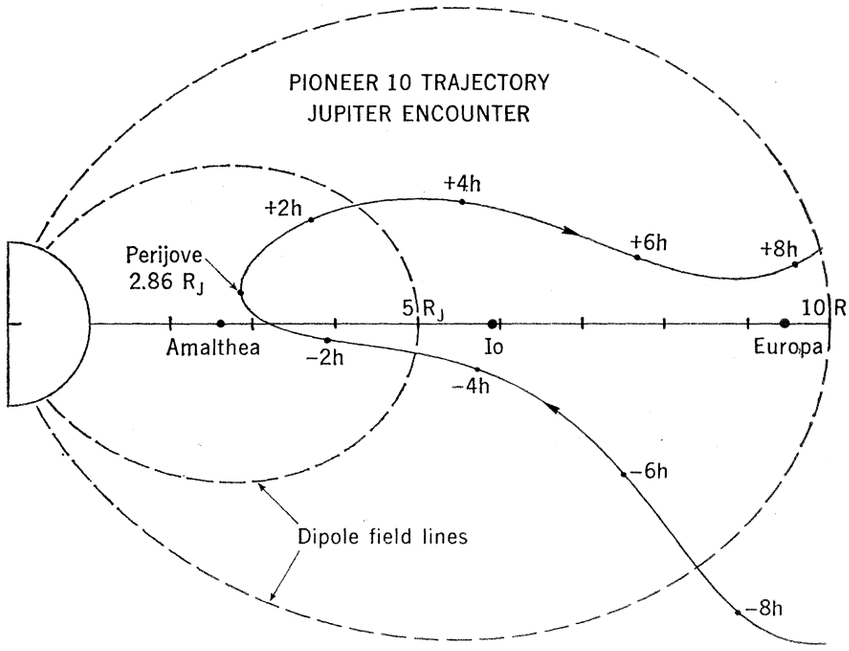

Fig. 2. Trajectory of Pioneer 10 as a function of radius and magnetic latitude. The satellites Amalthea, Io, and Europa are shown as points in the equatorial plane, although each oscillates in magnetic latitude with amplitude 10°. Times in hours (h) before (−) and after (+) perijove are indicated along the trajectory.

interaction of the electrons and protons with electric field fluctuations generated by an atmospheric-ionospheric dynamo. The rate of radial diffusion should be approximately the same for protons and electrons. By fitting the observed radial distribution of Jupiter's decimeter radio emission (*5*) to a model of trapped electrons emitting synchrotron radiation, we have estimated (*4*) the electron radial diffusion coefficient to be $D = (1.7 \pm 0.5) \times 10^{-9} (R/R_J)^{1.95 \pm 0.5}$ R_J^2/sec. (The best-fit value $\mu_0 = 770$ Mev/gauss also comes from this analysis.) We assume that this value of D can be extrapolated out to 20 R_J, although the radio emission is insignificant beyond 4 R_J.

The energy degradation term in Eq. 1 is due to synchrotron radiation emission, which is effective only in the region 1 to 4 R_J. At 1.85 R_J, the center of the synchrotron emission region, a 10-Mev electron loses half its energy via synchrotron radiation in approximately 6 months. Because of their much greater mass, protons with comparable energies do not emit synchrotron radiation, and consequently there is no such energy degradation term in the proton equation.

The remaining factor in Eq. 1 represents particle absorption by the satellites Amalthea, Io, Europa, and Ganymede. We assume that these four moons sweep up in snowplow fashion any particles which lie in their paths (*2*). The electrical conductivity of these satellites is taken to be sufficiently low that they do not distort the electromagnetic fields in Jupiter's magnetosphere, and thus trapped particles cannot slip around and past the satellites.

Figure 2 shows the trajectory of Pioneer 10 in magnetic coordinates. At perijove (the position of closest approach to Jupiter), 0225 U.T. on 4 December 1973, the spacecraft will be 2.86 R_J from the center of the planet at a magnetic latitude of 7.6°. The period of greatest danger to the spacecraft appears to lie during the 5 hours just before perijove passage, when the spacecraft will be inside 7 R_J at magnetic latitudes from −9° to +8°. This is the latitude region where the moons are least effective in absorbing radiation belt particles and where fluxes are expected to be the most intense. We have calculated the absorption effect as a function of magnetic latitude and averaged the reduction factor over this portion of the trajectory. The average fluxes should be about a factor of 100 less than they would be if there were no absorbing moons. This may be enough to prevent serious radiation damage to the spacecraft (*6*).

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References and Notes

1. See, for example, various articles in *Proceedings of the Jupiter Radiation Belt Workshop*, A. J. Beck, Ed. (Technical Memorandum 33-543, Jet Propulsion Laboratory, Pasadena, Calif., 1972).
2. G. D. Mead and W. N. Hess, *J. Geophys. Res.* **78**, 2793 (1973).
3. N. Brice and T. R. McDonough, *Icarus* **18**, 206 (1973); S. A. Jacques and L. Davis, Jr., unpublished manuscript; F. V. Coroniti, *Astrophys. J.*, in press; K. G. Stansberry and R. S. White, *J. Geophys. Res.*, in press.
4. T. Birmingham, W. Hess, T. Northrop, R. Baxter, M. Lojko, *J. Geophys. Res.*, in press.
5. G. L. Berge, *Astrophys. J.* **146**, 767 (1966).
6. A more complete presentation of the technique and results outlined here is in preparation by W.N.H., T.J.B., and G.D.M.

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Hyperactivity and Brain Catecholamines in Lead-Exposed Developing Rats

Abstract. *Newborn rats that suckled mothers eating a diet containing 4 percent lead carbonate display hyperactivity, aggressiveness, and excessive stereotyped behavior starting at 4 weeks of age. There is an eightfold increase in the concentration of lead in brain, no change in norepinephrine, but a 20 percent decrease in dopamine relative to coetaneous controls. This suggests a relationship between central nervous system dysfunction due to lead and dopamine metabolism in brain.*

The nervous system is very sensitive to the toxic effects of lead, and it is the young who are particularly susceptible to cerebral dysfunction produced by lead (*1*). Although the histopathological findings of lead encephalopathy are well documented (*2*), practically nothing is known about the biochemical changes in brain following acute lead intoxication or chronic low-level exposures to lead during early developmental years. An experimental model with morphological alterations closely resembling those occurring in humans with lead toxicity has been described by Pentschew and Garro (*3*). This model utilizes the suckling rat and in some ways is analogous to pica seen in clinical

cases of lead poisoning. We have modified the experimental design so that paraplegia and extensive cerebellar vascular damage do not develop as previously described (*3, 4*), but the lead-exposed neonates display (i) hyperactivity, (ii) aggressiveness, and (iii) stereotyped repetitive behavior as manifested by excessive self-grooming. It has been suggested that the catecholamines regulate these behavioral functions (*5*). Therefore, we have measured brain tissue levels of norepinephrine and dopamine in lead-exposed young rats prior to and during hyperactive episodes.

Timed pregnant Sprague-Dawley rats, obtained from ARS/Sprague-Dawley Co. (*6*), at approximately 16 days of pregnancy, were caged individually and housed in temperature-controlled rooms illuminated from 8 a.m. to 8 p.m. Powdered Ralston Purina Laboratory Chow Meal and tap water were freely available to them. After parturition the experimental females were given powdered chow containing 4 percent lead carbonate ($PbCO_3$). The food consumption of experimental mothers was measured each day and the average amount eaten was provided in the form of normal chow to pair-fed control lactating mothers. On day zero the litters were adjusted to ten animals and at 5 days of age they were culled to six sucklings per nursing mother.

Pentschew and Garro (*3*) reported that lactating rats eating a diet containing 4 percent $PbCO_3$ produce milk containing 40 parts of lead per million. Michaelson and Sauerhoff (*7*) have observed that 18-day-old experimental animals suckling leaded milk are capable of gaining access to and consuming some of the solid-lead–containing diet intended for the mother and thereby have a 1000-fold increase in lead exposure. Therefore, to maintain a continuous 40-ppm lead exposure in the present study the mother's diet was changed from 4 percent $PbCO_3$ to that containing 40 ppm lead on the 17th experimental day. Neonatal rats from experimental and control mothers were weighed each day and there was no difference between experimental and control groups. During the fourth week of development the lead-exposed rats displayed hyperactivity and aggressiveness and exhibited excessive stereotyped self-grooming behavior relative to coetaneous controls. Spontaneous activity was quantitated with the use of a Selective Activity Meter (*8*). Six experimental or six control young rats

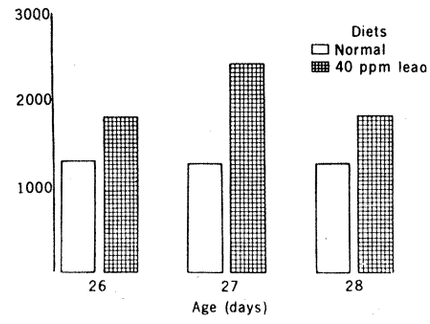

Fig. 1. Comparison of spontaneous locomotor activity in young rats raised on control diet and lifetime exposure to diet containing 40 parts of lead per million (see text for details). Ordinate: mean activity counts (crossings of a magnetic field) per colony of six rats per hour during a 24-hour test period.

were removed from their mother and placed in a cage on the Selective Activity Meter for a 24-hour period with free access to food and water. Twenty-five to 28-day-old experimental and control groups were tested on alternate days. A 40 to 90 percent increase in spontaneous movement in lead-exposed animals relative to control animals is shown in Fig. 1. At the completion of the test period the animals were decapitated and their brains were rinsed in ice-cold deionized water, separated longitudinally into two halves, frozen on Dry Ice, and weighed. One half of the brain was dissolved in tetramethylammonium hydroxide (24 percent in methanol) and its lead content was estimated by flameless atomic absorption spectroscopy (*9*). The second half of the brain was analyzed for norepinephrine and dopamine according to the method of Anton and Sayre (*10*).

In a concurrent study experimental and control rats were killed at 5, 13, 21, and 29 days of age and their brains were treated as described above. Brain lead and catecholamine content of experimental and control animals at various ages of development and during the period immediately after assessment of hyperactivity is shown in Table 1. Exposure to lead via the regimen described leads to a significant increase in the amount of lead in brain within 5 days of the onset of exposure (compare columns A and E, Table 1). The brain lead content of exposed animals increases another 33 percent after an additional 3 weeks of exposure. It is interesting to note that there are no observable differences in the norepinephrine content between control and lead-exposed animals (compare columns B and F, Table 1). Dopamine content is similar in 5- and 13-day-old experimental and control animals; however, there is a statistically significant decrease in dopamine at 21 and 29 days in the leaded animals relative to controls (compare columns C and G, Table 1). Furthermore, a comparison of dopamine to norepinephrine ratios in experimental animals relative to control animals (compare columns D and H, Table 1) shows an approximate 20 percent depression of dopamine in the 21- and 29-day-old experimental animals.

Hyperactivity in children is characterized by a high level of motor activity, short attention span, low frustration tolerance, hyperexcitability, and impulsiveness (*11*). Lead poisoning can, in some instances, be followed by hyperactivity (*12*). In a study of hyperactive children, David *et al.* (*13*) found a higher incidence of lead exposure in hyperactive children relative to controls. These investigators (*13*) report that blood lead was 15 percent higher in hyperactive children than in controls and that following a single provocative challenge with penicillamine, 60 per-

Table 1. Lead, norepinephrine (NE), and dopamine (DM) concentrations (in micrograms per gram wet weight) in brains of developing rats eating leaded diet (40 parts of lead per million) and in brains of pair-fed controls. Values are means ± standard deviation.

Age (days)	Pair-fed controls				Lead-exposed experimentals			
	(A) Lead	(B) NE	(C) DM	(D) Ratio C/B	(E) Lead	(F) NE	(G) DM	(H) Ratio G/F
5 ($N = 7$)	0.10 ±.01	0.156 ±.010	0.266 ±.049	1.70	0.60 ±.10	0.156 ±.020	0.261 ±.024	1.67
13 ($N = 6$)	0.10 ±.01	0.168 ±.020	0.318 ±.026	1.89	0.70 ±.15	0.160 ±.020	0.300 ±.024	1.87
21 ($N = 6$)	0.10 ±.01	0.230 ±.020	0.560 ±.020	2.43	0.78 ±.15	0.238 ±.020	0.467* ±.046	1.96
29 ($N = 6$)	0.10 ±.01	0.298 ±.020	0.560 ±.033	1.88	0.88 ±.11	0.293 ±.035	0.450* ±.041	1.54

* $P = .01$ by Student's t-test.

cent of these children had urinary lead levels in the toxic range. Penicillamine is an effective heavy-metal–chelating agent capable of mobilizing bone stores of accumulated lead and was chosen (*13*) because of its comparatively low toxicity and oral mode of administration. This suggests that a large burden of body lead existed and probably represents earlier exposure to subtoxic levels of lead. We are presently unaware of any definitive studies on catecholamine metabolism in hyperactive children. However, it has been suggested that brain catecholamines regulate activity and influence aggressiveness and stereotyped repetitive behavior (*13*). It is interesting to note that in the present study hyperactivity in lead-intoxicated rats is associated with an altered norepinephrine to dopamine ratio. The experimental design as described provides a model of central nervous system dysfunction due to lead exposure without debilitating histopathologies (*3, 4*). The observation that hyperactivity following lead exposure is accompanied by an effect on dopamine metabolism in the brain could offer an avenue to further study, and possibly explain some of the behavioral sequelae of high-level and, more importantly, low-level environmental exposure to lead.

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References and Notes

1. R. A. Goyer and B. C. Rhyne, *Int. Rev. Pathol.* **2**, 1 (1972).
2. A. Pentschew, *Acta Neuropathol.* **5**, 133 (1965).
3. ——— and F. Garro, *ibid.* **6**, 266 (1966).
4. J. A. Thomas, F. D. Dallenbach, M. Thomas, *Arch. Pathol. Anat.* **352**, 61 (1971); I. A. Michaelson, *J. Toxicol. Appl. Pharmacol.*, in press.
5. J. J. Schildkraut, *Am. J. Psychiatry* **122**, 509 (1965); O. Benkert, H. Gluba, N. Yatussek, *Neuropharmacology* **12**, 177 (1973); M. Goldstein, A. F. Battista, T. Ohmoto, B. Anagnoste, K. Fuxe, *Science* **179**, 816 (1973).
6. ARS/Sprague-Dawley Co., Madison, Wisconsin.
7. I. A. Michaelson and M. W. Sauerhoff, in preparation.
8. Selective Activity Meter, model S, Columbus Instrument Co., Columbus, Ohio.
9. L. Murthy, E. E. Menden, P. M. Eller, H. G. Petering, *Anal. Biochem.* **53**, 365 (1973); S. Gross and E. Parkinson, in preparation.
10. A. H. Anton and D. F. Sayre, *J. Pharmacol. Exp. Ther.* **138**, 360 (1962).
11. R. S. Paine, *Pediatr. Clin. North Am.* **15**, 779 (1968).
12. R. K. Byers and E. E. Lord, *Am. J. Dis. Child.* **66**, 2171 (1966).
13. O. David, J. Clark, K. Voeller, *Lancet* **1972-II**, 900 (1972).
14. Supported by grants NIEHS-ES-00042 and NINDS-NB-06053.

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Electrophoretic Variation in Escherichia coli from Natural Sources

Abstract. *At each of five loci in 829* Escherichia coli *clones from 156 samples from diverse natural sources, electrophoretic analysis reveals a prominent mobility class (frequency over 0.70) and 2 to 11 distinct mobility classes at lower frequencies. The frequency distribution of the classes argues against the importance of neutral mutations in allozymic variation. Heterosis is not the universal cause of genic polymorphism.*

For a variety of reasons, it is of interest to study natural genetic variation in *Escherichia coli*. Is there genic polymorphism in a species that is haploid and thus has essentially no heterosis? Does each host animal species harbor a genetically unique strain? To what extent do the *E. coli* within a host vary genetically? And finally, can one use *E. coli* to distinguish between predictions based on the "neutral" (or non-Darwinian) hypothesis from those based on the "strong selectionist" hypothesis of electrophoretic variation (*1, 2*)?

Studies of allozymes at five loci lead to the following conclusions. (i) Genetic variation is not very different from that observed in the many diploid species studied. (ii) There is no obvious parallel between any allele and the phyletic position of its source. (iii) There is often a great deal of genetic variation among the *E. coli* within a host. (iv) The results are inconsistent with the current form of the neutral hypothesis (*3, 4*).

The neutral hypothesis attributes most observed electrophoretic variation, and most amino acid substitutions over the course of evolution, to the random genetic drift of the frequencies of various alleles at a locus, all of practically equivalent adaptive value. Thus a newly arisen neutral allele has a small but finite chance of rising in frequency, even to the point of replacing its predecessor. Since populations are large and time is abundant, even this improbable event occurs often. On the way to replacement, and in other cases that never go that far, two or more alleles coexist, each at a substantial frequency. The neutralists point out that there is no known quantitative relation between the number of allelic substitutions and most evolutionary changes at higher phenotypic levels. Thus, we cannot be certain that all the genic polymorphisms we detect by electrophoretic analysis result from the same process of natural selection that shapes morphological and physiological evolution. Perhaps only 1 percent of this allelic variation is involved, the rest being "evolutionary noise" (*2*).

Naturally, not all mutations are neutral; a nonsense mutation near the amino end of a polypeptide will annihilate it. Selection will eliminate such mutations and many others in essential polypeptides. But perhaps many amino acid substitutions cause charge- and electrophoretic mobility changes without altering substantially the value of the polypeptide to the organism. Limitations on the number of such substitutions at any one time have been described by Fitch in the form of the covarion theory (*3, 5*). For most higher organisms, the number of alleles at frequencies above 1 percent is likely to be well below ten at any locus because of these limitations on acceptable substitutions and because of limiting population size and limiting duration of existence of the species (or existence since passing through a bottleneck of small numbers) (*6, 7*). Thus it is hard to distinguish between the predictions of the neutral and the strong selection hypotheses. But in *E. coli*, whose numbers are astronomical and whose existence doubtless extends back 100 million years, there must be a veritable tree of covarions, and the number of alleles at any locus anticipated by the neutral hypothesis would be very great. A convenient table has been prepared by Kimura (*8*).

The allelic diversity in a population is most usefully thought of in terms of the likelihood of encountering the same allele at a locus twice in a row. Extremely rare alleles are negligible; thus, instead of the total number of alleles in a population, we determine the effective number, n_e, which is the reciprocal of the sum of the squared frequencies of all the alleles.

For a given total number of alleles at a locus in a population, n_e is maximal when all their frequencies are equal. The expected n_e in a population is $4N_e u + 1$, where N_e is effective population size, and u the mutation rate