COPROPORPHYRINS IN LEAD AND MERCURY WORKERS

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Coproporphyrin determination by the Askevold method is simple, easy and inexpensive. Coproporphyrin determination may be better than urinary lead analyses when well equipped laboratories and technicians with experienced training are not available, especially in small industries or perhaps in developing countries. For the screening periodic examination of lead workers to obtain information of lead exposure, and to prevent lead poisoning of those workers, it was demonstrated in the present study that one might skip urinary lead analyses which should be carried out for suspected cases. Because urinary coproporphyrin increase nicely paralleled that of lead, and in the coproporphyrin determination there could be considered no such fear of contamination or analytical errors which might yield in the determination of urinary lead.

According to the combined observations of the present as well as the previous study, the threshold limit value of lead in air was assumed to be around 0.08 to 0.12 mg/m³ for coproporphyrin increase.

In mercury absorption coproporphyrin was found to increase to some extent, and mercury exposure as a group will be detected by plotting data on paper of logarithmic normal probability, provided a standard distribution curve obtained from normal subjects.

The qualitative estimation of coproporphyrin in urine has already become one of routine laboratory tests in periodic medical examination of lead workers. It is also an established fact that increased excretion of coproporphyrin as well as of delta-aminolevulinic acid (ALA) are the earliest signs after exposure to lead. The signs may be called an inapparent symptom or nonpathological response, since many lead workers, who excrete remarkably increased coproporphyrin or ALA over a long period of time, develop no clinical symptoms of lead poisoning. The author questioned if it was possible that the quantitative determination of coproporphyrin could be a substitute for urinary lead analysis, in order to indicate the grade of lead exposure. However, according to Maloof,¹⁾ the quantitative determination will never supplant urinary lead analyses, although his data showed reasonable correlation between urinary lead and coproporphyrin. Nevertheless, it is well known that contamination of urine samples by lead is almost inevitable when they are collected in the factories or plants. In a given urinary sample it is not

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infrequent that there is a great discrepancy between lead and coproporphyrin amount. One of the causes of this discrepancy excluding individual biochemical differences, is considered due to the accidental error in the lead analyses. The analytical method of lead in biological material is not simple, and insidious error may accompany the process, in either the dithizone mixed color or polarographic method.

On the other hand, the coproporphyrin determination by the Askevold method²⁾ as modified by With and Petersen³⁾ is not only much simpler than lead analysis, but the results are accurate and consistent. Thus, when it is possible to exclude other pathological conditions, in which coproporphyrin excretion is increased, the coproporphyrin estimation should be more strongly considered for periodic medical examination of lead workers, if it is really a good index of lead exposure and correlates with urinary lead.

From the point of view mentioned above, the present paper describes a correlation between urinary coproporphyrin and lead of workers exposed to lead. It will also demonstrate the association between lead concentration in air and the corresponding percentage of coproporphyrinuria, and threshold limit value⁴⁾ (TLV) of lead in air is discussed from these results.

In addition to the study concerning lead exposure, distribution of coproporphyrin concentration among mercury workers was observed to deviate significantly from the among normal adults.

METHOD OF STUDY

Ninety urine samples were collected from lead workers in a storage battery plant and a lead oxide firm, where lead concentrations in air varied from 0.2 mg up to several milligrams per cubic meter. The urine samples were brought back to our laboratory on the day collected, and coproporphyrin determination was made by the method³⁾ mentioned above. Urinary lead was analyzed thereafter over a period of three months, keeping urine in polyethylene bottles, by the U. S. P. H. S. mixed color method.⁵⁾

Urinary lead and coproporphyrin of a patient from the storage battery plant who had been hospitalized one month after a convulsion which was finally diagnosed due to lead poisoning, were also analyzed almost daily during his 40 day stay in our hospital.

For the study of the correlation between urinary coproporphyrin and mercury, urine samples were taken from mercury workers engaging in thermometer production. Urinary mercury was analyzed by the Natelson's method,⁶⁾ as modified by the author *et al.*⁷⁾

In order to indicate a distribution curve of the normal excretion of coproporphyrin, male doctors from our laboratory and medical students were subjected

for the study.

The TLV of lead in air was calculated, according to the data previously published,⁸⁾ by applying the Bliss's Probit Method,⁹⁾ which is generally used for deriving LD₅₀.

RESULTS

Relationship between urinary coproporphyrin and lead concentration of ninety samples from ninety lead workers was shown in Fig. 1. As seen in the figure,

Fig. 1. Relationship between urinary coproporphyrin and lead amount.
(N=90)

which was drawn on logarithmic (both abscissa and ordinate) paper, a very close correlation between the two urinary components was observed, $r=0.92$ ($t=20.74$). From the data, a regression formula, $\log y = 1.35 + 0.32 \log x$ was obtained, where y is coproporphyrin concentration and x is lead concentration in micrograms per liter of urine. As the figure shows almost all the dots, which are below 100 μg/l of coproporphyrin, do not exceed the value of 0.12 mg to 0.15 mg in lead concentration, which is generally considered upper normal limit. The result implies that coproporphyrinuria is not only a pertinent index of lead exposure, but its grade will follow the urinary lead concentration.

A male lead worker of 42 years of age in the same storage battery plant had a convulsion during his work, and was admitted to a hospital close to the factory. He was then transferred to our hospital about one month after the accident, with a diagnosis of suspected lead poisoning. He was closely examined and finally diagnosed as lead poisoning. His urine was analyzed every day for coproporphyrin and lead during his 40 day hospitalization. During this period treatments such as administration of CaEDTA or other therapies enhancing excretion of lead were

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Fig. 2. Coproporphyrin and lead in urine of a case with lead poisoning.

not carried out. As shown in Fig. 2, on about the tenth day of admission both coproporphyrin and lead started to increase in excretion, due to perhaps mobilization of lead to blood from bone. On the 16th day it reached a peak, decreased, then rose again to its second peak on the 30th day. His general condition had been improved from about the 20th day and about a week before discharge he did not complain of any subjective symptoms nor reveal positive laboratory findings. Fig. 2 nicely illustrates the direct relationship between the excretion of coproporphyrin and lead. The correlation between the two substances was indicated in Fig. 3. The correlation was significant, $r=0.565$, and a regression formula, $\log y=1.627+0.47 \log x$ was obtained. In this case, coproporphyrin is somewhat less than that of lead in proportion, in comparison with the observation seen in Fig. 1. It might be due to individual difference, namely coproporphyrinuria following lead absorption might be different in amount from person to person. Some persons are very sensitive and some are less so against the same dose of lead intake.

In respect to the correlation between coproporphyrinuria and lead concentration in air of working environment, data, which had been used by the author somewhere else,⁸⁾ were rearranged as shown in Fig. 4. As will be mentioned below, the upper limit of coproporphyrinuria in normal subjects was $53.1 \mu\text{g}/1$ in 95% level of significance. Fig. 4 means that lead concentration in air was taken on logarithmic

Fig. 3. Urinary coproporphyrin and lead in a case with lead poisoning.

Fig. 4. Proportion of coproporphyrinuria ($>50 \mu\text{g/l}$) corresponding to lead concentration in air. N: No. of subjects.

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abscissa and a percentage of the abnormal (more than 50 $\mu\text{g}/\text{l}$) coproporphyrinuria was plotted against the corresponding lead concentration. The percentages were transformed to the Bliss's Probit Values,⁹⁾ which Horiuchi *et al*¹⁰⁾ applied to calculate TLV of lead in air, and the ordinate of the figure was scaled by probit value. The five dots fall almost on a straight line, and a regression formula, $Y=4.83+1.88(X-1.67)$, was significant for linearity ($\chi^2=3.96 < \chi^2_{(0.05)}=7.81$). When an abnormality percentage of 5%, transforming to the probit value, is put on y in the above equation formula, x is obtained as 0.08 mg/m^3 lead in air. Since there was 5% coproporphyrinuria more than 50 μg (upper limit 53 $\mu\text{g}/\text{l}$, in 95% level of significance) in normal subjects, 0.08 mg/m^3 should be a TLV, as far as coproporphyrinuria more than 50 $\mu\text{g}/\text{l}$ is considered to be indicative of lead absorption hazard.

Increased coproporphyrin in urine following mercury exposure has been reported by Suzuki.¹¹⁾ He observed coproporphyrinuria among mercury workers, and in his experience the increase of coproporphyrin is limited to a certain extent and is not as high as that seen in lead absorption. The present author made analyses of urinary mercury and coproporphyrin of thermometer workers. Fig. 5 shows a

Fig. 5. Relationship between urinary coproporphyrin and mercury amount. (N=39)

correlation between the two substances in urine. There was a significant correlation ($r=0.572$), which was not as close as that observed in lead absorption. But proportional increase of coproporphyrin with mercury was not observed by the concentration beyond about 100 $\mu\text{g}/\text{l}$. In Fig. 6, distributions of coproporphyrin concentration of both normal and mercury subjects were shown. In the normal group the curve is quite consistent with logarithmic normal distribution (a straight

Fig. 6. Distribution of urinary coproporphyrin amount. (Males; 20~40 yrs of age), ●: Normal; ▲: Exposed to mercury.

line on logarithmic normal probability graph). On the contrary, the distribution of that in mercury workers does not show any regular pattern. Thus, if one preliminarily provides accumulated percentage curve of normal distribution on paper of logarithmic normal probability, and plots the data obtained from mercury workers on the same paper, and a deviation of the latter plotting from the standard curve is found, it should imply that mercury absorption of workers as a group is significant.

COMMENT

As already mentioned, Maloof states in his article¹⁾..... "Since coproporphyrinuria is not specific for lead poisoning, a test for it will never supplant urinary lead analysis"..... The present author agrees with him, if it is feasible to carry out lead analyses under strict supervision of collection of urine samples, in properly equipped laboratories and by well trained technicians. It is, however, not so easy to meet the mentioned conditions, particularly in small industries, or in small towns, or in developing countries. Furthermore, among normal workers it is rare to find such a patient who excretes increased coproporphyrin due to other pathological condition than poisonings by lead, mercury etc. In fact, as shown in Fig. 5, distribution curve of coproporphyrin concentration in healthy adults gives

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a quite good logarithmic normal pattern. Horiuchi *et al*¹²⁾ also reported the similar observation on normal subjects in much more number. Thus, it is not very dangerous to adopt coproporphyrinuria as an indicator of lead exposure instead of lead analyses, and to use it for the purpose of prevention of lead hazard and for health care of lead workers. If physicians find workers with increased coproporphyrinuria, basophilic stippled red cells, and perhaps slight anemia by screening examination, further detailed laboratory tests including lead analyses of blood and urine should, of course, be carried out. It is also needless to add that complete examinations should be conducted on a suspected case of lead poisoning.

Lead analysis, *per se*, does not necessarily indicate the extent of lead poisoning, but it is significant to give informations of lead exposure. The present author would like to point out that lead analyses are not necessarily indispensable in periodic examinations of lead workers. Coproporphyrin determination is much simpler, easier, more accurate, less expensive, and feasible to be conducted with less experience of technical training, in comparison with lead analysis.

The threshold limit value derived by the mentioned method is a little lower than that reported elsewhere,⁷⁾ which was computed by another statistical method using the same source. But in that report the data only from a printing mill workers, which are seen as a dot (N=143) on Fig. 4, had been used. By that method TLV was 0.12 mg/m³ for the coproporphyrinuria more than 50 µg/l. It should be noted that, generally speaking, probability of response or death rate against both high or low level doses may be rather varied in the Bliss's Probit Curve. Hence, the TLV for coproporphyrinuria resulted from lead exposure should exist at the level of 0.08–0.12 mg/m³ taking into account the previous results.⁷⁾

When TLV is determined, would the increase of coproporphyrin in urine above normal limit be an appropriate index of a hazardous response? It depends on a criterion which is considered to be hazardous to human being. Haeger-Aronsen¹³⁾ found a positive correlation between delta-aminolevulinic acid and lead excretion in the urine. If the increase of ALA, for example, is made an index for criterion as early sign of lead exposure, one may use it for determining TLV. In this study, a TLV of lead in air was determined by an index, coproporphyrinuria, which has been proved to be a valuable tool in the prevention of inorganic lead intoxication by Zielhuis.¹⁴⁾ It should be also bore in mind there are by no means signs, which may develop specific for lead absorption. Some workers may insist that coproporphyrinuria could be produced by other morbid states, and not specific for lead. Nevertheless, ALA is neither specific for lead absorption.¹⁵⁾

In the present paper discussions on etiological point of view concerning coproporphyrinuria following lead or mercury absorption shall not be made. However, according to the following reason it will be assumed that biochemical mechanism of increased excretion of coproporphyrin caused by lead by and mercury absorption

Fig. 7. Difference of the empirical curve from the expected between Coproporphyrinuria and urinary lead as well as mercury.

may be different in each case. The expected regression curves between urinary coproporphyrin and lead as well as mercury, already shown in Figs. 1 and 5 were duplicated in Fig. 7, and they were compared with the empirical. In mercury exposure there is a threshold of coproporphyrin increase at the level of about $100\mu\text{g/l}$, as was mentioned, while urinary mercury shows tendency toward increasing. On the contrary, in lead absorption, the rise of urinary lead becomes rather depressive, from the observation, at the level of about 0.3 mg/l of lead concentration, as coproporphyrin continues to increase with the same proportion.

In conclusion the present paper presented an important role of coproporphyrin determination in the field of occupational health. The coproporphyrin determination is an important item for periodic medical examination of lead and mercury workers to obtain information of exposure grade to these industrial chemicals. When the results of determination are evaluated, however, significance should depend on the chemical involved.

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