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Elevation of Blood Lead Concentration by Confinement in the Rhesus Monkey

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In the course of behavioral studies in this laboratory of chronic, low-level lead (Pb) intoxication in rhesus monkeys, fluctuations over time in the concentration of Pb in the blood (PbB) were observed. These fluctuations were not explicable on the basis of well-known influences on Pb metabolism (e.g., age, diet, or length of Pb exposure), but seemed to correlate with changes in behavioral testing routines, particularly those associated with signs of distress in the experimental animals. Since no data in the literature could be found relating effects of psychological stress on Pb metabolism, we decided to examine the PbB of monkeys with a known history of Pb exposure under conditions known to be stressful. The magnitude and time course of this stress response was assessed by following the serum cortisol concentration, the principal component of the adrenal steroid secretions associated with psychological stress in monkeys (MASON 1968).

Confinement in an unfamiliar chamber was adopted as the stressor since previous studies in monkeys in this laboratory had shown it to be an effective means of inducing a stress response (SHELTON and DAVENPORT unpublished observations). In other studies, prolonged isolation of a single chimpanzee had been shown to induce a prolonged psychoendocrine response which did not habituate over a 30-day period (SABBOT et al. 1972). This animal showed ionic imbalances similar to those seen in hyperparathyroidism (e.g., hyperphosphaturia and hypocalciuria) in addition to chronically elevated excretion of corticosteroids. The more severe responses to physical restraint (PYKE et al. 1968) and to continuous 72-hour avoidance of electric shock (MASON 1968) were avoided in an attempt to produce levels of stress not dissimilar from those previously observed in the ongoing behavioral studies.

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METHODS

Subjects. Sixteen rhesus monkeys (Macaca mulatta), 28-32 months of age, were used. Ten (5 male, 5 female) had been exposed experimentally to Pb acetate during the first year of life (BUSHNELL et al. ms. under editorial review) and six (4 male, 2 female) served as untreated controls. All animals were housed in individual stainless steel mesh cages, 40 X 54 X 54 cm (width/depth/height) and were maintained on 120-140 gm laboratory chow[1] offered daily at 0700 hours with water available ad libitum.

Apparatus. Six identical sound-attenuating chambers, 46 X 65 X 64 cm (w/d/h), containing a response lever, liquid reinforcement dispensing tube, and stimulus lights used for operant conditioning studies (DAVENPORT et al. 1972), were used as confinement chambers.

Procedures. Lead dosing. The procedures followed for the ten lead-treated animals have been described in detail (BUSHNELL et al. ms. under editorial review); briefly, the treatments were as follows. A chronic-only group (2 male, 2 female) was dosed with Pb acetate ($Pb(CH_3COO)_2 \cdot 3H_2O$) in water via nasogastric intubation at 10 mg Pb/kg body weight on day 8 or 9 postpartum, and with 1.0 mg/kg/day for the next five days. Beginning on day 14 or 15, Pb was administered daily at 0800 hours, in 100 ml of a milk formula[2] at a dose of about 0.70 mg/kg/day, with dose adjustments made to maintain PbB values in a range of 80±10 ug Pb/dl whole blood for the first year of life. A pulse-only group (1 male, 1 female) received no Pb until day 29 postpartum, at which time 10 mg Pb/kg/day in water was intubated to each animal for three consecutive days, followed by 1-3 mg/kg/day for 12 days. This treatment resulted in a brief pulse in the PbB with a peak level of about 300 ug/dl obtained during week 7 of life. Finally, a pulse-chronic group (2 male, 2 female) received Pb acetate in water at 10 mg/kg via nasogastric intubation on day 8 or 9 postpartum, followed by 0.5-1.0 mg/kg/day for five days, 10 mg/kg on day 29, and 1.25-3.25 mg/kg/day for the next 12 days. Chronic exposure to Pb in milk commenced on

[1]Purina Monkey Chow, 12% protein, Ralston Purina Co. St. Louis, Mo.

[2]Similac with Iron, Ross Laboratories, Columbus, Oh.
[3]Tang, General Foods, White Plains, New York.

day 14 or 15 postpartum as for the chronic-only group, above. These treatments resulted in elevation of the PbB to about 80 ug/dl within the first month, followed by a PbB pulse with a peak level of about 300 ug/dl during week 7 postpartum, and a chronically-elevated PbB of about 80 ug/dl for the remainder of the first year of life.

Confinement. At the start of the present experiment, the 16 monkeys were divided randomly into three squads of 5, 5, and 6 animals, with the restriction that each treatment condition was approximately balanced within squads. Squads were run successively through the confinement procedure as follows. Each animal was placed in a test chamber at 0700 hours Monday with its daily ration of food and 500 cc tap water, and left undisturbed for five days, except for the following interruptions. At 0900 hours each day except day 4, each animal was removed briefly for blood drawing. Chambers were cleaned at 0700 and 1530 hours each day, at which time each animal was fed (0700 hours) and watered (0700 and 1530 hours) but not removed from the apparatus. Food and water intake, and fecal production were recorded during the cage cleaning operations. Between 1100 and 1500 hours each day, house lights were illuminated and the conditioning apparatus was activated, permitting each animal to work for liquid reinforcement[3] on an FR1 schedule. The chambers were otherwise dark.

Blood drawing. Blood samples were obtained from each animal 6 days prior to confinement, on days 1, 2, 3, and 5 of confinement, and 3 days after the end of confinement. Beginning at 0900 hours the animals in each squad were removed from the home cage or the confinement chamber in a random order and restrained. A first blood sample of 6 cc was drawn via femoral venipuncture into an evacuated collection tube; a second sample of 2 cc was then drawn through the same needle into an evacuated tube containing 40 ul EDTA to prevent clotting. "Sampling time" was recorded for each animal as the time interval between opening its cage and withdrawal of the needle from its vein. The first sample was centrifuged for 10 minutes within 30 minutes after drawing, and serum was drawn off and stored at -20 °C until assayed for cortisol concentration. The second sample was stored at 5 °C as whole blood until assayed for lead concentration.

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Chemical determinations. Cortisol concentrations were assayed by a modified competitive protein-binding procedure (BOWMAN and DELUNA 1968) which used outdated human serum instead of rabbit serum as the protein source, 1,2-di^{3}H corticosterone instead of 1,2-di-^{3}H cortisol as the radioisotope, and 40 mg florisil instead of 15 mg Fuller's earth as the adsorbent. Blood lead concentrations (ug Pb/dl whole blood) were determined using a modification of the Delves Cup technique (EDIGER and COLEMAN 1972) on a Perkin-Elmer Model 306 Atomic Absorption Spectrophotometer.

RESULTS

Food and water intake, fecal production, and sampling times did not differ significantly among treatment groups at any time during the study. Sampling times averaged 1.73 minutes (range, 0.80-7.47 minutes) and were observed to be unrelated to any other measure taken in the experiment. Food and water intakes remained relatively constant in a normal range across the confinement period.

Mean serum cortisol concentrations for each group rose 60-90% above baseline after 2 hours of confinement (Fig. 1), and declined subsequently

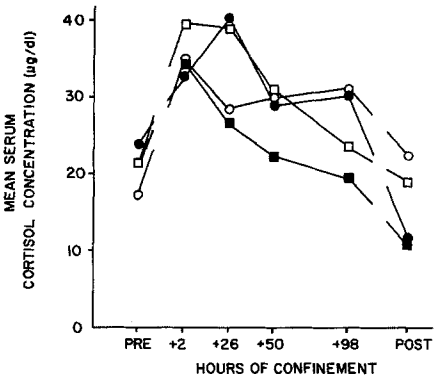

Fig. 1. Effect of confinement on serum cortisol concentration in the four groups of monkeys. Symbols: o, control; □, chronic-only; ●, pulse-only; ■, pulse-chronic.

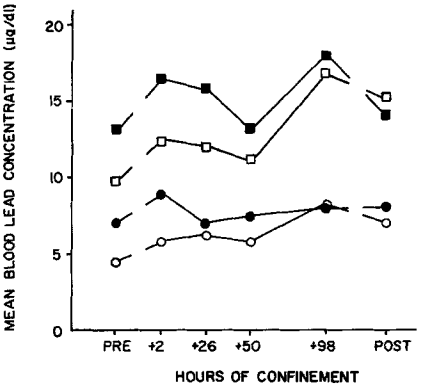

Fig. 2. Effect of confinement on blood lead concentration in the four groups of monkeys. Symbols as in Fig. 1 .

across the week of the experiment. This change was highly significant by analysis of variance (MYERS 1971) ($F(5,60) = 12.49$, $P < .001$). However, lead treatment did not differentially affect cortisol concentrations, since neither the main effect of lead treatment nor its interaction with time periods was significant (both P values $> .20$). Across all animals, cortisol concentrations were elevated significantly above baseline after 2, 26, and 50 hours of confinement, while the levels observed after 98 hours and from the post-confinement sample did not differ from baseline (Fisher's LSD).

Overall, PbB levels differed among groups ($F(3,12) = 17.94$, $P < .001$), with mean PbB values during the experiment reflecting uncleared lead acquired from previous exposure during the first year of life (Fig. 2). Mean PbB values rose in all four groups during confinement ($F(5,15) = 6.70$, $P < .005$) in a manner not differentially affected by prior lead history (interaction $F = 0.90$). Contrasted to baseline, PbB values obtained after 2 and 98 hours of confinement, and that of the post-confinement sample, were significantly elevated across the 16 animals, while those obtained after 26 and 50 hours of confinement were not (Fisher's LSD).

DISCUSSION

The effectiveness of the confinement procedure in inducing a stress response in these monkeys was clear from the elevation of serum cortisol concentrations observed (Fig. 1). The time course of this response was not dissimilar to that obtained in rhesus monkeys under conditions of continuous operant shock avoidance (MASON 1968), suggesting a qualitative similarity in the response of this species to the two stressors. However, the monkeys in the present study continued to eat and drink and were not adversely affected by the confinement in a clinical sense.

Since the monkeys' diets were not changed during the experiment, the Pb added to the blood during the confinement period must have been derived from body stores. Since treatment with chelating agents mobilizes Pb preferentially from bone (HAMMOND 1971), and since the bones contain more than 90% of the Pb body burden (BARRY and MOSSMAN 1970, GROSS et al. 1975), it is likely that the source of the Pb introduced into the blood in the present study was skeletal. Two peaks in the curves relating PbB to time

were obtained (Fig. 2), one 2 hours after confinement, the other at the end of the week. This pattern suggests (1) the involvement of more than one physiological process in the mobilization of stored Pb, and/or (2) the existence of more than one pool of stored Pb with different accessibilities to mobilization during stress.

The physiological processes involved in Pb mobilization were probably hormonal, and may reflect mobilization of Pb along with Ca from the bone matrix by parathyroid hormone (PTH), or an indirect effect of adrenal steroids. The very rapid response in PbB (25-35% increase within 2 hours after confinement) suggests the action of PTH, which is known to exert immediate effects on bone Ca (JANDE 1972). The later elevation in PbB may possibly have resulted from interference with bone mineral accretion by adrenal steroids (COLLINS et al. 1962), or by enhancing bone catabolism (STOREY 1963, EISENBERG 1964), or both. However, no correlation between various measures of adrenal response and indices of mobilization of Pb could be demonstrated, suggesting no direct action of cortisol on Pb mobilization under these conditions. However, glucocorticoids have been shown to inhibit Ca absorption, both when administered orally (HARRISON and HARRISON 1960) and parenterally (KIMBERG et al. 1971). If this inhibition resulted in a negative Ca balance during confinement, as commonly results from the hypersecretion of adrenal steroids in Cushing's syndrome (VAUGHAN 1970), bone resorption and consequent mobilization of stored Pb may have occurred as a means of maintaining serum Ca concentrations in the face of net Ca loss.

When expressed as a percent increase from baseline, the PbB data of Fig. 2 suggested that the Pb burdens of the different groups were not equally accessible to mobilization by the response to confinement. The magnitude of elevation from baseline at 98 hours after confinement was greatest for the control group (100% increase) and least for the pulse-only group (14% increase), with the chronic-only and pulse-chronic groups showing elevations of 80% and 50%, respectively. While not statistically different, the ordering of these peak percent increases in PbB reflect accurately the length of time (0 months for the control group, 16-18 months for the chronic-only and pulse-chronic groups, and 26-28 months for the pulse-only group) between each group's acquisition of the bulk of its Pb burden and the point at which the animals were subjected to the confinement procedure. A similar relationship between

the fraction of skeletal Pb excreted in response to chelation and the time interval between a single Pb dose and a subsequent chelator dose has been described for rats and rabbits (HAMMOND 1971).

The present data suggest that studies of Pb metabolism should consider possible effects of stressing experimental animals on the PbB or other measures of body Pb. In addition, clinical examination and screening program personnel may wish to consider the psychological atmosphere under which the blood sampling is performed, to minimize any effects of anxiety or apprehension which may elevate the PbB above its resting level.

SUMMARY

Rhesus monkeys were exposed to lead (Pb) acetate under various regimens during the first 12 months of life. At 30 months of age, these animals and unexposed controls were confined to an unfamiliar experimental chamber for one week. Serum cortisol concentration and Pb concentration in whole blood (PbB) were measured prior to, during, and after this confinement. Cortisol concentrations rose 60-90% within 2 hours of confinement, and declined to baseline levels after 98 hours of confinement. Mean baseline PbB levels reflected the state of clearance of the previously-ingested lead, rose 25-35% within 2 hours of confinement, and reached mean maximum levels as much as 100% above baseline after 98 hours of confinement. The data are discussed in terms of hormonal mobilization of Pb stored in bone, and subgest (1) that this storage is multicompartmental, (2) that more than one process is probably involved in its mobilization, (3) that cortisol probably does not directly affect PbB levels, and (4) that stress should be considered a potential factor in determining the PbB in studies of Pb metabolism.

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