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# Urinary Porphyrins in Lead Absorption

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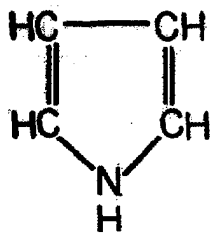
## 1. INTRODUCTION

There is a great need for a simple chemical test which will give an early indication of dangerous lead absorption or incipient lead poisoning. Quantitative analyses of urine for lead require specific and expensive equipment besides trained personnel, and the methods are tedious and time-consuming. Enumeration of stippled cells in blood films is laborious and not entirely specific. The newer knowledge of porphyrin metabolism resulting from the work of Watson in America, Rimington in Britain, and Waldenström in Sweden has shown the need for a careful evaluation of urinary prophyrins in health and disease.

## 2. DEFINITION AND DESCRIPTION OF PORPHYRINS

Porphyrins are pigment compounds which are widely distributed in nature and consist chemically of four pyrrole rings linked together by four methene (CH) bridges. The pyrrole ring has the configuration shown in Figure 1, and four such rings joined together by methene bridges form porphin (Fig. 2). In the naturally occurring porphyrins, the eight hydrogen atoms of the pyrrole rings are substituted by methyl ($CH_3$), ethyl ($C_2H_5$), vinyl ($CH_2.CH$), propionic acid ($CH_2 \cdot CH_2COOH$), and acetic acid ($CH_2COOH$) groups. Coproporphyrin may exist in four isomeric forms, depending on the sequence of methyl (M) and propionic acid (P) groups, and since only Types I and III isomers have so far been isolated in clinical conditions, it will suffice to represent them in Figure 3. The uroporphyrin isomers contain acetic acid (A) and propionic acid (P) groups, and Types I and III isomers are represented in Figure 4. The terms copro- and uro- porphyrin were introduced by Fischer [6] and imply that these pigment compounds occur separately in the feces and urine. It is now known that they occur normally as well as in pathological conditions in both excreta.




**PYRROLE**
Figure 1

## 3. CHEMICAL PROCEDURES

(*a*) *Qualitative Test for Urinary Coproporphyrin.*—According to de Langen and ten Berg [1] the detection of coproporphyrin in abnormal amounts in the urine is a more reliable method than the degree of basophilic stippling of erythrocytes in the early diagnosis of lead poisoning. The method is simple and rapid and can be made semiquantitative as a screening test where large numbers of workers are exposed to lead dust and fume. A small sample of urine (5, 10, or 20 ml.) is acidified with a few drops of glacial acetic acid, a few milliliters of ether (2 to 5 ml.)

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added, and, after shaking the stoppered tube, the ether layer is inspected under an ultraviolet lamp. A blue, pink, or red fluorescence may be observed.

In searching the literature, no less than seven modifications of the original de Langen and ten Berg test were noted. The modifications are summarized in Table 1 and show the quantity of urine taken for the test, the reagents added, the procedure, and the scheme for reading the result according to the intensity of the fluorescence under an ultraviolet lamp. Meek, Mooney, and Harrold [2] suggested the addition of 3% hydrogen peroxide (2 drops) to change any interfering substances which might cause doubtful reactions.

In my experience, ether alone gives a blue-violet fluorescence under ultraviolet light, and the use of hydrogen peroxide is seldom necessary. Other solvents besides ether, such as isopropyl ether, ethyl acetate, and amyl

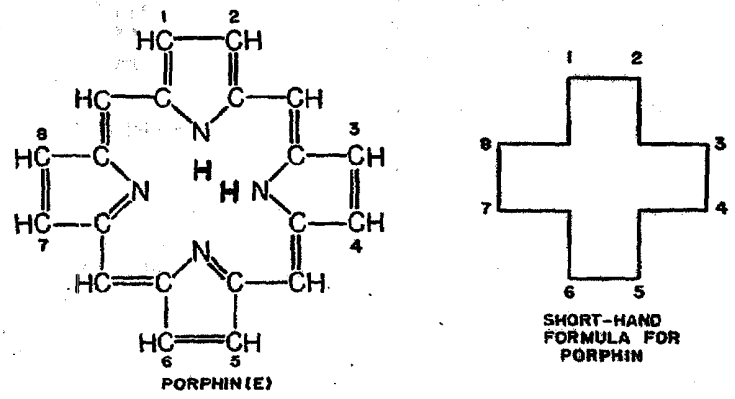


Figure 2

TABLE 1.—*Modifications of Test for Coproporphyrin*

| Method | Quantity of Urine for Test, Ml. | Reagents Added | Procedure | Positive Result |
|---|---|---|---|---|
| 1. de Langen and ten Berg [1] | 20 | Few drops glacial acetic acid; 2 ml. ether | Shake several times; examine ether layer under U.V.L. | Rose to deep red fluorescence |
| 2. Meek, Mooney, and Harrold [2] | 20 | 2 ml. glacial acetic acid; 2 drops 3% $H_2O_2$; 2 ml. ether | Shake several times; examine ether layer under U.V.L. | Light pink to red fluorescence |
| 3. Waldman and Seideman [7] | 10 | 2 drops glacial acetic acid; 2 drops 3% $H_2O_2$; 1.5 ml. ether | Shake stoppered tube on long axis 20 times; examine under Wood Lamp | Fluorescence in ether layer |
| 4. Maloof [8] | 5 | 6 drops 6 N acetic acid; 5 ml. ether | Shake and view in dark room with Black Light Lamp | Fluorescence with various shades of red |
| 5. McCord [9] | 10 | 2 drops glacial acetic acid; 2 drops 3% $H_2O_2$; 2 ml. ether | Stopper 25 ml. test tube; shake on long axis 20 times; allow layers to separate and read after 10-15 min. | Red fluorescence; slight, moderate, marked, and very marked |
| 6. Brooks [10] | 5 | 1 ml. glacial acetic acid; 5 ml. ether; 3 drops $H_2O_2$ | Invert tube several times; allow to stand for 10 min. | Violet = + Pink = ++ Light rose = +++ Deep rose = ++++ |
| 7. London School of Hygiene and Tropical Medicine * | 10 | 2 ml. glacial acetic acid; 20 ml. ether | Shake 1 min.; discard lower layer; wash ether twice with D.W.; shake with 0.25% HCl in lots of 2, 2, and 1 ml.; adjust acid extracts to 5 ml. in Monax tube | Match against tube containing standard sol. in fluorescence comparator |
| 8. U. S. Naval Shipyard Method [11] | 5 | 3 drops 3% $H_2O_2$; 6 drops glacial acetic acid; 5 ml. ethyl ether | Stopper tube and shake 30 sec.; allow layers to separate; examine under U.V.L. | Pink to orange-red fluorescence |

* Sherwood, R. J.: Personal communication to the author.

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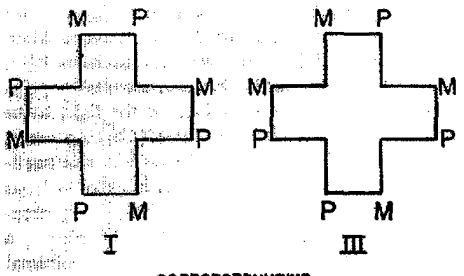



COPROPORPHYRINS

Figure 3

acetate, have been tried, but the best solvent is ethyl acetate which of itself does not show any fluorescence under ultraviolet light. I use the following test:

To 2.5 ml. of urine in a test tube, 3 to 5 drops of 6 N acetic acid are added to bring the urine to pH 6, testing with hydrion test paper of range 1 to 11. Two milliliters of ethyl acetate * is added; the stoppered test tube is inclined on its long axis 10 times (violent shaking is unnecessary) and allowed to stand for two or three minutes. The fluorescence of the ethyl acetate layer is observed under radiation from an ultraviolet lamp.† The addition of acetic acid, not hydrochloric acid, is necessary to permit the ethyl acetate layer to extract coproporphyrin from the urine. If hydrochloric acid is used, the ethyl acetate layer appears a greenish blue at pH 6 under ultraviolet light, and this coloration persists at pH 5, pH 4, and pH 3, while at pH 7 up to pH 12 the coloration is blue.

With use of the technique described, it has been found repeatedly that a violet or heliotrope fluorescence in the ethyl acetate layer under ultraviolet light corresponds to approximately 5γ of coproporphyrin per 100 ml. of urine.

It can be shown that the intensity of the fluorescence varies with pH, for at pH 5 the violet changes to pink and this, in turn, changes to red at pH 4 and pH 3. At pH 8, the violet changes to faint blue and at pH 9 and pH 10 to a blue coloration indistinguishable from that obtained with normal urine. Since urine contains uroporphyrin as well as coproporphyrin, the blue coloration is due to its presence when the coproporphyrin has been suppressed in the alkaline range.

(*b*) *Quantitative Determination of Urinary Coproporphyrin and Uroporphyrin.*— The determination of coproporphyrin quantitatively requires the following:

(*i*) Use of a fluorescence attachment to the Uvispek Spectrophotometer, with a sensitivity down to 0.5γ per 100 ml. of 1.5 N HCl

(*ii*) Preparation of standard solutions of coproporphyrin ‡

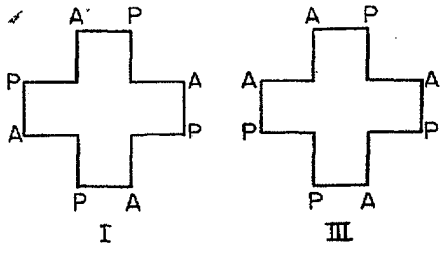
(*iii*) Preparation of calibration curves (Fig. 5), plotting coproporphyrin concentration (micrograms per 100 ml.) against per cent transmission (% T)

In Schwartz, Zieve, and Watson's method,[8] 5 ml. of urine is pipetted into a Squibb separatory funnel, 5 ml. of buffered acetic acid added, and the coproporphyrin removed by extracting with ethyl acetate. The method permits the removal of ether-insoluble porphyrins by washing with 1% sodium acetate and the conversion of precursors to porphyrins by shaking with dilute iodine solution. The coproporphyrin is finally extracted from the ethyl acetate in 1.5 N HCl (20 ml.). The per cent transmission is measured in the spectrophotometer, and the coproporphyrin concentration estimated from the graph of

* Ethyl acetate $CH_3 \cdot COO \cdot C_2H_5$ has the same price as ether (ethyl ether) $[(C_2H_5)_2O]$.

† The Hanovia mercury-arc Inspectolite (Hanovia Chemical & Manufacturing Co., Newark, N. J.) is eminently satisfactory. This lamp has a special dark-glass filter, practically opaque to visible light generated in the mercury-arc quartz tube, but freely transmits light in the region of 3660 A., the most powerful wave lengths causing fluorescence.

‡ Supplied by Dr. S. Schwartz of the University of Minnesota.




UROPORPHYRINS

Figure 4

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10γ per 100 ml. or of 100γ per 100 ml. of 1.5 N HCl solution.

Uroporphyrin has been determined quantitatively by Rimington's method § which consists in calcium chloride precipitation of uroporphyrin from a small amount of urine, in the presence of alkali and excess calcium ions, and its subsequent measurement in the Soret band region.

The methods used for these estimations have been as follows:

An apparatus has been constructed, with the aid of the University instrument maker, in which an AH 4 mercury-vapor lamp (supplied by the General Electric Co., Toronto), as a source of suitable wave lengths of ultraviolet light, has been inserted in a metal housing (manufactured by Hilger & Watts, London), with a condensing lens to transmit "parallel" light. The primary filter consists of a 405 mμ interference filter (obtained from Baird Associates, Cambridge, Mass.) combined with a blue auxiliary filter (Corning Auxiliary Filter 4-72). The combination of the interference filter, transmitting 30.5% of the 405 mμ exciting light, with the blue filter, which has a transmission value of 62% at 406 mμ, permits 19% of the light to be transmitted. A secondary red filter (Corning 2-61), which transmits light above 620 mμ and has negligible transmission below 600 mμ, is inserted in front of the photoelectric cell of the Uvispek Spectrophotometer. With this combination of filters, a blank solution of 1.5 N HCl gives only a minimal deflection of 0.5 mm. when a 10γ solution of coproporphyrin I is set to read 100% T. This gives the sensitivity of the Uvispek.

The intensity of fluorescence is determined by inserting a quartz cell containing 1.5 N HCl as a blank into the adapter of the Uvispek, setting the transmission at zero, and centering the galvonometer needle by means of the zero knob on the potentiometer of the Uvispek. The porphyrin standard is next set to give a transmission reading of 100 by adjusting the check knob. The transmission reading of the unknown is then determined. (No use is made of the light source or monochromator of the Uvispek Spectrophotometer in fluorescence determinations.)

§ References 4 and 5.

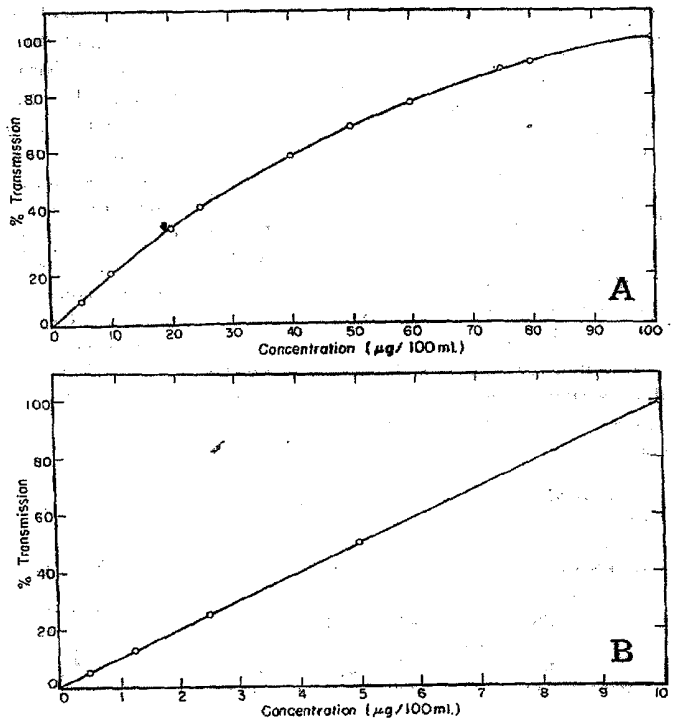


Fig. 5.—Calibration curves. *A*, curve prepared by plotting average per cent transmission readings against coproporphyrin concentrations 5γ to 100γ per 100 ml. of 1.5 N HCl. *B*, curve prepared by plotting average per cent transmission readings against coproporphyrin concentrations 0.5γ to 10γ per 100 ml. of 1.5 N HCl.

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## 4. RESULTS OF PORPHYRIN DETERMINATIONS ON URINE SAMPLES

The urine samples were analyzed quantitatively from the following:

(*i*) Presumably normal, healthy individuals

(*ii*) Employees with a mild exposure to lead in an industrial process, e. g., repair of motor-vehicle bodies and glazing of wall tiles

(*iii*) Employees liable to a severe exposure to lead in a storage-battery plant

(*i*) To obtain data on the values of urinary coproporphyrin and uroporphyrin excreted normally, morning urine samples were obtained from fourth and fifth year medical students during March and April, 1954. Sixty-six samples were examined. Qualitative tests for coproporphyrin, for urobilinogen and porphobilinogen using Ehrlich's aldehyde reagent, for urobilin using Schlesinger's test, and for bile pigments using Ehrlich's diazo reaction were performed prior to the quantitative determinations of porphyrins. On tabulating the results, it was noted that urines with strongly positive reactions for urobilinogen had higher uroporphyrin values than urines with negative or weakly positive reactions for urobilinogen. Twenty-two out of sixty-six samples had this feature and were tabulated separately. The remaining samples were selected at random, and two groups of 22 samples formed.

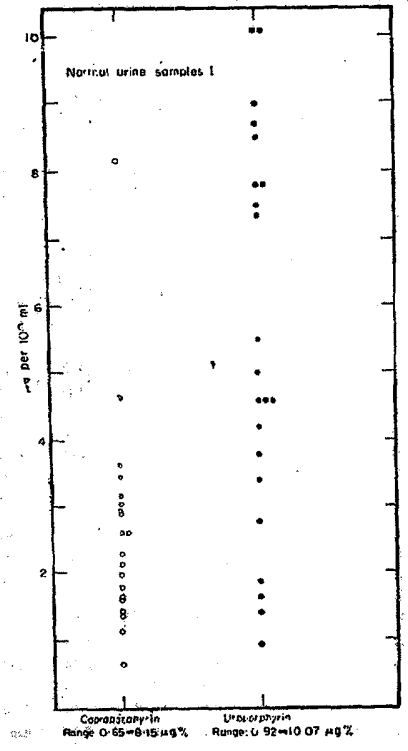


Figure 6

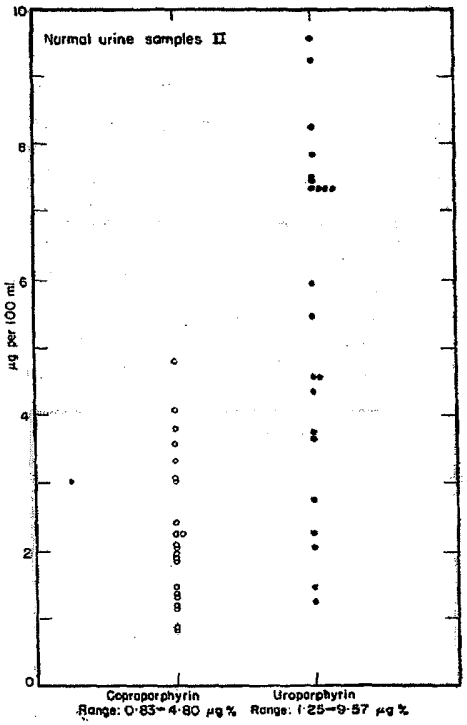


Figure 7

Figures 6 and 7 show the range of values for the first and second normal groups and Figure 8 the range for the group with high uroporphyrin values. The results are given in Table 2. The average uroporphyrin value for the third group is approximately three times that for either the first or the second group, while the average coproporphyrin value is only slightly increased over that for the first or the second group.

(*ii*) Six healthy men, aged 54, 23, 34, 40, 38, and 23 years, respectively, were

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TABLE 2.—*Results of Porphyrin Determinations on Urine Samples*

| Group | Values | Copro-porphyrin, γ/100 Ml. | Uro-porphyrin, γ/100 Ml. |
|---|---|---|---|
| 1st | Range | 0.65 — 3.15 | 0.92 — 10.07 |
| Normal | Average | 2.76 ± 0.38 | 5.45 ± 0.61 |
| 2d | Range | 0.33 — 4.80 | 1.25 — 9.57 |
| Normal | Average | 2.80 ± 0.23 | 5.52 ± 0.53 |
| 3d | Range | 1.06 — 7.13 | 11.44 — 25.18 |
| Normal with high uroporphyrin | Average | 3.31 ± 0.33 | 15.97 ± 0.80 |

allowed to begin work in an industrial process involving exposure to lead dust. Ten days later the collection of weekly samples of urine was commenced and continued for six weeks, the period extending from Sept. 9 to Oct. 16, 1953. The urine samples were collected in chemically clean bottles of 16 oz. capacity, each containing 0.5 gm. of pure, dry sodium carbonate. The men were instructed to pass urine directly into the bottles on rising at 7 a. m. on Wednesday of each week. Simultaneously, blood films for the estimation of stippled cell counts ‖ were taken weekly from each workman. So far as is known, the workmen had not been engaged previously in an atmosphere where there was a lead hazard, and blood films taken prior to employment showed negligible stippled cell counts.

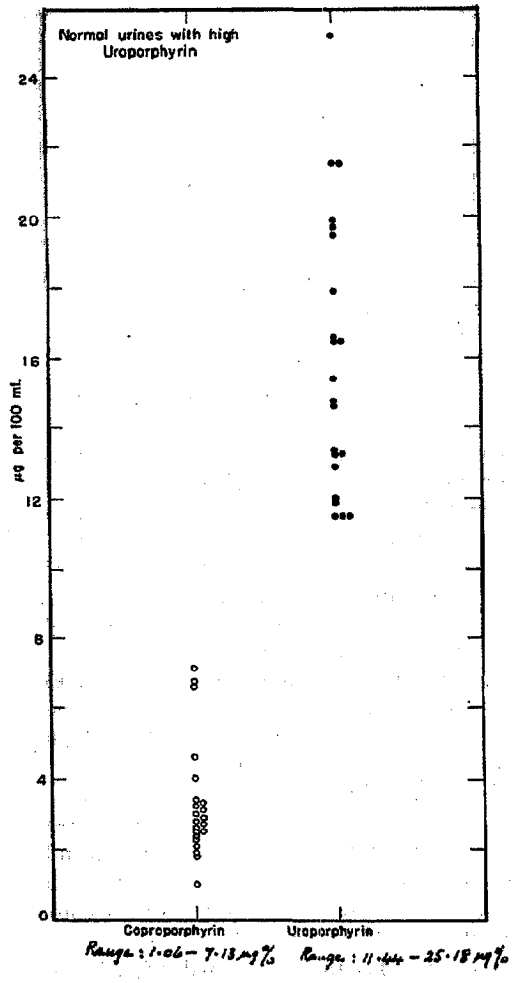


Figure 8

The new type of work consisted in repairing motor-vehicle bodies in which buffing and spray painting were executed. Due precautions in wearing respirators and protective clothing were observed, so that the exposure to lead was considered minimal.

Coproporphyrin determinations were carried out on the urine samples on the same

‖ The blood films were stained and the stippled counts estimated in the Industrial Hygiene Laboratory of the Ontario Department of Health.

TABLE 3.—*Porphyrin Determinations on Morning Urine Samples Submitted Weekly from Three Workmen with Light Exposure to Lead*

| | J. R. G. Porphyrin, γ/100 Ml. | | W. V. Porphyrin, γ/100 Ml. | | E. E. Porphyrin, γ/100 Ml. | |
|---|---|---|---|---|---|---|
| Date | Copro- | Uro- | Copro- | Uro- | Copro- | Uro- |
| 4/14/54 | 2.36 | 6.67 | 3.15 | 6.18 | 2.13 | 3.24 |
| 4/20/54 | 2.82 | 5.72 | 4.51 | 6.49 | 3.33 | 6.41 |
| 4/23/54 | 3.91 | 1.14 | .... | .... | 6.41 | 5.04 |
| 5/ 6/54 | 4.49 | 1.87 | 11.30 | 3.66 | 3.06 | 2.52 |
| 5/12/54 | 3.06 | 2.29 | 6.86 | 9.15 | 3.56 | 3.70 |
| 5/19/54 | 1.71 | 0.46 | 4.07 | 0.21 | 4.50 | 1.66 |
| 5/26/54 | 6.76 | 2.06 | 11.32 | 3.43 | 7.78 | 2.50 |
| 6/ 2/54 | 7.18 | 7.99 | 11.7 | 5.04 | 3.10 | 9.15 |
| 6/10/54 | .... | .... | 3.62 | 12.10 | 4.14 | 4.12 |
| 6/18/54 | 7.20 | 5.04 | 6.62 | 4.74 | 5.00 | 3.49 |
| 6/23/54 | 3.74 | 2.52 | 6.90 | 4.85 | 5.34 | 2.06 |
| 7/ 7/54 | 3.32 | 2.98 | .... | .... | .... | .... |
| 7/14/54 | 6.30 | 5.20 | 8.94 | 12.30 | 3.78 | 5.49 |
| 7/22/54 | .... | .... | 6.44 | 6.03 | .... | .... |
| 7/28/54 | 6.78 | 5.04 | 7.86 | 6.87 | 4.22 | 4.81 |
| 8/13/54 | 3.10 | 4.16 | 4.55 | 7.01 | 2.59 | 1.46 |
| 8/19/54 | 4.12 | 5.27 | 4.06 | 8.70 | 4.28 | 2.52 |
| 8/27/54 | 3.03 | 1.87 | 7.32 | 6.87 | 15.60 | 8.53 |
| Average values | 4.4 | 3.8 | 7.15 | 6.45 | 5.55 | 4.8 |

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day or on the following day after being received in the laboratory. The 36 observations on coproporphyrin content and stippled cell counts were plotted as shown in Figure 9. Twenty-two observations, with counts less than 1000 stippled cells per 1,000,000 erythrocytes, had a range from 0.6γ to 7.5γ of weekly samples of urine examined for their porphyrin content. In all, 16 weekly samples were examined from April to August, 1954, and the average values calculated, as shown in Table 3. During this period the stippled cell counts were less than 1000 per 1,000,000 erythrocytes.

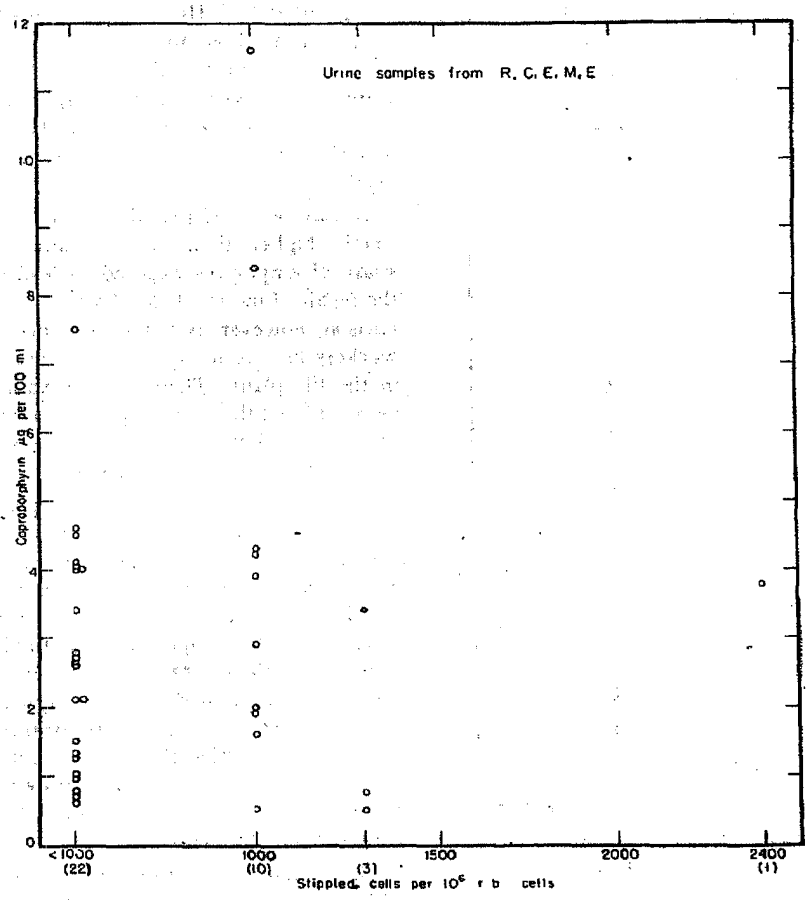


Figure 9

coproporphyrin per 100 ml. of urine, the average value being 2.69γ±0.35γ per 100 ml. Thirteen observations, with counts of 1000 to 1500 stippled cells per 1,000,000 erythrocytes, had a range from 0.5γ to 11.6γ of coproporphyrin per 100 ml. of urine, with an average value of 3.54±0.86.

Three of these workmen were followed for a subsequent period of four months, and It may be noted that, although there are wide variations in the weekly determinations, the average coproporphyrin values for J. R. G., W. V., and E. E. are higher than the average value for counts between 1000 and 1500 stippled cells per 1,000,000 erythrocytes (4.4, 7.15, and 5.55 as compared with 3.54γ per 100 ml.). There is, nevertheless, an indication that the coproporphyrin

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values increase as the stippled cell counts increase from less than 1000 to 1000 and 1500 stippled cells per 1,000,000 erythrocytes (Fig. 9).

The uroporphyrin determinations have also been carried out weekly, and the average values calculated. The copro-/uro- porphyrin ratio is on the average 1 : 1, although there are wide differences in the individual ratios.

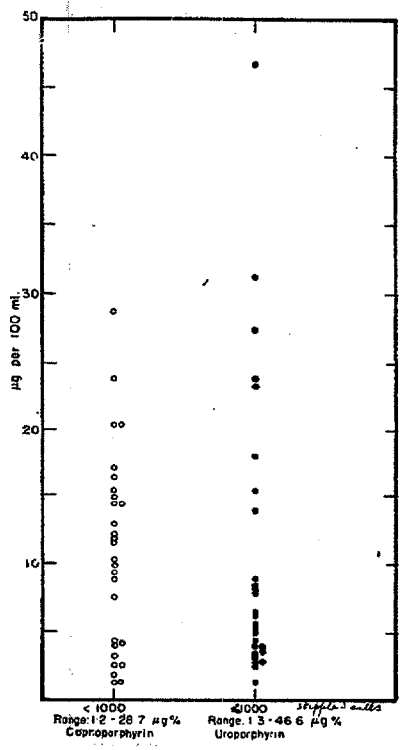


Figure 10

A number of workers with a mild exposure to lead in a tile-manufacturing plant where the tiles are sprayed with a lead glaze before firing in kilns, have been under medical supervision for a number of years. Morning urine samples obtained weekly on several occasions have been analyzed for porphyrin content. As blood films for stippled cell counts are taken only at three-month intervals in this plant, the porphyrin determinations on urine samples, submitted the next day after the blood films were taken on Aug. 18, 1953, and on March 16, 1954, were considered suitable for study of a correlation between stippled cell count and amount of urinary coproporphyrin. Twenty-eight urine samples had a coproporphyrin range from 1.2γ to 28.7γ per 100 ml., the average value being 10.862±1.316, and a uroporphyrin range from 1.3γ to 46.6γ per 100 ml., with an average value of 10.57±2.012. Figure 10 shows the results graphically. All these employees were reported to have counts less than 1000 stippled cells per 1,000,000 erythrocytes.

It may be noted that these values are distinctly higher than those obtained in the study of employees exposed to lead dust in the repair of motor-vehicle bodies. The comparison, however, is not valid, since the tile workers had been exposed for several years in the tile plant. Their average values may be considered therefore to provide a norm for persons working for several years in an atmosphere with a mild exposure to lead.

(*iii*) A number of urine samples were obtained from employees in a storage-battery-manufacturing plant on the morning following the day when blood films were taken for stippled cell counts in August, 1953, and April, 1954. Six employees had stippled cell counts less than 1000, two had counts of 1000, and seven had counts varying from 1600 to 9800 per 1,000,000 erythrocytes. Figure 11 shows that there is a definite tendency for increased coproporphyrin excretion when stippled cell counts exceed 1000 per 1,000,000 erythrocytes.

The range for coproporphyrin excretion with stippled cell counts of 1000 and less than 1000 per 1,000,000 erythrocytes was 3γ—120γ per 100 ml., the average value being 55.21±15.04. For uroporphyrin, the corresponding range was 2.5γ—75.15γ per 100 ml., the average value being 34.52±9.29. These average values are distinctly higher than those obtained in a mild lead industrial exposure.

It is interesting to note the copro-/uroporphyrin ratio, which has not been referred

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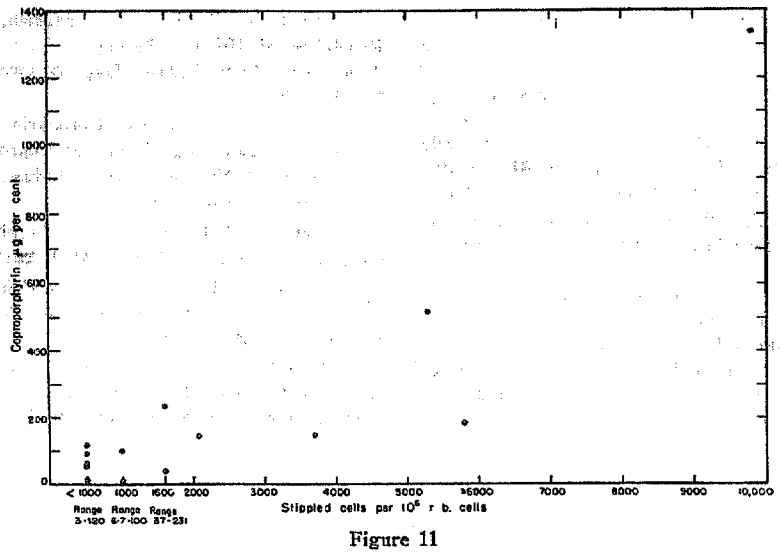


Figure 11

to previously in the literature. With stippled cell counts of less than 1000 and counts of 1000 per 1,000,000 erythrocytes, the copro-/uro- porphyrin ratio seldom exceeds 2:1, but as the stippled cell counts increase, the copro-/uro- porphyrin ratio becomes 3:1, 4:1, 5:1, and 6:1, as indicated in Table 4.

TABLE 4.—*Change in Ratio of Coproporphyrin and Uroporphyrin with Increase in Stippled Cells*

| Name | Stippled Cells | Copro-porphyrin, γ/100 Ml. | Uro-porphyrin, γ/100 Ml. | C/U |
|---|---|---|---|---|
| T. | 4,000 | 60.0 | 27.9 | 2:1 |
| H. | 1,000 | 98.0 | 44.52 | 2:1 |
| P. | 1,600 | 231.0 | 70.5 | 3:1 |
| B. | 1,600 | 37.0 | 12.0 | 3:1 |
| Tob. | 5,800 | 181.0 | 46.3 | 4:1 |
| Top. | 3,700 | 144.0 | 28.0 | 5:1 |
| A. | 5,300 | 509.0 | 96.3 | 5:1 |
| C. | 9,800 | 1,881.0 | 215.0 | 6:1 |

## 5. SUMMARY AND CONCLUSIONS

(*i*) In porphyrin analysis of morning urine samples from apparently normal persons, it has been observed that urines with a strongly positive qualitative test for urobilinogen generally have high uroporphyrin values. There is evidence that this feature occurs in persons on a high-vegetable diet as well as in certain liver diseases.

(*ii*) An attempt has been made to correlate stippled cell counts with quantitative coproporphyrin determinations in a group of employees mildly exposed to lead for the first time in buffing and spray painting of damaged motor-vehicle bodies. As the stippled cell counts increase to 1000 and 1500 per 1,000,000 erythrocytes, the coproporphyrin values tend to increase. With continuing mild exposure to lead, although the stippled cell counts may not exceed 1000 per 1,000,000 erythrocytes, the urinary coproporphyrin values tend to increase.

Employees with a mild exposure to lead for several years have higher urinary coproporphyrin and uroporphyrin values than those found in normal persons.

(*iii*) Employees liable to a severe exposure to lead show high values for urinary coproporphyrin as the stippled cell counts exceed 1000 or 1500 per 1,000,000 erythrocytes. The uroporphyrin values are also increased. It is interesting to note that in this type of exposure the copro-/uroporphyrin ratio increases from the normal 1:1 or 2:1 to 3:1 and may reach 6:1.

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