The results of the two experiments demonstrate that treatment with dibutyryl cyclic AMP hastened the return of sensorimotor functioning in rats after nerve damage.

Others have reported effects of nerve growth factor on the regeneration of neurons. Scott and Liu (*7*) reported evidence for enhanced regenerative growth of afferent neurons in kittens after the administration of this material. Our report here is the first to show that a derivative of a cyclic AMP nucleotide may affect the return of function after peripheral nerve injury. It has been shown that there is a large increase in the concentration of cyclic AMP below the site of crushing of a sciatic nerve within 6 hours after surgery (*8*). Such an increase in concentration of cyclic AMP might be the mechanism for the normal regenerative process.

Our finding raises some interesting possibilities. Dibutyryl cyclic AMP or other nucleotides may have clinical utility in accelerating reinnervation by peripheral nerves of sensory and motor receptor sites. Recent reports (*9*) that nerve growth factor may mediate regenerative processes in the central nervous system open the possibility that cyclic AMP might affect as well the return of function after injury to central neurons.

MICHAEL PICHICHERO
Department of Pharmacology, School of Medicine and Dentistry, University of Rochester, Rochester, New York 14642

BERNARD BEER, DONALD E. CLODY
Squibb Institute for Medical Research, Princeton, New Jersey 08540

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2a. *Note added in proof*: Two recent reports have questioned the mediation of the in vitro effects of nerve growth factor by cyclic AMP [D. B. Hier, B. G. W. Arnason, M. Young, *Science* **183**, 79 (1973); W. A. Frazier, C. E. Ohlendorf, L. F. Boyd, L. Aloe, E. M. Johnson, J. A. Ferrendelli, R. A. Bradshaw, *Proc. Nat. Acad. Sci. U.S.A.* **70**, 2448 (1973)].
3. Lesions made by crushing and hemisection rather than transection afford better alignment for reinnervation. Crushing resulted in complete axonal separation, as demonstrated in nontreated rats by lack of muscular contraction after electrical stimulation of the nerve fibers above the lesion immediately after surgery. In histological studies, we observed clear Wallerian degeneration of the nerve distal to the site of either kind of lesion.
4. A 50 mg/kg dose of dibutyryl cyclic AMP was selected because it did not produce any behavioral effects when tested in rats in a general observational screen. Because the animals with hemisected nerves were expected to take longer to recover than those with crushed nerves, dibutyryl cyclic AMP (50 mg/kg) was injected twice daily. It is unlikely that the foot-flick results were due to any stimulating properties of dibutyryl cyclic AMP because dibutyryl cyclic AMP decreases motor activity and abdominal tone in rats when administered as a single dose (100 to 400 mg/kg) intraperitoneally.
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6. These animals treated with dibutyryl cyclic AMP developed foot infections after considerable self-mutilation. Two of these animals were killed. The death of the third animal was possibly due to the infection. All surviving animals appeared to be healthy during the prolonged daily administration of dibutyryl cyclic AMP.
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10. We thank I. Miller for technical assistance. Request reprints from B.B., Department of Pharmacology, Squibb Institute for Medical Research, P.O. Box 4000, Princeton, New Jersey 08540.

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Lead Metabolism in the Normal Human: Stable Isotope Studies

Abstract. *Kinetic and metabolic balance studies in a healthy man fed a diet normal in lead content and labeled with lead-204 indicated that approximately two-thirds of his assimilated lead was dietary in origin; the remainder was inhaled. Kinetic analysis shows that the isotopic data can be interpreted by a three-compartment model.*

There has been considerable discussion in the scientific and general literature concerning environmental contamination by lead. Evaluation of possible dangers from lead contamination has been impeded by the relative lack of data concerning normal human metabolism of lead during typical urban exposure, and the relative contributions to the total lead intake of various sources of environmental lead. Previous methods of studying lead metabolism in human subjects have involved either abnormally high exposures to lead (*1*) or the use of radioisotopes with half-lives that are short compared to the transfer times for lead within body tissues (*2*).

These limitations were overcome by using stable isotopes of lead as tracers. This permits a prolonged metabolic study without the introduction of long-lived radioisotopes into the body. Moreover, with modern methods of mass spectrometry (*3*) the concentration and isotopic composition of microgram quantities of lead can be accurately measured (standard error less than $\pm$ 1 percent). This report presents the use of this technique in one subject to assess (i) the kinetics of lead metabolism under steady-state conditions; (ii) the relative contributions of respired and dietary lead to the total lead intake; and (iii) possible homeostatic responses to an abrupt decrease in lead intake to subnormal quantities.

A healthy 53-year-old white male weighing 70 kg was fed a constant diet with a low lead content (156 μg/day), for 160 days while he lived in a metabolic unit and underwent metabolic studies by standard balance techniques (*4*). The diet provided 2500 kcal/day and abundant quantities of protein, vitamins, and the required minerals. During the first 104 days of the experiment, each meal was supplemented with lead-204 nitrate to increase the total dietary lead intake to 367 μg/day (the subject's approximate intake before the study) so that a metabolic steady-state condition was maintained. Then lead-204 nitrate was replaced with lead-207 nitrate for 10 days. For the next 46 days, the subject received only the diet low in lead.

The concentration and isotopic composition of lead were determined serially in the diet, feces, blood, urine, facial hair, and atmosphere by mass spectrometric isotope dilution analysis. During the course of study, two samples of bile and gastric secretions, one sample of sweat, and a skeletal biopsy containing both cortices of the ilium, from a point 2 cm below the iliac crest, were analyzed for the concentrations of the four stable isotopes of lead.

The kinetics and distribution of lead in the body were analyzed in terms of a three-compartment model (Fig. 1). Compartment 1 can be considered to be primarily blood [principally erythrocytes (*5*)] and possibly some soft tissues which exchange rapidly with blood. This compartment receives isotopically labeled lead from the gut and unlabeled lead from the atmosphere and exchanges lead with compartments 2 and 3. Lead is excreted from compartment 1 into the urine. Compartment 2 includes primarily soft tissue and possibly the more actively exchanging parts of the skeleton. Compartment 3 includes the skeleton, which contains most of the lead in the body (*6*), and the remainder of the soft tissue. Because of the

large quantity of lead in compartment 3, the quantity of labeled lead from this pool which moved back into the blood during the course of the experiment was negligible; hence, compartment 3 can be considered as a time-independent source of normal lead and as a sink of labeled lead coupled to compartment 1.

The differential equations for the change in concentration of ^{204}Pb in the two time-dependent compartments are

$$\frac{dq_1}{dt} = \frac{A}{Q_1} - \lambda_1 q_1 + \lambda_{21}\frac{Q_2}{Q_1} q_2 \quad (1)$$

$$\frac{dq_2}{dt} = \frac{Q_1}{Q_2}\lambda_{12} q_1 - \lambda_2 q_2 \quad (2)$$

where q_i is the concentration of ^{204}Pb in compartment i; λ_{ij} is the exchange constant between compartments i and j; Q_i is the mass of compartment i; λ_i is the sum over j of λ_{ij}; and A is the quantity of ^{204}Pb absorbed daily from the gut.

Although Eqs. 1 and 2 have the form of first-order kinetic equations, their use does not involve this assumption. Rather, it is only assumed that the isotopic composition of the efflux of a pool and its content are identical. Analysis of the data in terms of the rare isotope ^{204}Pb avoids problems of contamination of samples with normal lead.

General solutions of Eqs. 1 and 2 have been derived, giving q as a function of time in terms of λ, Q, and A. The measured concentration of ^{204}Pb in the blood (Fig. 2) can be used to evaluate some of the parameters appearing in these equations and their solutions, the pool sizes, and mean lives $(1/\lambda)$.

It is found that 8.3 ± 0.4 percent of the ingested ^{204}Pb is absorbed into the blood, where it has a mean life $(1/\lambda_1)$ of 27 days. Fifty-four percent of this lead is lost from blood through the urine, which has an isotopic composition identical with whole blood; the remainder is transferred to compartments 2 and 3. The initial portions of the curves describing the growth of ^{204}Pb and ^{207}Pb and the loss of ^{204}Pb from blood fit a simple expression [1 − exp $(-\lambda t)$] which corresponds to a one-pool model. However, a complete fit of blood and lead output data requires that lead be transferred to and return from another compartment of similar half-life which contains less lead.

With the exception of blood, most tissues and fluids cannot be assigned exactly to a particular compartment. If one assumes that the lead in hair, sweat, nails, and alimentary tract secretions is from compartment 2, then 37 percent of the daily efflux (approximately 12 μg/day) from this compartment leaves the body by this route. The rest of the efflux goes to compartment 1. The measured quantity of ^{204}Pb in the cortex and trabeculae of the iliac bone suggests that this part of the skeleton receives lead at about three times the average rate for compartment 3, as determined by the losses from the blood compartment.

To determine absorption of dietary lead, it was necessary to assess endogenous fecal lead excretion. The latter was estimated from the degree to which lead was labeled in bile, gastric secretion, and feces after removal of ^{204}Pb from the diet. It was found that 8 μg/day were secreted from the alimentary tract into the feces. Moreover, dietary lead exceeded total fecal lead by 25 μg/day. These observations indicate that 33 μg/day of ingested lead, or 9.4 percent of the total lead intake, was absorbed in the gut.

It is noteworthy that blood lead (Fig. 2) did not become entirely labeled with dietary lead even though the duration of the study with ^{204}Pb exceeded several mean lives of the blood compartment. This suggests input into the blood from other nonlabeled sources of lead—the skeleton and the atmosphere. Since the blood was 51 percent labeled with dietary lead on day 104, a strict upper limit of 49 percent can be set on the fraction of the daily lead intake which was derived from an unlabeled source. Moreover, projecting the growth for labeled blood lead to a time when a steady state is achieved in both labile compartments 1 and 2, a value of 37.5 percent or 20 μg/day is obtained. Analysis of the flux of lead out of the blood indicates that approximately 7 μg/day of lead enters the skeleton. Since medical examination revealed no evidence of bone loss and since calcium and phosphorus balances were not negative, it may be inferred that the skeletal mass was in a steady state. If blood and skeletal lead were also in a steady state, it can be concluded that bone yields approximately 7 μg/day of unlabeled lead to the blood. Thus, 13 μg/day or 28 percent of the total lead

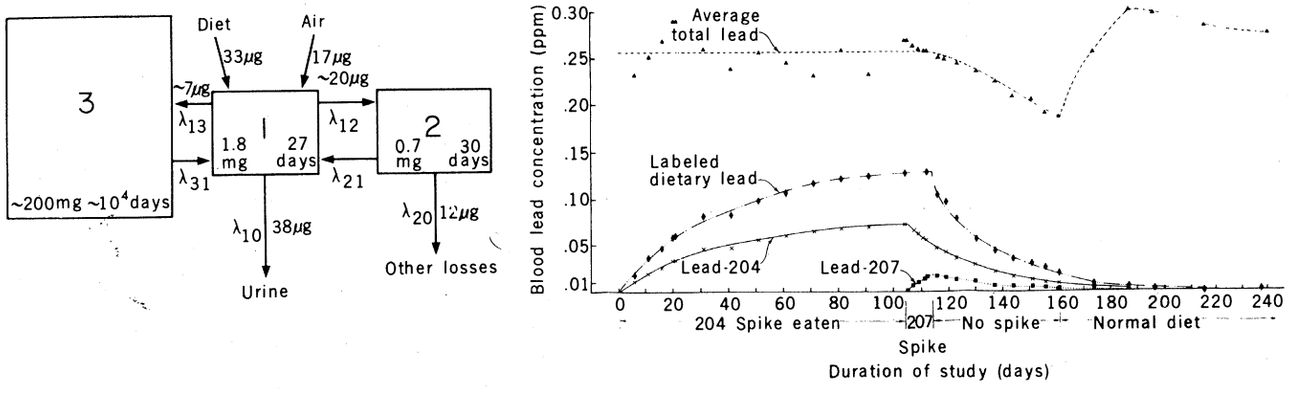

Fig. 1 (left). A three-compartment model for human lead metabolism derived from analysis of tracer and balance data in a healthy man. The lead content and mean life of each pool, the rates of flux (micrograms per day), and the exchange constants (λ) are shown. Loss of lead from the body via compartment 2 (λ_{20}) is from integumentary structures (hair, nails, and sweat) and alimentary tract secretions (bile and gastric juice). Fig. 2 (right). Blood lead concentrations in a man consecutively fed a low-lead diet supplemented with ^{204}Pb, a low-lead diet supplemented with ^{207}Pb, a low-lead diet with no lead supplement, and a normal diet. The concentrations in the blood of total lead (triangles), ^{204}Pb (crossed lines), and ^{207}Pb (squares) are shown. In addition, the concentration in the blood of lead with an isotopic composition identical to that present in the low-lead diet supplemented with ^{204}Pb is shown (diamonds). Not all of the blood lead would become labeled by a dietary input because of sources of unlabeled lead, such as the atmosphere and bone.

assimilated was derived from the atmosphere.

The results of the balance data agree with these findings. The net absorption of lead was 25 μg/day. Mean urinary excretion of lead was 38 μg/day and loss of lead from hair, nails, and sweat was approximately 4 μg/day. Thus, total lead output exceeded dietary intake by 17 μg/day, which presumably came from the atmosphere. This value is in general agreement with that derived from the analysis of the isotopic blood data. This is also consistent with a value of about 16 μg/day calculated from the measured indoor concentrations of aerosol lead (about 2 $\mu g/m^3$) and reported values for absorption rates of inhaled lead (*7*). The subject smoked eight cigarettes per day of a brand which contained 0.9 μg per cigarette, about one-fourth the amount in the average American brand. From previous reports (*8*) we estimate that this subject absorbed about 1 μg/day from tobacco; the remainder of the absorbed aerosol component is presumed to come from the exhaust of leaded gasoline (*9*).

When the subject ingested the low-lead diet without supplemental lead, the absorption rate of dietary lead did not change. The lead excreted daily in the urine and the blood lead concentration each decreased in proportion to the change in the total amount of lead assimilated (Fig. 2), and the ratio (λ_{10}) of lead excreted daily in the urine to blood lead remained constant. When the subject was fed an uncontrolled hospital diet and smoked cigarettes at will (days 160 to 220), the lead concentration in the blood, urine, and feces increased to approximately the values before the study (Fig. 2). Thus, over this small range in exposure, no homeostatic mechanisms for maintaining lead concentrations in the blood were seen.

MICHAEL B. RABINOWITZ
GEORGE W. WETHERILL
Institute of Geophysics and
Department of Planetary and Space Science, University of California, Los Angeles 90024

JOEL D. KOPPLE
Medical and Research Services, Veterans Administration, Wadsworth Hospital Center, and *Department of Medicine, School of Medicine, University of California, Los Angeles*

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Temperature Sensitive Programming of the Silkmoth Flight Clock: A Mechanism for Adapting to the Seasons

Abstract. *When males of the silkmoth* Antheraea pernyi *were exposed to 12°C during adult development and then tested for flight activity at 25°C, the time of the onset of the activity was advanced to the early part of the night. This change in the time of activity was stable and persisted through the life of the moth. Females showed a corresponding shift in the time of sex pheromone release when they were treated in the same fashion.*

The behavior of the wild silkmoths is a striking example of temporal coordination between the sexes (*1*). These insects have developed an elaborate system of chemical communication which brings the partners together from distances of over a mile. During a specific portion of the day or of the night the female assumes a "calling" posture and releases pheromone. This behavior coincides with the time of male flight activity (*2*), and the coincidence of female pheromone release with male flight activity increases the probability that a male will encounter the odor plume from a female and that attraction and copulation will occur. We here report that the time of flight activity is not rigidly programmed, but it varies in a way that is related to the temperature of the environment during the development of the moth. This temperature sensitivity presumably allows the species to adjust its activity to anticipate seasonal fluctuations in daily temperature patterns.

Chilled pupae of the oak silkmoth, *Antheraea pernyi*, were exposed to a photoperiod consisting of 16 hours light and 8 hours dark (LD 16 : 8) at 25°C to terminate diapause. When adult development began 3 to 4 days later, groups of animals were exposed for various times to a LD 16 : 8 cycle at 12°C. The remainder of development occurred at 25°C. Shortly before emergence the moths were placed in activity-monitoring devices (*3*) with a photoperiod of LD 16 : 8 at 25°C.

The environmental temperature during the development of adult moths had a marked effect on the subsequent time of flight of male moths. Male *A. pernyi* exposed to temperatures of 25°C throughout development had a single peak of intense flight activity (Fig. 1). Flight began an average of 5.3 hours after lights off and ended abruptly with lights on. Males that were exposed to temperatures of 12°C during development and which were subsequently tested at 25°C began flying 1.3 hours after lights off. Their activity was very dispersed and there was intermittent flight through most of the night. Flight often ceased prior to lights on. This shift in the onset of activity was relatively stable: through the 7- to 10-day life-span of the adult there was only a slight drift in the time of flight initiation.

In an attempt to define a stage in development which was important in the determination of the timing of flight activity, the 20 days of adult development were arbitrarily divided into three equal portions (*4*) and groups of *A. pernyi* were exposed to all combinations of temperatures of 25°C and 12°C during these three time periods. No single developmental period was solely responsible for the resetting of the clock (Table 1). None of these treatments resulted in as prominent a shift in the time of the initiation of flight activity as that observed when the moths were exposed to temperatures of 12°C throughout development.

When the moths were exposed to the lower temperature during two of the developmental periods, the responses were more pronounced. Exposure to 12°C during the last two-thirds of development was almost as effective as exposure to 12°C throughout development. The temperature experienced through day 7 of development was