These experiments indicate that cellular aggregation of the dissociated, glutaraldehyde-fixed, sponge cells depends primarily on the presence of intact disulfide groups and on protein integrity. The importance of the dithiol groups in the process suggests the need for an investigation of cysteine and disulfide linkages in the cell-binding protein. It is suggestive that our amino-acid analysis of acid hydrolyzates of six sponge extracts revealed the presence of cysteic acid and cysteine (*18*). Margoliash *et al.* (*9*) found that each molecule of glycoprotein in their purified aggregating principle contained 3 moles of sulfate. However, presence of cysteine was not determined.

Although none of the glycosidases destroyed the activity of the crude extract, the participation of sugars in the mechanism of the cell aggregation cannot be ruled out definitively because release of sugars has not been shown to occur without impairing the aggregating activity of the extract. However, because one of the enzyme mixtures (clostridial glycosidases) used is very active in removing sugars in other related cell-surface systems (*14, 19*), one could assume that such a removal, complete or incomplete, took place in our material.

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Spontaneous Mutation Rates at Five Coat-Color Loci in Mice

Abstract. *Examination of 1.5 million mice yielded natural mutation rates estimated from 5.2 million gene reproductions at five specific coat-color loci. The average rates were 11.1 × 10^{-6} for forward mutations and 2.7 × 10^{-6} for reverse mutations. Differences between the frequencies of mutations at the individual loci were evident.*

Spontaneous or natural mutation rates are the rates of the occurrence of gene changes under normal conditions, excluding the deliberate use of mutagenic agents. Mutations may be either somatic or germinal. Germinal mutations occur in any cell of the germ line and result in a new allele not present in either parent. Somatic mutations involve body cells in any stage of development in an individual, and these cannot be transmitted to the next generation if they occur after the germ cells have been differentiated. The random changes induced by germinal mutations create an alteration in the genetic constitution of an individual and thus increase the genetic variability of a population. The change may be perpetuated for many generations and may, under the proper conditions of natural selection, appreciably alter the characteristics of a population. Information concerning the rate at which these gene changes occur under normal conditions can be obtained by studying specific loci at which a mutation is detectable.

The forward (wild-type to mutant) and reverse (recessive mutant to wild-type or to mutant of higher dominance) spontaneous mutation rates at five specific coat-color loci of mice are under investigation at the Jackson Laboratory (*1*). The mice in 18 inbred strains maintained by brother-sister matings and six F_1 hybrids produced by crossing various pairs of these strains are examined two to four times between birth and weaning for deviations from the expected coat color and other external characteristics. The variant mice are scored as mutations only if the trait is transmitted, as determined by genetic tests. Fifteen of the strains are homozygous for specific coat-color alleles, and, in particular, sufficient numbers of mice are reared and examined for mutations at the nonagouti (*a*), brown (*b*), albino (*c*), dilute (*d*), and leaden (*ln*) loci to warrant the calculation of reverse spontaneous mutation rates at these loci. In addition, these five loci are tested for forward mutations in the F_1 hybrids produced by crosses between wild-type and mutant-carrying strains.

This report summarizes the findings, based on an examination of 1.5 million mice for 5.2 million gene reproductions at the five specific coat-color loci, collected between August 1963 and January 1965. The strains, their coat-color genotypes, and the numbers of mice examined in each strain are shown in Table 1. A reverse mutation at any of these loci in the inbred strains will be visibly detectable in the offspring of the generation following its occurrence. The number of mutations m can thus be used to calculate directly the reverse mutation rate, v, by $v = m/2n$, where n is the number of animals examined. For instance, the DBA/2J strain yields information on the *a*, *b*, and *d* loci. The number of animals examined in this strain is included in the n used in calculating the

rates for each of these loci. On the other hand, A/He and the seven other albino strains yield information for only the *c* locus, since the expression of the other coat-color genes, say of *a* and *b* in the A/He, is masked in the albino offspring. The hybrids also furnish information about reverse mutations where the two strains crossed possess the recessive allele at the same locus. Since the two gametes that unite to produce the offspring both carry the recessive allele, two gene reproductions are tested in each mouse examined.

Forward mutations at each of these five loci are detectable in the F_1 hybrids, and the number of mutations can again be used directly to calculate the forward mutation rate, u, by $u = m/n$. In the case of forward mutations, each mating tests one gene reproduction, since only one of the two uniting gametes carry the wild-type allele at the locus. The albino CAF_1 gives information at neither the *a* nor *b* loci. All matings are pedigreed so a single mutational event will not be counted more than once if it appears in two or more collateral lines. This problem has not occurred to date, nor is there any evidence for mutations occurring in clusters.

Table 1. Numbers of mice examined and the coat-color genotypes of the inbred strains and F_1 hybrids used to estimate spontaneous mutation rates at five coat-color loci. Includes data given in earlier paper (*1*). Abbreviations: *a*, nonagouti; *b*, brown; *c*, albino; *d*, dilute; *ln*, leaden; A^w, white-bellied agouti; c^{ch}, chinchilla; +, wild-type allele at each locus.

Strain	Coat-color genotype				No. of mice examined
	Inbreds				
A/HeJ	*a/a*	*b/b*	*c/c*		37,047
A/J	*a/a*	*b/b*	*c/c*		119,742
AKR/J	*a/a*		*c/c*		157,784
BALB/cJ		*b/b*	*c/c*		54,960
C57BL/6J	*a/a*				357,656
C57BL/10J	*a/a*				11,062
C57BR/cdJ	*a/a*	*b/b*			7,003
C57L/J	*a/a*	*b/b*		*ln/ln*	47,314
C58J	*a/a*				9,581
DBA/1J	*a/a*	*b/b*		*d/d*	45,354
DBA/2J	*a/a*	*b/b*		*d/d*	246,326
RF/J			*c/c*		10,167
SJL/J			*c/c*		12,073
SWR/J			*c/c*		7,122
129/J	A^w/A^w		c^{ch}/c^{ch}		14,636
	Hybrids				
$AKD2F_1$	*a/a*	*b/+*	*c/+*	*d/+*	19,669
$B6AF_1$	*a/a*	*b/+*	*c/+*		16,965
$B6D2F_1$	*a/a*	*b/+*		*d/+*	226,940
CAF_1	*a/+*	*b/b*	*c/c*		32,555
$C3D2F_1$	*a/+*	*b/+*		*d/+*	14,066
LAF_1	*a/a*	*b/b*	*c/+*	*ln/+*	66,291
Total					1,514,313

Table 2. Estimates of the spontaneous mutation rates at five specific coat-color loci in mice.

Locus	No. of gametes tested	No. of mutations	Mutation rates* (multiplied by 10^6)	95% confidence limits† (multiplied by 10^6)
		Forward		
a	14,066	1	71.1	1.8 to 396.0
b	277,640	0	0	0 to 13.3
c	102,925	1	9.7	0.2 to 54.1
d	260,675	5	19.2	6.2 to 44.8
ln	66,291	1	15.1	0.4 to 84.0
Total	721,597	8	11.1	4.8 to 21.8
		Reverse		
a	2,108,322	10	4.7	2.3 to 8.7
b	824,576	0	0	0 to 4.5
c	862,900	0	0	0 to 4.3
d	583,360	2	3.4	0.4 to 12.4
ln	94,628	0	0	0 to 39.0
Total	4,473,786	12	2.7	1.4 to 4.7

* Mutations per locus per gamete. † Stevens (*5*).

Table 2 summarizes the number of gametes or gene reproductions tested for forward and reverse mutations at each of the five loci, and the estimates of the spontaneous mutation rates. Of the eight forward mutations found, one was from + to A^y (yellow), one from + to c^p (platinum), one from + to *ln*, one from + to d^s (slight dilute), four from + to d^l (dilute lethal). Of the 12 reverse mutations, eight were from *a* to a^t (black and tan), two from *a* to A^w (white-bellied agouti), and two from *d* to +.

The average forward mutation rate at the five loci was 11.1×10^{-6} mutation per locus per gamete, which is considerably higher than the average reverse mutation rate of 2.7×10^{-6}. This fourfold difference was statistically significant at the 1-percent level of probability. The average forward mutation rate was slightly higher, but not significantly so, than the rate of 10×10^{-6} reported by Searle and Phillips (*2*), and 7.5×10^{-6} reported by Russell (*3*) for seven specific loci in the mouse (*a, b, c, d, p, s,* and *se*).

It appears that differences between the rates at the specific loci will be found. The rate of reverse mutation at the *a* locus is significantly higher ($P < 0.05$) than the rates at the *b* and *c* loci. Differences between the forward rates are not so evident because of the smaller number of mice examined and the consequently larger confidence interval. However, the *b* locus appears not to be as mutable as the other four loci.

It has been demonstrated that the incidences of some human diseases, still births, and neonatal deaths are related to the age of the father (*4*). In the mice of this study, the parental age at the time of littering was calculated for all the reverse mutations and compared with the average parental age for litters that did not bear mutants. The average parental age for a sample of 5920 matings that produced litters containing only normal offspring was 127 ± 1 days, while the average parental age for 23 litters bearing dominant mutants was 145 ± 10 days. Although mutations occurred in litters of older parents, the differences between litters of older parents and those of younger parents were not statistically significant.

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Polymorphism of Lactate Dehydrogenase in Gelada Baboons

Abstract. *In a group of 21 gelada baboons, three different lactate dehydrogenase patterns were observed after starch-gel electrophoresis of tissue extracts. The patterns indicate that a mutation has occurred in this population in the gene responsible for the A-subunit. The normal and variant homozygotes each contain five isozymes, those containing the A subunit having characteristically different electrophoretic mobilities. The heterozygote contains the expected 15 isozymes.*

The five isozymes of lactate dehydrogenase (LDH) usually observed in mammals are tetramers formed by combinations of two different polypeptide subunits, A and B (*1*). Genetic evidence from deer mice (*2*) and man (*3, 4*) indicates that synthesis of the polypeptides is controlled by two genetic loci. Genetically determined variants of human LDH have been described (*3, 4*) in which alterations in electrophoretic mobility occurred in either the A- or B-subunits, and, ex-