Studies of Human Lead Metabolism by Use of Stable Isotope Tracers

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Dynamics of lead metabolism were studied by replacement of a portion of the dietary lead with stable isotope tracers, and maintaining subjects on controlled diets for about 6 months. Results for one subject have been previously reported. Preliminary data are now available for a second subject.

Although the data on the two subjects are basically similar, there are also significant differences. The two subjects have different blood lead concentrations (0.25 and 0.18 μg/g). Both subjects received the same dietary and similar atmospheric lead exposure, and the lead concentration of their blood was shown to be nearly in a steady state. The difference in blood lead concentration appears primarily attributable to differences in the fraction of lead absorbed from the gut, although there are also differences in other physiological parameters, as well as probable small differences in their intake of atmospheric lead.

The quantity of lead absorbed from a typical urban atmosphere (Pb concentration = 1–2 μg/m³) has been shown by our isotopic data and balance studies to be 15±3 μg/day. Measurement of the contribution of atmospheric lead to the lead intake of the second subject was also carried out by removal of lead from the atmosphere. Decline in the blood concentration of lead of normal isotopic composition was shown to be equivalent to the removal of 15 g/day.

Measurements made during the course of a day show complexities in the absorption and distribution of lead, which are averaged out on a time scale of ca. 5 days.

Introduction

In two earlier publications (*1*, *2*) we have described an experiment in which the kinetics of human lead metabolism were studied by using stable lead isotope (^{204}Pb and ^{207}Pb) tracers. In that work, a normal, healthy man (Subject A) ate a constant low lead diet (156 μg/day) in a metabolic unit. For 104 days this diet was supplemented with ^{204}Pb nitrate to restore his dietary intake to 361 μg/day, his prestudy intake, thereby maintaining a steady state. Subsequent removal of the ^{204}Pb and maintenance of the steady state by substitution of ^{207}Pb permitted measurement of endogenous fecal excretion of lead. Finally, the isotopic supplements were removed from the diet, in order to observe if homeostatic mechanisms tended to maintain a constant blood lead concentration despite the change in lead intake.

Mass spectrometric isotope dilution was used to determine the concentration and isotopic composition of lead in the diet, feces, blood, urine, facial hair, and the atmosphere, as well as in two samples of bile and gastric secretions, one sample of sweat, and a sample of iliac bone obtained by biopsy.

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The experimental data were analyzed by use of a three-compartment model. A schematic representation of this model is shown in Figure 1, together with the rates of transfer of lead between the compartments of the model as inferred from the data.

We have now nearly completed a similar study on a second male subject (Subject B, age 49 yr, weight 89 kg). Although complete data are not available for this subject as yet, some results have been obtained which will be modified only slightly by complete treatment of the data.

Steady-State Blood Lead Concentration

The two subjects had different blood lead concentrations, both before and during the study (0.25 and 0.18 $\mu g/g$, respectively). The concentration of blood lead is frequently used as an indicator of lead exposure, and attempts have been made to correlate this with occupation and other variables (*3*, *4*). It is therefore of interest to learn whether internal, physiological, variables also offect the total lead content of the blood or whether these differences can be essentially entirely related to exposure. In these experiments the external inputs of lead from the diet were maintained constant and the atmospheric exposures were nearly the same for both subjects. Furthermore, the length of the experiment was long compared to the half-life characteristic of the blood's response to changes in exposure (ca. 30 days, as determined by these experiments). It is conceivable that exposure to high levels of lead earlier in the life of the subject may have resulted in the deposition of large quantities of lead in long-lived sites, such as the skeleton, which now serve as internal sources of lead to the blood. Such a condition may influence blood lead concentrations in some individuals. However, balance studies carried out along with these experiments show that for these two subjects a simple imbalance in the input and output from long-lived compartments cannot be the sole explanation of the difference in blood lead concentrations. This is shown by the fact that the total imbalance between ingested and excreted lead for the second subject was within 2 μg of the directly measured atmospheric contribution. Indicating he had no significant unbalanced internal source of lead. Although the atmospheric lead contribution was not directly measured for the first subject, his imbalance between ingested and excreted lead was the same as that of the second subject. This proves that regardless of whether or not differences in exposures occurred, the combined effect of the atmospheric contribution and possible net release from unbalanced internal pools could not be greater for the first subject and therefore cannot account for his higher blood lead concentration. Consequently, the observed difference in the blood lead concentrations of the two subjects must be accompanied by physiological differences. In addition, calcium, phosphorus, magnesium, and nitrogen balances in both subjects were neutral or slightly positive, indicating that body protein and bone were in a steady state. This is consistent with the result that lead concentrations in the major body pools were nearly in equilibrium.

Even though data on the second subject are preliminary, they suggest that the difference in blood lead concentrations is primarily a consequence of difference in the absorption of lead from the gut. The ab-

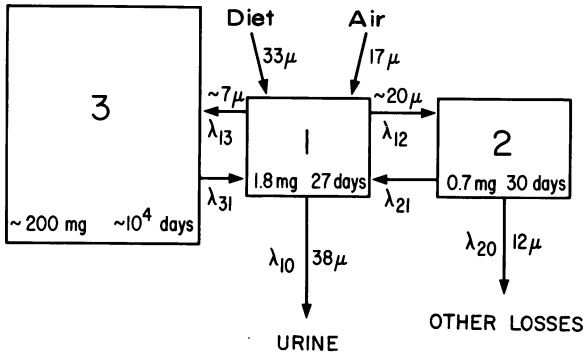

FIGURE 1. Three-compartment model for human lead metabolism. The mean life and quantity of lead in each pool, the flux (μg/day) and the exchange constants (λ) are shown for Subject A. Loss of lead from the body via compartment 2 (λ_{20}) is from integumentary structures (hair, nails, sweat) and alimentary tract secretions (bile and gastric juice).

sorption was determined from the difference between the quantity of lead in the diet and in the feces. This measurement is somewhat difficult for lead of normal isotopic composition, because the two quantities are of similar magnitude, and small amounts of lead contamination introduced in the handling of the sample can have an important effect on the result. In addition, a significant quantity of lead is excreted into the gut, primarily in bile and other gastric and intestinal secretions. Although in our experiments this endogenous fecal excretion was measured, the experimental error in this determination introduces a significant uncertainty in the measurement of the absorption of normal lead.

These difficulties are greatly reduced when the ^{204}Pb tracer, which comprised about half of the lead ingested by the subjects during the experiment, is measured. Because of its characteristic isotopic composition, totally unlike that of any naturally occurring lead, the problem of contamination during analysis is negligible. At the beginning of the experiment the endogenously excreted lead is entirely normal in isotopic composition and does not contribute to the fecal ^{204}Pb concentration. By the end of the experiment there is some contribution from this source. However this can be measured directly, following removal of ^{204}Pb from the diet, and appropriate corrections can be made. The measured absorption rates of ^{204}Pb were markedly different for the two subjects. For the first subject (Subject A) it was 8.3± 0.4%, whereas for the second subject (Subject B), data obtained near the beginning of the experiment indicate a value of 6.3%. Later in the experiment this value apparently decreased to about 5.5%. However, this is what would be expected from the endogenous excretion of ^{204}Pb, and it is anticipated that when data on this are available at the end of the experiment and corrections are made for this effect, the average value will be about 6.3%. Therefore there is a clear difference between the two subjects in their absorption of lead as nitrate, the form in which the ^{204}Pb was administered with the meals.

If one simply assumes that lead in food is absorbed to the same extent as that in lead nitrate, then these two subjects will absorb different quantities of lead from their diets. Since these experiments provide evidence that there is little physiological regulation of blood lead level in this concentration range, this difference in lead absorption will cause a proportional change in the blood lead concentration, other variables being equal. Whether or not food lead is absorbed to the same extent as lead nitrate is a valid question, the answer to which is not entirely clear. That there is no great difference is shown by the fact that no significant change in blood lead concentration was found when ^{204}Pb was substituted for lead from food at the beginning of the experiment on Subject A. However an unexplained but apparently random fluctuation in blood lead concentration, 5–10% in amplitude, precludes very precise conclusions from this observation. This variation could mask an absorption factor difference of about 20%.

Possible differences in absorption of food lead and lead nitrate cannot yet be assessed for Subject B because we intentionally "prestabilized" his blood lead concentration to a steady state by placing him on our low lead diet 31 days before the beginning of the experiment. At the same time, we supplemented this diet with lead nitrate of normal isotopic composition and reduced his lead intake from smoking cigarettes to a negligible level. This was considered desirable because his prestudy blood lead content resulted from the combination of a previous low-lead diet (~200 μg/day) for several months with the effect of his smoking more than 20 ordinary cigarettes per day. At the end of the experiment we plan to give him a diet providing 360 μg/day of lead from natural food. Comparison of the response of his blood lead concentration to this change with that caused by the addition of the ^{204}Pb and ^{207}Pb supplements will provide an additional measurement of his gastrointestinal absorption for the half of his dietary lead provided from natural food. Experiments

are also being carried out to measure the absorption factor of Subject B for isotopically labeled lead in different chemical forms.

Direct measurement of the absorption of natural food lead in Subject A, limited in accuracy by the difficulties described earlier, suggest that the absorption of this lead may be as high as 10%% for this subject. However, the uncertainties in this measurement do not exclude it being the same as the absorption factor of 8.3% for ^{204}Pb nitrate.

Differences in gastrointestinal absorption factors are not the only possible cause of differences in the blood lead concentration. This may be seen most easily by consideration of the steady state blood concentrations in a simplified version of model shown in Figure 1, in which compartments 1 and 2 are combined to form a single time-dependent compartment. This is a satisfactory approximation for description of the blood lead concentrations because if only the blood lead data are considered, parameters can be found which fit the data of the simplified model as well as other parameters fit the more general model. The more general model is introduced primarily to include the time dependence of lead in other fluids and tissues, i.e., bile and hair.

These "combined compartment" parameters which fit the blood data of the two subjects are given in Table 1. A steady state is reached when the external input from the diet and atmosphere equals the output:

$$A+B=C_bQk\lambda \qquad (1)$$

where A and B are the absorbed dietary and atmospheric contributions, respectively, C_b is the steady-state blood lead concentration, Q is the mass of the compartment, k is the ratio of the mean concentration in the compartment to that of the blood, and λ is the rate constant for loss of lead from the compartment. The steady-state blood lead concentration will be

$$C_b=\frac{(A+B)}{Qk\lambda} \qquad (2)$$

The difference in absorption for the two subjects causes the difference in the contributions of dietary lead shown in Table 1. However it should also be noted that there are other physiological differences in the two subjects as indicated by the differences in Qk and λ. The product of Qk and λ is nearly the same for the two subjects, causing the effect of these differences on C_b to be small. If this product of about 225 g/day is actually constant from one subject to another, this will prove significant in understanding the mechanisms of lead metabolism. However it would be premature to generalize on the basis of only two individuals.

Substitution of the parameters in Table 1 into eq. (2) yield steady-state blood concentrations about 10–15% lower than those observed. The probable reason for this is that eqs. (1) and (2) do not properly take into consideration the steady-state contribution of deeper compartments, such as the skeletion, when parameters are used which are based on data for an experiment of short duration compared to the time scale of these deeper compartments. The effect of these deeper compartments can be accounted for by adding 7 and 5 μg/day to the values of $(A+B)$ for Subjects A and B, respectively, which then brings the observed and calculated steady-state blood lead concentrations into agreement. These are reasonable values for the contribution from the deeper compartments based on all the data measured for these subjects. It is also

Table 1. Combined compartment parameters.

Subject	Blood lead, μg/g	Gut absorption, %	Absorbed diet lead (A), μg/day	Air lead (B), μg/day	Qk, kg	λ $days^{-1}$
A	0.25	9.0	33	15	7.7	0.029
B	0.18	6.3	23	15	11.5	0.020

possible that differences of a few micrograms per day in the contribution of atmospheric lead may be important in this regard.

The Contribution of Atmospheric Lead

In our earlier work, the contribution of atmospheric lead to the total daily lead intake of Subject A was measured in two ways: from the failure of the blood lead to become completely labeled during the course of the experiment, and from the lead balance data. These results are shown in the first two columns of Table 2. These values are in satisfactory agreement with those estimated from the product of the observed atmospheric concentration on respiratory absorption factors of lead from aerosols, ~40% based on earlier studies (*5,6*), and an estimate of the volume of air respired (20 m^3/day). This value is given in the third column of Table 2. Of course this estimate is very crude, as it does not take into account possible differences in the respired volume of the two subjects or the amount of time they spent in environments of somewhat different lead concentrations—their sleeping quarters, other rooms in the metabolic unit, and the grounds outside the building. In the first subject the calculation of the atmospheric contribution of lead depended on the assumption that long-lived internal sources of lead, principally the skeleton, were in a steady state. Although this assumption seems plausible, it was not demonstrated. This difficulty has been avoided in the case of the second subject by measuring the atmospheric contribution more directly. This was done by installation of an air-filtering system which removed lead aerosols from the atmosphere after day 109. In this way the measured concentration of atmospheric lead in the room was reduced to 0.07 $\mu g/m^3$. At the same time, the subject's diet was supplemented with ^{207}Pb in addition to the ^{204}Pb supplement received throughout the experiment. The quantity of ^{207}Pb (185 μg/day) was so chosen that the fraction of ^{207}Pb absorbed into the blood would approximate that removed from the atmosphere, thereby maintaining the blood lead nearly constant.

Because these experiments have shown that the blood lead responds proportionately to variations in intake of lead, this precaution was not actually necessary. In any case, the ^{207}Pb served as a useful calibration of the response of blood lead to changes in the input of lead.

The results of this experiment are shown in Figure 2. Prior to day 109, the total blood lead was essentially in a steady state. The component of the blood lead of normal isotopic composition was declining slightly with time because the ^{204}Pb concentration of the blood had not yet quite reached its steady-state value. On day 109 the atmospheric lead was removed by the filtering system, and the ^{207}Pb supplement initiated. The concentration of lead of normal isotopic composition in the blood immediately began to drop, while that of ^{207}Pb began to rise. The ^{207}Pb was continued for 15 days. Comparison of the measured rise in ^{207}Pb concentration with the decline in normal lead concentration, together with the known input of ^{207}Pb (11.5 μg/day) leads to the conclusion that the contribution of atmospheric lead to this subject was 15 ± 3 μg/day. This value is shown in the last column of Table 2. Again it is seen that this result is in

Table 2. Atmospheric lead input.

Subject	Atmospheric lead input, μg/day			
	By blood isotopic data	By lead balance	By calculated lung deposition	By response to filtered air
A	13	17	16	
B	<22[a]	17	16	15

[a] Actual value available upon completion of experiment.

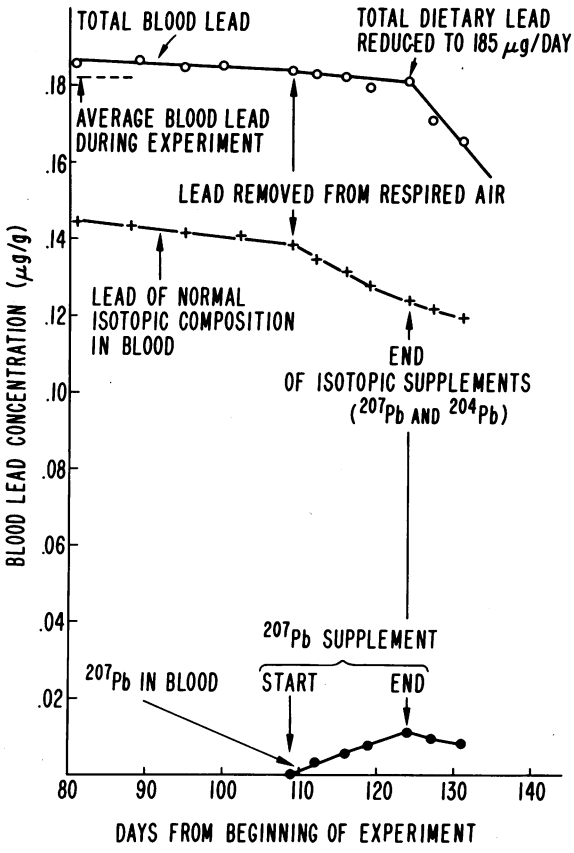

FIGURE 2. Measured concentrations of total lead, lead of normal isotopic composition, and ^{207}Pb in the blood of Subject B between day 80 and day 131 of the experiment. The removal of lead from the respired air on day 109 causes the concentration of normal lead to decrease following that date. The additional removal of the isotopic supplements on day 124 causes the total blood lead concentration to drop.

agreement with those obtained from the balance data and that estimated from the air lead concentrations. The subject has now been returned to an environment in which he is exposed to a more normal exposure to atmospheric lead. The anticipated increase in the concentration of normal lead in his blood will permit an additional measurement of the contribution of the atmospheric source. More extensive sampling of the air in his environment, making use of four air samplers deployed in the several areas which he occupies are being used accurately to document his atmospheric exposure. A final value for the quantity given in the first column must await completion of the experiment, and complete evaluation of such factors as exogeneous fecal excretion. However the data obtained so far allow an upper limit of 22 μg/day to be placed on the atmospheric contribution, and it is plausible that the final value will be in agreement with the other data.

After day 124, both the ^{204}Pb and ^{207}Pb supplements were discontinued, reducing the total lead intake to about 1/3 of that maintained throughout the experiment. The total lead concentration dropped abruptly, in agreement with the observation on the first subject that there appears to be no tendency for the blood lead concentration to be maintained at a constant value when the intake varies.

Therefore, the data for both subjects shows that ca. 15 μg/day is received by the body from the atmosphere, and this quantity is similar to, although somewhat less than, that obtained from the diet.

Short-Time Response of the Blood and the Urine

In the earlier treatment of our data, we have emphasized the changes in lead concentration which take place on time scales of weeks and months. On this time scale the blood lead concentrations increase and decrease smoothly, and the isotopic composition of the urine follows that of the blood very closely. These results, while correct, represent a simplification of what is actually occurring. The long time required for the concentration of lead in the blood to change is a consequence of the fact that a relatively large quantity (ca. 1 mg) of lead is in the red cells (*7*) as well as a similar quantity in tissues and fluids which exchange rapidly with the red cells (*1*). At a typical rate of about 50 μg/day, about 40 days are required to exchange this mass of lead.

On the other hand, the quantity of lead in the plasma is only about 100 μg. Replacement of this pool can occur much more rapidly as a consequence of the smaller size of the pool and its rapid rate of exchange with

much larger pools of lead. Therefore, the concentration of lead in plasma can respond to changes in input in minutes or hours, in contrast to the days or weeks required to effect a similar change in the concentration in the red cells or the whole blood. Because the mechanism for excretion of lead in the urine involves its separation from the plasma, rather than from whole blood, it may also be expected that the urine lead concentration will also be rapidly responsive to changes in lead input.

Previous human kinetic studies (*5*, *6*) in the normal range of blood lead concentrations involved use of radioactive ^{212}Pb (half-life = 10.6 hr). These studies emphasized lead kinetics in plasma and urine, because the radioactive isotope tracer decayed long before the red cells reached their steady state.

We are planning experiments with stable isotope tracers to study these short-term effects. The purpose of these experiments is to provide a link between our work and the earlier studies with ^{212}Pb and to extend the study of short-term effects into the intermediate time scale of a few days, i.e., the time required for ingested material to pass through the alimentary tract.

Table 3. Lead in blood fractions 6 hr after ingestion of ^{204}Pb tracer.

Fraction	^{204}Pb tracer, ng/g	Normal lead, μg/g
Plasma	0.032	0.040
Red cells (light 1/3, young)	0.49	0.35
Red cells (heavy 1/3, old)	0.43	0.38

Some of these experiments have now been carried out on Subject B. Table 3 shows the distribution of lead between red cells and plasma on the first day that the ^{204}Pb supplement was added. On this day, the total daily intake of ^{204}Pb was taken with breakfast as opposed to the routine procedure of ingesting a fraction of it with each meal. Blood was drawn for lead analysis 6 hr after ingestion. The concentration of lead of normal isotopic composition in red cells and plasma differed by a factor of about 10. A larger ratio is found for the ^{204}Pb. This suggests that ^{204}Pb can be rapidly transferred from the plasma into the red cells, but that there is a delay in its return to the plasma. In addition, the red cells were separated into three density fractions, following the procedure of Perry et al. (*8*). These workers showed that the density fractions contained cells of different age, the youngest being the least dense. If our repetition of their procedure for centrifuging the blood was successful in reproducing this separation, our data show that the lead is concentrated in both the young and old cells within 6 hr of ingestion. This is in marked contrast to the results found by Perry et al. for iron, which was concentrated only in the newly formed young cells. We are continuing this work, including verification that the separation into young and old cells has actually been achieved.

A subsequent similar experiment carried out on day 174 again showed no difference in lead isotopic composition between the light and heavy red cell fractions. In this case, hematological examination showed that reticulocytes were enriched by a factor of 4 in the light fraction. This experiment showed that the young cells, formed after ^{204}Pb had been removed from the diet, nevertheless incorporated an equilibrium concentration of ^{204}Pb.

The excretion of ^{204}Pb in the urine on the same day is shown in Figure 3. It is seen that a significant fraction of the lead is passed rapidly from the alimentary tract into plasma and then into urine. The rapid drop in the rate of excretion represents depletion of ^{204}Pb in the plasma on a similar time scale. During the initial 24-hr period only 0.3% of the ingested ^{204}Pb was excreted in the urine. Thus, the decay in the plasma concentration was not caused by urinary excretion, but by transfer of ^{204}Pb to other fluids and tissues, including the red cells.

These results are in general agreement with those found by previous workers using radioactive ^{212}Pb, although there may be significant differences. For example, the uri-

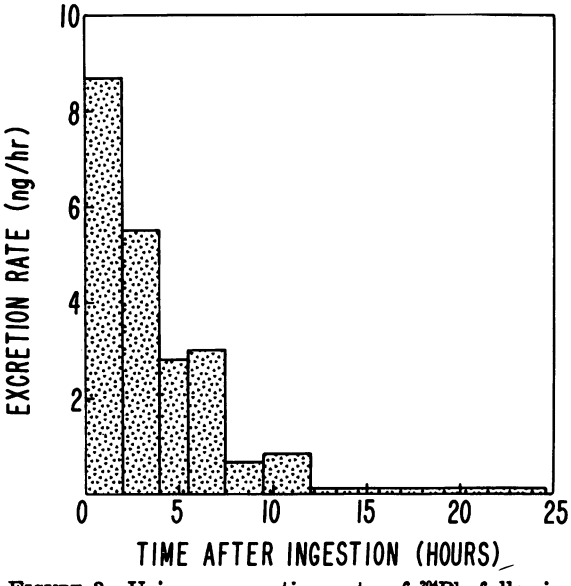

FIGURE 3. Urinary excretion rate of ^{204}Pb following the first ingestion of this isotopic tracer on day 1.

nary excretion during the first 24 hr is only 1/3 the lowest value found by Hursh and Suomela for ingested lead (*9*).

If we confine our considerations to our own data on Subject B there are also some unresolved problems. From the data in Tables 1 and 3 it may be calculated that after 6 hr, the quantity of ^{204}Pb in compartment Q was 2.5 μg. On the other hand, our absorption data show that 11.6 μg of the 185 μg of ^{204}Pb should be absorbed. It is possible that the remainder has not yet been absorbed, and the initial "pulse" of absorption occurring shortly after ingestion will be followed by further absorption from the alimentary tract. On the other hand, it is also possible that the lead has temporarily "disappeared" into some portion of Q external to the blood and will be returned to the blood sometime during the next day or two. If this is the case, a model suitable for analysis of lead kinetics on this short time scale will require that Q be divided not simply into plasma plus the remainder of the compartment. It will also be necessary to distinguish between the red cells and that portion of Q external to the blood.

Measurements in progress will extend these studies to include the full time required for the ingested lead to pass through the gut. In addition, compartmental modeling of these rapidly exchanging compartments will be used to facilitate evaluation of the short time scale variations of the lead concentrations obtained as the blood, plasma, and urine become progressively more labeled with ^{204}Pb during the course of the experiment.

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