J Ind Hyg 23 (1941) 313-321

RENAL EXCRETION OF INDUSTRIAL CHEMICALS*

I. Urinary-Lead Concentration and Blood-Lead Clearance

Sherman S. Pinto, Hervey B. Elkins and John F. Ege, Jr.

Massachusetts Division of Occupational Hygiene, Department of Labor and Industries, Boston, Massachusetts

TOXIC chemicals when absorbed by the body eventually act on individual cells and produce changes which are designated as the particular pathological changes produced by that chemical. From the concentration in the blood of the chemical under investigation we may infer the approximate concentration to which the tissue cells are subjected, but there is no way of studying the concentration and effect of that substance in the individual cell. Therefore, we feel that the blood level of a substance is of great significance since it is the closest approach we can make toward learning the concentration of that substance to which the cells are subjected. In the case of lead even this approach is open to some doubt, since by our methods of analysis we are not able to determine whether the lead as determined in the blood is in a form that might affect the fixed cells of the tissues.

Smith, Rathwell, and Marcil (11) have studied the distribution of lead between serum, cell, and fibrin fractions of blood in acute and chronic lead poisonings. These studies indicate that there are changes in the lead content of the several fractions under various conditions. Whether this indicates a change in the chemical nature of the lead-bearing compound has not been determined.

In spite of the doubts raised by our lack of knowledge of the exact form which lead takes when present in the blood stream, we feel that blood lead values are of greater significance than are the values for lead concentration in excreta. From a practical industrial viewpoint it is much easier to get a sample of urine from a workman than it is to get a sample of blood. Therefore, if a urinary test can be devised which will show a definite relationship between blood-lead and urinary-lead levels, a great deal more significance can be placed on the urinary lead test than is possible at present.

Since only 10% 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 (15). It has been shown by practical experience based on statistical analysis of large amounts of data that some idea of the amount of lead in the blood can be determined by this means. However, numerous examples can easily be found where the apparent lead concentration in the urine did not

*Received for publication March 1, 1941.

coincide with the clinical picture which the patient presented (12, 13). It is the purpose of this paper to develop formulas by which a closer correlation between blood-lead and urinary-lead concentrations can be shown.

Analytical Methods

Determination of lead in urine

To 50 cc. of the sample, 50 cc. of glass-distilled water is added. After bringing the pH to about 4.5 (brom cresol green) with acetic acid, 5 cc. of saturated ammonium oxalate is added. If necessary the pH is readjusted and 0.5 cc. of 10% calcium chloride is added while swirling. After transferring to long test tubes and allowing to stand overnight, the supernatant liquid is siphoned off. The precipitate is transferred to smaller tubes with glass-distilled water, centrifuged, washed, and oxidized by the Ross-Lucas method (14).

The solution is transferred to a separatory funnel, 5 cc. of 10% citric acid and 0.5 cc. of 25% sodium cyanide are added. After bringing the solution to a pH of 8.5 (phenol red), the lead is extracted with dithizone. After complete extraction, the combined extracts are stripped with 10 cc. of 1 N hydrochloric acid and after washing the aqueous layer with chloroform, 0.5 cc. of 25% cyanide is added to the partially neutralized solution. The pH is brought to 8.5 (phenol red) and the lead is titrated by extraction with successive portions of dithizone (equivalent to approximately 3γ lead per cc.).

Determination of lead in blood

Syringes and needles are treated in the manner suggested by Willoughby and Wilkins (8).

The sample is collected in a cle flask to which 1 mg. of heparin added for each 5 cc. of blood (6).

The volume of blood is measure out into a Vycor evaporating dish an then placed in a muffle furnace. Th temperature is gradually brought t 650°C. and maintained at this tempera ture for half an hour or until all organi materials are destroyed.

The dish is removed from the fur nace and 20 cc. of 10% citric acid an enough 6 normal hydrochloric acid ar added to obtain solution on heating. After adding 3 drops of a 70% solution of hydroxylamine hydrochloride, the solution is transferred to a separatory funnel, extracted, and titrated as in the determination of lead in urine.

The Estimation of Average Urinary-Lead Concentration from Spot Samples

In a fundamental study of kidney function, Van Slyke and others have shown that, at least in the case of urea, elimination of a substance from the blood through the kidneys into the urine can be expressed by either of the two formulas (1) or (2) depending on the rate of urine formation per minute.

$$(1)\ C = \frac{U}{B} \times \sqrt{V} \text{ or } (2)\ C = \frac{U}{B} \times V$$

Formula (1) is used when less than 2 cc. of urine are excreted per minute; formula (2) when more than 2 cc. per minute are formed. (C) is the clearance of the substance under study, (U) is urinary concentration in milligrams per cent, (B) is blood concentration in milligrams per cent, and (V) is the volume of urine excreted per minute (4, 5).

The term "clearance" has been defined as designating the volume of

blood which 1 minute's excretion of urine suffices to clear of the substance under investigation. Since all the blood flowing through the kidneys is only partially cleared during its passage through these organs, the volume expressed is virtual rather than real. "Clearance" may also be defined as the minimum volume of blood required to furnish the quantity of substance excreted in the urine in 1 minute's time (2).

An attempt was first made to apply this formula to lead excretion in the urine. Kehoe has shown that during a 24 hour period the blood-lead is constant if no strenuous attempts are made to change it (7). Smith, Rathmell and Marcil have shown that nourishment and violent exercise do not affect the blood-lead level of normal individuals (11). Therefore we based the first of our experiments on the premise that the blood-lead levels would remain fairly constant for at least 24 hours. If we may assume that the rate of excretion of lead through the kidneys is constant, (this assumption was made only to test the validity of the rest of the hypothesis, as no proof of such an assumption has been shown as yet), then in the formula $C = \frac{U\sqrt{V}}{B}$ the expression $U\sqrt{V}$ should also be a constant. If any two components of such an equation are fixed, the third part must of necessity be constant also.

The urine in each case was collected from individuals who were recovering from lead poisoning. The acute episode had occurred from 2 weeks to 3 months prior to the urine sample collection. Each individual sample during a test period was collected separately and analyzed individually for lead content, and the period of collection varied from 12 to 44 hours. The ordinary urinalyses (including microscopic examination) were done and no subjects were used in these experiments if it was thought the kidneys were abnormal. When the results of lead excretion of the individual samples during any test period are expressed as milligrams per liter (we prefer gamma per 100 cc. of urine) quite a variation from the average lead content of the entire pooled sample is to be found. Barnes has shown this to vary from 82% to −40% (10). Our results verified these figures. Since we knew the time periods represented by each individual sample of any test period, we were able to calculate the lead concentration of anyindividual sample by the expression $U\sqrt{V}$ in which U = urinary lead concentration in γ/100 cc. and V = volume of urine excreted per minute. As stated above, if the volume per minute was less than 2 cc. per minute, the square root formula was used. If greater than 2 cc. per minute, the figure was used directly as in the expression $U \times V$. From a study of eight series of samples it is seen that calculation by the formula yielded less erratic results than expression of results simply as gamma per 100 cc. In table 1 are shown the scattering about the mean when results are expressed by the two methods. In almost every instance it will be seen that the standard deviation of the calculated concentration is smaller than is that of concentration expressed as gamma per 100 cc. The standard deviation expressed as percentage of the mean is also shown. Barnes (10) showed very clearly that the expression of results of lead excretion as gamma

per hour was less subject to variation than expression as milligrams per liter. In his 3 subjects having excessive lead exposure, we have calculated the standard deviation of the gamma per hour excretion. In Subject A the standard deviation was 16.8% of the mean, in Subject B, 18.3%, and in Subject C, 29%. The lead concentration of the samples shown in table 1 has also been calculated as gamma of lead excreted per hour. The standard

TABLE 1

EXTENT OF SCATTER OF URINARY-LEAD CONCENTRATIONS ABOUT THE MEAN WHEN DETERMINED BY SPOT SAMPLES

The standard deviations from the mean when results are expressed as γ/100 cc., calculated by the formula $U\sqrt{V}$, or determined as γ/hr. are shown. The standard deviation is also shown as percentage of the mean.

SAMPLE NO.	CALCULATING RESULTS AS γ/100 cc.		CALCULATING RESULTS BY FORMULA $U\sqrt{V}$		CALCULATING RESULTS AS AS γ/HR.	
	Std. dev.	Std. dev. expressed as % of mean	Std. dev.	Std. dev. expressed as % of mean	Std. dev.	Std. dev. expressed as % of mean
285-290	±7.4	±63.0	±3.85	±42.0	±1.44	±38.0
291-295	12.8	37.5	8.97	36.0	4.23	38.0
320-326	2.8	12.3	2.42	8.4	3.7	33.0
378-384	8.66	28.8	3.76	17.0	4.2	36.0
397-400	7.87	29.0	6.56	25.0	5.5	41.0
423-429	9.25	29.0	8.95	34.0	6.95	51.0
430-435	6.27	29.0	6.04	31.0	7.37	64.0
436-441	6.9	37.4	4.9	23.8	4.53	30.0

TABLE 2

EXTREMES OF URINARY-LEAD CONCENTRATION DEVIATIONS FROM THE MEAN WHEN EXPRESSED AS γ/100 cc. AND WHEN CALCULATED BY THE FORMULA $\gamma/100$ cc. $\times\sqrt{V}$

The ratio between the two means is also shown

SAMPLE NO.	TIME PERIOD OF COLLECTIONS	LEAD CONCENTRATION OF INDIVIDUAL SAMPLES EXPRESSED AS γ/100 cc.			CALCULATED LEAD CONTENT $\gamma/100$ cc. $\times\sqrt{V}$			RATIO ACTUAL MEAN / CALCULATED MEAN
		Highest	Lowest	Mean (actual)	Highest in period γ/100 cc.	Lowest in period γ/100 cc.	Mean (calculated)	
	hours							
285-290	21.5	25.0	5.0	11.75	15.5	4.6	9.23	1.27
291-295	39.0	51.0	21.0	34.2	39.2	14.0	24.8	1.38
320-326	28.5	27.0	18.0	22.8	23.6	17.2	20.4	1.12
378-384	44.0	40.0	16.0	31.2	26.1	17.0	22.2	1.40
397-400	12.0	52.0	24.2	27.2	30.6	18.0	25.6	1.06
423-429	34.0	44.4	21.0	31.8	44.4	13.5	27.0	1.18
430-435	33.3	27.8	10.6	21.8	26.6	8.6	19.4	1.12
Mean								1.22
Standard deviation								±0.12

deviation of each series has been determined and also expressed as percentage of the mean. From this comparison it can be seen that calculating results by the formula $U\sqrt{V}$ shows less scatter about the mean than does calculating results as either γ/100 cc. or γ/hr.

Table 2 shows the extremes of urinary-lead concentrations for each test period when expressed as γ/100 cc., or γ/100 cc. $\times\sqrt{V}$. It so happens that in all the series studied, the volume of urine excreted was less than 1 cc. per minute. The individuals whose urines were examined were carrying on normal light activities, and it has been our general experience that workmen engaged in their usual tasks excrete on the average less than 1 cc. of urine per minute. Consequently, lead concentration values calculated as γ/100 cc. $\times\sqrt{V}$ will usually be lower than the concentration when expressed as γ/100 cc. The ratio Actual

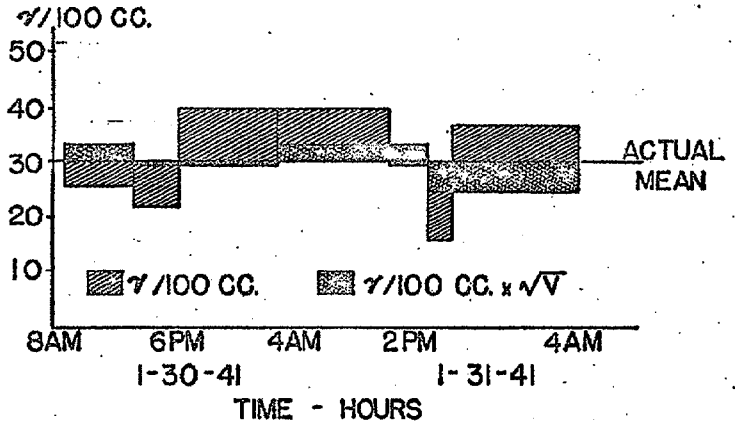

FIG. 1. Graphic representation of the variations found when expressing results of urinary lead concentration γ/100 cc. and by calculation from the formula γ/100 cc. $\times\sqrt{V}$. The two means have been superimposed.

TABLE 3

DETAILED DATA ON INDIVIDUAL SAMPLES CONSTITUTING ONE COLLECTION PERIOD

SAMPLE NO.	VOL.	$\sqrt{\text{VOL./MIN.}}$	γ/100 cc.	DEVIATION FROM MEAN	$U\sqrt{V}$	DEVIATION FROM MEAN
	cc.					
378	345	0.98	26	−5.2	25.4	+2.6
379	233	0.985	22	−9.2	21.6	−1.2
380	160	0.56	40	+8.8	22.4	−0.4
381	238	0.65	40	+8.8	26.0	+3.2
382	157	0.87	30	−1.2	26.1	+3.3
383	135	1.06	16	−15.2	17.0	−5.8
384	139	0.46	37	+5.8	17.0	−5.8
Mean.....			31.2		22.8	

Mean: Calculated Mean was found to be 1.22 ± 0.12. This must be borne in mind in the interpretation of results.

In figure 1 a typical example of the two methods of expressing urinary-lead concentration is shown. The mean of the calculated values has been superimposed on the mean of the plain gamma per 100 cc. figures in order to show the extent of variation about both means. Table 3 gives in detail the data from which figure 1 was constructed.

Calculation of lead concentration by the formula does not invariably give a result closer to the mean of the total sample than does expression of results as simply γ/100 cc. In our total of 47 individual samples studied, the calculation of results by the formula was closer to the mean in 32 instances than was the expression of results as γ/100 cc. Expressing results of the individual samples as γ/100 cc. was closer to the mean of the total sample in 15 instances.

Blood-lead Clearance

An attempt was then made to apply the accepted formulas for blood clearance to the excretion of lead. Again it should be stated that this was done on an empirical basis, and the calculations used because the formulas seemed to apply to the problem at hand. The formula $C = \frac{U\sqrt{V}}{B}$ was used in which the symbols have the same significance as above.

There is a constant relationship between amount of kidney tissue and body surface area. Therefore, in order to make all clearance figures comparable they should be corrected to the figure for an "ideal man." The ideal man has a body surface area of 1.73 sq. m. From the height and weight of an individual the surface area may be obtained from a table such as is shown by Osgood (9). This correction factor $\frac{1.73}{\text{actual surface area}}$ is determined, and the urine volume per minute multiplied by it before the square root of that figure is taken. In our series the effect of not making this correction was to raise the calculated clearance value by 6.5% in the case of a man 6 ft. 2 in. tall and weighing 205 lbs. At the other extreme was an individual who weighed 130 lbs. and was 5 ft. 2 inches tall. Here the effect of not making the correction was to lower the clearance value by 2.8%. All other corrections were less than these two and raise the question as to whether it is worthwhile making such calculations. In the interest of exactitude we felt the calculation was justified.

All subjects studied for clearance values were exposed to lead in battery companies and the degree of exposure was high. This accounts for the high blood-lead values observed, and should not be considered as being due to faulty blood-lead analyses. We feel fortunate in obtaining as varied blood-lead levels as we did, since this allows us to study clearance figures over quite a range of blood-lead concentrations. Table 4 shows the fundamental data from which some blood clearance values were calculated. Figure 2 shows the relationship of blood-lead levels and clearance values. From this graph it is seen that there is a slight tendency for the clearance values to fall as the blood-lead level is elevated over about 80 γ%. If the greatest part of the lead in the urine

is excreted through the kidney tubules such a result is to be expected. The tubular excretory mechanism has an upper limit (1) and as the blood level of any substance is elevated beyond the capacity of the tubules to excrete that substance, the clearance value falls. This does not mean that the actual amount of the substance excreted is any less, however.

TABLE 4

TYPICAL DATA FROM WHICH SOME BLOOD CLEARANCE FIGURES WERE DERIVED

SAMPLE NO.	URINE VOLUME	TIME OF URINE SAMPLE	URINARY LEAD CONCENTRATION	BLOOD LEAD CONCENTRATION	$\frac{1.73}{\text{SURFACE AREA}}$	CLEARANCE $C = \frac{U\sqrt{V}}{B}$
		min.	*γ/100 cc.*	*γ/100 cc.*		
401	69	185	52	84	1.09	0.38
402	141	190	40	28	0.955	0.12
403	102	145	34	67	0.88	0.40
404	22	150	42	60	1.05	0.28
405	75	160	34	56	0.99	0.42
406	75	160	42	77	0.98	0.37
407	268	135	15	95	0.89	0.21
408	298	210	24	60	0.91	0.45
409	73	190	33	91	1.11	0.24
410	68	170	37	101	0.93	0.22
411	134	165	20	74	0.955	0.24
412	138	185	17	56	0.94	0.25
413	92	150	45	101	0.95	0.34
414	89	130	23	105	0.805	0.16
Mean						0.292
Standard deviation						±0.102
σ of mean						±0.027

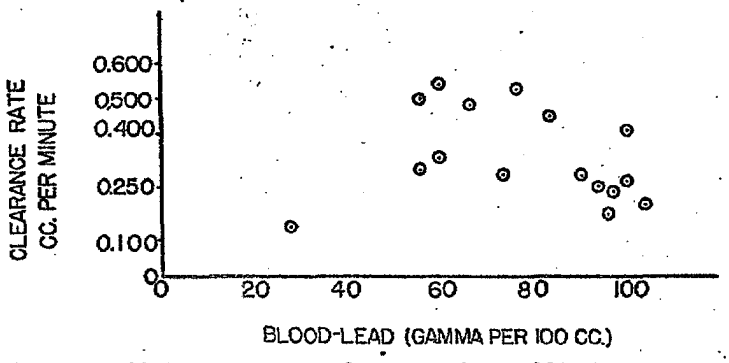

FIG. 2. Relationship between blood-lead concentrations and blood clearance rates

The average clearance figure of all samples determined was 0.284 ± 0.103.

In other words, even in the ideal man the blood-lead clearance value is a range, more properly speaking, than a constant. More data should be acquired from patients especially with higher blood-lead levels than we have studied in order to study the clearance values over a greater blood-lead range.

Relationship Between Blood-Lead Levels and Urinary-Lead Concentration

From Table 4 and additional data the average clearance value was found to be 0.28. If this average value for (C) is used as a constant in the formula $C = \frac{U\sqrt{V}}{B}$ it should be possible to estimate the blood-lead concentration from the urinary lead concentration, providing the period of time during which the urine was collected is known. This would be expressed by the formula $B = \frac{U\sqrt{V}}{C}$.

Since (C) is not exactly a constant, the blood-lead level as determined by this procedure is just an approximation. However, it is a useful approximation since it serves to depict the approximate concentration of lead to which the body tissues of a given individual are subjected. In the range of blood-lead values below 100 γ/100 cc. of blood such figures are useful from a clinical point of view. When the blood-lead level is much higher than 100 γ/100 cc. of blood, definite symptoms of lead poisoning appear (3). However, as an example, a blood-lead level estimated to be 80 γ/100 cc. would indicate that the individual so affected was unduly exposed to lead and precautions should be taken against more exposure.

Summary and Conclusions

1. In calculating the lead concentration of urine, the formula $U\sqrt{V}$ should be used when the volume of urine excreted is less than 2 cc. per minute. U = urinary lead concentration in gamma per 100 cc., V = volume of urine excreted per minute.

2. The concentration of lead in urine has been compared when results are expressed as γ/100 cc., γ/100 cc. $\times\sqrt{V}$, and γ/hr. Of these three methods the use of the formula γ/100 cc. $\times\sqrt{V}$ showed less scatter about the mean than did the other two methods.

3. The clearance of blood-lead through the kidneys is expressed by the formula $C = \frac{U\sqrt{V}}{B}$ or $C = \frac{UV}{B}$. Blood lead values are expressed as gamma per 100 cc. of blood.

4. The clearance for lead has been determined to be 0.284 ± 0.103.

5. It is possible to determine approximately the blood-lead level by the formula (1) $B = \frac{U\sqrt{V}}{0.28}$.

We wish to express our gratitude to Dr. Joseph C. Aub for his continued interest and helpful criticisms in connection with this study.

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