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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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

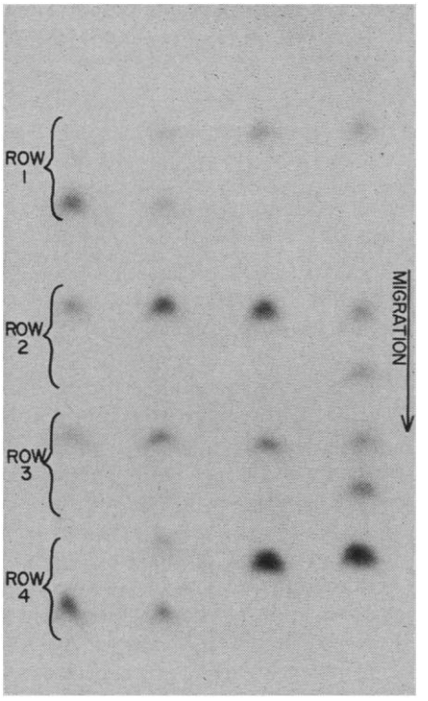

Fig. 1. 6-Phosphogluconate dehydrogenase allozymes of two mobility classes, + and S-3. The samples were spotted in four rows, rather than in a single row as in most techniques. Columns 1 and 3 contain single samples; columns 2 and 4 contain paired samples, which produce a single spot if they are identical in mobility. The small letters refer to the sources of the samples: (row 1) +, +/S-3a, S-3a, S-3a/S-3b; (row 2) S-3b, S-3b/S-3c, S-3c, S-3d/+; (row 3) S-3d, S-3d/S-3e, S-3e, S-3e/+; (row 4) +, +/S-3f, S-3f, S-3c/S-3d. The S-3f bands in positions 2 and 3 of row 4 are from the same homogenate, illustrating the imprecision of single lateral comparisons.

to neutral alleles (*6, 8*). But two conditions are of importance here. (i) The population must be in a steady state, which requires that it have been at its present size for $4N_e$ generations (*6*). A lesser population size may be chosen to match a known period of existence: this will lead to a minimum estimate for n. (ii) The number of possible equivalent allelic states (that is, the number of different neutral alleles) must be very large.

Escherichia coli has certainly exceeded a species size—and an effective population size—of 10 billion for 40 billion generations (40 million years at three generations per day). The evolution of the horse took 60 million years. As to the mutation rate to neutral alleles, the neutral hypothesis would at the very least require it to be one-hundredth of the overall mutation rate per locus, which in turn should be greater than 10^{-6}. We can therefore predict that n_e will be more than $4 \times 10^{10} \times 10^{-8}$, or 400. The actual number of alleles would be even greater.

The selection hypothesis rejects the notion of many neutral alleles at a locus and would predict a small effective number of alleles, and great genetic similarity among many individuals. (An alternative selectionist prediction, that of host-species specificity, is not fulfilled.) In fact, there are very few electrophoretically different alleles among the samples studied so far, and they are listed in Table 1.

Each sample represented a clone plated from a homogenized fecal sample. Standard microbiological tests were performed (*9*) to identify the bacteria as *E. coli*, which were then cultured, centrifuged, resuspended, washed, sonicated, and subjected to electrophoresis for about 45 minutes at *p*H 8.8 on Gelman Sepraphore III cellulose acetate strips, with the use of the Adamkewicz multiple applicator (*10*). Standard staining techniques were used (*11*). Mobility differences were striking, and each sample was tested electrophoretically in several combinations, both singly and mixed with another. Each new mobility variant was also classified by coelectrophoresis with a standard (for example, all new "slow" allozymes were compared with the one or more slow allozymes known to be distinct from one another). Mobility differences were generally quite clear (Fig. 1).

Within a mobility class, many different amino acid sequences of similar net charge can theoretically occur (*12*). This means that exhaustive analysis of allozyme variation cannot be achieved by electrophoretic analysis, no matter how fine its resolution. Furthermore, ionization is not an all-or-none phenomenon for a single ionizable group, much less for an entire protein molecule. The distribution of possible net charges on different allozymes at a given *p*H is therefore essentially continuous, not discontinuous. In this context, then, the present results are striking. A clear conclusion emerges from the preponderance in the present experiment of one mobility class for each enzyme, and this is the critical point: At equilibrium, the neutral hypothesis predicts that the various mobility classes should approach a normal distribution. If one distinct class contains 0.70 of the total, then it should be flanked

Table 1. Frequencies of electrophoretic mobility classes. Mobility classes are sets of electrophoretically indistinguishable allozymes (*p*H 8.8) listed in order of increasing mobility for each locus. Only in the case of 6-PGDH do some bands overlap when subjected to coelectrophoresis; they are S-6/S-5, S-5/S-4, and S-3/S-2. Nevertheless, members of these pairs are clearly and reliably distinguishable. Source animals include humans, mice, assorted primates, assorted small mammals, assorted domestic animals, and a lizard, among others. Source locales include New Guinea, Alberta, Santa Cruz Island (California), the Netherlands, Woodland Park Zoo (Seattle), and Iowa. No obvious geographical specificity was encountered. The mobility range is the distance after a standard run between slowest and fastest band. Overall range for the five enzymes is 27 mm. Abbreviations: ADH, alcohol dehydrogenase; AP, alkaline phosphatase; G-6-PDH, glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase: MDH, malate dehydrogenase; US, unclassified slow; and UF, unclassified fast.

Mobility class	Enzyme				
	ADH	AP	G-6-PDH	6-PGDH	MDH
S-9				1	
S-8				9	
S-7				7	
S-6				16	
S-5				6	13
S-4				21	1
S-3				66	2
S-2			1	32	30
S-1	15	1	2	21	2
+	595	633	791	590	770
F-1	139	1	10	27	1
F-2		8	13	29	2
US	9	31	3	2	8
UF	5	14	7	0	0
Unclassified	66	141	2	2	0
Band width (mm)	1	1.5	0.8	0.5	1.5
Mobility range (mm)	8	12	6	14	12
n_e	1.56	1.18	1.09	1.92	1.16

by adjacent classes of equal width, each containing almost 0.15 of the total, as inspection of a normal probability table will confirm. The observed distribution is radically different for each of the five loci; indeed, adjacent positions empty in a number of cases. We must conclude that the neutral hypothesis is incorrect: instead, the number of allelic states equivalent or nearly equivalent to the best one is very few.

These observations, drawn from geographically and phyletically diverse hosts, include numerous sets of 10 to 20 clones, each set being taken from a single fecal sample. The variation within most sets is great enough to suggest that recombination takes place regularly within hosts. Returning to the major consideration, one can compile the data according to a very conservative procedure, in which each mobility class is counted only once per set. The prominence of the most common mobility class remains striking. This being the case, the selectionist hypothesis is favored.

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Hepatitis A: Detection by Immune Electron Microscopy of a Viruslike Antigen Associated with Acute Illness

Abstract. *Spherical 27-nanometer particles were visualized in stools obtained from hepatitis A patients in the acute phase of the disease. The particle was serologically specific for this disease, and every hepatitis A patient tested demonstrated a serologic response to this antigen. The findings suggest that it is the etiologic agent of hepatitis A.*

The detection of hepatitis B antigen (Australia antigen) in the serums of certain hepatitis patients and the discovery of its association specifically with hepatitis B (serum hepatitis) provided a diagnostic tool making possible the serologic identification of this important disease (*1*). Attempts to find a virus or antigen in the serums of patients with hepatitis A (infectious hepatitis) have been unsuccessful, possibly because the viremic stage of hepatitis A appears to be of short duration and of low magnitude. In contrast, there is epidemiologic and experimental evidence that feces of patients with hepatitis A are infectious from approximately 2 weeks before until 2 weeks after onset of clinical symptoms (*2*). We, therefore, examined stool specimens from patients with hepatitis A for viruslike antigens, using the technique of immune electron microscopy (IEM) (*3*). This method was used successfully in our laboratory for the detection in a stool filtrate of a viruslike agent (Norwalk) and also for the demonstration of its association with acute infectious nonbacterial "Norwalk" gastroenteritis (*4*). We report here the detection of a morphologically similar viruslike particle that is serologically associated with hepatitis A infection.

Stool specimens (as 20 percent saline extracts) were supplied, under code, by Dr. D. Gibson. They consisted of specimens obtained before inoculation or during acute illness from each of four

Table 1. Antibody to antigens associated with hepatitis A, hepatitis B, and Norwalk gastroenteritis in paired serums from patients with these diseases. Antibody to the hepatitis A antigen was detected by immune electron microscopy (IEM) and was rated on a 0 to 4 scale, depending on the amount of antibody coating the particles. Antibody to the gastroenteritis antigen was determined in the same way. Antibody to hepatitis B antigen was measured by radioimmunoassay (RIA). The first serum of each serum pair was obtained prior to exposure, except in the case of four serums which were obtained during the acute phase of illness (acute). The second serum of each pair was obtained approximately 1 to 6 months after the onset of illness. Serums were tested by IEM at an initial dilution of approximately 1 : 10. Serums were tested by RIA at a dilution of 1 : 4 or 1 : 10. Abbreviations: NT, not tested; −, negative; +, positive.

Patient No.	Antibody to indicated antigen in first and second serum samples					
	Hepatitis A antigen		Hepatitis B antigen		Norwalk gastroenteritis antigen	
	First	Second	First	Second	First	Second
	Experimental hepatitis A (MS-1), New York					
1	0	1–2	−	−	NT	NT
2	0	1–2	+	+*	NT	NT
	Experimental hepatitis A (MS-1), Illinois					
3	0	3–4	−	−	1	1
4	0	3–4	−	−	1	1
5	0	1–2	−	−	NT	NT
6	0	3–4	−	−	NT	NT
	Naturally acquired hepatitis A, Massachusetts					
7	0–1 (acute)	3–4	−	−	NT	NT
8	0 (acute)	3	−	−	NT	NT
9	0 (acute)	3	−	−	NT	NT
	Naturally acquired hepatitis A, American Samoa					
10	0	2	−	−	NT	NT
11	0	3	−	−	NT	NT
12	1–2 (acute)	3–4	−	−	NT	NT
	Naturally acquired hepatitis B					
13	0	0	−	+	NT	NT
14	0	0	−	+	NT	NT
	Experimental nonbacterial gastroenteritis					
15	0	0	−	−	1	4
16	2–3	2–3	−	−	1	4

* No antibody increase.