

Urinary Coproporphyrin Isomers I and III in Lead Workers and a Control Group

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The excretory pattern of coproporphyrin I and III isomers has been studied in urine from nine healthy subjects and 10 lead-exposed workers with no clinical evidence of lead poisoning.

In normal urine it was found that coproporphyrin I exceeded coproporphyrin III, whereas in lead workers coproporphyrin III predominated, although this was accompanied by an increase in the absolute amount of coproporphyrin I, indicating active haemopoiesis in lead absorption.

Improvements in analytical methods during recent years have made it possible to gain a general picture of the patterns of porphyrin excretion seen in the different porphyria diseases. A clearer view of normal porphyrin metabolism has also been obtained.

It is thought that during the formation of porphyrin III in haemoglobin synthesis, porphyrin I is also formed by a side reaction. This porphyrin, being of no use for haem formation (Rimington, 1936), is excreted in the urine, bile, and faeces as coproporphyrin I, the amount of which is a good index of erythropoietic activity. Porphyrin III is excreted as coproporphyrin III, but only in excess if there is some toxic action on the blood, as in poisoning with lead, arsenic or sulphonamide drugs. This excretion appears to be due to the blocking of a stage in the production of haemoglobin and not to the destruction of haemoglobin itself.

A review of the literature revealed that very little has been published on the pattern of porphyrin excretion in lead poisoning and none at all on the excretion after lead absorption. In qualitative studies, Watson (1936) and Holeček and Pěničková (1957) reported a greater predominance of isomer III over isomer I in urine in lead poisoning. A quantitative study by Kench, Lane, and Varley (1952) on urine from seven lead-poisoned workers showed that coproporphyrin I excretion in urine is concomitantly increased with coproporphyrin III, and that the former constituted an average of 30% of the total coproporphyrin.

In normal urine the proportion of the two isomers has been the subject of study by several authors who followed various procedures for the separation of the two isomers. As an example of the variability of figures recorded in the literature, Watson, Hawkinson, Schwartz, and Sutherland (1949) found by a fluorescence quenching technique at least 60% of the total to be coproporphyrin I, while Comfort, Moore, and Weatherall (1954), using the Chu-Green paper chromatographic method (1951), obtained a preponderance of coproporphyrin III and Holeček and Pěničková (1957) obtained equal amounts. The most widely accepted view is that the coproporphyrin I isomer predominates.

The present study examines by a paper chromatography technique the excretory pattern of these two metabolites in urine from recently exposed lead workers and a control group.

Ten lead workers have been studied. They had been exposed to lead for periods varying from two to six months with an average of three months. None of them showed clinical evidence of lead poisoning. For comparison, nine spot urine samples from healthy non-exposed subjects have been collected and analysed for coproporphyrin I and III isomers.

Methods

Haemoglobin Haemoglobin was determined by the oxyhaemoglobin method (Britton, 1963) using a Hilger electrophotometer, model H. 810.301 at 550 m μ . The results were obtained from standard curves and tables (100% = Hb 14.8 g./100 ml.).

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Punctate Basophilic Cells These were counted under dark-ground illumination (Lane, 1949).

Urinary Lead Urinary lead was determined by a modified dithizone method (Dick, Ellis, and Steel, 1961).

δ -Amino-laevulinic Acid in Urine. This was determined by the method of Mauzerall and Granick (1956).

Separation and Determination of Coproporphyrins I and III The method of Chu, Green, and Chu (1951) was the basis of the following procedure. For lead workers, 20 ml. of urine, and for normal subjects, 100 ml., were taken, and the total coproporphyrin (I and III) was determined by the Rimington and Sveinsson method (1950). (For normal subjects it is best to extract about 100 ml. of urine before proceeding with the isomer analysis, even though the latter can be accomplished with as little as 2 μ g. of the total porphyrin, since considerable loss of porphyrin may occur during the subsequent purification procedure.) Sodium acetate in 0.5 g. portions was added to the hydrochloric acid extract of total coproporphyrin in a separating funnel and shaken until dissolved. This was repeated until the pH of the solutions was neutral to bromophenol blue, used as an external indicator. An equal volume of ether was added to the separating funnel and shaken for one minute. The acid layer was run off. The ether was transferred to a 100 ml. beaker and taken to dryness under reduced pressure. Methanolic hydrochloric acid (10% conc. HCl in methyl alcohol), 5 ml., was added and allowed to stand overnight. The solution was transferred to a separating funnel with water washes and made up to about 50 ml. with water. The porphyrins were extracted with 5 ml. portions of chloroform. The chloroform extracts were shaken twice with water, once with 0.1 M sodium carbonate, and finally twice with water. The concentration of the porphyrins was ad-

justed in the chloroform by comparing the fluorescence of standard solutions of porphyrins.

Three drops of the chloroform solution were chromatographed on Whatman No. 1 paper with propanol/light petroleum (Analar grade, 1/5, v/v) for 45 to 60 minutes. The papers were viewed under ultraviolet light to find the porphyrins, and the spots were cut out, and the porphyrins were extracted with chloroform/acetic acid (5/1, v/v), and estimated in the electrophotometer (Unicam SP500) at 406 m μ .

If the urine contained x μ g./l. of total coproporphyrin it contained: $xz/(x + y)$ μ g. coproporphyrin I/l. and $yz/(x + y)$ μ g. coproporphyrin III/l., where x and y were the optical densities of the chloroform solutions of coproporphyrins I and III respectively.

Results

The results of analyses for lead, total coproporphyrin, and δ -amino-laevulinic acid in urine, haemoglobin, and basophilic stippled cells in blood in the lead workers indicated a considerable degree of lead absorption compared with similar analyses from 25 control subjects (Mehani, 1965) (Table I). The highest values for urinary lead (219 μ g./l.), total coproporphyrin in urine (2,625 μ g./l.), and basophilic stippled cells in blood (12,000/million red blood cells), and a relatively low haemoglobin value (12.87 g./100 ml. of blood) were found in worker no. 6 who had worked in the lead industry for four months following a period of eight years in a shipbuilding yard. It is not known whether his previous job had exposed him to lead.

In Table II the percentages of coproporphyrins I and III for control no. 4 have been omitted in calculating the means, as they are more than

TABLE I
LEAD AND δ -AMINO-LAEVULINIC ACID IN URINE, AND HAEMOGLOBIN AND BASOPHILIC STIPPLED CELLS
IN BLOOD FROM LEAD WORKERS AND A CONTROL GROUP

Worker No.	Exposure (mths.)	Urine		Haemoglobin (g./100 ml.)	Punctate Basophils (per million R.B.C.)
		Lead (μ g./l.)	δ -ALA (μ g./l.)		
1	2	91	630	14.94	4,000 SML
2	2½	133	66,130	14.94	5,000 SML
3	3	178	7,340	14.50	—
4	2	162	5,840	14.06	650 SM
5	3	58	21,020	14.50	None
6	4	219	6,980	12.87	12,000 SML
7	6	171	5,460	16.42	1,000 SM
8	4	69	5,980	16.98	None
9	3	155	3,940	16.98	—
10	4	77	5,840	12.13	550 SM
Average	3	131	12,916	14.83	2,900
Average results of 25 non-exposed men		15	2,019	15.60	None

S = small; M = medium; L = large.

TABLE II
COPROPORPHYRINS I AND III AND TOTAL URINARY COPROPORPHYRINS FROM 10 LEAD WORKERS
AND NINE CONTROLS

Lead Workers	Total Coproporphyrin ($\mu\text{g.}/\text{l.}$)	Coproporphyrin III		Coproporphyrin I	
		$\mu\text{g.}/\text{l.}$	% of total	$\mu\text{g.}/\text{l.}$	% of total
1	1,125	984	87	141	13
2	740	598	81	142	19
3	342	230	67	112	33
4	550	434	79	116	21
5	630	568	90	62	10
6	2,625	2,289	87	336	13
7	683	484	71	199	29
8	230	199	87	31	13
9	340	253	74	87	26
10	416	348	84	68	16
Average	768	639	81	129	19 S.D. \pm 7.8% S.E. \pm 2.4%
Controls					
1	31	17	55	14	45
2	23	12	52	11	48
3	43	19	44	24	56
4	40	29	73*	11	27*
5	45	13	29	32	71
6	29	12	41	17	59
7	29	9	31	20	69
8	54	25	46	29	54
9	54	17	31	37	69
Average	39	17	41	22	59 S.D. \pm 10% S.E. \pm 3.5%

* These values have been omitted in calculating the means as they are over three standard deviations from them.

3 standard deviations from them. Therefore, in normal human urine coproporphyrin I isomer comprised from 45 to 71% of the total coproporphyrin with an average of $59\% \pm 10\%$ (S.D.) or $59\% \pm 3.5\%$ (S.E.). These results indicate that (1) in normals the mean value of coproporphyrin I is significantly ($P < 0.05$) greater than 50%; (2) about one in six normals will give percentages less than 50% as the S.D. is 10%; this is not inconsistent with the results obtained by workers who find equal amounts of both isomers, but is inconsistent with any suggestion that coproporphyrin III greatly exceeds coproporphyrin I in normals.

In the lead workers, coproporphyrin III predominated with an average of 81%. Even though coproporphyrin I was increased about sixfold it only constituted $19\% \pm 7.8\%$ (S.D.) or $19\% \pm 2.4\%$ (S.E.) of the total coproporphyrin, which was increased 20-fold (Table II).

Discussion

In the small groups examined it was found that in normal human urine coproporphyrin I consti-

tuted 59% of the total coproporphyrin. This is in agreement with Hoerburger and Fink (1935), Watson (1936), Grotepass (1938), Watson *et al.* (1949), Kench *et al.* (1952), and Lockwood and Bloomfield (1954) who reported that the type I isomer predominates in normal urine. It is not inconsistent with that of Holeček and Pěničková (1957) who found that isomers I and III are formed in equal amounts in the urine of healthy subjects. It does not support the work of Comfort *et al.* (1954) who reported that isomer III much exceeded isomer I in normal urine. These authors admitted that, while extracting porphyrins from large volumes of normal urine, they failed to eliminate impurities from the material, thus probably causing defective separation of the two isomers.

In lead workers, despite the reversal of the normal isomer ratio, *i.e.*, coproporphyrin III predominated, it was found that the absolute amount of coproporphyrin I regularly increased with increase of coproporphyrin III (Table II, column 5). The predominance of coproporphyrin III in urine from lead workers was in accord with the widespread view that lead exerts a toxic action on the enzymatic

system at certain stages of haem biosynthesis, resulting in decreased utilization of coproporphyrin III isomer, defective haemoglobinization, and short survival of red blood cells. Thus, to compensate for the threatened anaemia and to supply sufficient coproporphyrin III for the requirements of haemoglobin formation, the whole level of porphyrin synthesis has to be raised, and relatively large quantities of the useless coproporphyrin I are formed and eliminated. Its amount is an index of the degree of haemopoietic activity, which varies with the intensity and duration of lead absorption.

The present finding is in agreement with that of Kench *et al.* (1952) who have shown that, with the increase of urinary coproporphyrin III in lead poisoning, there is an increase in coproporphyrin I which constitutes an average of 30% of the total coproporphyrin. But in this study coproporphyrin I constituted only 19% of the total coproporphyrin. As urine from recently exposed lead workers was examined in the present study, this indicated that the haemopoietic activity was greater in poisoning than absorption.

A similar conclusion has been reached by Sweeney (1963) who, in South Africa, studied the pattern of faecal porphyrin excretion in normal subjects and porphyric patients. Sweeney reported that in normal persons 70% of the faecal coproporphyrin is of the isomer I series, whereas in porphyric patients 75 to 85% of the faecal coproporphyrin was isomer III. He also reported that in an acute attack there appears to be a relative increase in coproporphyrin I, which forms up to 50% of faecal coproporphyrin.

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