November 16, 1960

Memo to: Dr. Kehoe

From: T. Sterling

Subject: Deposition of Lead

The reanalysis of the lead data was motivated mainly by the question whether one could assess the amount of lead deposited in the tissues of individuals who are exposed to lead in quantities greater than usual. I can now submit a solution to you of which I am reasonably certain.

This report will deal with the bare bones of the problem, as we have discussed it during the year. It does not cover other aspects of last year's work which are in varying states of completion. Figures, tables, and a list of the symbols used and their meanings can be found at the end. I have also summarized some prospective work which appears imminently necessary. Conclusions or applications were avoided until further discussion with you.

I would like to add one note of caution. In computing actual values I have been willing to generalize from the observation done on six subjects, under somewhat varying conditions. It is encouraging that a fairly consistent picture emerges at the end despite individual differences. Yet, the calculations are limited by this fact.

Finally let me acknowledge the patient help and cooperation that was given by Mr. Shaefer (in generous quantities) by Mr. Creech, and by John Phair, who was willing to toss the problem around almost endlessly.

Some Preliminary Discussion

We start with a simple descriptive model (Fig. 1). In this model lead may be ingested or inhaled. Excretion of lead is through feces and urine. Lead may be deposited or returned to the body fluid compartment. Lead may also be lost through sweat or may be taken in without being measured. However the statement may heuristically be made that unmeasured intake and output counterbalance each other. A long term comparison of lead, in and out, as measured by the experimental procedures shows that over a long duration lead in(PbI)is approximately equal to lead out (Pb0). Table 1 gives the comparison for a number of subjects.

Primary Equilibrium

An equality of PbI and Pb0 during the pre-experimental period may be taken to indicate that the subject is in a state of equilibrium in which the amount of lead excreted approximately equals the amount of lead ingested. Since some of PbI comes into the body compartment one cannot assume that the equilibrium is due to direct elimination of PbI. However, even if some of the PbI enters the body and even if some fraction of that amount is deposited it is still possible to obtain such an equilibrium between PbI and Pb0. The logical steps by which such an equilibrium is possible can be conveniently discussed by a physical analogy.

Let us take a beaker of water B in which the volume of fluid V is kept constant. A sample of fluid, S, is removed each day and an equal amount of clear fluid added.

Case 1

Let us now add a constant amount X of a soluble salt, to the beaker each day. The first day some of the salt will be removed

with S. The precise amount removed will be $S\left(\frac{x}{v}\right)$ or that proportion of X in V that falls into S. It is obvious that after adding X once, only a fraction will have been removed. The level of the salt in the beaker will increase until the amount removed is equal to the amount of salt added. An equilibrium will be reached when

$$S\left(\frac{X}{V}\right) = x \qquad (1)$$

Case 2

Let us assume next that we disturb this equilibrium by removing a constant amount of X, dX, through some other channel without modifying V. Again it is obvious that the process will go to equilibrium at a time when

$$S\left(\frac{X(1-d)}{V}\right) = (1-d)x \qquad (2)$$

Case 3

Let us assume that only part of x is added to B and that the remainder is placed directly in S. Where $k_1 x$ is added to S and $k_2 x$ is added to B and where

$$k_1 x + k_2 x = x$$

so that

$$k_1 + k_2 = 1$$

equilibrium when

$$S\left(\frac{X}{V}\right) + k_1 x = x \qquad (3)$$

or when the amount of salt removed in the sample S equals to the amount added. Of course in the latter case

$$S\left(\frac{X}{V}\right) = k_2 x$$

Case 4

Let us assume that x is not a constant quantity but a random variable, taking on values between $x_{min.} \leq x_j \leq x_{max.}$. When the amount of x_j varies at random $S\left(\frac{X}{V}\right)$ will vary so that the amount of salt removed is S_j will fluctuate between $x_{min.} \leq S_j\left(\frac{X}{V}\right) \leq x_{max.}$. If the best unbiased estimate of $x_j = E(x) = \frac{\sum_1^n x_j}{n}$ is considered. It follows that over a duration of n samples

$$\frac{\sum_1^n S_j\left(\frac{X}{V}\right)}{n} = \frac{\sum_1^n x_j}{n} = E(x)$$

and that

$$\sigma^2_{s_j} = \sigma^2_{x_j}$$

or that the average output equals to average intake and that the Variance of output, $\sigma^2_{s_j}$, equals to the Variance of input, $\sigma^2_{x_j}$.

If the variable input of x is considered for Case 3 it is still true that

$$\frac{\sum_1^n \left(\frac{S_j X}{V} + k_1 x\right)}{n} = \frac{\sum_1^n x_j}{n}$$

but now

$$\sigma^2_{s_j} > \sigma^2_{x_j}$$

because of the additivity of Variances. A similar statement applies to Case 2.

Recovering Unknowns

Let us assume that one can measure only the amount of salt removed in the sample S, while V, and x are unknown.

From our discussion of Case 1 it is clear that if one permits the process to go to equilibrium one can then know X, the amount of salt added. If the amount of salt itself is added in variable quantities the average amount of salt removed during equilibrium emerges as the best estimate of $E(x)$, the average amount added. Cases 2 and 3 present difficulties in that two unknowns have to be evaluated from a single measurement. However, a solution here is possible if we change the level of equilibrium. For instance, in Case 2 if a known amount Δx is added to the beaker a new equilibrium will be reached when

$$S\,\frac{(X + \Delta X)(1-d)}{V} = (x + \Delta x)(1-d) \qquad (4)$$

in measuring the proportionate increase of salt in the sample the constant d and the original value of x can be computed.

In case 3 a simple comparison of two equilibria is not sufficient to recover the unknowns k_1 and k_2. A number of methods could be used here. One could, for instance compare the rate with which the new equilibrium is reached for a number of different increments of x, Δx_1, Δx_2, etc. It is obvious that the rate with which the salt in S reaches each new equilibrium is a function of Δx and of k_1 and k_2.

The discussion of the physical analogue points to the following conclusions:

1. A model constructed along its lines should show the same properties presently exhibited by the data.

2. The analogue can be co-ordinated almost point for point with a reasonable picture of how the exchange of inorganic lead takes place.

   a. The soluble salt in variable quantities is equivalent to the daily inorganic lead intake.

   b. The constant volume in the beakers is equivalent to the body fluid.

   c. The sample, S, is equivalent to the day by day excretion.

   d. Case 2 describes a deposition process that may possibly remove some of the lead from the body fluid and store it in tissue and bone.

   e. Case 3 describes a process that may be similar to the direct fecal excretion of a fraction of lead ingested daily.

   f. Case 4 allows for treatment of the data even under variable conditions of intake and output.

3. The obvious advantage of the model is that some important conclusions can be reached from knowledg of:

   a. lead removed by excretion
   b. lead intake
   c. additional lead added in known increments.

4. The major concern will be with the consequence of adding an increment of lead through the lungs. Both the increment and the possible amount of that increment that is deposited can be evaluated from the data by use of a model similar to the analogue.

We return now to the flow of lead intake and excretion of Figure 1. The pre-exposure period will be evaluated to begin with. During this period the subject's lead ingestion and excretion is measured but he is not yet exposed to an increment of lead in air.

Let $Pb0_o = f(PbI, PbV)$ where the function is linear

so that

$$PbU_j + PbF_j = k_1 PbI_{j-i} + k_2 PbV_j + a = Pb0_j \qquad (5)$$

where

$Pb0_j$ = total excretion of lead on the j th day.

PbF_j = fecal excretion of lead on the j th day

PbU_j = urinary excretion of lead on the j th day

PbI_{j-i} = ingestion of lead on the i th day before the j th day of measurement

PbV_j = amount of lead in body fluid on the j th day

a = constant. a, may consist of three parts.

1. d(PbV) = a fraction, d , of lead in body fluid that is deposited.
2. D = difference between unmeasured input and loss of lead.
3. e = error of measurement.

assuming that in the long run $\frac{\sum_1^n e}{n} = 0$, or that the error is not correlated to any of the other variables, then a = d PbV + D. [It is assumed that PbU, excretion of lead through urine, is simply a linear function of PbV and of urine volume VU.

$$PbU_j = c_1 PbV_j + c_2 VU_j + e \qquad (6)$$

where again,

$$\frac{\sum_1^n e}{n} = 0$$

The last assumption appears to be reasonable, During the experimental period both urinary lead (PbU) and blood level of lead

into the body, so that

$$L = L_b + L_s \qquad (7)$$

The amount of lead excreted after the first day is given by

$$Pb0_1 = k_1(PbI_{-i} + L_s) + k_2[PbV_o + (1-k_1)L_s + L_b] + a$$

The amount of lead excreted after the second day is given by

$$Pb0_2 = k_1(PbI_{-i} + L_s) + k_2[PbV_o + (1-k_1)(1-k_2)L_s + (1-k_1)L_s + (1-k_2)L_b + L_b] + a$$

if we let

$$b_1 = (1-k_1)$$
$$b_2 = (1-k_2)$$

$$Pb0_2 = k_1(PbI_{-i} + L_s) + k_2[PbV_o + (b_1L_s + L_b)(1+b_2)] + a$$

Note that PbV_o stands for the amount of lead in total body fluid at the onset of the new additional increment. After the nth day, the amount of lead excreted will be given by the general expression (8a)

$$(8a) \quad Pb0_n = k_1(PbI_{-1} + L_s) + k_2[PbV_o + (b_1L_s + L_b)(1 + b_2 + b_2^2 + \ldots + b_2^{n-1})] + a$$

but

$$\sum_1^n [1 + b_2 + b_2^2 + \ldots b_2^{n-1}] = \frac{1 - b_2^n}{1 - b_2}$$

and

$$\lim_{n \to \infty} \frac{1 - b_2^n}{1 - b_2} = \frac{1}{1 - b_2}$$

so that (8b)

$$Pb0_n = k_1(PbI_{-i} + L_s) + k_2\left[PbV_0 + \frac{b_1L_s + L_b}{1 - b_2}\right] + a \qquad (8b)$$

The increment in excreted lead is now given by the difference between the amount of lead excreted without the increment L, or $Pb0_o$, and the amount of lead excreted on the nth day, or Pbo_n, (9)

$$\Delta Pb0 = Pb0_n - Pb0_o \qquad (9)$$

$$= k_1 L_s + k_2 \left(\frac{b_1 L_s + L_b}{1 - b_2} \right)$$

but

$$1 - b_2 = k_2$$

so that

$$\Delta Pb0 = k_1 L_s + (1 - k_1) L_s + L_b = L_s + L_b = L$$

So that after n days, the increment of lead excreted each day is equal exactly to the additional increment of lead taken in each day.

If now conditions of Case 2 hold and some of the lead present in the body is stored, it follows that the increment of excreted lead is given by (10)

$$\Delta Pb0 = (1-d)L \qquad (10)$$

where d = constant of deposition.

To find the constant of deposition one simply takes

$$1 - \frac{\Delta Pb0}{L} = d \qquad (11a)$$

or

$$1 - \frac{Pb0_n - Pb0_o}{L} = d \qquad (11b)$$

where

$Pb0_n$ = the daily total lead excretion after equilibrium has been reached

$Pb0_o$ = the daily total lead excretion of the previous equilibrium, before the increment, L, was given daily.

L = the daily increment of lead

d = rate of deposition of L.

Use of the Deposition Theorem in a Practical Solution

Because of the variability of actually measuring intake and output of lead for human subjects, the theorem has to be modified to conform to conditions of Case 4. (12) and (13)

$$\Delta Pb0 = E(Pb0_n) - E(Pb0_o) \tag{12}$$

$$d = 1 - \frac{E(\Delta Pb0)}{E(L)} \tag{13}$$

and where

$$E(Pb0) = \frac{\sum_{1}^{m} Pb0_i}{m}$$

$$E(L) = \frac{\sum_{1}^{m} L_i}{m}$$

or the arithmetic average of daily intake or daily output, measured during periods of equilibrium. One could now obtain the necessary estimates to solve for the conditions of the lead chamber experiments by measuring excretion of lead previous to the experimental exposure and after the exposure period has gone on for a sufficient time span to permit a new equilibrium.

In practice such an attempt will not work, unfortunately. All subjects show a change in food intake during the experiment which, while possibly due to changes in daily regimen, makes it impossible to use the mean excretion during the pre-experimental period as an estimate of $E(Pb0_o)$. This difference is shown in Table 2. We could use another approach to get an estimate of $Pb0_o$. It is reasonable to assume that input and output balance. In fact, the theorem states explicitly that the amount of lead ingested and absorbed from other

sources and not measured in the experiment is counterbalanced by lead lost through other sources and not measured by the experiment, (such as sweat). One could therefore take the average intake measures during equilibrium periods for the best estimate of $Pb0_o$. The validity of this procedure is testified by figures in Table 1. In short, one can take the average amount of lead ingested daily during the experimental period (at equilibrium) to estimate the average amount that would have been excreted due to ingested lead. Table 3 gives the best estimated of $Pb0_o$, $Pb0_n$, as well as the best estimate of L, as measured directly from the amount of air respired by each subject and from the chamber concentration. The constant of deposition, d, can now be recovered by the following expression

$$E(d) = 1 - \frac{E(Pb0_n) - E(Pb0_o)}{E(L)}$$

The calculations and resulting proportion of storage are given in Table 4. (The values of L were obtained from measurements done by Mr. Shaeffer.)

Lawful relation between d and L

It is unlikely that d is a constant for any amount of lead absorbtion. A number of factors appear to speak against such an assumption. For instance, the lead and tissue may interact as a function of combined surface area or volume. Deposition of lead may increase the likelihood of additional deposition. Deposition of lead in some tissues may inhibit the elimination of lead from body fluid.

Variations in d values for different subjects became orderly when, at the suggestion of John Phair, the impact of L on d was considered. Table 5 shows that there appears to be an orderly and lawful relation between d and L. Increases in L are associated quite obviously with exponential increases in d. With one single

exception, the d values for all subjects fall on the equation (12).

$$d = e^{-e^{-5.17(L) + 1.4}} \qquad (12)$$

An independent check exists partially for the validity of this expression. When L equals zero, or before the individual starts his daily exposure in the chamber, the value of d, refering now simply normally to ingested lead, should equal to $e^{-4.1}$ or .02. This value is very close to the actual observed difference between daily intake and excretion of lead for those subjects for whom $PbI_o > Pb0_o$.

Additional Empirical Validation

Neither the reasonableness of the model nor the satisfactory computation of the deposition constants are by themselves completely sufficient to validate the constructs here developed. A very simple experiment could decide this issue satisfactorily.

This experiment will use 5 groups of animals, each group consisting of 4 to 5 subjects. A control group will be fed ad libitum, and the only measurements made will be of the lead consumed and excreted. The other 4 groups will be given, as a dietary supplement, specified increments of lead. Depositions constants, d, will be computed as was done here and verified by completely ashing the animals and measuring their total lead content. In each case, the differences between control and experimental animals should be an amount of lead equal to $d\sum_{1}^{n} L$ and the d's themselves should show an exponential association to the varying increments.

If this experiment has the predicted outcome, it will be desirable next to establish d as a function of body size, blood level of lead, and of other and related variables.

Since the model should apply to other inorganic substances as well, another, and perhaps more easily measurable agent could be used.

Table 1.

Observed average daily intake (PbI) and output (Pb0) of lead during the last 22 weeks of the control period preceeding exposure to lead in the chamber's air.

(In mg. of lead)

Subject	$E(PbI_o)$ for each day	$E(Pb0_o)$ for each day	Difference $E(PbI_o)-E(Pb0_o)$
Creech exp. 1	.329	.294	+.035
Creech exp. 2	.145	.141	+.004
Barber exp. 1	.240	.229	+.011
Barber exp. 2	.198	.241	-.043
Blackstone	.188	.203	-.015
Svetlik	.171	.169	+.002

Mean Difference = .001

Table 2

Observed average daily intake E (PbI), during the last 22 weeks of control and experimental periods.

(In mgs.)

Subject	$E(PbI_o)$ for each day	$E(PbI_n)$ for each day	Difference $E(PbI_o)-E(PbI_n)$
Creech exp. 1	.329	.206	+.113
Creech exp. 2	.145	.138	+.007
Barber exp. 1	.240	.182	+.058
Barber exp. 2	.198	.125	+.073
Blackstone	.188	.186	+.002
Svetlik	.171	.220	-.049
	Mean Difference =		+.034

Table 3

Observed values of daily food intake during the last 22 weeks of the experimental period. These values are taken as the best estimate of $E(Pb0_o)$. Observed values of average daily total excretion, $E(Pb0_n)$, and of inhalation of lead, $E(L)$ during the same period

Subject	$E(PbI_n) = E(Pb0_o)$	$E(Pb0_n)$	$E(L)$
Creech exp. 1	.2058	.3290	.1488
Creech exp. 2	.1384	.3205	.3392
Barber exp. 1	.1819	.2347	.0627
Barber exp. 2	.1253	.3006	.2402
Blackstone	.1862	.3336	.3552
Svetlik	.2201	.3932	.2706

Table 4

Computation for Δ (Pb0) and d, based on the figures given in Table 3.

$$\Delta (Pb0) = E(Pb0_n) - E(Pb0_o)$$

$$1 - d = \frac{\Delta (Pb0)}{E(L)}$$

Subject	$\frac{E(Pb0_n) - E(Pb0_o)}{E(L)}$	(1 - d) (proportion of L excreted each day.)	d (proportion of L deposited each day)
Creech exp. 1	$\frac{.3290 - .2058}{.1488}$	.8280	.1720
Creech exp. 2	$\frac{.3205 - .1384}{.3392}$	.5368	.4632
Barber exp. 1	$\frac{.2347 - .1819}{.0627}$	.8421	.1579
Barber exp. 2	$\frac{.3006 - .1253}{.2402}$	.7298	.2702
Blackstone	$\frac{.3336 - .1862}{.3552}$	.4150	.5850
Svetlik	$\frac{.3932 - .2201}{.2706}$	.6390	.3410

Table 5

Observed relation between the L and d of each subject

Subject	Rank	L	D
Blackstone	1	.3552	.5850
Creech exp. 2	2	.3392	.4632
Svetlik	3	.2706	.3410
Barber exp. 2	4	.2402	.2702
Creech exp. 1	5	.1488	.1720
Barber exp. 1	6	.0627	.1579

$$d = e^{-e^{-5.17(L) + 1.4}}$$

Abbreviations and Symbols:

PbI_i Ingested lead on the i th day, in mgs.

$Pb0_j$ Total excreted lead on the j th day, in mgs.

PbF_j Lead excreted in feces in the j th day, in mgs.

PbU_j Lead excreted in urine on the j th day, in mgs.

PbV_j Amount of lead in body fluid on the j th day, in mgs.

VU_j Volume of urine on the j th day, in gm.

L Constant increment of lead taken in through the atmosphere

L_s Part of L, reaching the body via the stomach

L_b Part of L reaching the body directly through the lungs.

$\Delta Pb0$ Difference between excretion solely due to PbI and excretion due to PbI + L

k_1 Proportion of PbI removed directly in feces

k_2 proportion of lead in body fluid that is removed through feces and urine

d Constant of deposition: Proportion of L that is deposited in tissues

$b_1 = (1 - k_1)$

$b_2 = (1 - k_2)$

Figure 1