gest that more attention should be given to experimentally induced amnesias from the point of view of failures of retrieval.

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7. Mann-Whitney U tests were used for all statistical comparisons.
8. A reviewer has raised the possibility that had we not had an arbitrary cut-off point of 180 seconds and had we included a group receiving FS and no ECS, reminder shock might have increased these latencies also. This might have happened, but it could only be accounted for by an enhancement of an already established memory and would therefore in no way change the interpretation that the increase in latency from T_1 to T_2 in group 5 is due to a recovery of the original memory.
9. D. Quartermain and B. McEwen, *Nature*, in press.
10. Cycloheximide (Actidione, Upjohn) at a dose of 3 mg blocked incorporation of [^{3}H]leucine into total cerebral proteins by 94 percent at 30 minutes after subcutaneous administration in saline. Incorporation returned to normal within the ensuing 7 hours. With this dosage less than 5 percent of the mice showed any sickness at the time of testing. Those which did were easily recognized and discarded from the experiment. These results and a more detailed description of the experimental procedure can be found elsewhere (*9*).
11. We have also shown that memory can be recovered after ECS when RS is given 1 hour after T_1, and T_2 follows 4 hours rather than 24 hours later.
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13. We often observe that when rats are placed in the start compartment for T_1, they show a brief freezing response often accompanied by defecation and urination. After a few seconds they relax, explore the start box, and then tentatively walk out into the large compartment.
14. Supported by research grants MH-13189, NB-07080, and GM-1789. We thank Neal E. Miller for critically reading the manuscript.

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Fitness of an Escherichia coli Mutator Gene

Abstract. *Competition experiments between* Escherichia coli mutT1 *and* mut+ *populations show that the mutator gene confers selective advantage on the strain that carries it. The observed increase in fitness varies, with an average increase in mutator growth rate of 1.4 percent when mutator and wild-type strains are grown together in chemostats.*

Mutator genes are genes that increase the mutation rate of other genes. They have been studied in *Drosophila*, maize, several strains of bacteria, and bacteriophage (*1*). One such gene, the Treffers mutator gene of *Escherichia coli*, increases the mutation rate at least 100-fold at most, if not all, chromosomal loci (*2*, *3*). Because this mutator gene (the *mutT1* allele) preferentially increases transversions of adenine-thymine (AT) to cytosine-guanine (CG) (*2*), the DNA isolated from a *mutT1* population exhibits a growth-dependent increase in the buoyant density of its DNA, and a decrease in the frequency of thymidylate sequences within that DNA (*3*). Both changes are interpreted to mean that the GC content of the *mutT1* DNA has increased, a consequence of a unique mutational pressure (*3*).

We have examined the fitness of a population carrying the *mutT1* allele and report here that *mutT1* populations consistently outgrow coisogenic *mut*+ populations when the two are grown together in a chemostat.

Cultures were grown in phosphate minimal medium (*4*) with limiting glucose as the sole carbon source in chemostats similar in design to those described by Monod (*5*, *6*). Samples were removed from the chemostat at appropriate intervals and assayed for *mut*+ and *mutT1* cells. So that large populations could be screened rapidly for *mutT1* cells, a *mutT1*-induced clone unable to ferment lactose (Lac−) was isolated and purified. Changes in the *mutT1*/*mut*+ ratio in a population were then followed by plating samples on tetrazolium-lactose plates (*7*). If we are to infer the selective advantage of *mutT1* from this experiment, we must establish that the Lac− mutant so used is selectively neutral. In order to show that the Lac− allele was neutral, we grew *mutT1* Lac+ cells with *mutT1* Lac− siblings under our standard conditions (Fig. 1 and Table 1). For a variety of growth conditions in which cell density and generation time were varied, the Lac− mutant chosen [mutated in the permease gene (*6*)] was at a slight disadvantage in the chemostat. It is therefore unlikely that the results that we will attribute to the *mutT1* allele are in fact attributable to the Lac− mutation.

When a *mutT1* Lac− population competes with a *mut*+ Lac+ population in a glucose-limited chemostat the mutator strain consistently outgrows the strain without the mutator (Fig. 2). The increase in the *mutT1* population is characteristic and reproducible and occurs under a variety of cell densities, generation times, and *mutT1*/*mut*+ ratios (Table 1). Similar results have been obtained with strains that were not coisogenic, and under conditions where the culture vessel contained a linearly increasing concentration of casein hydrolysate (chemostat 5, Table 1).

Do these results have a trivial explanation? We have already shown that the Lac− allele used to monitor the mutator level puts the mutator population at a slight selective disadvantage, and therefore the increased fitness of these strains is not a consequence of the Lac− mutation. In fact, it is likely that our estimates of the fitness of the mutator gene are for this reason conservative. In any case, when *mut*+ Lac+ and *mutT1* Lac+ populations are followed in the chemostat and assayed directly for mutator activity (*8*) the conclusion is the same (chemostat 19 of Fig. 2 and Table 1). Further checks on each chemostat showed that the cultures remained free of bacteriophage and colicins, and the *p*H of the culture medium remained constant. Each population was also assayed at the middle and end of each experiment to guarantee that all Lac− colonies were mutator active and all Lac+ colonies were mutator inactive.

In two chemostats we did find mutator active Lac+ colonies at the termination of the experiment. In both cases however, the Lac+ mutator activity was cotransducible with leucine at the same frequency as the *mutT1* allele, showing that the mutator gene present was evidently *mutT1* (*6*, *8*). We therefore assume that in these two cases the Lac− allele had reverted to Lac+ in the *mutT1* background and was selected for, an assumption that is consistent with the data of Fig. 1.

Included in Table 1 is a measure of the fitness of the *mutT1* population estimated from the rate of increase in the *mutT1*/*mut*+ ratio. This value, *K*, is related in a rather complex way to the growth and death rates of the two strains, competition between them for food and space, and cross effects such

as the feeding or poisoning of one by the other (*9*). This value represents the difference in fitness between the two populations, so that if we assign a fitness of 1.0 to the *mut*+ population, then our estimate of the fitness of the *mutT1* population assumes a value of 1.004 to 1.032. This observed range in fitness values is weakly correlated with cell density, but not with generation time (Table 1).

There are two general classes of explanation for these results: (i) either a population carrying the *mutT1* allele is intrinsically more fit because of some peculiarity of the *mutT1* product that allows faster doubling times; or (ii), the *mutT1* allele increases by mutation the variance in fitness, thereby supplying a larger variety of phenotypes for testing in this environment. These two possibilities can be distinguished by an experimental series in which the initial *mutT1* cell density is lowered while the *mut*+ population is held constant. If the increase in the mutator population observed here is a consequence of mutation to increased fitness, then as the population size falls, so will the probability of mutation to a more fit phenotype. Clearly a population size will soon be reached in which the probability of there being a more fit cell in the chemostat will be small or zero. At this point the *mutT1* population will either be lost or will remain constant until a more fit cell finally appears. On the other hand, if the *mutT1* allele is intrinsically more fit, then the mutator cells will outgrow wild type regardless of their initial density. Our data do not permit us to distinguish between these two possible models, since the range of fitness values observed in these experiments could be due to variables not yet recognized. Experiment 15 does suggest that the *mutT1* population is intrinsically more fit, since the initial cell density in this experiment was 10^5 *mutT1* cells per milliliter, a population size which would not have been predicted to have contained a more fit mutant. However, this suggestion assumes some knowledge of the total number of loci that can mutate to increased fitness. Although it seems likely that this number can be estimated from experiments of this design, at present it is unknown, and therefore the data are only suggestive, and any definitive conclusions must depend on further experiments.

These results are of general interest for two reasons. First, they show that many mutations in these strains must not be strongly selected against and may even be of neutral selective value (*3*, *10*). If this were not the case, we would have expected these mutator populations to accumulate an intolerable number of lethal or semilethal mutations over the course of a thousand generations, and therefore to have been selected against. That they have not is in agreement with earlier experiments with the *mutT1* allele which showed that the GC content of *mutT1* DNA increased during repeated subculturing, an increase that did not noticeably change the mutator doubling time (*3*). Second, these results are important because they provide an experimental demonstration of the evolutionary importance of a high mutation rate that at the same time confers increased fitness, whatever the reason for this increase. Although we do not understand *mutT* action in detail, we do know that it is tightly coupled to DNA replication (*11*), that this allele is recessive (*8*), and that it can mutate some phages but not others (*8*). Given this information, and the possibility of further understanding the mode of action of the *mutT* gene in

Table 1. The chemostats are of a simple design similar to the Bactogen of Monod (*5*, *6*). Each growth vessel contained approximately 40 ml of minimal media (*4*) supplemented with sufficient glucose to achieve the desired cell density. The inoculums were taken from cultures growing logarithmically in the same medium. The competitor ratio was determined by plating on tetrazolium-lactose plates (*7*). K was calculated from the expression $K = [\ln (N_{1,g}/N_{2,g})(N_{2,0}/N_{1,0})]/g$ where $N_{1,g}$ and $N_{2,g}$ are the cell densities at the gth generation of the *mutT1* and *mut*+ populations, respectively; similarly for $N_{1,0}$ and $N_{2,0}$ at $g = 0$. The value K is then a measure of the slope of the rate of increase of the *mutT1* population as a function of generation time. It reflects the difference in fitness between the mutator and nonmutator populations (*6*).

Chemostat	Competitors	Competitor ratio		Generations	Generation time (min)	Cells/ml	K
		Initial	Final				
6	*mutT* Lac⁻/*mutT* Lac⁺	1.1	1.5	80	186	2×10^7	
9	*mutT* Lac⁻/*mutT* Lac⁺	1.1	0.011	1035	150	3×10^8	
17	*mutT* Lac⁻/*mutT* Lac⁺	1.0	0.03	307	108	1.4×10^7	
18	*mutT* Lac⁻/*mutT* Lac⁺	1.1	0.37	368	150	8×10^6	
5	*mutT* Lac⁻/*mut*⁺ Lac⁺	0.99	23	99	150	2×10^9	0.0318
8	*mutT* Lac⁻/*mut*⁺ Lac⁺	1.00	102	165	90	6×10^8	.0281
10	*mutT* Lac⁻/*mut*⁺ Lac⁺	0.89	34	777	180	8×10^6	.0046
14	*mutT* Lac⁻/*mut*⁺ Lac⁺	0.0008	0.014	715	102	2×10^7	.0039
15	*mutT* Lac⁻/*mut*⁺ Lac⁺	0.01	0.24	810	90	1×10^7	.0039
19	*mutT* Lac⁺/*mut*⁺ Lac⁺	0.56	19	368	90	1×10^7	.0097

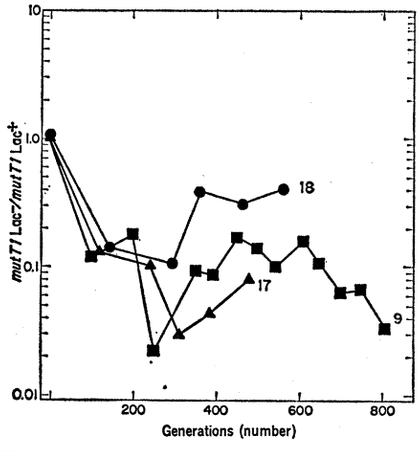

Fig. 1. Control chemostats. The ratio of Lac⁻ to Lac⁺ cells is plotted as a function of the number of generations. The Lac⁻ allele is a spontaneous *mutT1*-induced mutation in the *y* gene and is presumed to differ by a single mutational step from its Lac⁺ parent (*6*). The *mutT1* allele was introduced by P1 transduction into a Leu⁻ Azi^S derivative of W3110, a strain of *Escherichia coli* K12. The P1 lysate had been prepared on W3110 *mutT1* Azi^R; selection was for Leu⁺. Azide-sensitive, P1-sensitive cotransductants with and without the *mutT1* allele were purified and used in this and subsequent experiments. Other parameters relevant to this experiment are listed in Table 1.

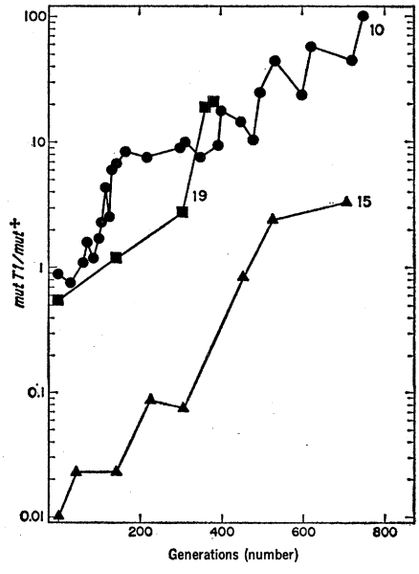

Fig. 2. The growth of *mutT1* and *mut*⁺ strains of W3110 in the same chemostat. Strains and conditions are described in the text and in Fig. 1.

both mutant and wild state, it seems to us likely that the relation between the population biology of a gene and the molecular biology of a gene can be established in detail.

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Lesch-Nyhan Syndrome: Preventive Control by Prenatal Diagnosis

Abstract. *The Lesch-Nyhan syndrome was detected in a fetus at a time sufficiently early to allow termination of the pregnancy. The feasibility of a preventive program for control of a severe sex-linked neurological disease through prenatal diagnosis is thus demonstrated.*

Children with the Lesch-Nyhan syndrome (X-linked uric aciduria) (*1*) have a syndrome consisting of mental retardation, choreoathetosis, muscle spasticity, and a compulsive tendency to self-mutilation, whereby the lips and distal finger are bitten away. The syndrome is inherited as a sex-linked recessive trait. Such children also show excessive purine synthesis and usually die before puberty. Since the description of the disease in 1964, approximately 150 cases of this condition have been detected, and it is likely that more remain to be discovered (*2*). No treatment is available for the neurological aspects of this severe disease. Adenine therapy (*3*) and a combined regimen of folic acid and adenine (*4*) have failed to prevent the development of the neurological features in affected children. The demonstration of a gross deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT; E.C. 2.4.2.8) in the erythrocytes and fibroblasts of affected children (*5*) has permitted the early prenatal detection of a heterozygote female fetus (*6*) and of affected twin male fetuses (*7*) late in pregnancy, which suggests the feasibility of a program for the preventive control of this disease by termination of pregnancies carrying an affected fetus. So far, however, there has been no report of detection of an affected male child at a time sufficiently early in pregnancy to allow termination of the pregnancy.

We now report the prenatal detection of a fetus with the Lesch-Nyhan syndrome before the 22nd week of pregnancy, the successful termination of the pregnancy, and confirmation of the enzyme defect in the aborted fetus. The mother had previously given birth to a normal male child and in her second pregnancy to an affected child. Radioautography performed on her fibroblasts with [^{3}H]hypoxanthine had shown two populations of cells, one with HGPRT and one without, as would be expected for the carrier of a sex-linked recessive trait by random inactivation of one X chromosome at an early stage of somatic development proposed in the Lyon hypothesis (*8*). Amniotic fluid (approximately 20 ml) was obtained in the 18th week of her third pregnancy without incident by transabdominal amniocentesis. A portion of the cells was examined for heterochromatin bodies. In addition, cells were cultured in 40 percent fetal calf serum and a mixture of medium 199 and medium NCTC 109 (1 : 1) (*9*) for radioautographic studies of their ability to fix [^{3}H]hypoxanthine. Accumulation of the radioactive label requires the presence of HGPRT, while lack of accumulation of radioactivity in the cells reflects absence of the enzyme (*6*).

Examination of the amniotic cells failed to disclose cells that contained sex chromatin, an indication that the fetus was male and that it had a 50 percent chance of expressing the disease. This was confirmed by the consistent recovery of male karyotype in amniotic cells cultured for 2 weeks. After the cells were grown in culture for 3 to 4 weeks they were subjected to radioautography with [^{3}H]hypoxanthine (*6*). Cul-

Table 1. Activity of hypoxanthine-guanine and adenine phosphoribosyltransferases in a fetus with the Lesch-Nyhan syndrome and in two other fetuses aborted for other reasons. Tissues were homogenized at 0°C in 0.05*M* tris buffer, *p*H 7.4, and 0.005*M* $MgCl_2$. Homogenates were dialyzed for 2 hours in the cold against 200 volumes of 0.01*M* tris buffer, *p*H 7.4, containing 0.005*M* $MgCl_2$ with a change of buffer at 1 hour. Assay tubes contained 50 μl of sample; 10 μl of 5-phosphoribosyl 1-pyrophosphate (PRPP), 9 m*M*; 10 μl of 0.4*M* tris buffer, *p*H 7.4, which was 0.04*M* with respect to $MgCl_2$; either 15 μl of [8-^{14}C]hypoxanthine (3.7 m*M*, 4 mc/mmole) or 15 μl of [8-^{14}C]adenine (1.9 m*M*; 17.3 mc/mmole); and 15 μl of water. Incubation was at 37°C for 20 minutes. Reactions were stopped by the addition of 10 μl of cold 42 percent perchloric acid with immersion in an ice bath. The tubes were brought to neutrality by addition of 10 μl of 0.5*M* tris buffer, *p*H 7.0, and 10 μl of 4.4*M* potassium hydroxide. Assay tubes were then centrifuged at 4000 rev/min for 10 minutes, and 3 μl of the supernatant was applied to a thin-layer chromatography cellulose sheet previously treated with 12 μg of the appropriate purine carriers (inosinic acid, inosine, and hypoxanthine for the HGPRT enzyme assay). The plates were developed for 10 minutes in 1.6*M* LiCl. The nucleotide and nucleoside spots were identified under ultraviolet light and cut out; the radioactivity was counted in toluene phosphor with 160 ml of Liquifluor per liter. Protein was determined by the method of Lowry *et al.* (*14*). The lower limit of detection of the assay was: the conversion of 0.3 nmole of [^{14}C]hypoxanthine to [^{14}C]inosine acid and the conversion of 0.08 nmole of [^{14}C]adenine to [^{14}C]adenylic acid. A control tube containing 10 μl of 0.1*M* EDTA instead of PRPP was run for each assay. Subtraction of the sum of the radioactivity in inosinic acid and inosine in this tube from the sum of the radioactivity in inosinic acid and inosine in the test tube gave the PRPP-dependent activity. All assays were performed in duplicate. HGPRT and APRT activities are expressed as the number of nanomoles of substrate converted per milligram of protein per hour at 37°C.

Tissue	HGPRT activity after delivery			APRT activity		
	Lesch-Nyhan	Normal		Lesch-Nyhan	Normal	
	34 hr	24 hr	48 hr	34 hr	24 hr	48 hr
Basal ganglia	< 0.3	59.7	30.6	9.0	1.2	< 0.08
Cortex	< 0.3	83.5	40.1	1.0	1.2	< 0.08
Cerebellum	< 0.3	44.2	41.3	2.6	1.4	< 0.08
Testis	< 0.3	67.0	24.0	1.6	1.2	< 0.08
Blood	< 0.3	11.2		11.2	1.4	
Skin	< 0.3	40.0	23.9	14.6	1.6	< 0.08