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A SIMPLE PORTABLE APPARATUS FOR THE SEMI-QUANTITATIVE DETERMINATION OF THE COPROPORPHYRIN CONTENT IN URINE*

W. F. DONATH

Department of Industrial Medicine of the Netherlands Institute for Preventive Medicine, Leyden

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A simple portable instrument for rapid semi-quantitative determination of coproporphyrin excretion in urine is described. The instrument is suitable for use by the industrial physician in screening an industrial population exposed to lead.

The method is essentially the comparison of the fluorescence colour of the porphyrin content as developed in ether and glacial acetic acid with that of a series of paperstrip of graded degree of fluorescence.

Technique and reliability are discussed.

Introduction

Recently the increased excretion of coproporphyrin III in urine has come to be considered one of the earliest symptoms both of increased lead absorption and actual lead intoxication.

An increase in coproporphyrin excretion should never be considered in isolation, but in close relationship with the clinical symptoms that may arise with increased lead intake. In doubtful cases fluctuations in coproporphyrin excretion should be taken into account, and repetition of the examination is advisable. When there is a copious excretion, the diagnosis of increased lead absorption or of lead intoxication may only be made when the clinical symptoms are present or when the lead content in blood and urine is also above normal. Support for such diagnoses may often be found in further haematological examination (fall in haemoglobin content, pronounced polychromatism and increased basophilic punctation of the erythrocytes).

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The industrial physician can, however, utilize the determination of coproporphyrin excretion in order to get an insight into the extent of possible increase of lead intake in various departments of the factory. But to do this he must be provided with a simple rapid method, suitable for being used on the spot.

It is clear from the literature that in cases of lead poisoning, the increased porphyrin excretion is mainly due to coproporphyrin III, which is soluble in ether, usually accompanied by varying quantities of coproporphyrin I. One must commence with a method for isolating the porphyrins soluble in ether from the urine samples of persons suspected of an increased lead absorption. There are several known methods, among which are those of Hymans v. d. Bergh (1), Gorter en de Graaff (2), Ten Berg (3), Weidner (4), Brugsch (5) and Maloof (6). They are all based on bringing the coproporphyrins into solution in ether and evaluating their red fluorescence in ultra-violet light, followed by either the spectroscopic determination of coproporphyrin or the comparison with standard solutions or glasses. Our method is the same in principle, except that we have attemped to determine the amount of coproporphyrin excretion semiquantitively, so that the industrial physician may obtain an idea as to the degrec of abnormal excretion.

Methods and Results

Determination principle

Samples of urine treated with a mixture of glacial acetic acid are examined under ultra-violet light and their fluorescence compared with that of a series of paper strips of graded fluorescence.

Construction of the apparatus

In view of the necessity of maintaining the same illumination conditions at the fluorescent surfaces, the ultra-violet light source (Philips analysis lamp H. P. W. 125 W.) was built in at a fixed location in the apparatus. About 25 cm below the lamp there is a table with two similar orifices. In one of them the test-tube can be inserted and retained at any desired height by a spring clamp under the table. Under the other there is a rotating disc carrying strips of fluorescent paper of varying grades, so that the rotation of the disc brings each grade of paper successively under the orifices.

This set-up facilitates the comparison of the urine sample and the standard fluorescent strips under standard conditions. The position of the rotating disc may be read off through a third smaller orifice in the table. A Philips 58205 CH/03 choke operating at 220 V is housed in the base of the apparatus. To enable the simultaneous testing of a number of urine samples before measurement proper, three test-tube clips are fitted into the backplate of the apparatus. In the cover there are six test-tube clips and a bottle of extraction reagent solution. Pipettes of 10 cc and 2 cc capacity complete the equipment (fig. 1 and 2).

Choice of scale

When calibrating the scale, care was taken that the scale covered the following three regions of coproporphyrin concentrations: that in which the excretions of coproporphyrin could be considered as normal, that in which there is doubt as to the

existence of too high an absorption of lead, and that in which there is no doubt of it. A preliminary survey was, therefore, made of the coproporphyrin content in urine specimens from a large number of normal persons. Values of more than 200 γ/L were not found.

At the same time, there appeared to be no quotations of normal values higher than 200 γ/L. (BRUGSCH (7), SUNDERMAN and BOERNER (8), TEN BERG (9) quote values not higher than 100 γ/L). In practice it has been found in the lead-using branches of industry that values higher than 200 γ/L are regarded with suspicion.

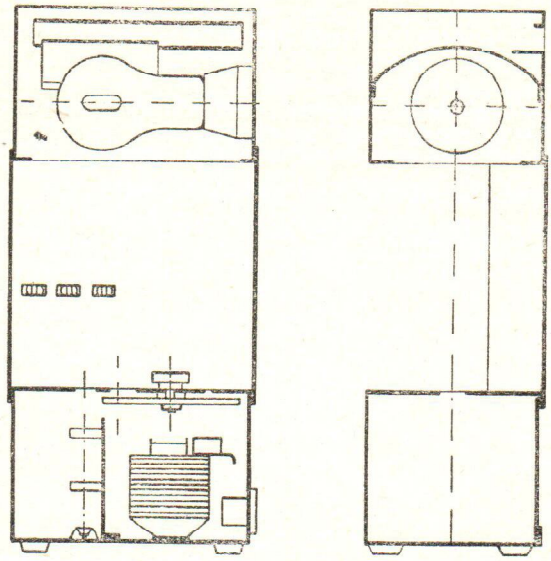

Fig. 1

The scale was accordingly adjusted, so that the zone in which the industrial physician should suspect a high lead absorption and continue to check up on the worker, corresponded to coproporphyrin excretion between 200 γ/L and 400 γ/L. Values below this zone are considered non-pathological. Values above this zone are generally taken to indicate that there has been an increased lead absorption. The 400 γ/L borderline was taken from experience and is supported by reports in the literature (10).

Preparation of the fluorescent paper-strips

The paper used was thin typewriter copy-paper of pure bleached cellulose, weighing 35 g./m², exhibiting no fluorescence in ultra-violet light. The strips were treated for 15 minutes in a bath of 160 mg/L solution of »Uvitex R. P« (11). Superfluous solution was absorbed by blotting with filter paper and drying at 105° in an oven, hanging freely in clips. At this stage the sheets exhibited a light blue fluorescence in ultra-violet light. The sheets were then placed for varying periods of time in colouration baths of hydrochloride of tetra-ethyl-diamin-ortho-carboxyphenyl-xanthyllium (-ion) (Rhodamin B). They were once more blotted off and dried in the oven. The basic solution contained 2 g. Rhodamin per litre and was diluted according to the desired scale. Depending on the concentration of the solution and the length of time in the bath, fluorescence from blue to orange-red is exhibited in ultra-violet light.

Calibration of the paper strips

The positioning of the paperstrip in the fluorescence scale is determined by means of coproporphyrin-free urine to which known quantities of coproporphyrin III* have been added.

A known weight of pure coproporphyrin is first dissolved in 5% HCl, and varying quantities of this solution are added to samples of 10 cc of urine samples. The porphyrin urine mixtures are then neutralized with a saturated solution of sodium acetate until Congo paper is on the point of turning from blue to red. Each 10 cc quantity of urine is then shaken up with ether and glacial acetic acid, as in the instructions for use, and the tube placed in the apparatus.

The fluorescence colours of the treated paperstrips are compared with those of the ether layers in the test-tubes, and should correspond with them. Strait's method (13) may then be utilised as a quantitative check on the amount of coproporphyrin, fluorescence being determined in a fluorometer,[1] using the three R-filters as for the measurement of red fluorescence. We have produced 8 paperstrips by the above procedure, corresponding with 8 graduations in a fluorescence scale as follows:

Degree of fluorescence	Concentration in γ/L of coproporphyrin
1	0 — 50
2	50 — 100
3	100 — 200
4	200 — 400
5	400 — 800
6	800 — 1600
7	1600 — 3000
8	3000 — 5000

The determination of porphyrin in urine: technique

a) *Taking the sample*

Coproporphyrin is easily changed by light. For the collection of 24-hour samples it is therefore advisable to use dark coloured bottles and to add a little thymol. In practice, however, the collection of 24-hour sample is often fraught with difficulty. In screening an idustrial population, we therefore prefer to utilise samples of fresh urine and directly determine the degree of fluorescence. This implies that one occasionally has to do measurements with low-density samples. When the value found falls in the »suspicious« zone, one can either measure a second sample taken later in the day, or determine the creatine content in the low-density sample (14).

One may also convert the concentration to a density of 1.024, as suggested by LEVINE and FABY (15).

b) *Determination of the degree of fluorescence*

10 cc of fresh urine is transferred by a pipette to a nonfluorescent test-tube. To this is added 2 cc of an extraction solution consisting of 90% peroxide-free ether and 10% glacial acetic acid. The tube is closed with a rubber stopper and shaken vigorously 3 times. A foamy layer of ether is formed on the surface of the urine, in which the coproporphyrin is now contained. After carefully extracting the stopper, the tube is lowered into the appropriate orifice in the table of the instrument far enough to ensure that the urine layer is no longer visible, since the urine itself may fluoresce and so disturb the observation of the fluorescence colour of the ether layer. The

* We express our thanks to Dr. Martin STRELL, Org. Chem. Institut der Technischen Hochschule at München, for supplying us with a small amount of pure coproporphyrin III.

[1] Manufactured by Kipp Ltd., Delft, Netherlands.

Fig. 2

Apparatus for determination of coproporphyrin

ether layer is then subjected to ultra-violet radiation for a period of *not less than* 10 *minutes*. The fluorescence colour of the ether layer is then compared with those of the 8 degrees of the scale by rotating the disc so that each in turn is next to the tube. The selection of the corresponding degree of the scale needs very little experience. When the colour of the ether layer falls between two degrees, for instance > 2 and < 3, there is no difficulty in reading. Determination should preferably be carried out in a darkened room or with diffused light. The industrial physician will evaluate the results as follows: the values of coproporphyrin lower than degree 4 on the scale will be considered non-pathological; degree 4 (or > 3 and < 5) will require regular checking of the employee concerned. The »zone of suspicion« is therefore about 200–400 γ coproporphyrin per litre. Degree 5 or above indicates a high lead intake, and renders it advisable to determine the lead content in a 24-hour urine specimen and in blood.

Reliability of the observed values

a) *Relation between the scale readings and the quantitative determination of coproporphyrin content.*

Both scale readings and quantitative determinations were carried out the same day, on the same urine specimens from 80 persons selected at random. Fig. 3 is a graphic representation of the relationship between the values obtained by the two methods. The scale readings are plotted horizontally (X) against the logarithms of the quantitively determined porphyrin content (Y, vertically).

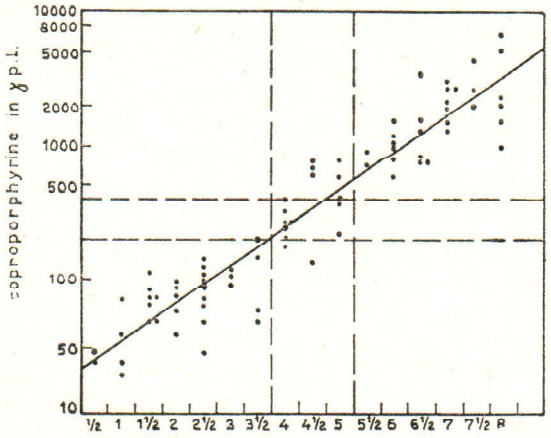

Fig. 3

The calculation of the correlation coefficient (BRAVAIS-PEARSON method) shows a highly significant linear relationship;

$r = 0.948$ while Y (log content) $= 1.28 + 0.28$ X.

The convertibility of scale reading accurately to porphyrin content is practically unimportant. Table I may readily be derived from fig. 3 on

the basis of the assumption that the observed porphyrin content of 400 γ/ litre indicates a high lead absorption and that values between 200 γ/ litre and 400 γ/litre are to be considered dubious.

Table 1

Scale-reading

		0—3½	4, 4½ and 5	5½ and more	total
Porphyrin content in urine according to analysis, in γ/L	401 and >	0	6	30	36
	201—400	0	8	0	8
	< 200	34	2	0	36
	Total	34	16	30	80

Thus the check on 80 persons establishes that for all scale readings below 4 (42½%) the porphyrin content was in fact < 200 γ/litre (1 case was exactly 200 γ/litre), and that for all readings above 5 (37½%) the content was in fact > 400 γ/litre.

Only the readings 4 and 5 and observations between them (20%) fail to provide an unequivocal verdict.

When the subjects examined may be considered a representative sample of the population examined, it is reasonable to expect clear decisions regarding 80% of the tests and doubtful ones in only 20%; this latter group however tends to a high content, so one may consider it dubious.

b) *Reproducibility of the results*

Scale readings of 29 persons were taken successively by 4 observers working completely independent of each other in the same consecutive order of test persons and under the same conditions: no significant differences in the results could be detected. Two of the observes were skilled and two unskilled.

Table 2

Comparison of the readings by 4 observers

Date	Person no.	Scale readings of the observers				Differences between the observers					
		1	2	3	4	1—2	1—3	1—4	2—3	2—4	3—4
6—1—'54	1	1/2	1	1/2	1/2	+ 1/2	0	0	+ 1/2	+ 1/2	0
	2	1 1/2	2	1 1/2	1 1/2	+ 1/2	0	0	+ 1/2	+ 1/2	0
	3	1 1/2	2	1 1/2	1	+ 1/2	0	— 1/2	+ 1/2	+ 1	+ 1/2
	4	1/2	1/2	0	0	0	— 1/2	— 1/2	+ 1/2	+ 1/2	0
	5	1/2	1	1/2	1	+ 1/2	0	+ 1/2	+ 1/2	0	— 1/2
	6	3	3	3	3	0	0	0	0	0	0
	7	2 1/2	3	2	2 1/2	+ 1/2	— 1/2	0	+ 1	+ 1/2	— 1/2
	8	3	3	2 1/2	3	0	— 1/2	0	+ 1/2	0	— 1/2
	9	3 1/2	4	4	3 1/2	+ 1/2	+ 1/2	0	0	+ 1/2	+ 1/2
	10	1/2	1/2	1/2	1/2	0	0	0	0	0	0
	11	1 1/2	2	1 1/2	1 1/2	+ 1/2	0	0	+ 1/2	+ 1/2	0
	12	1	0	1/2	1	— 1	— 1/2	0	— 1/2	— 1	— 1/2
	13	0	1	0	1/2	+ 1	0	+ 1/2	+ 1	+ 1/2	— 1/2
	14	1/2	1/2	1/2	1/2	0	0	0	0	0	0
	15	1	1	1/2	1	0	— 1/2	0	+ 1/2	0	— 1/2
8—1—'54	31	1 1/2	2	1 1/2	1 1/2	+ 1/2	0	0	+ 1/2	+ 1/2	0
	35	6	5 1/2	5	6	— 1/2	— 1	0	+ 1/2	— 1/2	— 1
	38	3	3	2 1/2	2 1/2	0	— 1/2	— 1/2	+ 1/2	+ 1/2	0
	39	2	2	2	1 1/2	0	0	— 1/2	0	+ 1/2	+ 1/2
	42	2 1/2	3	2	2 1/2	+ 1/2	— 1/2	0	+ 1	+ 1/2	— 1/2
	44	5	5	4 1/2	5	0	— 1/2	0	+ 1/2	0	— 1/2
	45	1/2	1/2	1/2	1/2	0	0	0	0	0	0
	32	4	4 1/2	4 1/2	4	+ 1/2	+ 1/2	0	0	+ 1/2	+ 1/2
	33	6 1/2	5	5 1/2	7	— 1/2	— 1	+ 1/2	— 1/2	— 2	— 1 1/2
	34	2 1/2	3	2 1/2	2 1/2	+ 1/2	0	0	+ 1/2	+ 1/2	0
	36	5	5	4	5	0	— 1	0	+ 1	0	— 1
	37	2 1/2	3	2 1/2	2 1/2	+ 1/2	0	0	+ 1/2	+ 1/2	0
	40	5	5	5	5 1/2	0	0	+ 1/2	0	— 1/2	— 1/2
	43	5 1/2	5 1/2	5 1/2	5 1/2	0	0	0	0	0	0
Total: n = 29						+ 4	— 6	0	+10	+ 4	— 6
+ or —						+ 0.14	— 0.20	0	+ 0.34	+ 0.14	— 0.20
Observers 1 = D 2 = Di 3 = R 4 = J.	Numbers				+	13	2	4	18	15	4
					0	13	16	21	9	10	13
					—	3	11	4	2	4	12
	Total					29	29	29	29	29	29
	Significance*					*	*	—	**	*	—

* According to the signtest — = not significant
* = 5% level of significance
** = 1% level of significance

Table 2 summarises the results and indicates a close agreement between the readings of the four observers. In fact, the unskilled observers occasionnally differed from the skilled observers, but to such a slight degree that the variation is negligeable. Observers should, of course, have a good colour perception for red-blue.

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Sadržaj

JEDNOSTAVNI PRIJENOSNI APARAT ZA POLUKVANTITATIVNO ODREĐIVANJE KOPROPORFIRINA U URINU

Opisan je jednostavni prijenosni instrumet za brzo polukvantitativno određivanje izlučivanja koproporfirina u urinu. Instrument je prikladan za potrebe tvorničkih liječnika pri brzom provjeravanju ekspozicije olovu.

Opisana metoda temelji se na uspoređivanju fluoroscencije, koju razvija porfirin u eteru i ledenoj octenoj kiselini s nizom standardnih papirića, koji su tako pripravljeni, da fluoresciraju određenim intenzitetom.

Autor prikazuje tehnički postupak i pouzdanost metode.

Odjel za industrijsku medicinu,
Holandski institut za preventivnu medicinu,
Leyden

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