EVALUATION OF URINARY LEAD DETERMINATIONS*

I. The Significance of the Specific Gravity

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I

THE determination of urinary lead is a tool of considerable importance in the control of industrial exposures to that metal. Much effort has been devoted to increasing the reliability of this tool through improvements in methods of sampling and analysis, and through better interpretation of analytical data.

Relatively large scale field work such as occupies this Division, especially with respect to lead, the chief health hazard in Massachusetts industry, sets certain practical limitations to otherwise desirable methods of sampling. For example, we have found it impossible to arrange routinely for twenty-four hour samples, widely advocated as more reliable than instantaneous samples. Having accepted "spot" samples, we at one time hoped that it would be possible to note the time rate of secretion so that results could be reported as mass per unit time, as is also widely advocated. This, too, proved to be impractical. Hence for the past five years we have been taking spot samples and reporting the results in terms of weight by volume concentration, i.e., milligrams per liter.

Whatever defect may inhere in this method of sampling and reporting, the findings have been extremely useful. Their general validity was established by Elkins et al. (2) who showed high correlation between urinary and atmospheric findings in 19 plants having lead exposures. Although this work concluded that "measurement of either atmospheric or urinary lead will give a true picture of the lead hazard in the majority of industrial processes," we have continued to make both measurements, as a check, one on the other. The good correlation has continued.

Earlier Barnes investigated the "Possibilities of Control of Lead Exposure by Examining Less than 24 Hour Urine Samples" (3). His method was to study the variation in lead excretion by the same exposed subject from whom several spot samples had been obtained in two days. The variation was sufficiently low to satisfy him that spot samples were adequate for the purpose.

Received for publication May 18, 1945.

Meek, Collins, and Harrold (4) made a similar investigation, analyzing 23 eight-hour samples which were composite parts of the nine 24-hour samples. The standard deviation of the spot sample findings from the 24-hour finding was ±0.026 mg. per liter. It was concluded that the percentage error for spot samples was large for low lead samples (normal range) but satisfactory on urines indicating absorption.

Notwithstanding the general validity and established utility of urinary lead determinations, including spot samples, they have been subjected to criticism for which remedies have been and should be sought. This criticism is based on the fact that urinary lead concentrations are not as constant as blood lead levels over short time intervals in cases of constant exposure or mild or advanced plumbism. According to Kehoe, et al. (5), reporting on results of lead ingestion by human adults, "...lead concentration in the blood of the subjects was subject to insignificant variation during the course of a day, and to only minor variations from day to day, while the urinary lead concentration, as well as output per unit of time, varied widely in accordance with the volume of water excreted by the kidneys."

Thus stated, the criticism applies not only to spot samples, but also to 24-hour samples, and to findings based on the time rate of lead excretion.

If this is the case, the evaluation of spot samples on the basis of how they compare with 24-hour samples, carried out by Barnes, Meek, and others, afford only limited comfort. Indeed, Barnes (3) warns that "it must be borne in mind that 24- or 48-hour specimens from the same person show quite a wide range of lead concentration and of lead excreted per day..."

Webster (6) believes that "the total 24-hour urinary lead output has more significance than any other measure of lead made on fractional-day samples," and finds against spot samples whether reported as lead concentration or time rate of excretion.

Attempts have been made to correlate urinary lead findings with other properties of the urine

in the hope that this would provide a basis for reporting or correcting findings, resulting in diminished variation. Webster found considerable diurnal variations, unrelated to specific gravity, pH, time, volume, and blood-lead content. He found some correlation with total phosphate in the urine.

Barnes (3) looked for, but failed to find, a correlation between lead concentration on the one hand, and specific gravity, acidity, nitrogen, and total volume, on the other. He did find a decrement in variability when results were calculated in γ per hour. Pinto and others (1), using similar data, studied the variability of findings from individuals recovering from plumbism, calculating the results in three different ways for comparison, as follows: (1) as γ/100 ml., (2) as γ per 100 ml. times the square root of the volume excreted per minute, and (3) as γ per hour. They found that the second method gave the lowest coefficients of variability. Urinary findings were sufficiently constant for them to deduce an empirical formula giving the approximate blood-lead level in terms of the urinary finding. The standard deviation of the ratios urinary lead to blood lead was 36 per cent of the mean.

Unfortunately, however, it was not practical to integrate the results of this work into our routine studies because it required notation of the time interval during which each sample was secreted. This would negate the advantages of spot sampling.

Kehoe and co-workers (5), in their studies of lead ingestion cited above, showed a high negative correlation between the volume of urine excreted and the lead concentration. They stated, "The urinary lead concentration. . .varied over a wide range during each 24-hour period, and the plotted curves of these concentrations are almost exactly opposite, throughout their course, to the corresponding curves of urinary volume. It is apparent . . .that *no factor other than the volume of available water* exerted any important role in the induction of variations in urinary lead concentration during these 24-hour periods." (Our emphasis.)

If the volume of available water is responsible for the variations in lead concentration, it is also responsible, in whole or part, for variations in the concentration of other normal constituents of the urine, for example, total dissolved solids. This raises the interesting question whether greater constancy could be expected if the results were calculated as mass of lead per unit mass of total solids. This would be a very simple matter to do, since total urinary solids is directly proportional, roughly, to the specific gravity minus unity (7). This relation is discussed in greater detail below.

II

It is the purpose of this paper to present data which constitute evidence of the following:

1. That the specific gravity is an important property of a urine specimen from the viewpoint of evaluating urinary lead excretion and that its measurement can and should be integrated with the analytical findings.

2. That when the specific gravity is properly so integrated, the instantaneous, or spot sample is at least as valid as the 24 hour sample calculated conventionally.

3. That when an individual with an abnormally high, fairly constant blood lead level (a condition which obtains for long periods during recovery from plumbism) is subjected to a series of lead urinalyses, greater constancy of excretion is found when the results are adjusted for the specific gravity of the samples, than when calculated either as weight per unit volume, or as weight per unit time. The constancy is approximately equal to that found when calculating by the volume-time formula $U\sqrt{\frac{v}{t}}$ (1).

4. That the mass of lead excreted per unit mass of total urinary solids is more constant, under conditions where the blood lead level is fairly constant, than the mass of lead excreted per unit of volume of urine, or per unit of time.

III

This laboratory has appreciated the importance of reporting the specific gravity concomitantly with results of urinalysis. The specific gravity reading has therefore always been taken and noted in our reports to medical and industrial establishments. This practice is, in fact, quite general. But it may well be asked what use is made of this information. It would seem that a certain allowance is made mentally for the specific gravity in evaluating the lead finding. For example, a distinction is made mentally between a finding 0.2 mg. per liter on a sample of gravity 1.010 and the same finding on one of 1.030. It is recognized that the former may well be more serious. But however valuable this supplementary information may be, it must be admitted that its use constitutes reliance on a

highly subjective kind of mental reservation or allowance.

An interesting illustration of the kind of subjective "correction" that is relied on in evaluating findings is contained in the remark of Meek and co-workers (4), viz.: "Previously Barnes (3) has reported good results using instantaneous samples, *particularly when the specific gravity of the sample was noted.*" (Our emphasis.) It does not seem to us that the results themselves were improved simply by noting the specific gravities, since no other use was made of them.

Now, if it be conceded that the specific gravity does have some bearing on the results, and that certain modifications, mental or otherwise, are in order contingent on their notation, then it is worth while to try to ascertain as precisely as may be just what that bearing is and how it can be integrated into the result itself.

In an effort to do this, we undertook an analysis of the accumulated data for the past three years on lead urinalyses of spot samples. A total of 1157 samples were run in this period. In every case there was at least some basis for suspicion of lead exposure, and in the overwhelming majority of cases there was, in fact, greater or less exposure. The data for each sample consist of its specific gravity and its lead content in micrograms per 100 ml. The specific gravity was determined with a floating spindle urinometer graduated for use at 60° F. The correction involves adding or subtracting 0.0001 for each degree F. above or below 60 degrees. However, we did not correct our readings for temperature. The majority of our readings are probably correct to within ± 0.002.

The samples were analyzed by the dithizone titrimetric extraction of the oxidized calcium oxalate coprecipitate (9).

Our object was to determine whether or not a correlation existed between the specific gravity and the lead content.

A table was constructed for each year, noting the lead content of each sample under its specific gravity. As would be anticipated, the number of samples falling in the various specific gravity columns tended to form the curve of normal distribution, with the mode the same for each year, namely at $G = 26$ (i.e., specific gravity = 1.026. This notation will be used henceforth).

In the vicinity of the extremes, $G = 3$ and $G = 40$, there were naturally too few samples for a valid mean to be taken, and classes of gravity were established so that about 50 samples would fall into each class per year. For the three years, each gravity class would have roughly 150 samples, a number that would provide a satisfactory average lead content figure. The average lead content of the samples in each gravity class is shown in Table 1, Column 4. It is seen that for each class the average lead concentration increases as the average specific gravity increases. This is shown graphi-

TABLE 1

SPECIFIC GRAVITY AND LEAD CONTENT, 1157 SPOT SAMPLES

1	2	3	4	5	6	7	8
No. of Samples	G Specific Gravity Class	$\overline{G}$ (Average Gravity)	$\overline{U}$ (Ave. Pb) γ/100 ml.	$\frac{\overline{U}}{\overline{G}}$	$\overline{U}_G$ (Ave. Pb corrected for gravity)	$\overline{U}_G$ (Same using Eq. 2)	$\overline{U}_g$ (Ave. Pb, in γ/gram solids)
167	3–16	12.5	9.4	0.75	17.8	17.0	2.89
147	17–20	19.0	12.9	0.68	16.2	16.1	2.60
161	21–23	22.0	14.0	0.64	15.1	15.2	2.44
145	24–25	24.5	17.4	0.71	16.9	16.9	2.73
266	26–28	26.9	18.6	0.69	16.5	16.5	2.65
137	29–30	29.7	18.7	0.63	15.0	14.8	2.42
134	31–40	32.7	19.9	0.61	14.5	14.6	2.34
Mean.....		23.8	15.9	0.67	16.0	15.9	2.58
Standard Deviation		±6.52	±3.55	±0.058	±1.03	±0.93	±0.175
Coefficient of Variability.....		±27.4	±22.4	±6.8	±6.4	±5.8	±6.8

cally in Figure 1. The correlation between the two averages was calculated by Pearson's formula for the correlation coefficient, r:

$$r = \frac{\dfrac{(\bar{U} - U_1)(\bar{G} - G_1) + (\bar{U} - U_2)(\bar{G} - G_2) + \cdots + (\bar{U} - U_n)(\bar{G} - G_n)}{N}}{\sigma_U \sigma_G}$$

$$r = +0.94$$

Perfect correlation is $r = +1$.

The high correlation of + 0.94 between the average specific gravities and lead contents of seven groups of spot samples classified by specific gravity, embracing an aggregate of 1157 samples largely from exposed workers, is evidence that the specific gravity influences urinary lead concentrations and should be integrated with the findings.

We have considered three ways in which the specific gravity may be applied in calculating results. The first and simplest is to calculate all results to the mean gravity of all samples, $\bar{G} = 24$. That is,

$$(1) \qquad U_G = U \frac{24}{\bar{G}}$$

Perhaps a few words should be said about the fact that the mean gravity of our spot samples is considerably higher than mean normal urinary gravities cited in the medical literature. We believe this is adequately explained by the contrasting conditions under which our samples were obtained and those under which in all probability the clinical or hospital data were compiled. Practically all our samples were taken from workers who were on the job during the secretion of the sample. The increased metabolism of persons at work would be expected to result in increased renal excretion of solids and in higher urinary concentrations of solids.

Mean urinary lead concentrations in γ/100 ml. (U_G) corrected for gravity by Equation (1) were calculated for the seven classes of samples and appear in Column 6, Table 1. It is seen that the coefficient of variability of the corrected values is 6.4 per cent, that for the uncorrected is 22.4 per cent.

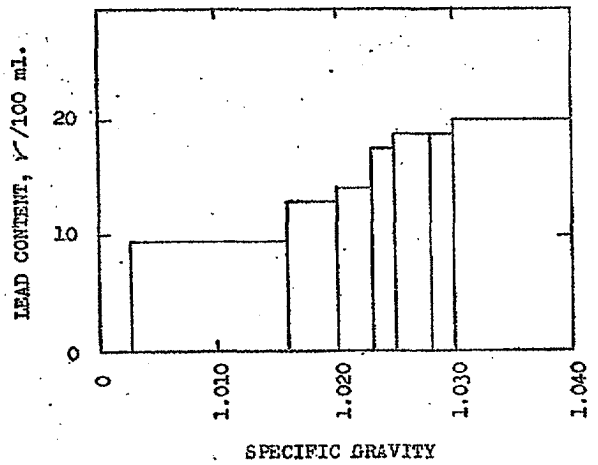

FIG. 1. Specific gravity and mean lead content, 1157 samples.

The ratios of the mean lead concentrations to the mean gravities $\left(\frac{\bar{U}}{\bar{G}}\right)$ of the several classes are shown in Column 5, Table 1. This ratio is fairly constant, the coefficient of variability being only 6.8 per cent. Application of the mean of this ratio, 0.[illegible] in the following equation is a second method of integrating the specific gravity with the urinary

(2) If $G < 24$, $U_G = U + 0.67\,(24 - G)$
If $G > 24$, $U_G = U - 0.67\,(G - 24)$

Column 7 gives the corrected results calculated in this manner.

The third method of making the correction is to calculate the result as mass of lead per unit [illegible] of total solids.

Total urinary solids may be roughly estim[illegible]

from the volume and the specific gravity by application of Long's formula,

$$S = 2.6\,G \qquad (7)$$

where S is total solids in grams per liter. Then

$$U_s = \frac{10\,U}{2.6\,G} = \frac{3.8\,U}{G},$$

where U_s is micrograms of lead per gram of solids (or parts per million), and U is the urinary lead concentration in micrograms per 100 ml. It is evident that the value U_s is directly proportional to U_a of Equation (1), for

$$\frac{U_a}{U_s} = \frac{U\frac{24}{G}}{\frac{3.8\,U}{G}} = 6.3$$

The values for U_s for the averages of the seven groups of samples appear in Column 8, Table 1.

The reduced variability coefficient $\bar{U}_s$ compared with $\bar{U}$ is evidence that lead excretion calculated on the basis of total solids is more valid than when calculated on the basis of urine volume. There is every indication that the improved validity which many investigators have obtained with 24-hour samples as compared with spot samples is due primarily to the fact that the 24-hour sample tends, of course, to approach the mean concentration of urinary solids, or what is substantially the same thing, tends to approach the mean specific gravity. But if the spot sample is adjusted to mean specific gravity, or calculated on the basis of its solids, then it should have a validity equal to or greater than the conventionally calculated 24-hour sample. Data specifically confirming this are not available, but it appears to us to follow as a corollary of the demonstrated correlation between specific gravity and lead concentration. The high negative correlation found by Kehoe and co-workers (5), referred to above, between the volume of urine excreted and the lead concentration, is a further indication that "the volume of available water" can induce lead variations in 24-hour samples that will be compensated for by adjusted spot samples.

The foregoing three methods of correcting for specific gravity are equivalent, as may be noted from the tabular values and from the nearly equal coefficients of variability.

Probably the first method (Equation 1) is to be preferred because of its simplicity and because it involves the least radical departure from conventional methods of reporting results.

A word should be said about samples of exceptionally low specific gravity. The question will arise whether it is valid to triple, or more than triple the lead concentrations found in samples of $G \lesssim 8$. Only 20 samples out of 1157 had specific gravities of 1.008 or less, and their mean lead concentration was 0.058 mg. per liter. In our opinion, the only consideration that militates against making the adjustment to mean gravity is the fact that the analytical precision drops sharply as the lead concentration of a sample decreases. The sensitivity of any method is a fixed quantity, say ± 1 microgram. For a 50 ml. sample having 0.2 mg. lead per liter the probable error is ± 10 per cent. For the same size sample containing 0.05 mg. per liter, the error is ± 40 per cent. The adjustment would obviously not be justified in this case. However, the precision may be increased by taking three or four times the usual quantity of sample for analysis.

The method of adjusting the concentration to mean specific gravity (Equation 1) was used in the calculations involving the data presented below as additional evidence of the validity of this adjustment.

IV

It has been assumed that the best test as to the reliability of the basis for calculating and reporting results of urinary lead determinations is the degree of constancy that can be shown under conditions where constancy is to be expected, namely, where the exposure is constant, or where the blood lead concentration is constant, or where recovery from plumbism is occurring by the gradual excretion of lead stored in the tissues. The appeal to this criterion is general throughout the literature on this subject. Under such conditions of expected constancy, there is no apparent reason for wide variations in renal lead excretion within relatively short time intervals, except where there is impairment of renal function. The evidence is that such reported variations are due to fallacies inherent in the determination which can be eliminated by further study.

It is true that other reasons for variations have been suggested. Pinto and co-workers (1), seeking a relation between urinary and blood lead, have said that "since only 10 per cent of the total lead excreted by the body is eliminated through the kidneys, the question can very well be raised as to

how significant urinary lead determinations may be."

However, Kehoe and co-workers (8) in their later report on lead ingestion experiments state "... that lead evacuated from the alimentary tract is largely lead which has been swallowed, and that the true alimentary excretion of lead is small. A large proportion of the lead actually absorbed into the body is excreted by way of the kidney. Certainly the renal lead excretion is quantitatively as great as the alimentary, and probably it is greater. On this point, clearly, the generally accepted idea of the means by which lead is excreted will have to be revised." This is the highest tribute yet paid to the significance of urinary lead excretion.

The work just cited also considerably weakens the theory that dietary factors may induce wide variations in renal lead excretion. Summarizing the effects of induced dietary changes the authors express "grave doubt" that the normal, spontaneous elimination of the toxic agent can be "materially accelerated by artificial means." And they conclude that their tests show that "... within the limits of lead absorption dealt with ..., the lead metabolism is exceedingly stable,—so stable, in fact, that it is not importantly influenced by gross changes in the acid-base equilibrium or by the calcium and phosphorus metabolism of the body."

This test of constancy was applied to the data for ten series of samples taken from ten subjects, three of whom (data of Barnes (3)) were spray painters with a presumably constant lead exposure of long duration, and seven of whom (our data) were recovering from lead poisoning. The series for each individual consisted of six or seven samples (in one case, five) collected over a period of about 12 to 48 hours. The lead excretion was calculated by two methods, simply as concentration in γ per 100 ml., and as γ per 100 ml. corrected to mean specific gravity by the factor $\frac{24}{G}$. It should be recalled that the latter method is the equivalent of reporting the result on the basis of mass of lead per unit mass of total urinary solids.

The variation within each series for each method was evaluated as the coefficient of variability. This term is simply the standard deviation of each series expressed as a per cent of the mean and is the mathematical measure of relative variation, or dispersion about the mean. Table 2 shows the coefficients of variability for the two methods. It is seen that by integrating the specific gravity into the results, the variability decreased in eight series and increased in two. There was a mean decrease in variability of 13 per cent.

Barnes calculated the results of his three series (A, B, and C, Table 2) as micrograms per hour. Pinto et al. (1) calculated our[1] data both as micrograms per hour and in terms of volume per unit

TABLE 2

COEFFICIENTS OF VARIABILITY OF 10 SERIES OF URINARY LEAD DETERMINATIONS

SUBJECT	NO. OF SAMPLES	ANALYSIS REPORTED AS γ/100 ML. UNADJUSTED FOR SPECIFIC GRAVITY	ANALYSIS REPORTED AS γ/100 ML., ADJUSTED FOR SP. GR. BY FORMULA $U_G = U\frac{24}{G}$	PER CENT DECREASE IN VARIABILITY
A	6	±36.9	±20.8	43.6
B	6	31.0	43.1	−39.0
C	7	28.3	24.3	14.1
M	5	37.0	35.3	4.6
N	7	19.6	14.3	27.0
O	7	28.9	21.6	25.2
P	7	47.0	39.0	17.0
Q	7	27.2	23.6	13.2
R	6	29.8	30.1	−1.0
S	6	35.8	27.0	24.6
Mean...		±32.2	±27.9	13.0

TABLE 3

MEAN COEFFICIENTS OF VARIABILITY OF FOUR METHODS OF CALCULATING URINARY LEAD DETERMINATIONS

U (10 SUBJECTS)	γ/HR. (11 SUBJECTS)	$U\sqrt{\frac{V}{t}}$ (8 SUBJECTS)	U_G (10 SUBJECTS)
±32.2	±34.5	±27.1	±27.9

time by the formula $U\sqrt{\frac{V}{t}}$. Thus we have a basis for comparison of the variability of four methods of calculating the results. Table 3 shows the mean coefficients of variability of the four methods. It will be noted that the method of adjusting results to mean gravity (U_G) and the method based on the formula $U\sqrt{\frac{V}{t}}$ show approximately equivalent variability, and effect a decrease of about 13 per cent compared with the result as a simple concen-

[1] i.e., data of this Division, under whose auspices the cited work was carried out.

tration. The result expressed as micrograms per hour shows no decrease in variability, confirming the finding of Webster (6).

Summary

1. A high correlation was found between specific gravity and lead concentration for 1157 spot urine samples obtained largely from workers with more or less exposure to lead.

2. A decrease in variation of urinary lead excretion of series of spot samples taken from subjects recovering from lead poisoning was found when the results were adjusted to mean specific gravity, as compared with the unadjusted concentration, or with results expressed as time rate of lead excretion.

3. These findings constitute evidence that renal lead excretion calculated as mass of lead per unit mass of total urinary solids is more representative of true exposure than when calculated as mass per unit volume or per unit time.

4. Calculated in this manner, the evidence is that spot samples are not less representative than 24-hour samples calculated in terms of weight per unit volume.

5. It is recommended, therefore, that urinalyses for lead be reported as "milligrams per liter adjusted to mean specific gravity", a figure obtained by multiplying the analytical finding in mg. per liter by the factor $\frac{24}{G}$, where G represents the significant figures of the specific gravity, i.e.

$$G = 1000 \text{ (sp. gr. } - 1).$$

Grateful appreciation for reviewing this paper is expressed to Dr. Joseph C. Aub, Massachusetts General Hospital, Captain Hervey B. Elkins, Chemical Warfare Service, Army of the United States, and Dr. Robert A. Kehoe, Director, Kettering Laboratory of Applied Physiology.

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