related to coproporphyrin. In view of the fact that alumina may cause partial saponification of porphyrin esters (Rimington, personal communication) the possibility that *D* and *G* are column artifacts requires further investigation.

The main hexacarboxylic fraction (e.g. 592) appears, like the tricarboxylic fraction, to consist of two components, one moving rather like coproporphyrin III on two-dimensional kerosene papers (fraction *E*) and one much more slowly (fraction *F*). Fraction *E* is differentiated from coproporphyrin III both by the behaviour of the hydrolysed porphyrin on lutidine papers and also by a consistently slightly lower R_F on one-dimensional kerosene papers.

The physiological significance of this large array of related substances is difficult to assess especially in view of the finding of one of us (Weatherall, 1952) that little if any of the ether-extractable porphyrin is present as such at the moment of excretion. The relative proportions of the porphyrins containing various numbers of carboxyl groups seem to differ little and there is no evidence that lead intoxication does more than provoke a general proportionate increase. Its effect on isomer distribution compared with the normal has not been studied by us, but we find very little if any coproporphyrin I in the lead-treated rabbit.

SUMMARY

1. The porphyrins present in the urine of lead-treated rabbits have been examined chromatographically.

2. The chief porphyrin present was coproporphyrin III. Coproporphyrin I was not convincingly demonstrable in any of the material, and if present constituted a very small fraction of the total.

3. Evidence was obtained for the presence also of porphyrins containing two, three, six and perhaps five carboxyl groups. It is suggested that the tri- and hexa-carboxylic porphyrins exist in two position-isomeric forms.

4. Evidence was also obtained for the presence of another tetracarboxylic porphyrin not identical with coproporphyrin I or III.

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REFERENCES

Chu, T. C., Green, A. A. & Chu, E. J.-H. (1951). *J. biol. Chem.* **190**, 643.

Kench, J. E., Lane, R. E. & Varley, H. (1952*a*). *Biochem. J.* **51**, ix.

Kench, J. E., Lane, R. E. & Varley, H. (1952*b*). *Brit. J. industr. Med.* **9**, 133.

Nicholas, R. E. H. (1951). *Biochem. J.* **48**, 309.

Nicholas, R. E. H. & Comfort, A. (1949). *Biochem. J.* **45**, 208.

Nicholas, R. E. H. & Rimington, C. (1949). *Scand. J. clin. Lab. Invest.* **1**, 12.

Nicholas, R. E. H. & Rimington, C. (1951). *Biochem. J.* **48**, 306.

Schwartz, S. & Zagaria, R. (1951). In *Toxicology of Uranium*, p. 290. Ed. by Tannenbaum, A. New York: McGraw-Hill.

Sveinsson, S. L., Rimington, C. & Barnes, H. D. (1949). *Scand. J. clin. Lab. Invest.* **1**, 2.

Weatherall, M. (1952). *Biochem. J.* **52**, 683.

Weatherall, M. & Comfort, A. (1952). *Nature, Lond.*, **169**, 587.