

A simplified method for the quantitative determination of urinary coproporphyrin in lead workers

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Soulsby, Joan and Smith, R. L. (1974). *British Journal of Industrial Medicine*, 31, 72-74. A simplified method of estimating urinary coproporphyrin is described, based on the method of Rimington (1971). Coproporphyrin and coproporphyrinogen are extracted into ether from acidified urine; the ether is then shaken with a solution of iodine in hydrochloric acid to oxidize any coproporphyrinogen to coproporphyrin and to extract the coproporphyrin. The solution is examined spectrophotometrically for coproporphyrin at the peak of the Soret band and at wavelengths on either side to correct for any impurities present.

A comparison with the method of Rimington (1971) in 94 urine samples with coproporphyrin levels up to 2.5 mg/l showed good agreement. Correlation coefficient (r) = +0.986. One hundred estimations can be carried out in five hours.

The estimation of urinary coproporphyrin is used in the assessment of inorganic lead absorption (Lane *et al.*, 1968). Waldron (1971) found it to be a suitable screening test to monitor a lead hazard. The quantitative method of Rimington (1971) is satisfactory but time consuming, and we have found semiquantitative tests, based on the red fluorescence of coproporphyrin, difficult to interpret. The following procedure was therefore devised.

Method

Reagents

Glacial acetic acid. Anaesthetic ether. Hydrochloric acid (approximately 5g/100ml) 120 ml concentrated acid diluted to one litre with distilled water. 1% iodine in ethanol, stored at 4° in the dark. Hydrochloric acid-iodine solution prepared freshly each day by mixing 1 ml 1% iodine and 200 ml 5% hydrochloric acid.

Procedure

Place 2 ml of mixed urine in a glass-stoppered tube (125 mm × 15 mm) and add acetic acid (0.2 ml) and anaesthetic ether (5 ml). Shake for 15 seconds and then allow the

phases to separate. Remove the lower aqueous layer; add hydrochloric acid-iodine solution (5 ml) to the ether layer and shake for 15 seconds. Allow the phases to separate, remove the upper layer of ether, and place the unstoppered tube in a water bath at 37° for 5 minutes. Re-stopper the tube and shake. Measure the optical density in a spectrophotometer, using a 1 cm cuvette, at wavelengths of 380 and 430 nm and at the peak of the Soret band (about 401 nm).

Calculation of results

If D_{max} = optical density at peak of Soret band

D_{430} = optical density at 430 nm

D_{380} = optical density at 380 nm

Then

$[2D_{max} - (D_{430} + D_{380})] \times 2.093 \times 1.064 = \text{mg coproporphyrin/litre.}$

Comparison with the method of Rimington (1971)

One hundred random urine samples were collected from lead workers over a period of several months. Coproporphyrin estimations were carried out by the method of Rimington (1971) and by the simplified

method. All estimations were carried out in duplicate and both methods were undertaken on the same day to avoid differences due to deterioration of the specimen with time and exposure to light.

Ninety-four of the samples had coproporphyrin levels up to 2.5 mg/l. The results from these samples are shown in the Figure. The values for the remaining six urines are shown in the Table. In all cases the values shown are the means of duplicate estimations.

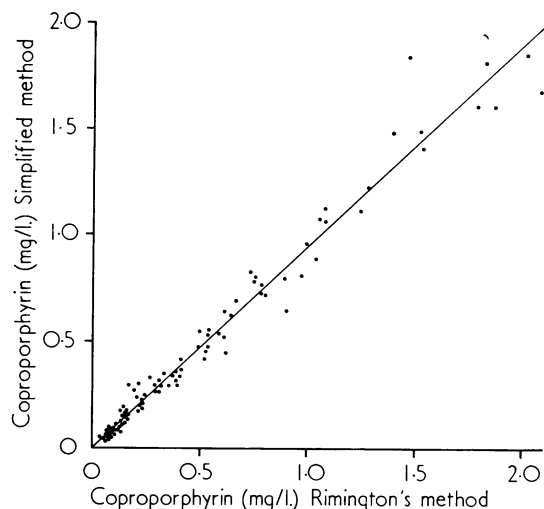


FIGURE Comparison of urine coproporphyrin estimated by the method of Rimington (1971) (X) and the simplified method (Y). The regression line of Y on X is given ($Y = 0.939X$), $n = 94$, $r = +0.986$.

The correction factor of 1.064 has not been applied to the values for the simplified method.

TABLE

URINE COPROPORPHYRIN ESTIMATION BY THE METHOD OF RIMINGTON (1971) AND THE SIMPLIFIED METHOD (VALUES NOT SHOWN IN FIGURE)

Diagnosis	Urine coproporphyrin (mg/l)	
	Rimington's method	Simplified method
Lead worker	3.269	2.753
" "	3.576	2.731
" "	3.677	3.250
" "	4.377	3.491
" "	6.391	5.236
" "	7.075	5.055
Acute intermittent porphyria	1.010	1.113
Acute intermittent porphyria	0.310	1.447

The correction factor of 1.064 has not been applied to the values for the simplified method.

The results show two distinctive features. First, with urine coproporphyrin levels over 2.5 mg/l, the simplified method gave significantly lower answers than Rimington's method, giving a pronounced curve in the overall pattern of the results. For clinical use the exact value of these high concentrations is not very important, so for the purposes of fitting a suitable equation these high values were ignored.

Secondly, the variance of the observations increases with increase of the concentration of coproporphyrin. Consequently, a weighted least squares analysis was used to fit a straight line through the origin.

The regression details are as follows:

Regression equation $Y = 0.939X$

Coefficient of correlation = 0.986

No. of observations = 94

Standard error of slope of regression line = 0.014

Standard error about the line = $\sqrt{X/10}$

The slope of the regression line (0.939) differs significantly from 1 ($p < 0.05$).

The variance between duplicates for both methods increases with the concentration of coproporphyrin in the samples. For the simplified method the variance between duplicates (over the range 0.2-2.5 mg/l) was approximately equal to $X/1000$ which was about half that for the Rimington method.

Discussion

The main differences between this method and that of Rimington (1971) are as follows. Single extractions are used instead of repeated extractions. This would account for the fact that the simplified method gave, in general, lower results than Rimington's method. To allow for this it is suggested that, in calculating the results, the factor of 2.093 used by Rimington is multiplied by 1.064, the reciprocal of the factor obtained in the regression equation.

The sodium acetate wash was omitted, for in no sample during the execution of Rimington's method did the acetate wash show any red fluorescence under ultraviolet light. The iodine and hydrochloric acid were combined into one reagent. The excess iodine passed into the ether layer and was removed. Warming the acid solution to 37°C removed dissolved ether. Without this step, bubbles sometimes formed during the photometric readings.

Optical density readings for this comparative investigation were made manually. A more rapid method is to scan the acid extract over the range 380-430 nm in a recording spectrophotometer. The height of the peak above the baseline can be measured with a scale calibrated directly in milligrammes coproporphyrin per litre. Using this method, 100 estimations can easily be carried out by a single worker in one day.

Rimington's method and the simplified method were applied to two urine specimens from two cases

of acute intermittent porphyria. The results are shown in the Table. These results throw doubt on the reliability of the simplified method in cases of porphyria, although the second urine had been stored for six weeks before examination.

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References

- Lane, R. E. and 17 others. (1968). Diagnosis of inorganic lead poisoning: a statement. *British Medical Journal*, **4**, 501.
- Rimington, C. (1971). Quantitative determination of porphobilinogen and porphyrins in urine and faeces and erythrocytes. *Association of Clinical Pathologists Broad-sheet No. 70*.
- Waldron, H. A. (1971). Correlation between some parameters of lead absorption and lead intoxication. *British Journal of Industrial Medicine*, **28**, 195-199.

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