Urinary non-precipitable lead in lead workers

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Kawai, M. (1976). *British Journal of Industrial Medicine*, 33, 187-192. **Urinary non-precipitable lead in lead workers.** Sixty-six workers engaged in lead-glazing pottery with a presumed moderate exposure to lead were studied. The group comprised 20 men with long-term exposure to lead and positive laboratory signs of increased lead absorption (Group A); 22 with long-term exposure and negative laboratory signs (Group B); 11 with short-term exposure and positive laboratory signs (Group C); and 13 with short-term exposure and negative laboratory signs (Group D). In addition, 14 workers employed in casting the helmet alloys with presumed heavy exposure to lead (Group E) and seven healthy individuals (Group F) were included. Urine samples from all the subjects were analysed to determine, first, the total lead using the ashing technique, and then the precipitable lead using the coprecipitation technique of Cholak, Hubbard, and Burkey (1948), but modified slightly by us. Thus, the non-precipitable lead fraction in urine was the difference between the two measurements and this was also expressed as a percentage of the total lead. The mean total lead and the mean proportion of non-precipitable lead were 0.62 $\mu\text{mol/l}$ and 48.7%, 0.35 $\mu\text{mol/l}$ and 44.9%, 0.40 $\mu\text{mol/l}$ and 48.9%, 0.17 $\mu\text{mol/l}$ and 24.6%, 1.43 $\mu\text{mol/l}$ and 44.3%, 0.14 $\mu\text{mol/l}$ and 18.8% for Groups A, B, C, D, E, and F respectively, showing that a large part of urinary lead was eliminated as precipitable lead in Groups D and F who had normal lead excretion, while about half was eliminated as non-precipitable lead in the other four groups who had excessive lead excretion. No essential difference in the proportion of non-precipitable lead among Groups A, B, and C excluded the possibility that the proportion might be directly related to the period of exposure to lead and to the laboratory findings of excessive lead absorption. The mean proportion of non-precipitable lead for the physiological (up to 0.240 $\mu\text{mol/l}$), intermediate (0.241 to 0.721 $\mu\text{mol/l}$), and excessive (above 0.722 $\mu\text{mol/l}$) total lead levels was 26.7, 41.3, and 52.3% respectively, in the lead workers comprising Groups A, B, C, and E each showing increased lead excretion when grouped together. These data suggested that, when urinary lead is within the normal range, it is excreted largely as precipitable lead even in individuals exposed to lead, and that the principal conditions determining the excretion of non-precipitable lead would be the current or recent degree of lead absorption. The excretory mechanisms and the biological significance of the non-precipitable lead are also discussed.

Several analytical studies have been made relating to the chemical form in which lead exists in blood or urine. However, little work has been concerned with the diagnostic and clinical relevance to lead intoxication. Increased urinary excretion of a non-dialyzable mercury fraction has been observed in workers with a long-term occupational exposure to mercury vapour (Suzuki *et al.*, 1964). However, only

the work reported by Dinischiotu *et al.* (1960) deals with the diagnostic and clinical significance of the chemical form of urinary lead. These investigators studied subjects who had had varying degrees of exposure to lead. They made parallel determinations of lead in urine using the ashing technique for total lead and the coprecipitation technique of Cholak *et al.* (1948) for precipitable lead, giving the values

for non-precipitable lead in urine from the difference between both measurements. Thus, they found that in normal subjects urinary lead was excreted entirely as precipitable lead while in workers exposed to lead, especially in those with chronic lead intoxication, a considerable proportion of it was non-precipitable lead. On the basis of these data they assumed that the principal condition determining the excretion of non-precipitable lead was the degree of lead accumulation. They suggested that the absolute or relative values of the non-precipitable lead fraction in urine might serve as a more useful index for evaluating the degree of exposure to, and intoxication from lead than other laboratory or clinical findings.

Additional experiments conducted by Dinischiotsu *et al.* (1960) suggested that precipitable lead in urine was ionized, while non-precipitable lead was possibly in a natural chelate. These observations are of special interest not only in understanding the metabolism of lead, but also in indicating the possibility that the degree of lead accumulation and intoxication may be assessed by the analysis of lead in a single urine sample.

The present study was carried out in an attempt to determine whether the urinary non-precipitable lead fraction can give a good measure of the degree of exposure to and intoxication by lead.

Preliminary experiments

Dinischiotsu *et al.* (1960) observed that the coprecipitation method of Cholak *et al.* (1948), in which lead is precipitated directly from urine, gives almost quantitative determination of urinary lead excreted as an ionized form, but eliminates determination of it as an organically bound form. The analytical procedure is as follows: 1 ml of precipitating solution is added to 40 ml of urine in a centrifuge tube. The resulting precipitate is dissolved in hydrochloric acid and then diluted with distilled water. The dissolved solution is mixed with an alkaline solution of dithizone and chloroform. Thus the lead-dithizone complex is extracted with chloroform.

However, the additional experimental results presented by Dinischiotsu *et al.* (1960) comparing precipitative lead values obtained using the above method with those obtained either by adsorption on activated alumina or dialysis, appear to suggest that urinary precipitable lead can escape quantitative determination in varying degrees according to the urine samples using the coprecipitation technique.

The current preliminary experiments were undertaken to see if this method can effect complete precipitation of ionic lead in urine. The test was conducted in the following way: a freshly voided

human urine sample was divided into two portions, to one of which (A) was added known amounts of lead, but none was added to (B). The entire analytical procedure was followed for portion (A); for portion (B) known quantities of lead were added to the precipitate resulting from coprecipitation. The subsequent analytical procedures were then performed on the same urine sample, treated in accordance with the coprecipitation method, and corrected for the original lead present in the urine sample. A calibration curve was prepared by mixing 40 ml of distilled water containing added lead with the alkaline solution of dithizone; it was then extracted with chloroform. The analytical results are shown in Table 1. The mean lead recovery for 5 and 10 µg

TABLE 1
COMPARISON BY TWO METHODS OF RECOVERY OF
LEAD ADDED TO HUMAN URINE

Method	Pb added (µg)	Pb found (µg) Mean (Range)
A	5	4.1 (3.8-4.3)
	10	8.3 (7.8-8.7)
B	5	5.0 (4.9-5.1)
	10	9.8 (9.5-10.1)

Five determinations were made in each case.

The differences between the means for two methods were significant in each case ($P < 0.01$)

of added lead using method (A) was significantly lower than that using method (B), ($P < 0.01$). These recovery tests show that a considerable portion of lead ions present in urine can escape quantitative coprecipitation with the original method.

At least two attempts were made to improve the recovery of lead from urine by repeating the coprecipitation step.

Table 2 gives a comparison of the recovery of lead added to normal human urine using the coprecipitation method which was repeated once, twice, and three times to demonstrate that satisfactory lead recovery can thus be attained. The results of the preliminary experiments led us to adopt the original coprecipitation method modified by repeating it three times.

The urinary lead estimated by this modified method was defined as urinary precipitable lead in the present study.

Materials and methods

Two groups of lead workers, differing in the type and intensity of their exposure to lead were compared in this study. One group comprised 66 workers, who had been engaged in lead-glazing pottery, and had a presumed

TABLE 2

MEAN RECOVERY OF LEAD ADDED TO HUMAN URINE ACCORDING TO NUMBER OF COPRECIPITATION STEPS REPEATED

Urine sample (no.)	Pb added (μg)	No. of coprecipitation steps repeated		
		1	2	3
		Pb found (μg)		
1	5	3.7 (2.9-4.4)	4.3 (4.1-4.4)	4.8 (4.4-5.2)
2		3.3 (2.9-3.9)	4.0 (3.8-4.2)	4.7 (4.5-5.1)
3	10	7.6 (7.3-8.3)	8.9 (8.7-9.5)	9.9 (9.8-10.0)
4			9.1 (8.8-9.3)	9.9 (9.7-10.2)

Five measurements were carried out in each case. Figures in parentheses represent range

intermediate exposure to lead. This group was categorized according to the occupational history and laboratory data which had been obtained by periodical health examinations for lead poisoning, into four subgroups (A, B, C, and D). These four subgroups were determined from the possible combination of a short or long-term exposure to lead, and the presence or absence of the laboratory signs of increased lead absorption. The grouping is shown in detail in Table 3. The other group included 14 employees, who had worked the kelmet alloy (containing lead 20-45% and copper), with a presumed heavy exposure to lead, all showing the laboratory findings of excessive lead absorption (Group E). Seven healthy individuals without any occupational exposure to lead served as a control group (Group F).

Determinations were made on individual urine specimens by the dithizone colorimetry method after wet ashing for total lead, and by the coprecipitation method of Cholak *et al.* (1948) as modified slightly by the author for precipitable lead. All the determinations

were carried out in triplicate. The non-precipitable lead can be determined as the difference between the values thus obtained.

Results

Table 3 summarizes the results of the measurements obtained for six groups of subjects observed. Urinary lead was excreted largely as precipitable lead in Group F (normal subjects) and in Group D (those exposed to lead but with normal lead excretion), while as much as about half of the lead was excreted as non-precipitable lead in the other four groups with increased lead excretion.

No practical difference was observed in the mean proportion of non-precipitable lead between Groups A and B, which were almost equal in the duration of exposure to lead, but opposite in the presence of the laboratory findings of undue lead absorption. A similar finding was observed between Groups A or B and Group C, which were widely different from each other in the period of exposure to lead. These results demonstrate that the proportion of non-precipitable lead fraction is not directly related to the presence or absence of the laboratory signs of increased lead absorption or with the length of time exposed to lead.

The levels of non-precipitable lead showed a roughly linear increase with those of total lead as shown in Fig. 1 ($r = 0.793$). Such a relationship may imply that non-precipitable lead, as a percentage of total lead, is maintained nearly unchanged regardless of the increasing total lead excretion. This is consistent with the results shown in Table 3. There is no appreciable difference in the mean proportion of non-precipitable lead among Groups

TABLE 3

WEIGHTED AVERAGES OF URINARY LEAD DETERMINED BY TWO METHODS FOR SIX GROUPS OF SUBJECTS

Subject	Group	Cases (no.)	Laboratory signs of increased lead absorption	Years of exposure	TL ($\mu\text{mol/l}$)	PL ($\mu\text{mol/l}$)	NPL ($\mu\text{mol/l}$)	NPL (%)
Lead-glazing workers	A	20	(+)	19 (8-35)	0.62 (0.14-1.75)	0.32	0.30	48.7
	B	22	(-)	19 (10-52)	0.35 (0.15-1.25)	0.19	0.16	44.9
	C	11	(+)	3 (2-6)	0.40 (0.11-1.08)	0.20	0.19	48.9
	D	13	(-)	2 (1-5)	0.17 (0.07-0.41)	0.13	0.04	24.6
Casting workers	E	14	(+)	9 (1-22)	1.43 (0.22-3.48)	0.80	0.63	44.3
Normal subjects	F	7			0.14 (0.07-0.24)	0.10	0.03	18.8

TL = total lead; PL = precipitable lead; NPL = non-precipitable lead;

%NPL = non-precipitable lead as a percentage of total lead.

Figures in parentheses indicate range

FIG. 1. Relationship between total lead and non-precipitable lead for all subjects.

A, B, C, and E with the mean total lead varying from 0.35 $\mu\text{mol/l}$ to 1.43 $\mu\text{mol/l}$. However, the possibility that the proportion of non-precipitable lead depends on the total lead levels must also be considered in view of the findings that Groups D and F, low in the proportion had normal lead output. When a comparison of the mean proportion of non-precipitable lead was made for the three different levels of total lead classified as low (up to 0.240 $\mu\text{mol/l}$), moderate (0.241 to 0.721 $\mu\text{mol/l}$), and excessive (above 0.722 $\mu\text{mol/l}$) in all the lead workers (Fig. 2 and Table 4),

TABLE 4
WEIGHTED AVERAGES OF THE PROPORTION OF NON-
PRECIPITABLE LEAD ACCORDING TO TOTAL LEAD
LEVEL

Group	Total lead ($\mu\text{mol/l}$)					
	0-0.240		0.241-0.721		0.722-	
	N	%NPL	N	%NPL	N	%NPL
A+B+C +D+E	19	26.0	43	40.3	18	52.3
A+B+C+E	10	26.7	40	41.3	18	52.3

N = number of cases

the following findings became apparent: the mean proportion of non-precipitable lead decreased to about 26% for the total lead level up to 0.240 $\mu\text{mol/l}$, while it increased to about 40% for the total lead level of 0.241 to 0.721 $\mu\text{mol/l}$ with a wide

FIG. 2. Distribution of % non-precipitable lead by total lead levels for all the lead workers. Open circles, Group D; solid circles, Groups A, B, C, and E. Weighted averages indicated by the bar.

variation of individual values, and increased further to about 52% for the total lead level above 0.722 $\mu\text{mol/l}$ with a limited variation.

A similar relationship was observed for all the

lead workers excluding those comprising Group D with the low mean proportion of non-precipitable lead (Table 4).

Hence, it is conceivable that the proportion of non-precipitable lead is related to total lead levels on a group basis, and that when total lead is within the normal range lead will appear in urine mainly in the precipitable lead form, even in individuals with varying degrees of lead exposure.

Table 5 shows the distribution of subjects according to the levels of total lead in urine. These data together with those in Table 4, suggest that the main difference in the mean proportion of non-precipitable lead between Group D or F and each of the other four groups can be attributed to the marked dissimilarity in the relative distribution of the subjects by total lead levels; the absence of an observed practical difference in the mean proportion among the Groups A, B, C, and E can be ascribed to the close similarity in relative distribution.

TABLE 5
DISTRIBUTION OF NUMBERS OF SUBJECTS BY GROUP
AND TOTAL LEAD LEVEL

Group	Total	Total lead ($\mu\text{mol/l}$)		
		0-0.240	0.241-0.721	0.722-
A	20	2	13	5
B	22	5	16	1
C	11	1	9	1
D	13	10	3	0
E	14	1	2	11
F	7	6	1	0

Discussion

Although most blood lead is bound to red blood cells, about 5% is plasma bound (Goyer, 1971b) and available for excretion by the kidney by way of glomerular filtration and/or secretion through the tubular wall (Vostál and Heller, 1968). Lead in the plasma is believed to be present in two chemical forms, non-diffusible and diffusible, of which only the latter can actually pass into the urine by these two routes of renal excretion (Kety, 1942; Vostál, 1966). The diffusible lead fraction in plasma is thought to consist not only of simple lead ions but also of soluble lead complexes bound to small organic ligands (Kety, 1942; Vostál and Heller, 1968; Goyer, 1971a). Hence, if it is assumed that the diffusible lead in the plasma does not change in the process of its renal excretion, then lead appears in urine in the same two chemical forms as the diffusible lead in the plasma. In fact, the few studies so far reported confirm this. Nelson and Hamm (1958)

provided experimental evidence that lead in human urine was bound as a chelate or complex. The experiments carried out by Dinischiotsu *et al.* (1960) in an attempt to analyse the chemical nature of precipitable lead and non-precipitable lead fractions in urine suggested that precipitable lead is ionized, while non-precipitable lead is present as a natural chelate. As demonstrated by Dinischiotsu *et al.* (1960), we have also confirmed the presence of lead in two different chemical forms in urine, precipitable lead and non-precipitable lead.

Principal conditions in governing the excretion of non-precipitable lead

Dinischiotsu *et al.* (1960) found that the mean proportion of non-precipitable lead for normal subjects, and those exposed to lead with and without chronic lead poisoning was 0.93, 26.8, and 41.6%, the mean total lead for the corresponding group being 0.21, 0.63, and 1.24 $\mu\text{mol/l}$, respectively. When these data presented by Dinischiotsu *et al.* (1960) are considered in relating the proportion of non-precipitable lead to total lead levels, there is a close correlation. From figures given by Dinischiotsu *et al.* (1960) showing the relation of precipitable lead to total lead, it will be noted that urinary lead is excreted largely as precipitable lead at the level of total lead below 0.48 $\mu\text{mol/l}$ even in subjects exposed to lead with or without clinical lead poisoning. These findings seem to indicate the dependence of the proportion of non-precipitable lead on total lead level. The results of our present study showed that the proportion of non-precipitable lead for lead workers was not directly associated with the laboratory signs of increased lead absorption and the duration of lead exposure, but varied according to the graded level of total lead below 0.241, 0.241 to 0.721, and above 0.722 $\mu\text{mol/l}$. The total lead levels of less than 0.241, of 0.241 to 0.721, and of 0.722 $\mu\text{mol/l}$ or more may be regarded as representing normal, intermediate, and undue absorption of lead respectively, in the light of present knowledge that the urinary lead levels are indicative of the current or recent magnitude of exposure to or absorption of lead (Cantarow and Trumper, 1944). Thus, the most reasonable conclusion to be drawn from the results of the study is that the principal conditions determining the proportion of non-precipitable lead are not so much the degree of lead accumulation and the severity of lead intoxication as the magnitude of the current or recent absorption of lead.

Dinischiotsu *et al.* (1960) observed that the proportion of non-precipitable lead in cases of chronic lead poisoning decreased rapidly after an interruption of lead absorption. This observation gives

added support to our conclusion. All these arguments seem to rule out the supposition that the urinary non-precipitable lead may provide a satisfactory index of the degree of lead accumulation and intoxication.

Mechanisms by which the proportion of non-precipitable lead is governed according to the total lead levels

Teisinger (1966) assumed that lead was eliminated only by glomerular filtration within the range of normal blood lead concentration. He also found that most of the lead appeared as an inorganic form below normal blood lead levels. These observations led Goyer (1971b) to the suggestion that organically complexed lead is re-absorbed by renal tubules. Indeed, it has been experimentally established that lead filtered at the glomerulus is partly re-absorbed in the tubules (Vostál, 1966). We have observed urinary lead excretion largely in the precipitable form (that is, inorganic form) in normal individuals and in lead workers with urinary lead levels below $0.241 \mu\text{mol/l}$, suggesting physiological lead absorption. A possible explanation for these observations may be that most filtered organically bound lead is re-absorbed by the renal tubules.

The increased proportion of non-precipitable lead at the level of total lead above $0.241 \mu\text{mol/l}$, particularly above $0.722 \mu\text{mol/l}$, suggesting intermediate and undue lead absorption respectively, may be understood by assuming the possible increase in the transtubular secretion of the amount of organically bound lead with increasing blood lead levels, particularly at pathological levels (Goyer and Chisolm, 1972). Alternatively the organically bound lead in plasma increases to a level so high as to exceed the maximum capacity of its tubular re-absorption after abnormal lead absorptions.

The fact that the proportion of non-precipitable lead was raised at the total lead levels indicative of the existing excessive lead absorption appears to be of interest in considering the biological significance of non-precipitable lead. Non-precipitable lead, possibly a lead complex, is formed by complexing the chelating substances present normally in the plasma with lead ions entering circulating blood, thereby modifying toxic lead ions to an inert or less toxic form (Nelson and Hamm, 1958). As suggested by Dinischiotu *et al.* (1960), non-precipitable lead appears to be the main form in which lead is trans-

ported for excretion or accumulation particularly under conditions of a high rate of lead absorption.

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