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 $11.1 \times 10^{-6}$ mutation per locus per gamete, which is considerably higher than the average reverse mutation rate of $2.7 \times 10^{-6}$. 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 $10 \times 10^{-6}$ reported by Searle and Phillips (*2*), and $7.5 \times 10^{-6}$ 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-

cept for one case these variants were heterozygous individuals. The exception, an isolated case (*4*), was interpreted as a homozygous variant. So far, studies in humans or other higher primates have failed to uncover both the heterozygote and homozygous variant in any single pedigree. We now report genetic variation in LDH within a small group of gelada baboons (*Theropithecus gelada*), in which both heterozygous and homozygous variants are represented. The prevalent LDH pattern in these animals was identical to that found in humans and other catarrhine primates and is considered to be the normal homozygote.

The baboon tissues were shipped to us in dry ice by the Delta Regional Primate Research Center in Covington, Louisiana. The 21 animals (13 females and 8 males) were captured within a narrow radius in the vicinity of three villages in the southern part of Dessie Province, Ethiopia: Debre Sina, Debre Berhon, and Robi (*5*). Starch gels were prepared at a concentration of 12 percent in *p*H 7.0 buffer composed of 0.0126*M* tris and 0.0037*M* citric acid. Two milliliters of 0.006*M* nicotinamide adenine dinucleotide (NAD) were added to the starch solution after deaeration. The "bridge" solution consisted of 0.378*M* tris-0.141*M* citric acid at *p*H 6.0. After homogenization in 0.05*M* tris-maleate, *p*H 7.5, the livers and other tissues were centrifuged at 20,000*g* for 20 minutes. The supernatants were applied to the gels and subjected to electrophoresis overnight at 3°C at a gradient of 4 volt/cm. Day-to-day uniformity of isozyme migration was controlled by inclusion of dyed serum albumin in one of the sample slots in each gel. After electrophoresis the gels were stained for LDH activity (*6*).

Two types of variant LDH patterns were observed in the 21 animals examined (Fig. 1). Variant I occurred in only one baboon (a female) and contained the usual five main bands. The LDH-1 of this variant had a gel position identical with that of normal LDH-1, whereas LDH-2, LDH-3, and LDH-4 had distinctly more anodal positions than the corresponding normal bands had. In both the normal and variant I, LDH-5 moved to the cathode but the variant-I band had the more anodal position. The degree of anodal shift in the variant bands was greatest in LDH-5 and least in LDH-2. Shadow bands directly anodal to LDH-3 of the normal and variant I are indicated by arrows in Fig. 1. Although such bands are frequently encountered in LDH patterns their significance is not clear.

The other variant pattern, here called variant II, was observed in seven of the 21 animals. The LDH-1 of this variant was also in the normal position (Fig. 1). The LDH-2 consisted of two sub-bands of equal intensity, the more anodal being identical in position with LDH-2 of variant I, the other being in a position corresponding to that of normal LDH-2. The LDH-3 was composed of three sub-bands, the middle one more intensely stained than the other two. The most anodal sub-band had a gel position corresponding to LDH-3 of variant I, and the slowest one was identical in position with normal LDH-3. In LDH-4 four sub-bands were present, the inner two being more intensely stained than the outer two. The slowest sub-band had the same gel position as that of normal LDH-4, and the fastest one was identical in position with LDH-4 of variant I. The LDH-5 was composed of five sub-bands. The two outer sub-bands showed much less staining reaction than the middle three sub-bands. The most anodal sub-band was identical in position with LDH-5 of variant I, and the most cathodal sub-band had the same gel position as that of the normal LDH-5.

The pattern of sub-bands in LDH-2, LDH-3, LDH-4, and LDH-5 of variant II (Fig. 1) was the same in brain, lens, retina, erythrocytes, and plasma of this variant. Thus, the sub-bands are not a tissue-specific phenomenon like that described for mouse liver (*7*). Apoenzyme-coenzyme dissociation of the type described for mouse liver LDH (*8*) can also give rise to sub-bands. However, the sub-band patterns are altered when NAD or β-mercaptoethanol has been incorporated into the gel prior to electrophoresis. In our experiment, incorporation of NAD into the gels failed to alter the sub-band pattern in variant II.

The pattern of sub-bands in variant II is best explained on a genetic basis, an interpretation supported by (i) the segregation of the sub-bands into groups of two, three, four, and five components corresponding to LDH-2, LDH-3, LDH-4, and LDH-5, respectively, and (ii) the identical positions of the particular sub-bands described above with those of LDH-2, LDH-3, LDH-4, and LDH-5 in the normal and variant I. We suggest that the LDH

Table 1. Theoretical composition of the LDH tetramers for the three patterns observed on starch gel.

| Normal | Heterozygote | Homozygous variant |
|---|---|---|
| | *LDH-1* | |
| BBBB | BBBB | BBBB |
| | *LDH-2* | |
| BBBA | BBBA′ | BBBA′ |
| | BBBA | |
| | *LDH-3* | |
| | BBA′A′ | |
| | BBAA | BBA′A′ |
| BBAA | BBAA | |
| | *LDH-4* | |
| | BA′A′A′ | BA′A′A′ |
| | BAA′A′ | |
| | BAAA′ | |
| BAAA | BAAA | |
| | *LDH-5* | |
| | A′A′A′A′ | A′A′A′A′ |
| | AA′A′A′ | |
| | AAA′A′ | |
| | AAAA′ | |
| AAAA | AAAA | |

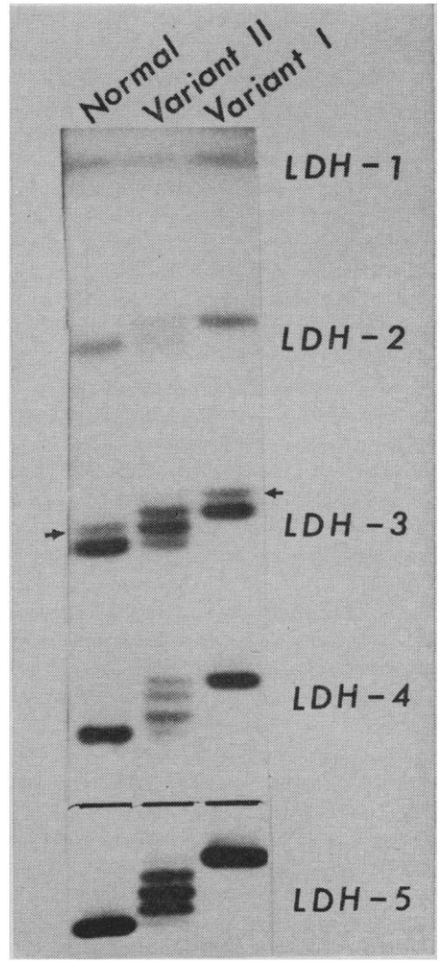


Fig. 1. Starch-gel electrophoresis patterns of liver LDH of the two types of variants and of a normal animal. Anode direction is upward from slots.

patterns for variant I and variant II are the result of a mutant A subunit and that the sub-bands in variant II are tetramers composed of combinations of normal A, mutant A (A′), and normal B subunits as shown in Table 1. Thus, variant II is the heterozygote and variant I the homozygous variant. Support for this hypothesis was obtained in dissociation-recombination experiments as originally described (*9*). When a mixture of isolated LDH-1 from a normal baboon and LDH-5 isolated from variant II was frozen and thawed in a medium containing 0.25*M* sodium chloride and 0.1*M* sodium phosphate, *p*H 7.0, the resulting LDH pattern on the starch gel was the same as the pattern of variant-II phenotype pattern. Recombinations of a mixture of isolated normal LDH-1, normal LDH-5, and variant-I LDH-5 could not be made because of lack of adequate amounts of variant-I tissue. The Delta Regional Primate Research Center is attempting to breed male gelada variant II with a female variant II, and it may eventually be possible to test the genetic hypothesis by examination of the progeny.

The incidence of variant LDH types in male and female gelada baboons is not significantly different, although of eight males only one is the type II variant, whereas of 13 females six are type II variants and one is type I variant. The high frequency of LDH variants in the total gelada group (8 out of 21) is in contrast to the rarity of LDH variants (15 out of 5158) in man (*10*). In other catarrhine primates two of 215 studied in our laboratory showed variants in the A subunit of LDH. These two were among 19 Thailand cynomolgus monkeys (*Macaca irus*), but we found no variants among the 107 animals of the same species from the Philippines. Among 17 animals of various Ceboid species we observed a variant LDH pattern of the heterozygous type in one of two Titi specimens.

In the gelada group, the A′ subunit variant is apparently either a case of local genetic drift or an example of more widespread polymorphism of an autosomal LDH-determining gene among the populations of *Theropithecus gelada*.

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## Medium for Selective Isolation of Cryptococcus neoformans

Abstract. *A medium has been developed that permits the selective recovery of* Cryptococcus neoformans *from heavily contaminated materials. It employs creatinine as a nitrogen source, diphenyl ($C_6H_5C_6H_5$) and chloramphenicol as mold and bacterial inhibitors, and* Guizotia abyssinica *seed extract as a specific color marker. The medium has proved to be effective in the direct isolation of* Cryptococcus neoformans *from pigeon nests and from the air.*

The development and use of a selective isolation medium for human pathogenic fungi has greatly facilitated diagnostic, ecological, and epidemiological studies (*1*). By incorporating the antibacterial agent chloramphenicol and the antifungal agent cycloheximide into Sabouraud's dextrose agar, pure cultures of most pathogenic fungi are obtainable from heavily contaminated substrata. One of the limitations of this otherwise practical medium lies in its failure to select for *Cryptococcus neoformans*, a yeast that is extremely sensitive to the inhibitory action of cycloheximide.

Staib (*2*) discovered that the purine creatinine, present in bird manure, is readily assimilated by *C. neoformans* and not by other species of the genus *Cryptococcus* or members of the following genera of yeasts: *Bullera, Candida, Debaryomyces, Lipomyces, Pichia, Rhodotorula, Torulopsis*, and *Trichosporon*. One isolate of *Cryptococcus laurentii* proved to be exceptional in that it assimilated creatinine. In addition he observed that a pigment derived from *Guizotia abyssinica* seeds (constituents of canary feed) was selectively absorbed by *C. neoformans*. Staib expressed the hope that these two properties of *C. neoformans* could be utilized in the detection and identification of this important human pathogen. *C. neoformans* grows well on a simple agar medium we made with glucose, creatinine, extract of *Guizotia abyssinica* seed, and chloramphenicol; and the colonies become distinctly brown. However, when plates of this medium were inoculated with material from pigeon nests, the medium was rapidly overgrown with saprophytic molds to such an extent that the *C. neoformans* present could not be isolated. A mold inhibitor was needed. Hertz and Levine (*3*) reported that diphenyl ($C_6H_5C_6H_5$) at a concentration of 100 parts per million suppresses the growth of a large variety of molds but does not inhibit the growth of yeasts. *C. neoformans* was not tested.

We determined that various strains of *C. neoformans* are not inhibited by diphenyl at 100 parts per million. Accordingly, a medium with the following composition was prepared: Glucose, 10.0 g; creatinine, 780 mg; chloramphenicol, 50 mg; diphenyl (dissolved in 10 ml of 95 percent ethanol), 100 mg; *Guizotia abyssinica* extract (*4*), 200 ml; distilled water, 800 ml; and agar, 20.0 g.

Plates of this medium when inoculated with pure cultures of *C. neoformans* supported good growth of pigmented colonies. At 37°C most isolates of *C. laurentii* did not develop; the few isolates that grew on this medium at 37°C did not become brown. *Candida albicans* cultures grew well but remained unpigmented. Several isolations of *C. neoformans* from a series of pigeon nests and from the air have demonstrated the efficacy of the medium.

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