Lead Pica Produced in Rats

Abstract. *Weanling rats eating a low calcium diet voluntarily ingested lead acetate solutions in much greater proportions than did iron-deficient or control weanlings. This increased ingestion occurred even with high concentrations of lead acetate which normal weanlings found extremely aversive. Chronic injections of lead acetate into weanlings did not change lead ingestion, indicating an absence of behavioral regulation of body lead levels. Female lead-injected weanlings did show a significant increase in calcium ingestion. Calcium deficiency may be one component of lead pica.*

Lead poisoning resulting from pica has been estimated to affect as many as 225,000 young children each year (*1*), leading to severe and irreversible retardation (*2–4*) and clear evidence of brain damage (*5*). Why children should voluntarily ingest lead is not clearly understood. The most common explanation is that the failure of the mother to provide proper supervision of the child or the proper emotional support of the child (*6*) coupled with a young child's predilection for mouthing and ingesting nonfood substances (*7*) leads to the maladapted behavior.

An alternative to the inadequate maternal care notion is that the pica might have a nutritional basis. Animal picas have frequently been determined to have a nutritional basis (*8*). There are two leading nutritional candidates for lead pica. Iron-deficiency anemia is commonly found in conjunction with lead poisoning and was suggested as a possible predisposing factor to lead pica (*9*). However, Gutelius *et al.* (*10*) failed to find evidence of iron deficiency in the diets of children with pica and failed to demonstrate a subsequent reduction of pica with iron injections (*11*).

The other nutritional candidate is calcium. There have been several indications of a metabolic interaction between lead and calcium. The addition of calcium lactate to the diet of lead-injected rats reduced their body lead levels (*12*). Rats placed on a low calcium diet showed an increased toxicity to lead exposure manifested by increased body lead in blood, soft tissues, and bone, and increased urinary levels of δ-aminolevulinic acid. Kidney damage was also found, yet the lead levels used did not produce damage in rats with normal dietary calcium levels (*13*).

The present experiments examine the possible nutritional basis of lead pica and the specific role of calcium. Three questions were asked: (i) Do normal weanling rats demonstrate any preferences for lead solutions? (ii) Does calcium deficiency or iron deficiency produce an increased lead ingestion? (iii) Do animals detect the presence of lead in their bodies and regulate their lead levels through their ingestive behavior either by reducing voluntary lead ingestion or increasing calcium ingestion?

Ninety-seven Sprague-Dawley male and female weanling rats were studied in a two-bottle preference situation. Five solutions were presented, with distilled water as the alternative, in a Latin square design that was repeated so that solutions appeared once on each side to control for possible side preferences. The test solutions were distilled water, 0.08, 0.16, 0.32, and 0.64 percent (wt/vol) lead acetate solutions. Since lead acetate began to precipitate as insoluble lead hydroxide at the highest concentrations, 5 percent acetic acid (3 ml per liter) was added as a buffer to all solutions, including distilled water.

All animals were received at weaning and adapted for 25 days before the start of any testing. During this time mineral-deficiency or injection manipulations were begun. All manipulations continued through the test period. The deficient diets used were General Biochemicals Low Calcium Test Diet (0.002 percent calcium) and Low Iron Test Diet; the normal diet was Wayne Lab Blox (1.2 percent calcium). The protein, fat, and carbohydrate content was similar in all diets. All diets were freely available to the rats. Twenty-four-hour fluid intake data were converted for analysis in three ways: (i) the milligrams of lead acetate ingested per 100 g of body weight per day; (ii) the amount of test solution ingested as a percentage of total fluid intake; and (iii) the total fluid ingested per 100 g of body weight per day.

Thirty-six nondeficient weanling rats were presented with the test solution series. They decreased their percentage intake of lead solutions with increased concentration (Table 1, top line), but the compensation was not precise. Thus, there was a significant increase in the lead ingested (in milligrams per 100 g) with increased concentration ($F = 10.46$, d.f. $= 3/177$, $P < .001$). This finding of increased lead ingestion with increased concentration was found with every group of rats tested, regardless of manipulation. The total daily fluid in-

Table 1. Voluntary ingestion of lead acetate solutions by weanling rats. Figures in parentheses indicate the number of milliliters of total fluid ingested per 100 g of body weight per day. Body weights are those on the last day of testing (56 days). All values are means.

Group	Lead acetate ingestion (mg per 100 g per day)				Test solution ingestion as percent of total					Body weight (g)
	Solution				Solution					
	0.08%	0.16%	0.32%	0.64%	Water	0.08%	0.16%	0.32%	0.64%	
Controls ($N = 36$)	4.00	6.20	9.60	12.60	53.9 (17.8)	26.1 (18.5)	22.3 (17.4)	12.9 (18.1)	11.2 (17.4)	211.1
Deficiency experiments										
Calcium deficient ($N = 27$)	7.15*	8.81*	12.52*	33.53*	52.1 (16.9)	57.4* (15.4)	44.6* (13.9)	33.0* (12.5)*	35.6* (15.3)	122.8*
Iron deficient ($N = 16$)	2.98	5.32	5.10	13.49	60.3 (17.4)	21.2 (17.4)	19.4 (17.4)	10.1 (16.0)	12.9 (15.8)	145.6*
Injection experiments										
Water injections ($N = 9$)	3.28	7.57	9.30	9.85	59.0 (22.2)	20.3 (20.9)	25.0 (20.1)	15.1 (19.7)	8.6 (18.5)	195.2
Lead injections ($N = 9$)	5.63	9.27	13.86	18.49	50.4 (21.0)	31.4 (22.6)	25.6 (23.9)	19.9 (21.8)	14.3 (22.0)	171.8

* Significantly different from control values by *t*-test, all P's $< .001$.

take remained constant over all solutions. Nondeficient rats do not show a preference for lead solutions and reduce the percentage intake to 11 to 12 percent with high concentrations.

The second series of experiments examined the nutritional deficiency hypothesis. Twenty-seven calcium-deficient and 16 iron-deficient rats were used. Intake data of these animals were analyzed with the data for nondeficient rats presented above. The results are presented in the second section of Table 1. There were no differences between the iron-deficient and control weanlings in milligrams of lead ingested per 100 g per day, in percentage of test solution ingested, or in total fluid ingestion. Body weights of the iron-deficient animals were significantly lower than those of nondeficient rats ($t = 6.968$, d.f. $= 50$, $P < .001$).

However, the calcium-deficient weanlings showed a significant increase in the amount of lead ingested per 100 g per day ($F = 10.43$, d.f. $= 1/61$, $P < .005$). They also ingested proportionally more lead at higher concentrations than did control animals ($F = 4.096$, d.f. $= 3/177$, $P < .001$). Calcium-deficient rats ingested a larger percentage of their fluid intake from lead solutions than did control animals ($F = 36.30$, d.f. $= 1/61$, $P < .001$) and showed a disproportionate increase at higher concentrations ($F = 10.13$, d.f. $= 3/177$, $P < .001$). The t-tests performed at each concentration between calcium-deficient and control animals showed a significant difference in each measure at every concentration (t's $>$ 4.21, d.f. $= 61$, P's $< .001$). Two subsequent replications with minor changes in procedure have produced the same pattern of results.

Calcium deficiency imposed on weanling rats reliably produced a significant increase in the amount of lead ingested per 100 g at all concentrations. The calcium-deficient weanlings ingested an average of 33.5 mg of lead at highest concentrations. If the values found from humans on absorption of lead from the intestines (4 to 10 percent) are used (*14*), the rats absorbed 1.34 to 3.35 mg of lead per 100 g per day. This level is greater than the 1.2 mg per 100 g per day injections used by Snowdon (*15*) which produced severe symptomatology and death in many animals. Thus the calcium-deficient rats in the present study voluntarily ingested lead at toxic levels. The calcium-deficient rats had lower body weights than either the control rats ($t = 11.586$, d.f. $= 61$, $P < .001$) or the iron-deficient rats ($t = 3.792$, d.f. $= 41$, $P < .001$).

The final series of experiments examined the effects of lead injections on the ingestive behavior of weanling rats. If animals can regulate the level of lead in their bodies through their ingestive behavior, then imposing an increased level of body lead on animals should produce a reduction of voluntary lead ingestion below the already low levels of normal animals. If calcium and lead are interrelated, then an animal with an increased load of body lead might compensate with an increased voluntary calcium ingestion.

Eighteen weanlings were divided into a lead-injected group and a water-injected group with the lead-injected animals receiving 0.8 mg of lead acetate per 100 g daily for 25 days prior to the start of testing. Water-injected rats received equal volumes of distilled water. No significant differences were found between the groups on any of the measures of ingestion (Table 1, third section). From these data it would appear that lead-injected rats cannot compensate for an increased lead load by reducing their voluntary lead ingestion.

Twenty weanlings were divided into lead-injected and water-injected groups. After 25 days of injection they were tested in the two-bottle situation with distilled water, 0.6, 1.2, and 2.4 percent solutions of calcium lactate in a repeated Latin squares design. Analysis of the data indicated an increased calcium ingestion in lead-injected rats ($F = 5.28$, d.f. $= 1/16$, $P < .05$), an increased calcium ingestion by female rats ($F = 6.52$, d.f. $= 1/16$, $P < .025$), and an increased calcium ingestion by lead-injected female rats ($F = 6.09$, d.f. $= 1/16$, $P < .05$). No sex effects were found in the previous results. The t-tests between injected and noninjected females indicated increased intake in milligrams of calcium lactate per 100 g per day for the 0.6 percent solution (mean for lead-injected rats $= 83.9$ mg, mean for water-injected rats $= 38.0$ mg, $t = 2.500$, d.f. $= 8$, $P < .05$) and for the 1.2 percent solution (mean for lead-injected rats $= 232.0$ mg, mean for water-injected rats $= 100.4$ mg, $t = 5.354$, d.f. $= 8$, $P < .001$) with the intake at 2.4 percent barely missing significance (mean for lead-injected rats $= 384.4$ mg, mean for water-injected rats $= 208.0$ mg, $t = 2.081$, d.f. $= 8$, $.10 > P > .05$).

While the appearance of a sex difference is puzzling, the increased calcium intake of lead-injected rats corresponds to reports of many children with lead pica drinking large amounts of milk (*2*, *11*). Indeed, it has been suggested that such milk ingestion might be the cause of iron-deficiency anemia found with lead pica (*16*).

These experiments demonstrate that weanling rats do not normally ingest lead when an alternative, nonlead solution is available, nor do they compensate for increased body lead loads by reducing still further their voluntary lead intake. The low level of lead ingestion by normal rats probably does not represent an active regulation of lead levels, but rather taste qualities of even low concentrations of lead are probably aversive. Osmotic factors were not responsible for the low lead intakes, since all solutions were in the hypotonic range.

Only a calcium deficiency imposed on weanling rats produced a markedly elevated voluntary lead ingestion. Deficiency, per se, did not lead to the increased ingestion, since iron-deficient weanlings did not differ from controls. This suggests an involvement of calcium in the production of lead pica. This is further supported by the finding that at least female weanlings will increase voluntary calcium consumption when injected with lead.

Aside from the finding of increased milk consumption in some children with lead pica, there is no direct evidence of calcium deficiency leading to lead pica in humans. However, lead pica is most common among low-income children, and a survey of studies of mineral nutrition in the United States (*17*) indicated that almost 60 percent of the low-income households surveyed provided less than the minimum recommended level of calcium, a degree of failure greater than for any other nutrient surveyed. Thus low-income families are likely to provide inadequate calcium levels for their children.

Recent research in specific hungers in animals suggests a mechanism whereby a lead pica might be maintained in the absence of an obvious, symptomatic calcium deficiency. A nutrient deficiency induces the organism to seek other food substances, sampling until one source relieves the aversive symptoms produced by the deficiency. Such an animal will continue to ingest this symptom-relieving food unless and until it also produces negative symptoms through nutritional inadequacies of its own (*18*). Such an interpretation of lead picas would argue that a child de-

ficient in calcium may discover that lead relieves some of the symptoms of calcium deficiency. Thus, lead becomes a highly rewarding substance which the child continues to ingest beyond the duration of the calcium deficiency. But lead brings negative symptoms of its own. Why then should pica continue to the point of severe poisoning? Another experiment suggests a possible solution. Calcium-deficient rats were allowed to drink calcium solutions to restore their deficiency. Injections of lithium chloride were then administered concomitantly with calcium ingestion, a procedure that normally induces a long-lasting aversion for the solution it is paired with. However, in the case of calcium-deficient rats drinking calcium, the aversive effects of lithium chloride did not reduce calcium intake (*19*). Possibly for the child with lead pica the reinforcing effects of lead ingestion relieving calcium deficiency are sufficiently powerful that the negative effects of continued ingestion cannot overcome the impetus to ingest lead.

CHARLES T. SNOWDON
BLYTHE A. SANDERSON
Psychology Department,
University of Wisconsin, Madison 53706

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20. Supported by Office of Education grant OEG 5-71-0052 (508) and funds from the University of Wisconsin Graduate School Research Committee to C.T.S. Part of this report formed the senior thesis of B.A.S. We thank V. Recupero for helping with some of the testing and S. Hodun and R. E. Keesey for helpful criticism of the manuscript.

20 August 1973

Antireceptor Antiserum Causes Specific Inhibition of Reactivity to Rat Histocompatibility Antigens

Abstract. *The antigen receptor of lymphocytes destined to form antibody appears to have the characteristics of the immunoglobulin produced. Antibody directed against the combining region of this immunoglobulin should interact with the combining region of the cell receptor for the antigen. Purified Lewis rat alloantibody prepared against Brown Norway (BN) rat histocompatibility antigens was used to immunize L × BN F_1 hybrids. The resultant antiserum has antireceptor activity because (i) it yields precipitin lines in gel diffusion when reacted against the immunizing alloantibody; (ii) it inhibits the hemagglutinin antibody response of Lewis rats to BN histocompatibility antigens; and (iii) it inhibits the local graft-versus-host response of Lewis lymphoid cells against $B\bar{N}$ antigens. This suggests that antireceptor antibody may inhibit cell-mediated responses as well as antibody responses to histocompatibility antigens and may play a role in the regulation of immune responses to such antigens.*

The interaction of antigen with antigen receptors on lymphoid cells initiates the immune response. Procedures that selectively interfere with this interaction suppress the response. Antibody specific for an antigen can cause selective suppression by preventing antigen from reacting with the receptor (*1*). The antigen receptor on those lymphoid cells that can produce antibody appears to have characteristics of an immunoglobulin (*2*). The combining region of the cell receptor and that of the immunoglobulin produced are presumed to be the same (*3*). Therefore antibody directed against the combining region of an immunoglobulin (anti-idiotypic antibody) should also be directed against the combining region of the corresponding cell receptor. This kind of antibody might therefore suppress the expected antibody response by preventing the interaction of the receptor with the antigen.

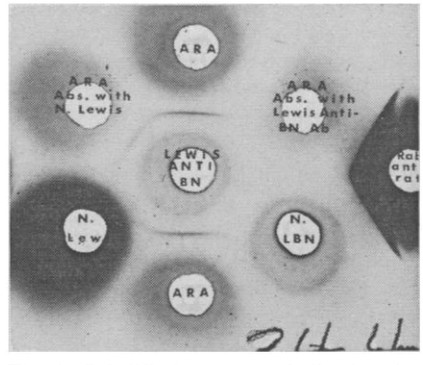

Fig. 1. Gel diffusion pattern indicating the reactivity of ARA. Antireceptor antibody produced in L × BN F_1 hybrids developed precipitin lines with the immunizing antiserum (Lewis alloantiserum to BN), but not with normal Lewis serum, normal LBN serum, or Lewis antiserum to sheep erythrocytes (not shown). Absorption of ARA with Sepharose-conjugated normal Lewis globulin (*ARA abs. with N. Lewis*) did not remove precipitin activity, whereas absorption with Sepharose-conjugated immunizing globulin (*ARA abs. with Lewis anti-BN*) did remove this activity. Sepharose absorption did not remove those immunoglobulins from ARA that were detected by the heterologous rabbit antiserum to rat gamma globulin.

Several observations support this concept (*4*), the most compelling being that presented by Cosenza and Köhler (*5*), who have studied the immune response of the mouse to phosphoryl choline. They have shown that anti-idiotypic antibody reactive with antibody to phosphoryl choline prevented an active immune response to phosphoryl choline when passively administered to intact animals or in cultures of mouse spleen cells before they were exposed to the phosphoryl choline antigen. The response of the mouse to phosphoryl choline is independent of thymus-derived cells, does not result in detectable cell-mediated immunity, and is associated with production of an IgM antibody that is apparently homogeneous.

Antibody to receptor (antireceptor antibody, ARA) could conceivably play a role in the regulation of an immune response. If ARA has such a role, it should be active in controlling immune responses that occur after immunization with complex antigens, as well as in controlling responses to simpler haptenic groups. In order to evaluate these possibilities, the biological effects of a putative ARA prepared against rat alloantibody have been studied.

Fibrosarcoma cells from Brown Norway (BN) rats were lysed in hypotonic solution and centrifuged. The cell membranes were then used to absorb the Lewis alloantiserum (*7*) to BN histocompatibility antigen (37°C for 30 minutes). The absorbed membranes were washed at room temperature,