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Histocompatibility mutations in mice: chemical induction and linkage with the H-2 locus

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SUMMARY

Young mice of the strains A/Y and B10.D2 were repeatedly treated with diethylsulphate (DES) in different doses (36 to 1100 mg/kg). At the age of 9 weeks they were mated to females of the strains A.CA ('A' group) and C57BL/10Eg ('D' group) respectively. 2101 progeny of these matings were tested for histocompatibility by skin grafting. The spontaneous H-mutation rates were $6{\cdot}96 \times 10^{-4}$ per gamete in the A group and $9{\cdot}6 \times 10^{-4}$ per gamete in D group. In progeny of treated males the H-mutation rates were 0 in A group and $5{\cdot}79 \times 10^{-3}$ per gamete in D group, showing apparent effect of paternal DES treatment on mutation frequency in the last group. Two mutations of the *H-2* locus were found, which together with the other three *H-2* mutations published so far yielded a mutation rate of $5{\cdot}18 \times 10^{-4}$ per gamete. The mutation rate of the *H-2* locus is higher than the expected rate per H-locus, indicating a great genetic complexity of *H-2*.

1. INTRODUCTION

Immunogenetic studies in the mouse have, over the past decades, yielded great progress in our knowledge of histocompatibility (H) loci in this species (Snell & Stimpfling, 1966). Despite this progress the organization and function of the whole H-gene system as well as the fine structure of individual H-loci yet remains uncertain.

Recently the data on histocompatibility mutations in mice were used to interpret the organization of the H-gene system and also the origin and function of the H-genes (Bailey, 1966, 1968). Besides, the study of mutation of a particular H-locus could serve as a useful method for the examination of its genetic structure. We feel that the latter task is quite feasible in respect of the *H-2* locus, which is known to be genetically complex.

The first attempt to induce H-mutations in mice by chronically irradiating mated pairs throughout a number of successive generations before graft-testing was made by Godfrey & Searle (1963). However, no mutations were detected in their experiments. Bailey & Kohn (1965) and Bailey (1966) tested for H-mutations a large sample of (C57BL/6JN × BALB/cAnN) F_1 mice which were progeny of acutely irradiated or unirradiated inbred mice. The estimation of spontaneous H-mutation

rate per gamete based on their data is $5{\cdot}4 \times 10^{-3}$; there was no detectable increase of mutation rate after paternal irradiation. The authors succeeded in isolating a number of mutant H-alleles, including two new *H-2* alleles (Bailey, 1969, 1970). Egorov (1967) also found a new mutant *H-2* allele, using hybrids of congenic resistant (CR) strain mice, C57BL/10Sn and B10.D2, differing at *H-2* only.

The present paper reports the results of our experiments aimed to test the mutagenic action of an alkylating agent, diethysulphate (DES), on the mouse H-loci, and to isolate new *H-2* mutant alleles.

2. MATERIALS AND METHODS

The mice of the inbred strain A/SnKlY ($H\text{-}2^a$), abbreviation A, and its CR partner A.CA ($H\text{-}2^f$), as well as of the inbred strain C57BL/10ScSnEg ($H\text{-}2^b$), abbreviation B10, and its CR partner B10.D2 ($H\text{-}2^d$) were used in our experiments. The history of the strains listed is given in Staats (1969).

A and B10.D2 homozygous mice were injected intraperitoneally with DES on the first day after birth followed by five injections at weekly intervals, the last one at the age of 35 days. The mice of the first series (series symbol 1) were given 6 mg/kg per injection, total DES dose 36 mg/kg; the mice of the second series (symbol 2) were given 100 mg/kg per injection, total dose 600 mg/kg; the mice of the third series (symbol 3) were given 100 mg/kg for the first injection and then 200 mg/kg per injection, total dose 1100 mg/kg. The mice of the control series (symbol 0) were not treated. The group of experiments with A males were designated by symbol A and the group with B10.D2 males were designated by symbol D. Each male was designated by the appropriate series and group symbols followed by a number; for example, 1A1, 1A2, etc.

At the age of 60–70 days every A male was mated to three A.CA females in a separate cage and every B10.D2 male was mated to three B10 females. They were allowed to breed for one year (or up to earlier death). Any infertile female was replaced by a new one.

Matings were started 60 days after the first DES injection and 25 days after the last injection. The majority of tested F_1 mice were sired when their fathers were 3 months old or older; that is, over 55 days after the last injection. Thus, the sperm tested descended from treated pre-meiotic and meiotic cell stages.

The combined F_1 progeny of sibs and half-sibs from one male were tested for histocompatibility when 6–8 weeks old by skin grafting. Two grafts from each offspring were placed on two different recipients, which were the progeny of the same male. The grafts were exchanged in a 'reciprocal circle' system used by Bailey & Kohn (1965). In this system, graft rejection should indicate the existence of mutations. If the standard F_1 hybrids reject the grafts from a tested mouse of the same kind, the latter should be classified as a gain (G) mutation bearer; if the tested mouse rejects the grafts from standard F_1 hybrids, it should be classified as a loss (L) mutation bearer; when both types of rejection occur the tested mouse should be classified as a gain and loss (GL) mutation bearer. A gain at any H-locus

could be detected by the method used. A loss at a locus which has identical alleles in the two parents will pass undetected. As the parental pairs (A.CA and A as well as B10 and B10.D2) differ in the *H-2* locus only, any L (and GL) mutations detected should be attributable to this locus.

Every mutant mouse was assigned a number used also as a provisional symbol of the corresponding mutant H-allele.

Suspected G and GL mutants were re-tested by placing their skin grafts on five or more standard F_1 hybrids. Type G mutant (B10 × B10.D2) F_1 hybrids were mated to B10 (or B10.D2) mice and progeny of this backcross were tested for mutant/normal phenotype by placing their grafts on standard F_1 hybrids. For *H-2* typing each backcross mouse received a B10.D2 skin graft (or B10 graft when progeny of F_1 × B10.D2 mating). Linkage of a mutant allele with *H-2* could be evaluated from the results of these two tests.

Suspected L mutants were re-tested by grafts of the standard F_1 donors. Type L mutant (B10 × B10.D2) F_1 hybrids were mated to B10 mice and the progeny of this backcross were challenged by B10.D2 and mutant parent grafts. When an antigenic component (or components) of the $H\text{-}2^d$ allele not shared by the $H\text{-}2^b$ allele was lost, all the backcross offspring should reject B10.D2 grafts while approximately one half of them should accept the mutant parent grafts. If there were no difference in graft survival, the mutant mouse was mated to a B10.D2 partner and their progeny were tested for the loss of $H\text{-}2^b$ components by B10 and mutant parent grafts.

Mutant (A.CA × A) F_1 hybrids were mated to A or A.CA mice and tested by the appropriate skin grafts.

All grafts were full thickness tailskin grafts placed on the dorsum of recipients. The grafts were 7–12 mm^2 size. The recipients were unbandaged on the 8th–9th postoperative days and were scored at frequent intervals for 30 days in primary G and L tests and for 80 days in tests for inheritance.

3. RESULTS

The total number of F_1 mice tested for histocompatibility was 2101 (Table 1). Sixteen mutants were detected. Among 718 (A.CA × A) F_1 hybrids tested (series 0 to 3A) there was only one mutant detected. Thus, DES treatment of the fathers had no apparent effect on the incidence of H-mutation in the A group of experiments. There was one mutant detected among 519 (B10 × B10.D2) F_1 tested hybrids of the control series (OD), and 14 mutants detected among 864 tested hybrids of the treated series (1 to 3D). The difference is statistically significant (at $P < 0{\cdot}05$), indicating the possible effect of mutagenic treatment of the fathers of mice in (1–3) D series. The incidence of mutants seems to be a little lower in males than in females but the sex influence is statistically insignificant.

In the progenies of some B10.D2-treated males more than one mutant appeared (Table 2), but the number of mutants did not exceed 1 per 25 tested offspring of one father. Clustering of mutants appeared in progeny of males 6 months old or older.

Table 1. *Spontaneous and induced mutations and mutation rates at H-loci**

Series	DES dose (mg/kg)	No. of offspring tested	No. of mutants found† G	L	GL	Total	No. of mutations‡	Mutation rates (× 10⁴)	95% confidence limits (× 10⁴)
0A	0	204	0	0	0	0	0	—	—
1A	36	343	0	0	1	1	1§	—	—
2A	600	98	0	0	0	0	0	—	—
3A	1100	73	0	0	0	0	0	—	—
(0–3)A	Subtotal I	718	0	0	1	1	1§	6·96	0·004–27
0D	0	519	1	0	0	1	1	9·6	0·002–37
1D	36	471	6	2	0	8	7	—	—
2D	600	280	4	0	0	4	1	—	—
3D	1100	113	2	0	0	2	2	—	—
(1–3)D	Subtotal II	864	12	2	0	14	10	57·9	28–100
	Total	2101	13	2	1	16	12	—	—

* In A series, homozygous A males were injected intraperitoneally with diethylsulphate (DES) on the first day after birth followed by five injections at weekly intervals, the total doses are given in the table. At the age of 60–70 days A males were mated to A.CA females to produce F_1 offspring to be skin graft tested. In D series, B10.D2 males were treated and mated to C57BL/10 females.

† G = gain, L = loss, GL = gain and loss in tissue antigenic specificity.

‡ For explanation see text.

§ Spontaneous mutation of the *H-2ᶠ* allele of an untreated A.CA female.

Table 2. *Mutants in progeny of individual B10.D2 males*

Series and male symbol	No. of offspring tested*	No. of mutants*	Type of mutants	No. of mutations†
1D1	82, 0 (82)	1, 0 (1)	G	1
1D2	51, 56 (107)	2, 0 (2)	G, L	2
1D3	78, 64 (142)	0, 2 (2)	G, G	2
1D4	78, 62 (140)	0, 3 (3)	G, L, G	2
2D5	45, 56 (101)	0, 3 (3)	G, G, G	0
2D6	83, 78 (161)	1, 0 (1)	G	1
2D7	18, 0 (18)	0	—	0
3D8	25, 0 (25)	1, 0 (1)	G	1
3D9	65, 23 (88)	1, 0 (1)	G	1
Total	525, 339 (864)	6, 8 (14)	—	10

* The sequence of numbers indicate offspring (mutants) sired when the male was under 6 months and those sired when the male was older than 6 months, the total numbers of offspring and mutants are given in parentheses.

† For explanation see text.

The data suggest that no B10.D2 fathers tested were heterozygous mutant carriers. Mothers giving rise to clusters cannot be identified with certainty because there were three or more mothers per cage. Prolonged serial skin-grafting tests of B10-strain mice and their derived hybrids constantly showed H-loci homogeneity in this strain.

Table 3 summarizes the data on tests of 'strength' of the detected incompatibilities and their inheritance. The majority of incompatibilities detected were of a

Table 3. *Strengths of the detected incompatibilities and tests of their inheritance*

Series and male symbol	Mutant symbol	Mutant sex	Fate of standard grafts on the mutant: Total grafts	Fate of standard grafts on the mutant: Grafts rejected*	Fate of the mutant's grafts on standard hybrids: Total grafts	Fate of the mutant's grafts on standard hybrids: Grafts rejected*	Mutant type	No. of BC_1 descendants tested	No. of mutant carriers	Proven carriers (%)	No. of carriers' grafts on standard hybrids	Rejection (%)
1A3	*506*	Female	2	2 (21)	15	15 (12–33)	GL	37	21	56·8	45	100
1D1	*507*	Female	2	0	8	4 (15–32)	G	54	34	62·9	161	65·8
1D2	*508*	Male	2	0	12	4 (17–31)	G	17	14	82·4	70	61·4
1D2	*509*	Female	2	2 (60)	2	0	L	9	0	0	—	—
1D3	*510*	Female	2	0	11	8 (33–35)	G	16	9	56·3	43	51·2
1D3	*511*	Female	2	0	9	9 (9–20)	G	15	12	80·0	64	75·0
1D4	*512*	Female	2	0	7	5 (41–54)	G	8	0	0	—	—
1D4	*513*	Female	2	2 (13)	2	0	L†	6	0	0	—	—
1D4	*514*	Female	2	0	6	5 (27–54)	G	8	0	0	—	—
2D5	*515*	Male	2	0	8	4 (9–14)	G	17	2	11·8	13	30·6
2D5	*516*	Male	2	0	7	3 (9–13)	G	35	12	34·3	44	79·5
2D5	*517*	Male	2	0	7	2 (9, 21)	G	16	4	25·0	26	30·8
2D6	*518*	Male	2	0	12	11 (13–14)	G	38	0	0	—	—
3D8	*519*	Male	2	0	11	5 (14–26)	G	28	6	21·4	32	28·1
3D9	*520*	Female	2	0	14	13 (9–21)	G	8	1	12·5	—	—
0D11	*521*	Female	2	0	13	5 (14–47)	G	10	1	10·0	—	—

* Graft survival time in parentheses.

† A histocompatibility mosaic: six grafts of the (B10 × B10.D2) F_1 female no. 513 were rejected by B10 mice; of six grafts on B10.D2 mice four were rejected on days 12 or 13 but two survived over 40 days. The mutant carriers were found among (BC_1 × BC_1) F_1 progeny.

Table 4. *Tests for allelism of* H-2^{fa}(506) *and* H-2^{a} *or* H-2^{f}

Crosses*	Progeny*					Total
	a/f	*fa/f*	*a/a*	*a/fa*	*fa/fa*	
a/fa × *f/f*	3	3	—	—	—	6
a/fa × *a/a*	—	—	13	18	—	31
a/fa × *a/fa*	—	—	9	19	14	42
a/fa × *fa/fa*	—	—	—	15	20	35
Total	3	3	22	52	34	114

* *a* = *H-2*^{a}; *f* = *H-2*^{f}; *fa* = *H-2*^{fa}. Histocompatibility phenotypes of the mice were tested by the skin-grafting technique.

'weak' type. We failed to find mutant phenotypes in progeny of three type-G mutants (*512*, *514* and *518*). The percentage of phenotypically mutant mice in progeny of other type-G mutants ranged from 12 to 82. It exceeded the expected 50 % in progeny of the mutant no. *508* statistically significantly (at $P < 0{\cdot}01$), and it was significantly (at $P < 0{\cdot}01$) lower than expected in progeny of the mutant no. *515*. Mutant alleles nos. *507*, *508*, *510*, *511*, *515*, *516*, *517* and *519* segregated independently of *H-2*. Linkage tests of the other alleles of type G were incomplete due to poor breeding of their carriers.

Two mice (nos. 509 and 513, Table 3) were originally classified as mutants of the L type. Incompatibility no. *509* has a long graft survival time – 60 days. No histo-incompatible descendants were derived from the female no. 509. Incompatibility no. *513* has a shorter graft survival time, 13 days. Moreover, the female no. 513 was shown to be a histocompatibility mosaic because six of her grafts were rapidly rejected on B10 hosts while of six grafts on B10.D2 hosts four were rejected on days 12 and 13 after grafting, but the remaining two grafts survived over 40 days (observation period). According to the laws of transplantation (Snell & Stimpfling, 1966) one should expect all the grafts of the F_1 donor to be rejected on parental strain mice. None of the six backcross offspring of the mating of the female no. *513* to a B10 male rejected the grafts of standard F_1 hybrids. Nevertheless few mutant bearers were detected among progeny of matings of those backcross mice. This odd type of incompatibility is still under investigation.

Female no. 506 was the only incompatible mouse among (A.CA × A) F_1 hybrids tested and it was classified as a mutant of GL type (Table 3). The exceptional female, *H-2*^{a}/*H-2*^{f}*506*/+, was mated to an A.CA male, *N-2*^{f}/*H-2*^{f} +/+, and produced six offspring which were challenged by A and 506 skin grafts. Three of them did not reject A grafts but rejected 506 grafts; three others rejected A grafts and did not reject 506 grafts. Later female no. 506 was mated to another male, this time of A strain, *H-2*^{a}/*H-2*^{a} +/+. Thirty-one offsprings produced were challenged by A.CA and 506 grafts. Thirteen of them rejected both A.CA and 506 grafts and 18 rejected A.CA grafts but did not reject 506 grafts. The data clearly showed *506* to be an *H-2* allele derived from *H-2*^{f} by mutation. Moreover, the mutation must be spontaneous because the *H-2*^{f}/*H-2*^{f} (A.CA) parent of female no. 506 was

not treated with DES. The proposed symbol of the mutant allele is $H\text{-}2^{f a}$ instead of the provisional symbol *506*. The inheritance of the mutant allele is shown in Table 4. The CR strain A.506 is now homozygous for this allele.

4. DISCUSSION

In the present study the F_1 hybrids of two CR strains differing only in the *H-2* locus and the short adjacent chromosome segment were used for the detection of H-mutations by the skin-grafting technique. The method used is a derivative of classic methods of study of H-genes introduced by Dr George D. Snell (1958). With the strain combinations used, L (and GL) mutations at the *H-2* locus could be distinguished immediately in the mice tested. The distinction between type G mutations at *H-2* and *non-H-2* loci is possible by linkage tests in the progeny of matings of the mutant mouse with the parent-strain mice.

The segregation of H-alleles in the strain tested due to residual heterozygosity or recently arisen mutations prevents an accurate estimation of mutation rates of H-loci (Bailey & Kohn, 1965). There was only one mutant in a sample of 718 (A.CA × A) F_1 hybrids tested and therefore the existence of residual heterozygosity in the parent strains is highly improbable at the present time. A decade ago residual heterozygosity was revealed in the A/Sn strain and its CR partner strains (Linder & Klein, 1960; Snell, 1960). Kindred (1963) also found some degree of genetic heterogeneity in her subline of A strain. Apparently, further inbreeding of the strains tested resulted in fixation of segregating alleles.

There were many mutants in a sample of (B10 × B10.D2) F_1 hybrids tested. The distribution of the number of mutants per progeny group (Table 2) showed that there were no mutant carriers among the B10.D2 fathers tested. Owing to the breeding system we were unable to identify B10 mothers giving rise to mutant clusters. Several years ago we tested B10 strain mice by skin grafting and found them to be homogeneous (Egorov & Medvedev, 1966). Repeated serial skin-grafting tests confirmed earlier results. However, some B10 females could be the heterozygous carriers of the recently arisen mutations. Theoretically, half of the descendants of such a carrier inherit the mutant allele. They might be detected as a cluster of phenotypically identical mutants in a progeny group. In fact, there were three phenotypically alike G mutants (nos. *515*, *516* and *517*) in the progeny of male 2D5 (Tables 2, 3). Unfortunately we failed to test the identity of their mutant alleles by skin grafts exchange (Counce *et al.* 1956; Egorov & Blandova, 1968) due to some technical trouble. The mutants of that cluster were excluded from our calculation of the mutation rates (Table 2).

There were three more mutant clusters in which mutants were phenotypically not alike (Tables 2, 3). Namely, in the progeny of male 1D2 there were the mutant no. *508* of type G and the mutant no. *509* of type L; in the progeny of male 1D3 there were two G mutants, nos. *510* and *511*, having different strengths of incompatibility; in the progeny of male 1D4 there were the mutant no. *513* of odd type L and two phenotypically similar G mutants, nos. *512* and *514*. As no histo-

incompatible descendants were derived from the last two mutants (Table 3) it is unlikely that the mutant allele was transmitted through their B10 mother. We treated them as two descendants of one mutation in the latest generation.

Our estimations of the spontaneous H-mutation rate are based on one mutation in each strain combination, A.CA × A and B10 × B10.D2, and therefore they should be treated as preliminary. The number of spontaneous mutations per gamete tested is estimated as $6{\cdot}96 \times 10^{-4}$ in the A series and $9{\cdot}6 \times 10^{-4}$ in the OD series (Table 1). Bailey (1966) recovered 17 H-mutations per 1567 (BALB/c × C57BL/6) F_1 hybrids tested, which yielded a spontaneous mutation rate of $5{\cdot}4 \times 10^{-3}$ mutations per gamete. The mutation frequency in Bailey's series is significantly (at $P < 0{\cdot}05$) higher than that in our A series and also higher (at $P \approx 0{\cdot}05$) than in the OD series. The difference could be attributed to different sensitivity of skin-grafting techniques employed in the two laboratories or to different mutability of the mouse strains tested.

No induced mutations were found in the A.CA × A strain combination, showing, perhaps, a very low mutability of those strains. In the B10 × B10.D2 strain combination ten mutations were found in the progeny of DES-treated males, series 1D, 2D, 3D (Tables 1, 2). Disregarding the DES dose delivered to fathers, the frequencies of mutations in the three series were similar. Malashenko (1971) obtained parallel dose-rate relationships in his experiments on DES induction of dominant lethals in mice. We do not know the reason for this relative mutagenic inefficiency of higher doses of DES in mice.

The mutation rate was calculated for the total of the treated series, (1–3)D, because they do not differ significantly. The number of mutations per gamete tested in the treated series is estimated as $5{\cdot}79 \times 10^{-3}$ (Table 1). The rate in the treated series is six times higher than the spontaneous rate in OD series ($9{\cdot}6 \times 10^{-4}$) and the difference is statistically significant at $P < 0{\cdot}05$. Recent studies from other laboratories (Godfrey & Searle, 1963; Bailey & Kohn, 1965; Bailey, 1966) showed that the number of H-mutations was not increased by parental X-ray treatment. Seemingly it is too early to speculate on the nature of this paradoxical difference in efficiency of DES and X-ray treatment.

The proportions of histo-incompatible descendants derived from the originally encountered histo-incompatible mice (Table 3) were usually not significantly different from the expected 50 %. In the case of mutation no. *508* the proportion was significantly (at $P < 0{\cdot}01$) greater than 50 %. Possibly the case could be attributed to better survival of the mutant heterozygous descendants over their normal homozygous sibs. In the case of the mutation no. *515* the proportion was significantly (at $P < 0{\cdot}01$) less than 50 %. There were no histo-incompatible mice in progeny of the mutant mice nos. 509, 512 and 514. After Bailey & Kohn (1965) the deficient proportions could be attributed to a low ability of weak antigens to elicit a rejection during the limited graft observation period.

This interpretation is not feasible in the case of male no. 518, which had rather a strong incompatibility: no incompatible mice were found in a sample of 38 offspring of this male (Table 3). One could speculate that the male was a mosaic, some

Table 5. *Mutations and mutation rates at the* H-2 *locus*

Strain combination	*H-2* alleles	No. of mice tested	No. of *H-2* muta-tions	Mutation rate ($\times 10^4$)	95 % con-fidence limits ($\times 10^4$)	Mutation	Refer-ences*
C57BL/10 × B10.D2	*b/d*	154	1	—	—	$d \rightarrow da$	1
C57BL × BALB/c	*b/d*	2572	2	—	—	$b \rightarrow ba$, $b \rightarrow bb$	2
C57BL/10 × B10.D2	*b/d*	1383	1	—	—	$b \rightarrow$?	3
A.CA × A	*f/a*	718	1	—	—	$f \rightarrow fa$	3
Total		4827	5	5·18	1·6–10·5		

* (1) Egorov (1967), (2) Bailey & Kohn (1965), Bailey (1969, 1970), (3) the present paper. The Table includes spontaneous and perhaps induced *H-2* mutations: alkylating agents were used in (1) and (3) and X-ray treatment in (2).

somatic tissues (and definitely the skin) of which could contain the mutant H-allele (and H-antigen) but the germ cells of which did not contain the mutant allele and, hence, did not transmit it to the progeny. Female no. 513, originally classified as an L mutant, was finally shown to be a histo-compatibility mosaic too: all the tested skin grafts of that female contained B10.D2 ($H\text{-}2^d$) antigens and in some grafts they were supplemented by B10 ($H\text{-}2^b$) antigens, but two grafts lacked the latter kind of antigens. The use of chemical mutagens in *Drosophila melanogaster* usually results in the production of mosaic or fractional mutants which are composed of mutant and normal tissue (Alderson, 1965; Epler, 1966; Jenkins, 1967*a*, *b*; Pelecanos & Alderson, 1964).

It was suggested that all the detected mutations of L and GL types should be mutations of the *H-2* locus. But in the female no. 509 a very weak antigen was lost by mutation and consequently we failed to study it further. Possibly, the B10 and B10.D2 strains could differ by an additional undetected weak H-locus and no. *509* was a mutation at this *non-H-2* locus. Nos. *506* and *513* were mutations at the *H-2* locus. None of the G-type mutations were shown to be *H-2* mutations.

Thus, there were two *H-2* mutations in our sample of 2101 mice tested (Table 5). Table 5 lists also three other *H-2* mutations published recently. In total, 4827 mice were tested for *H-2* mutations and five mutations were found, giving a rate of $5{\cdot}18 \times 10^{-4}$, slightly lower than the overall rates in the A and OD series but ten times lower than the rate in the (1–3)D series in Table 1 and than the rate published by Bailey (1966). Table 5 includes spontaneous and perhaps induced H-2 mutations because various mutagenic treatments were used in the tests listed in this Table. The true spontaneous rate at the H-2 locus may well be lower.

Recent estimates give at least 40 H-loci in the mouse (Bailey, 1970*a*), and possibly the true number is much higher. Assuming the rates of mutations of type G and L are equal at all H-loci, the mutation rate of the *H-2* locus is much higher than the expected average rate per H-locus; it is hundreds of times higher than the overall spontaneous visible mutation rate, which is 8×10^{-7} per locus per gamete (Schlager & Dickie, 1967). The data suggest that there are more mutation sites at the *H-2* than at an 'average' H-locus. Another explanation could be an

assumption that some mutant *H-2* alleles are products of unequal crossing over inside the *H-2* locus of homozygous mice. Some data indicate that duplications of small regions of the *H-2* locus do exist in a number of its alleles (Stimpfling & Richardson, 1965; Snell, Demant & Cherry, 1961). Both suggestions are not inconsistent. The *H-2* locus is likely to include two or more closely linked genes originating through duplication.

REFERENCES

Alderson, T. (1965). Chemically induced delayed germinal mutation in Drosophila. *Nature, London* **207**, 164–167.

Bailey, D. W. (1966). Heritable histocompatibility changes: lysogeny in mice? *Transplantation* **4**, 482–488.

Bailey, D. W. (1968). The vastness and organization of the murine histocompatibility-gene system as inferred from mutational data. In *Advances in Transplantation* (ed. J. Dausset, J. Hamburger and G. Mathe), pp. 317–323. Copenhagen: Munksgaard.

Bailey, D. W. (1969). Communication. *Mouse News Letter* **41**, 30.

Bailey, D. W. (1970). Analysis of a mutation at the *histocompatibility-2* locus in mice. Abst.: *Genetics* **64**, Supplement 2, **3**.

Bailey, D. W. (1970*a*). Four approaches to estimating number of histocompatibility loci. *Transplantation Proceedings* **2**, 32-38.

Bailey, D. W. & Kohn, H. I. (1965). Inherited histocompatibility changes in progeny of irradiated and unirradiated inbred mice. *Genetical Research, Cambridge* **6**, 330–340.

Counce, S., Smith, P., Barth, R. & Snell, G. D. (1956). Strong and weak histocompatibility gene differences in mice and their role in the rejection of homografts of tumors and skin. *Annals of Surgery* **144**, 198–204.

Egorov, I. K. (1967). A mutation of the *histocompatibility-2* locus in the mouse. *Genetika, Moscow* **3** (9), 136–144.

Egorov, I. K. & Blandova, Z. K. (1968). The genetic homogeneity of the inbred mice bred at the 'Stolbovaya' farm. II. Skin grafting tests. *Genetika, Moscow* **4** (12), 63–69.

Egorov, I. K. & Medvedev, N. N. (1966). Homozygosity of the C57BL/10ScSn, C57L/J, CC57BR and CC57W mouse strains as studied by the skin grafting method. *Genetika, Moscow* **2** (2), 130–136.

Epler, J. L. (1966). Ethyl methanesulfonate-induced lethals in *Drosophila* – frequency–dose relations and multiple mosaicism. *Genetics* **54**, 31–36.

Godfrey, J. & Searle, A. G. (1963). A search for histocompatibility difference between irradiated sublines of inbred mice. *Genetical Research, Cambridge* **4**, 21–29.

Jenkins, J. B. (1967*a*). The induction of mosaic and complete dumpy mutants in *Drosophila melanogaster* with ethyl methanesulfonate. *Mutation Research* **4**, 90–92.

Jenkins, J. B. (1967*b*). Mutagenesis at a complex locus in *Drosophila* with the monofunctional alkylating agent, ethyl methanesulfonate. *Genetics* **57**, 783–793.

Kindred, B. (1963). Skin grafting between sublines of inbred strains of mice. *Australian Journal of Biological Sciences* **16**, 863–868.

Linder, O. E. A. & Klein, E. (1960). Skin and tumor grafting in coisogenic resistant lines of mice and their hybrids. *Journal of National Cancer Institute* **24**, 707–720.

Malashenko, A. M. (1971). Sensitivity of mouse testis cells to the induction of dominant lethals by diethylsulphate. *Genetika, Moscow* **7** (1), 84–91.

Pelecanos, M. & Alderson, T. (1964). The mutagenic activity of diethylsulphate in *Drosophila melanogaster*. *Mutation Research* **1**, 173–181.

Schlager, G. & Dickie, M. (1967). Spontaneous mutations and mutation rates in the house mouse. *Genetics* **57**, 319–330.

Snell, G. D. (1958). Histocompatibility genes of the mouse. II. Production and analysis of isogenic resistant lines. *Journal of National Cancer Institute* **21**, 843–877.

Snell, G. D. (1960). Note on results of Linder and Klein with coisogenic resistant lines of mice. *Journal of National Cancer Institute* **25**, 1191–1193.

SNELL, G. D., DEMANT, P. & CHERRY, M. (1971). Hemagglutination and cytotoxic studies of *H-2*. I. *H-2.1* and related specificities in the *EK* crossover regions. *Transplantation* **11**, 210–237.

SNELL, G. D. & STIMPFLING, J. H. (1966). Genetics of tissue transplantation. In *Biology of the Laboratory Mouse* (ed. E. L. Green), 2nd ed., pp. 457–491. New York: McGraw-Hill.

STAATS, J. (1969). *Inbred Strains of Mice*, no. 6, pp. 1–126. Bar Habor.

STIMPFLING, J. H. & RICHARDSON, A. (1965). Recombination within the *histocompatibility-2* locus of the mouse. *Genetics* **51**, 831–846.