LINKAGE RELATIONS OF THE GENES HISTOCOMPATIBILITY-2 AND FUSED TAIL, BRACHYURY AND KINKY TAIL IN THE MOUSE, AS DETERMINED BY TUMOR TRANSPLANTATION¹

SALLY LYMAN ALLEN²

Department of Zoology, University of Chicago, Chicago, Ill., and Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

Received January 27, 1955

THE literature on the genetic and antigenic basis of tumor transplantation has been reviewed by BITTNER (1935), LITTLE (1941; 1947), HAUSCHKA, (1952) and SNELL (1953b, c). In mice, the genetic loci involved were named "histocompatibility" loci by SNELL (1948), the prefix "histo" being used to denote the probable similarity of genes determining reactions to neoplastic and normal tissue transplants. One of these histocompatibility loci, *H-2*, was shown to be closely linked to the *Fu* locus by GORER, LYMAN and SNELL (1948). *H-2* was shown to be identical with that gene determining antigen II present in A strain erythrocytes, which GORER had previously demonstrated (1936, 1937).

In addition to *H-2* and fused tail (*Fu*), one or two other loci have been identified on the ninth chromosome in mice: kinky tail (*Ki* or *Fu*^{Ki}) and brachyury (*T*). Fused tail is a dominant mutation, viable in the homozygous condition on certain genetic backgrounds, and characterized by the production of kinks and twists in the tail. Penetrance of *Fu* in heterozygous animals is incomplete with "normal overlaps" being found, the frequency of which varies with the genetic background and sex of the fused parent. The frequency of overlapping is greater when the female introduces the *Fu* gene than when the male introduces it. Kinky tail (*Ki* or *Fu*^{Ki}) is phenotypically similar to fused tail and may be an allele at the *Fu* locus (DUNN and GLUECKSOHN-WAELSCH 1954). Unlike fused, the homozygote is never viable, and there does not appear to be a sex difference in production of normal overlaps, but genetic background may considerably alter its expression. Hereafter, the gene symbol *Ki* will be used in referring to kinky. Brachyury (*T*) is a dominant mutation, lethal in homozygous condition. Typically, it produces a shortened tail but is highly variable in expression depending upon genetic background. It may produce kinks or, on certain genetic backgrounds, tailless animals, or, on still other backgrounds, normal overlaps (ALLEN and SNELL unpublished). For additional details or the original references pertaining to the tail mutants described, see GRÜNEBERG (1952).

Fu, *Ki* and *T* are closely linked loci contained within a chromosomal segment of approximately 8 units (DUNN and CASPARI 1945). The order of the loci was uncertain but their evidence pointed to the most probable order as either (1) *Ki T Fu*

¹ This investigation has been aided by a John M. Prather research fellowship from the University of Chicago and by a two year, three month predoctoral fellowship from the National Cancer Institute. Inbred mice and animal cages were provided by the Roscoe B. Jackson Memorial Laboratory with the financial assistance of a grant-in-aid (EG-14) from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.

² Present address: Department of Zoology, University of Michigan, Ann Arbor, Mich.

TABLE 1

Linkage experiments involving the ninth chromosome

Authors	Loci tested	Sex	Crossover		Number of mice
			percent		
			Halfvalue†	Total	
DUNN & CASPARI (1945)	<i>Fu-T</i>	♂	2.1	4.3	657
“ “		♀	0	0	137
DUNN & CASPARI (1945)	<i>Ki-T</i>	♂	1.2	2.5	1128
“ “		♀	3.0	5.9	542
“ “		♂	2.3	4.6	395
DUNN & GLUECKSOHN-WAELSCH (1953)		♂, ♀		3-5	73+
DUNN & CASPARI (1945)	<i>Fu-Ki</i>	♂	0	0	209
“ “		♀	1.7	3.4	296
DUNN & GLUECKSOHN-WAELSCH (1954)		♂	0	0	892
“ “		♀	0	0	79
SNELL (1952)	<i>H-2-Ki</i>	♂, ♀	no tested crossovers		
SNELL, SMITH & GABRIELSON (1953) & ALLEN (unpublished)		♂, ♀		4.0*	75
SNELL (1952)	<i>H-2-Fu</i>	♂, ♀		5.6	19
SNELL, SMITH & GABRIELSON (1953) & ALLEN (unpublished)		♂, ♀		8.3*	707
ALLEN (1955)		♂		4.1*	268
“		♀		6.9*	73
SNELL (1952)	<i>H-2-T</i>	♂		4.1	73
“		♀		12.5	8
SNELL, SMITH & GABRIELSON (1953) & ALLEN (unpublished)		♂, ♀		14.3*	394

* Crossover percent based only on animals with tail abnormality; crossovers not tested genetically.

† Frequency of one crossover class.

or (2) *Fu Ki T*. To account for the recombinants from a cross of *Ki T/Fu* × normal, DUNN and CASPARI (1945) concluded that *Fu* does not lie between *Ki* and *T*. The linkage relations of these loci, one to another and to *H-2*, are summarized in table 1, where a listing is made of the crossover values obtained by various workers for the 6 chromosome intervals.

By making use of the linkage of *H-2* with the genes *Fu*, *Ki* and *T* it has been possible to establish 9 alleles and 2 subgroup alleles at the *H-2* locus and to determine their distribution in 21 inbred strains and 7 isogenic lines of the A strain (SNELL 1951a, 1951b; SNELL and HIGGINS 1951; SNELL *et al.* 1952; SNELL, SMITH and GABRIELSON 1953; SNELL unpublished; ALLEN 1955; HOECKER, COUNCE and SMITH 1954). Six alleles have so far been found in only one strain or stock each (*H-2^{dk}* or *H-2^a*, *H-2^f*, *H-2^p*, *H-2^q*, *H-2^r*, and *H-2^s*), while 3 alleles are more commonly distributed (*H-2^b*, *H-2^d* and *H-2^k*). The 2 subgroup alleles are *H-2^{d'}* and *H-2^{k'}*. Strains

characterized by these alleles originally were classed as $H-2^d$ and $H-2^k$ but further genetic tests, on the basis of reaction to transplantation, indicated that the antigens produced by these alleles were slightly different in nature. Each of the alleles of $H-2$ exerts an effect in the heterozygote as in the case with alleles determining the M-N blood groups in man.

Originally the type allele characteristic of the A strain was designated simply $H-2$. Subsequently, however, extensive genetic crosses revealed that animals of the heterozygous genotype $H-2^d/H-2^k$, irrespective of the source of these alleles, grow strain A tumors, thus reacting as if $H-2 = H-2^d/H-2^k$ (SNELL 1951b; SNELL, SMITH and GABRIELSON 1953). Mice bearing other $H-2$ alleles in various combinations do not grow strain A tumors. Thus, the type allele belonging to the A strain is now redesignated $H-2^{dk}$, indicating the presence of two components responsible perhaps for producing two antigens or two antigenic factors. Eight antigenic factors have recently been demonstrated by a combination of tumor transplantation and red cell agglutination techniques (GORER and MIKULSKA 1954; HOECKER, COUNCE and SMITH 1954). Some of these factors are uniquely produced by one allele; others are common to several alleles. Under the scheme of these authors, $H-2^{dk} = H-2^a$ and produces antigenic factors (C)DEK. $H-2^d$ produces CD, $H-2^k$ —CEK, and $H-2^b$ —BE. Whether a single locus with components or two or more closely linked loci are involved in the production of these antigenic factors has not been determined.

MATERIALS AND METHODS

The *inbred strains* used were: A ($H-2^{dk}/H-2^{dk}$); BALB/c ($H-2^d/H-2^d$); AKR ($H-2^k/H-2^k$); C57BL/6 and C57BL/10 ($H-2^b/H-2^b$).

The *stocks* used were: A- $H-2^kKi$ ($H-2^kKi/H-2^{dk+}$); A- $H-2^lFu$ ($H-2^lFu/H-2^{dk+}$); A- $H-2^{dkT}$ ($H-2^{dkT}/H-2^{dk+}$). These stocks are isogenic lines of the A strain produced by repeated backcrossing of animals heterozygous for Ki , Fu or T to the A strain for 7 or more generations so that more than 99 percent of their genes are derived from the A strain with the exception of that chromosomal segment containing the locus Ki , Fu or T .

Transplantable tumor 15091a, native to the A strain, was used throughout these experiments. Originally diagnosed as a medullary adenocarcinoma of the mammary gland by CLOUDMAN (1932), it was transplanted for the first time on December 11, 1928. A recent report indicates that it is a sub-tetraploid tumor as determined by chromosome counts and by the desoxyribose nucleic acid phosphorus content per cell (HAUSCHKA and LEVAN 1953; LEVAN and HAUSCHKA 1953). Genetically it usually gives one factor ratios, $H-2$ being the locus involved. It does grow in a certain percentage of some foreign strains, although there appears to be a relationship between growth and the allele of $H-2$ carried by the particular strain (SNELL *et al.* 1953). In crosses it acts as a "good" genetic tumor (SNELL, SMITH and GABRIELSON 1953).

15091a was originally carried in the donor A strain by routine trocar inoculation. Because of difficulties encountered by infection, a more sterile technique had to be found. Therefore, for inoculating donor animals, the cytosieve, described by SNELL (1953a), was adopted. Experimental transplants, however, were always performed

FIGURE 2.—Plan of matings for establishing the order of the loci *Fu*, *T* and *H-2*. BC = backcross generation; TC = testcross generation. For the genotype of F₁ *Fu*/*T* it should be noted that only one of the three possible orders of the loci is represented.

strain and inoculated with 15091a. Half the offspring from "false negatives" would be expected to grow 15091a, whereas all offspring from true crossovers should fail to grow 15091a.

In the first cross a total of 196 normal-tailed offspring were inoculated with 15091a. There were 5 survivors. From the second cross, it appeared that 3 of these animals were "false negatives" (σ^788 , σ^91 , σ^500) and 2 were true crossovers (σ^90 , σ^501). A statistical test was run on these potential crossovers by comparing the ratios obtained with those expected for "false negatives," where:

$$t = \frac{\text{Observed value} - .5}{\text{S. E. } (q_o - q_T)}$$

Such testing revealed that the above supposition was correct, i.e. that only 2 of the 5 survivors were true crossovers. Therefore, it may be concluded that crossing over between *H-2* and *Ki* does occur, but with a low frequency. Where the heterozygous parent is the male, the indicated incidence is 1.02 percent (2/196). There are no data for crossing over in the heterozygous female.

ORDER OF THE LOCI *Fu*, *T* AND *H-2*

Plan of the crosses

The plan of matings made is represented in figure 2. After appropriate matings to test for possible crossovers, mice of proven genotype $H-2^{dk}+/H-2^fFu$ of the $A-H-2^fFu$ stock were crossed to animals from the $A-H-2^{dk}T$ stock (genotype $H-2^{dk}T/H-2^{dk}+$). F₁'s from such a cross, known to be *Fu*/*T*, were selected for matings to the C57BL/6 strain. Progeny tests of possible *Fu*/*T* animals were necessary since classification of these animals by tail type was not always certain. Seventy-eight F₁ animals were raised. Of the 21 animals tested only 5 proved to be both *Fu* and *T*.

The remainder carried either *Fu* or *T* only. In most animals proven *Fu*/*T* an extreme tail type was found; however, in one case, the tail was more typical of animals bearing *T* only.

Mice of the BC generation were saved and tested genetically by matings (1), (2) and (3). This was necessary because "false negatives" and "false positives" (to be described later) occur and tend to obscure results expected of particular genomes. At least 10 of the progeny of each BC mouse were inoculated, these mice being referred to as the testcross generation or TC. From the reaction of the TC mice in a particular progeny to the tumor inoculated, the genotype of the heterozygous BC parent could be determined. The number of TC young necessary to establish the parent's genotype beyond doubt varied from case to case, being dependent on both genetic and environmental conditions. As a rule 10 to 20 TC mice sufficed to prove a particular BC mouse a noncrossover. In a few cases TC young had to be saved and mated when testing of a BC mouse was incomplete or the results were open to question. In cases involving crossovers many more young were raised and inoculated. Two hundred ninety-five BC mice were mated, but, of these, 11 either died before sufficient data had been obtained or proved to be sterile, so that only 284 mice were actually tested.

It would have been desirable to have confined crosses to matings (2) and (3), but space and time did not permit this. Matings (2) and (3) are complementary tests for the possible separation of components *d* and *k*. In the case of a BC mouse in which separation had occurred, one but not the other of (2) and (3) would give tumor susceptible offspring. By the same BC mouse mating (1) would give young negative to the tumor. Thus, where (1) was not used, it was absolutely necessary to use both (2) and (3), otherwise the results might be misleading. Only one class was not tested for component separation; namely, noncrossover fused BC males.

For the classification of tail types, litters were inspected both at birth and at weaning. At weaning, profile drawings of the tails of all mice were made. For TC mice such classification was made "blind," i.e., without knowledge of the recorded tail type of the BC parent. This served to exclude any possible subjective bias in the classification.

TC mice were inoculated by trocar at 5 to 12 weeks of age and checked weekly thereafter, as described in the section on materials and methods.

Class	Alternative 1				Alternative 2				Alternative 3			
	$\frac{Fu}{+}$	$\frac{H-2^f}{xa}$	$\frac{+}{H-2^f kb}$	$\frac{+}{T}$	$\frac{H-2^f}{H-2^f kb}$	$\frac{Fu}{+}$	$\frac{+}{xa}$	$\frac{+}{T}$	$\frac{H-2^f}{H-2^f kb}$	$\frac{+}{xa}$	$\frac{+}{T}$	$\frac{Fu}{+}$
	<i>nc</i>	<i>xa</i>	<i>xb</i>	<i>dc</i>	<i>nc</i>	<i>xa</i>	<i>xb</i>	<i>dc</i>	<i>nc</i>	<i>xa</i>	<i>xb</i>	<i>dc</i>
<i>Fu</i>	-			+	-	+			-	+		
<i>T</i>	+			-	+	-			+	-		
<i>FuT</i>		+	-				-	+			+	-
+		-	+				+	-			-	+

FIGURE 3.—Pattern of response to inoculation of strain A tumor 15091a under three alternative orders of the loci *Fu*, *T* and *H-2*. Noncrossover = *nc*; crossover at region a = *xa*; crossover at region b = *xb*; double crossover = *dc*. Resistant to 15091a = -; susceptible to 15091a = +.

The pattern of response expected of the BC generation for each of the three possible alternative orders of the loci *Fu*, *T* and *H-2* is diagrammed in figure 3, which summarizes the classes expected under each of the alternatives. There are key classes which differentiate one alternative from the other two so that the most probable order of the loci may be established.

RESULTS

Penetrance of tail genes (See table 2)

In both the crosses involving *T* or *Fu* the total mortality between birth and weaning of TC mice was greatest when matings of BC parents were made to the C57BL/6 strain. The total mortality was less in matings to the AKR strain and least to the BALB/c strain. Since the BC generation was half C57BL/6 in genetic background, differences in mortality are probably explained on the basis of an increased homozygosity of the young in the first mating and an increased heterozygosity in the latter two matings. In almost all cases the total mortality was higher when *T* was segregating than when *Fu* was segregating. When the female parent was of a pure strain, the mortality was greater than when the female parent was heterozygous.

In all crosses involving *T*, the ratios obtained at birth of *T* to + very closely approximated the expected 1:1 ratio. At weaning differential mortality was evident

TABLE 2
Penetrance of T and Fu by age, sex and strain

Strain used in cross	Sex of BC parent	No. at birth			No. at weaning			% <i>T</i>	% Mortality		
		<i>T</i>	+	Total	<i>T</i>	+	Total		<i>T</i>	+	Total
1a C57BL/6	♂	992	994	1986	699	773	1472	47.5	14.8	11.1	25.9
b "	♀	772	761	1533	642	678	1320	48.6	8.5	5.4	13.9
2a BALB/c	♂	86	67	153	74	54	128	57.8	7.8	8.5	16.3
b "	♀	113	99	212	105	91	196	53.6	3.8	3.8	7.6
3a AKR	♂	49	50	99	31	45	76	40.8	18.2	5.1	23.2
b "	♀	126	130	256	111	129	240	46.3	5.9	0.4	6.3

Strain used in cross	Sex of BC parent	No. at birth			No. at weaning			% <i>Fu</i>	Mortality % Total	% <i>Fu</i> matings compared	χ^2 *	P
		<i>Fu</i>	+	Total	<i>Fu</i>	+	Total					
1a C57BL/6	♂	895	1344	2239	768	1042	1810	42.4	19.2	1a & b	158.2	<.0001
b "	♀	15	418	433	24	326	350	6.86	19.2	1b & 2b	91.6	<.0001
2a BALB/c	♂	80	142	222	73	131	204	35.8	8.1	2a & b	0.48	.50
b "	♀	327	831	1158	370	751	1121	33.0	3.2	2b & 3b	35.5	<.0001
3a AKR	♂	52	128	180	50	101	151	33.1	16.1	3a & b	8.78	<.01
b "	♀	202	1084	1286	269	956	1225	22.0	4.7	1b & 3b	40.0	<.0001

* Yates' correction applied.

in crosses to the AKR and C57BL/6 strains. With one parent from the AKR strain, *T* offspring are heavily selected against. A slight differential mortality is observed with the C57BL/6 strain. However, with one parent from the BALB/c strain, there appears to be no particular selection against *T*; animals carrying *T* or + are eliminated at equal rates.

It is not possible to measure the amount of differential mortality in crosses segregating for *Fu* because the expression of *Fu* at birth is often so slight that such animals may be classified as normal-tailed. Thus, the absolute number of recognized *Fu* is greater at weaning. There are both strain and sex differences in the penetrance of *Fu*. "Normal overlaps" are generally higher in crosses to certain strains. Where the female is the fused parent, there are very significant differences in the penetrance of *Fu* found in different strains. For the strains used the percent penetrance was: C57BL/6—6.86%; BALB/c—33%; and AKR—22%; where 50% is the expected value. There are significant differences in the penetrance of *Fu* between male and female in crosses to the C57BL/6 strain (42.4% *vs.* 6.86%) and in crosses to the AKR strain (33.1% *vs.* 22.0%) but not in crosses to the BALB/c strain (35.8% *vs.* 33.0%). The number of normal overlaps was so great in the mating of heterozygous females to C57BL/6 males that it had to be abandoned in favor of matings to BALB/c and AKR males.

Noncrossovers

Data obtained from noncrossovers are grouped by tail class, sex and mating in table 3. The results of tumor inoculation of the TC generation are totaled for each group. The first four subcolumns represent: (1) normal-tailed positive to A-strain tumor 15091a (++) ; (2) normal-tailed negatives (+-); (3) brachy-tailed (or fused-tailed) positives (*T*+); and (4) brachy-tailed (or fused-tailed) negatives (*T*-). Then follow two subcolumns representing total young positive (+) and total

TABLE 3
Data on noncrossovers

Classification of BC mice	Mating	Results of tumor inoculation of TC young					
		++	+-	<i>T</i> +	<i>T</i> -	+	-
62 <i>Fu</i> ♂♂	♀ C57BL/6	1	968	0	715	1	1683
	♀ BALB/c	0	49	0	27	0	76
	♀ AKR	3	19	0	14	3	33
72 <i>Fu</i> ♀♀	♂ C57BL/6	2	317	0	24	2	341
	♂ BALB/c	3	697	0	361	3	1058
	♂ AKR	10	906	2	265	12	1171
65 <i>T</i> ♂♂	♀ C57BL/6	51	671	517	142	568	813
68 <i>T</i> ♀♀	♂ C57BL/6	66	509	348	205	414	714
	♂ BALB/c	4	41	46	7	50	48
	♂ AKR	15	50	43	9	58	59

young negative (—). Individual progenies of BC animals within each tail class were tested by chi square for fit to the total values from all progenies of the class.

Fused noncrossovers are expected to produce offspring which are all negative to A-strain tumor 15091a no matter which alternative arrangement of the loci proves correct. The observed results show that this is the case with one qualification. "False positives" occur. These are animals genetically resistant to a tumor but which succumb to it due to a combination of residual heredity plus environmental factors of a largely unknown nature. In the present crosses there were relatively few "false positives" occurring in the fused-tail noncrossover class. The percentage of false positives varied according to the pure strain used in the matings. The frequency of false positives was highest in crosses to AKR (σ and φ *Fu* 15/1219 = 1.23%), next in crosses to BALB/c (σ and φ *Fu* 3/1137 = 0.26%) and lowest in crosses to C57BL/6 (σ and φ *Fu* 3/2027 = 0.10%).

The brachy noncrossover group is an extremely interesting one because it affords a second measure of crossing over between *H-2* and *T*. Under any of the alternative hypothetical orders of the three loci under consideration, brachy noncrossovers should throw *T* positive young. All matings of a BC *T* mouse may be expressed as the following: $H-2^{dk}T/H-2^b \times H-2^+ / H-2^+$. Thus, the noncrossover TC young expected would be normal-tailed negatives and brachy positives; crossovers would be normal-tailed positives and brachy negatives. That is exactly what is observed in the results of the inoculation of TC young by *T* noncrossover parents. Subcolumns (2) and (3) contain noncrossovers while (1) and (4) include crossovers (table 3).

In the crosses to C57BL/6 it soon became obvious that there were more negative animals in class (4) (*T*—) than expected; that is, the number of "false negatives" was high. Also, the number of negatives appeared to be higher in the first lots of mice tested than in the later ones. The data were therefore examined by month of inoculation, with the results depicted graphically in figure 4. The abscissa represents the percent positive to 15091a, while the ordinate represents the months of inoculation from February 1952 to July 1953. The total data are graphed as well as data from *T* TC young from heterozygous males and from heterozygous females. It may be observed that data from total animals fall below the expected 50 percent positive line and that data from *T* TC mice generally fall between 50 and 100 percent positive. It may also be noted that there is a marked decrease in positive animals during the summer months of 1952.

The tumor repeatedly became infected during these months; therefore, a switch was made on October 17, 1952 from the trocar technique of transplantation to the cytosieve technique in carrying the tumor in the donor line. Using the latter technique, the tumor was always free from infection. A consequent upward swing in percent positives may be observed. Similar reductions in the growth potential of transplanted tumors due to infection have been reported elsewhere (see, e.g., SCHREK 1936).

For comparing the effects of the two techniques of transplantation, the date, December 1, 1952, was chosen for dividing the data because of an initial retardation in tumor growth rate when the medium employed for tumor suspension was changed in these experiments. The number of positives produced by all BC *T* noncrossovers

FIGURE 4.—Percent positive to strain A tumor 15091a by month of inoculation among TC offspring of *T* noncrossovers $\times$ C57BL/6. Both the percent positive of the total animals inoculated and the percent positive of *T* animals are represented for each sex. 15091a was carried in the donor line by trocar before 10/17/52. After this date the cytosieve was used.

before versus after December 1, 1952 was analyzed statistically, and a very significant difference was found between the two populations ($P = <.0001$).

Previous to December 1, 1952 it was found that the age at inoculation had a slight influence on the number of positives. The number of positives was less among animals inoculated at 2 or more months of age than among those inoculated earlier ($P = <.01$). After December 1, 1952, most of the mice were inoculated at an earlier age. A comparison of the number of positives among mice inoculated at $1\frac{1}{2}$ months or older and among those inoculated earlier showed that the age effect was lost ($P = .50$).

When calculating crossover percents, it was necessary to take into account "false negatives." A method was devised for determining the proportion of "false negatives" among *H-2^{dk}* mice in the various divisions of *T* noncrossover data and then calculating the frequency of crossing over or "*c*". The method used was the following:

- (a) Let q = actual proportion of brachy
- x = proportion of false negatives
- c = frequency of recombination

(b) For each of the four classes (1) through (4) the absolute numbers were listed and the percentage contribution calculated. Algebraic expressions for the theoretical frequencies are shown for each class.

Classes	Theoretical frequency
(1) + +	$(1 - q)c - (1 - q)cx$
(2) + -	$(1 - q)(1 - c) + (1 - q)cx$
(3) T +	$q(1 - c) - q(1 - c)x$
(4) T -	$qc + q(1 - c)x$

(c) $x = \frac{(2) + (4) - (1) - (3) - (1 - 2q)(1 - 2c)}{1 - (1 - 2q)(1 - 2c)}$

(d) $c = \frac{(1) + (4) - qx}{(1 - x)}$

Table 4, part 1 summarizes the results of examining various crosses to the C57BL/6 strain for factors instrumental in altering the frequency of false negatives. Data divided at December 1, 1952 for heterozygous males and females show in both Cases 1 and 2 a higher frequency of false negatives before than after this date. In Case 1 there were 30.6 percent negatives before December 1, 1952 and 4 percent negatives after this date. In Case 2 the corresponding percentages are 28.9 and 15.3.

An influence of age at inoculation of TC young on the percentage of false negatives is demonstrated in Cases 3 to 6. In general the frequency of false negatives was higher in the older age groups. The data are listed separately for males and for females. Cases 3 and 4 group data obtained before December 1, 1952; while Cases 5 and 6 group data obtained after this date.

For any line of data in Cases 1 to 7 the values of q , x and c may be substituted in the theoretical frequencies (1) to (4). These give the exact observed frequencies, since there are no degrees of freedom, and are of interest in showing the extent of agreement from wholly independent determinations.

As indicated at the foot of table 4, a few T animals negative to the tumor or not inoculated were proved by matings to C57BL/6 (or BALB/c and AKR) to be genetically positive to the tumor. These were either not counted or not counted positive in Cases 1 to 6, already discussed. With the attempt to establish a sex difference in crossing over these animals were counted as positives in the remaining cases to be discussed. In this respect Case 7 is a revision of Cases 1 and 2. There is actually very little change in the calculated values of x and c .

Part 2 of table 4 is an attempt to demonstrate a sex influence on crossing over. All cases start with the observed frequencies found in Case 7. It was assumed first (Case 8) that there is a constant value of c in each sex, whether before or after December 1, 1952, but that the values of x may be different in the sexes, both "before" and "after". The final estimates were obtained by iteration between equations (c) and (d) using the weighted averages of the c 's "before" and "after" at each step until stability was reached. These estimates yield a total chi square of 3.448 with 2 degrees of freedom and a probability between .10 and .20. This hypothesis thus gives reasonably satisfactory results.

TABLE 4
Factors influencing the frequency of false negatives and crossovers produced by BC T noncrossomers mated to C57BL/6

PART 1

Influence of Age at Inoculation and Method of Inoculation

Case No.	Sex	Age at inoc.	Trocar vs. cytostieve ± 12/1/52	Response to tumor 15091a					Frequency of:	
				++	+-	T+	T-	N	z	c
1.	♂		Before After	17 34	286 403	165 351	95 47	545 835	.3057 .0402	.0859 .0811
2.	♀		Before After	61 5	448 61	286 54	197*7 16*1	992 136	.2880 .1527	.1684 .0895
3.	♂	2 mos. + -2 mos.	Before	11 6	176 92	114 51	77 18	378 167	.3443 .1997	.0897 .0765
4.	♀	2 mos. + -2 mos.	Before	51 10	388 60	221 65	177*6 20*1	837 155	.3287 .0924	.1730 .1574
5.	♂	1½ mos. + -1½ mos.	After	3 31	72 331	56 295	7 40	138 697	.0711 .0338	.0430 .0887
6.	♀	1½ mos. + -1½ mos.	After	1 4	31 30	31 23	11*1 5	74 62	.2307 .0609	.0406 .1252
7.	♂		Before After	17 34	268 403	166† 351	95 47	546 835	.3043 .0403	.0858 .0811
	♀		Before After	61 5	448 61	293*7 55*1	190 15	992 136	.2735 .1385	.1650 .0879

PART 2
Influence of Sex on Crossing Over

Case No.	Sex	Trocus vs. cytosieve 12/1/52	N	Frequency of:		χ^2	n	P
				π	c			
8.	♂	Before	546	.3041	.0829	.025	1	.80-.90
		After	835	.0405	.0829	.033		
	♀	Before	992	.2732	.1560	.305		
		After	136	.1352	.1560	3.390 3.085		
9.						3.448	2	.10-.20
	♂	Before	546	.2842	.0839	.452		
		After	835	.0538	.0839	.798		
	♀	Before	992	.2842	.1561	.352		
10.						2.783	1	.80-.90
	♂	Before	546	.3057 ± .0321	.0825 ± .0103	.031		
		After	835	.0390 ± .0198	.0825 ± .0103	.013		
	♀	Before	992	.2782 ± .0261	.1541 ± .0157	.414		
						2.376	1	.05-.10
		After	136	.0904 ± .0595	.1541 ± .0157	2.790		
						2.834		
						2.834		

* Negative when inoc.; tested genetically as +.

† Not inoc.; tested genetically as +; counted here only.

TABLE 4—Continued

PART 3

Method for estimating values of c , x_a , and x_b by the Method of Maximum Likelihood. The derivations of formulae for the first and second derivatives of c , x_a and x_b with respect to L are shown, where $L = \log P$

A	B	C	D	E	F	G = C X D
Class	p = theoretical proportions (separating terms in c)	$\frac{\partial p}{\partial c}$	$\frac{\partial}{\partial c} \log \frac{p}{\partial c} = \frac{1}{p} \frac{\partial p}{\partial c}$	$\frac{\partial^2 p}{\partial c^2}$	$\frac{\partial^2 p}{\partial x \partial c}$	$\frac{1}{p} \left(\frac{\partial p}{\partial c} \right)^2$
++	$p_1 = (1 - q)(1 - x)c$	$(1 - q)(1 - x)$	$\frac{1}{c}$	0	$-(1 - q)$	$\frac{(1 - q)(1 - x)}{c}$
+ -	$p_2 = (1 - q) - (1 - q)(1 - x)c$	$-(1 - q)(1 - x)$	$-\frac{1 - x}{1 - (1 - x)c}$	0	$(1 - q)$	$\frac{(1 - q)(1 - x)^2}{1 - (1 - x)c}$
T +	$p_3 = q(1 - x) - q(1 - x)c$	$-q(1 - x)$	$-\frac{1}{1 - c}$	0	q	$\frac{q(1 - x)}{1 - c}$
T -	$p_4 = qx + q(1 - x)c$	$q(1 - x)$	$\frac{1 - x}{x + (1 - x)c}$	0	$-q$	$\frac{q(1 - x)^2}{x + (1 - x)c}$
$H = D \times C' = D' \times C$ $\frac{1}{p} \left(\frac{\partial p}{\partial c} \right) \left(\frac{\partial p}{\partial x} \right)$						
A'	B'	C'	D'	E'	F' = F	G' = C' X D'
Class	p = theoretical proportions (separating terms in x)	$\frac{\partial p}{\partial x}$	$\frac{\partial}{\partial x} \log \frac{p}{\partial x} = \frac{1}{p} \frac{\partial p}{\partial x}$	$\frac{\partial^2 p}{\partial x^2}$	$\frac{\partial^2 p}{\partial c \partial x}$	$\frac{1}{p} \left(\frac{\partial p}{\partial x} \right)^2$
++	$p_1 = (1 - q)c - (1 - q)cx$	$-(1 - q)c$	$-\frac{1}{1 - x}$	0	$-(1 - q)$	$\frac{(1 - q)c}{1 - x}$
+ -	$p_2 = (1 - q)(1 - c) + (1 - q)cx$	$(1 - q)c$	$\frac{c}{(1 - c) + cx}$	0	$(1 - q)$	$\frac{(1 - q)c^2}{(1 - c) + cx}$
T +	$p_3 = q(1 - c) - q(1 - c)x$	$-q(1 - c)$	$-\frac{1}{1 - x}$	0	q	$\frac{q(1 - c)}{1 - x}$
T -	$p_4 = qc + q(1 - c)x$	$q(1 - c)$	$\frac{1 - c}{c + (1 - c)x}$	0	$-q$	$\frac{q(1 - c)^2}{c + (1 - c)x}$
q $\frac{q(1 - x)(1 - c)}{x + (1 - x)c}$						

$$\begin{aligned}
\sum p_i &= 1 \\
(1) \quad L &= \sum_{i=1}^4 \{n_{ia} \log \hat{p}_{ia}\} + \sum_{i=1}^4 \{n_{ib} \log \hat{p}_{ib}\}, \text{ where } \begin{cases} n_{ia} = \text{observed no. in class } ia \\ n_{ib} = \text{observed no. in class } ib \end{cases} \\
(2) \quad \frac{\partial L}{\partial c} &= \sum_{i=1}^4 \left\{ n_{ia} \frac{1}{\hat{p}_{ia}} \frac{\partial \hat{p}_{ia}}{\partial c} \right\} + \sum_{i=1}^4 \left\{ n_{ib} \frac{1}{\hat{p}_{ib}} \frac{\partial \hat{p}_{ib}}{\partial c} \right\} = 0 \\
(3) \quad \frac{\partial^2 L}{\partial c^2} &= \sum_{i=1}^4 \left\{ \tilde{n}_{ia} \frac{1}{\hat{p}_{ia}^2} \left[\frac{\partial^2 \hat{p}_{ia}}{\partial c^2} - \left(\frac{\partial \hat{p}_{ia}}{\partial c} \right)^2 \right] \right\} + \sum_{i=1}^4 \left\{ \tilde{n}_{ib} \frac{1}{\hat{p}_{ib}^2} \left[\frac{\partial^2 \hat{p}_{ib}}{\partial c^2} - \left(\frac{\partial \hat{p}_{ib}}{\partial c} \right)^2 \right] \right\} \\
(4) &= - \sum_{i=1}^4 \left\{ \tilde{n}_{ia} \left[\frac{1}{\hat{p}_{ia}} \left(\frac{\partial \hat{p}_{ia}}{\partial c} \right)^2 \right] \right\} - \sum_{i=1}^4 \left\{ \tilde{n}_{ib} \left[\frac{1}{\hat{p}_{ib}} \left(\frac{\partial \hat{p}_{ib}}{\partial c} \right)^2 \right] \right\} \quad \text{since } \frac{\partial^2 \hat{p}_{ia}}{\partial c^2} = \frac{\partial^2 \hat{p}_{ib}}{\partial c^2} = 0 \\
(5) &= -N_a \sum_{i=1}^4 \left[\frac{1}{\hat{p}_{ia}} \left(\frac{\partial \hat{p}_{ia}}{\partial c} \right)^2 \right] - N_b \sum_{i=1}^4 \left[\frac{1}{\hat{p}_{ib}} \left(\frac{\partial \hat{p}_{ib}}{\partial c} \right)^2 \right] \quad \text{since } \tilde{n}_{ia} = N_a \hat{p}_{ia}, \tilde{n}_{ib} = N_b \hat{p}_{ib} \\
&\quad \text{where } N_a = \sum_{i=1}^4 n_{ia}, N_b = \sum_{i=1}^4 n_{ib} \\
(6a) \quad \frac{\partial L}{\partial x_a} &= \sum_{i=1}^4 \left\{ n_{ia} \frac{1}{\hat{p}_{ia}} \frac{\partial \hat{p}_{ia}}{\partial x_a} \right\} = 0 \\
(6b) \quad \frac{\partial L}{\partial x_b} &= \sum_{i=1}^4 \left\{ n_{ib} \frac{1}{\hat{p}_{ib}} \frac{\partial \hat{p}_{ib}}{\partial x_b} \right\} = 0 \\
(7a) \quad \frac{\partial^2 L}{\partial x_a^2} &= -N_a \sum_{i=1}^4 \left[\frac{1}{\hat{p}_{ia}} \left(\frac{\partial \hat{p}_{ia}}{\partial x_a} \right)^2 \right] \\
(7b) \quad \frac{\partial^2 L}{\partial x_b^2} &= -N_b \sum_{i=1}^4 \left[\frac{1}{\hat{p}_{ib}} \left(\frac{\partial \hat{p}_{ib}}{\partial x_b} \right)^2 \right] \\
(8a) \quad \frac{\partial^2 L}{\partial x_a \partial c} &= \sum_{i=1}^4 \left\{ \tilde{n}_{ia} \frac{1}{\hat{p}_{ia}^2} \left[\frac{\partial^2 \hat{p}_{ia}}{\partial x_a \partial c} - \left(\frac{\partial \hat{p}_{ia}}{\partial c} \right) \left(\frac{\partial \hat{p}_{ia}}{\partial x_a} \right) \right] \right\} \\
(8b) \quad \frac{\partial^2 L}{\partial x_b \partial c} &= \sum_{i=1}^4 \left\{ \tilde{n}_{ib} \frac{1}{\hat{p}_{ib}^2} \left[\frac{\partial^2 \hat{p}_{ib}}{\partial x_b \partial c} - \left(\frac{\partial \hat{p}_{ib}}{\partial c} \right) \left(\frac{\partial \hat{p}_{ib}}{\partial x_b} \right) \right] \right\} \\
(9a) &= N_a \sum_{i=1}^4 \left[\frac{\partial^2 \hat{p}_{ia}}{\partial x_a \partial c} - \frac{1}{\hat{p}_{ia}} \left(\frac{\partial \hat{p}_{ia}}{\partial c} \right) \left(\frac{\partial \hat{p}_{ia}}{\partial x_a} \right) \right] \\
(9b) &= N_b \sum_{i=1}^4 \left[\frac{\partial^2 \hat{p}_{ib}}{\partial x_b \partial c} - \frac{1}{\hat{p}_{ib}} \left(\frac{\partial \hat{p}_{ib}}{\partial c} \right) \left(\frac{\partial \hat{p}_{ib}}{\partial x_b} \right) \right] \\
(10) \quad \frac{\partial^2 L}{\partial x_a \partial x_b} &= 0
\end{aligned}$$

TABLE 4—Continued
PART 4

Formulae for the standard errors of c , x_a and x_b

$\sigma c^2 = -\frac{Dcc}{D}, \quad \sigma x_a^2 = -\frac{D_{x_a x_a}}{D}, \quad \sigma x_b^2 = -\frac{D_{x_b x_b}}{D}$

where

$$D = \begin{vmatrix} \frac{\partial^2 L}{\partial c^2} & \frac{\partial^2 L}{\partial x_b \partial c} & \frac{\partial^2 L}{\partial x_a \partial c} \\ \frac{\partial^2 L}{\partial x_b \partial c} & \frac{\partial^2 L}{\partial x_b^2} & \frac{\partial^2 L}{\partial x_b \partial x_a} \\ \frac{\partial^2 L}{\partial x_a \partial c} & \frac{\partial^2 L}{\partial x_b \partial x_a} & \frac{\partial^2 L}{\partial x_a^2} \end{vmatrix}$$

and $D_{c\alpha}$, $D_{x_a x_a}$, $D_{x_b x_b}$ are the minors indicated by the subscripts.

PART 5

Difference/S.E. Difference of the frequency of crossovers and false negatives

$$\frac{c q - c c^2}{\sigma c q - \sigma c c^2} = \frac{.0716}{.0188} = 3.809 \quad \frac{x_b c^2 - x_b q}{\sigma x_b c^2 - \sigma x_b q} = \frac{.0275}{.0414} = .664 \quad \frac{x_a q - x_a c^2}{\sigma x_a q - \sigma x_a c^2} = \frac{.0514}{.0626} = .821$$

$$\frac{x_b c^2 - x_a c^2}{\sigma x_b c^2 - \sigma x_a c^2} = \frac{.2667}{.0354} = 7.534 \quad \frac{x_b q - x_a q}{\sigma x_b q - \sigma x_a q} = \frac{.1878}{.0649} = 2.894$$

Estimates were also obtained (Case 9) on the hypothesis that x is the same in males and females before December 1, 1952 and similarly for x after this date, as well as that there are single values of c within each sex. Weighted averages were used for x_a , x_b , c_{σ} and c_{φ} in the iteration process. Chi square came out slightly larger (4.385) but as there are now 4 degrees of freedom, the probability is raised to the .30 to .50 category for the set as a whole. It is evident that these hypotheses account for the data very satisfactorily. They do not, however, give standard errors for the c 's and x 's. These must be considerably larger than if there were no false negatives ($\sigma_c^2 = c(1 - c)/N$), or no crossing over ($\sigma_{x_a}^2 = x_a(1 - x_a)/Nq_a$). The method of averaging used does not, moreover, give the best estimates, since no cognizance is taken of the effects of x and c on reliability.

The estimates with single c 's for each sex were accordingly calculated by the method of maximum likelihood, iterating between the equations $\partial L/\partial c = 0$ and $\partial L/\partial x_a = 0$ and $\partial L/\partial x_b = 0$ (table 4, part 3). Standard errors were obtained by the method described by FISHER (1921). The formulae for the standard errors may be found in table 4, part 4. The results obtained by these methods are given in Case 10. Chi square is reduced to 2.834 which with 2 degrees of freedom gives a probability of .20 to .30 in comparison with .10 to .20 in Case 8. There is clearly a significant difference between males and females in recombination (males, $.0825 \pm .0103$; females, $.1541 \pm .0157$). Neither x_a nor x_b differs significantly between males and females but they differ significantly from each other in both sexes (table 4, part 5).

Crossovers

Data on 17 crossovers are listed in table 5. Positive and negative young, irrespective of tail type, are grouped according to mating for each crossover. Three classes of crossovers were definitely obtained and possibly a fourth, which were: fused positive ($Fu+$), brachy negative ($T-$), normal-tailed positive ($++$), and fused-brachy negative ($FuT-$). Chi square tests show that data from crossovers are clearly separated from data from the corresponding noncrossover groups.

The 3 males in the $Fu+$ group gave positive Fu young in all crosses. Since non-crossover Fu males mated to the C57BL/6 strain produced only 1 positive out of 1684 mice, the occurrence of positives in approximately half the mice in these crosses indicates crossing over between the loci $H-2$ and Fu .

There are two cases that require special consideration because of an apparent separation of the d and k components. φ 539 (Fu) gave 6 positive and 5 negative young from a cross with BALB/c ($H-2^d/H-2^d$), as expected for a crossover ($H-2^{dk}Fu/H-2^b+$), but gave no positives, 9 negatives from a cross with AKR ($H-2^k/H-2^k$), as if she were a noncrossover ($H-2'Fu/H-2^b+$). These results could be taken into account by supposing (1) that she is a ($H-2^{dk}Fu$) crossover but that the number of young from the cross with AKR was so small that no positives happened to appear; or (2) that a separation of the d and k components occurred in a crossover that implies the order $K D Fu T$ of the loci; or (3) that a mutation from $H-2'$ to $H-2^k$ occurred. The first hypothesis seems the simplest and is adopted.

σ 278 (T) gave 33 positives, 28 negatives from a cross with BALB/c, as expected for a noncrossover ($H-2^{dk}T/H-2^b+$), but gave no positives, 39 negatives from a cross

TABLE 5
Data on 17 crossovers

Class and no.	Results of tumor inoculation					
	C57BL/6		BALB/c		AKR	
	+	-	+	-	+	-
♂ <i>Fu</i> :						
290	11	11	21	25	16	23
346	20	21	16	25	23	24
529	12	22	20	19	15	14
♀ <i>Fu</i> :						
539			6	5	0	9
♂ <i>T</i> :						
278	0	19	33	28	0	39
356	0	37	1	51	0	31
♀ <i>T</i> :						
220	1	48	0	16	0	15
331	0	27	0	22	0	21
364	0	22	0	25	2	27
491	0	37	0	25	1	30
188	0	20	0	10	1	15
♂ <i>FuT</i> :						
521	0	30	0	47	6*	41
♂ +:						
336	29	46	33	25	14	11
465	18	16	13	7	12	13
♀ +:						
274	12	6	16	18	22	23
379	8	8	14	19	23	15
485	3	1	19	25	19	14

* Genetically resistant on basis of long survival.

with AKR, as if he were a crossover ($H-2^fT/H-2^b+$) or at least lacked the *d* component. The cross to C57BL/6 ($H-2^b/H-2^b$) gives further support to this hypothesis (0 positives, 19 negatives). In this case, chance absence of positives from these two crosses may be ruled out. The results can be accounted for most simply either by a separation of the *d* and *k* components in a crossover, that, in this case, implies the order $DKFuT$, or by a mutation from $H-2^{dk}$ to $H-2^k$ in a noncrossover.

There were 6 other *T* crossovers, 1 male and 5 females. Almost all *T* offspring were negative to the tumor, such positives as did occur being of the frequency of "false positives". Whether some of these crossovers also carried *Fu* could not be determined. The tail types were typically *T*, but *Fu* may also have been present.

Five normal-tailed crossovers were found, half of whose young were positive to 15091a. The genotype of this class was thus $H-2^{dk}+/H-2^b+$. In this class there were 2 males and 3 females. Large numbers of offspring were raised in order to exclude the possibility that any of these normal-tailed animals were actually normal overlaps (genetically *Fu*). A chi square test of the total number of normal-tailed young pro-

duced by suspected normal-tailed crossovers as compared with the total array of progeny produced by *Fu* mice, of each sex and mating type, revealed a significant difference in every case. Data on the results of tumor inoculation from all matings were pooled for each normal-tailed mouse. A good fit to a 1:1 ratio was found when t-tests were run.

A very real drawback in testing the genotypic array of the BC generation was the lack of a decisive test for the *FuT* class. There should have been some animals of this genotype unless they suffered a heavy mortality. There were 5 normal-tailed positives in 284 BC mice, so mice containing the complementary chromosome, *H-2'^{FuT}*, would be expected. These animals should produce all negative young. Of course, the possibility remains that this class could have been confused with two other classes, *Fu* noncrossovers or, more probably, *T* crossovers.

Male 521, suspected of being *FuT*, was subjected to rigorous testing. Despite the production of 57 offspring with affected tails, unequivocal classification as *FuT* or *T* was impossible. But, whatever the genotype of ♂521, the inoculation results indicate that he was negative to the tumor because all his offspring were negative, with the exception of certain offspring of AKR parentage. The expected number of "false positives" here is .012. More were found probably for the reason that these mice were inoculated at weaning (4 weeks). A true genetically resistant state is suggested because the tail-affected positive and 3 of the 5 normal-tailed positives survived an average of 12 or more weeks before dying, a time interval of some 7 weeks longer than the mode for animals which are genetically positive.

Finally, mention should be made of the distribution of crossovers among progenies of the *Fu/T* animals used in producing the BC generation. Little individual variation was evident in the male data, for the frequency of crossovers found in any one progeny approximated that found in the total data. Among 9 offspring produced by the one *Fu/T* female was one crossover.

Order of the loci established

The most probable order of the three loci is *H-2 Fu T*. Such a conclusion becomes apparent when observed and expected classes under each possible alternative arrangement of the three loci are compared (See figure 3 for expected classes). The key classes making the second alternative seem most likely are the occurrence of *Fu+* and *++* mice. Finding *Fu+* mice excludes alternative 1 because these mice would then have to be considered double crossovers. By the same reasoning, finding animals which are genetically *++* excludes alternative 3.

Table 6 listing the observed and calculated values for each of the three alternatives demonstrates the close agreement between these values for alternative 2. There, the genotype of ♂521 is considered *Fu T*. Very little difference is found in the chi square values if ♂521 is considered as *T* only. To include the greatest number of expected double crossovers, no interference of the loci was assumed. This is the most unfavorable assumption. Thus, the calculated double crossovers are the product of the total crossovers at region a times the total crossovers at region b. The results of chi square tests show a probability of <.0001 for alternatives 1 or 3 and a probability of .70 for alternative 2. It may be concluded that the order of the loci is *H-2 Fu T*.

TABLE 6
Observed and calculated values establishing the order of the three loci as H-2 Fu T

Classes	Alternative 1 $Fu \times a \quad H-2^{ab}T$		Alternative 2 $H-2^{ab}Fu \times bT$		Alternative 3 $H-2^{ab}T \times b \quad Fu$	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
Noncrossovers	267	256.66	267	267.25	267	261.36
Singles at (a)	0	10.33	11	10.76	11	16.65
Singles at (b)	6	16.35	6	5.76	0	5.63
Doubles	11	0.66	0	0.23	6	0.36
Total	284	284.00	284	284.00	284	284.00
	$Fu-H-2 = 0.0387$ (a) $H-2-T = 0.0599$ (b) $Fu-T = 0.0986$ $(a) \times (b) = 0.002318$ $(a) - 0.002318 = 0.036382$ $(b) - 0.002318 = 0.057582$		$H-2-Fu = 0.0387$ (a) $Fu-T = 0.0211$ (b) $H-2-T = 0.0599$ $(a) \times (b) = 0.000817$ $(a) - 0.000817 = 0.037883$ $(b) - 0.000817 = 0.020283$		$H-2-T = 0.0599$ (a) $T-Fu = 0.0211$ (b) $H-2-Fu = 0.0810$ $(a) \times (b) = 0.001264$ $(a) - 0.001264 = 0.058636$ $(b) - 0.001264 = 0.019836$	
$2 \times 2\chi^2 =$	152.8	$P < .0001$	0.2443	$P = .70$	71.3	$P < .0001$

* Yates' correction applied.

DISCUSSION

Three topics will be discussed: (1) Sex differences in crossing over; (2) Observed crossover values and the order of the loci; and (3) The nature of the *H-2* locus.

(1) The usual, but not invariable rule in the mouse is that crossing over is higher in the heterozygous female than in the heterozygous male (See review in GRÜNEBERG 1952). Chromosome IX is one of the chromosomes that conforms to this rule. DUNN and CASPARI (1945) found a probably significant difference between males and females for the interval *Ki-T*. In crosses where the heterozygous parent was *Ki+ / +T* there was 14/1128 normal-tailed crossovers produced by male heterozygotes and 16/542 by female heterozygotes. The differences between the percentages 1.24 ± 0.33 and 2.95 ± 0.73 was on the borderline of being significant ($P = <.04$). Data from early experiments of DUNN and CASPARI (1945), which were designed to test recombination between the loci *Fu* and *Ki*, can be analyzed by sex. It is found that the 5 crossovers observed were all progeny of female heterozygotes.

Recently, DUNN and GLUECKSOHN-WAELSCH (1954) found no recombinants in a cross between *Fu* and *Ki* among 971 mice. Only 79 of these mice were heterozygous females. It may be that a sex difference in crossing over would explain the various conflicting results with these two genes. It is suggested that recombination is virtually 0 in males for this chromosomal interval but that it may take place in heterozygous females.

In crosses demonstrating *H-2'* as a tenth allele at the *H-2* locus (ALLEN 1955), the observed values for the *H-2-Fu* interval were 6.85 percent for female heterozygotes and 4.1 percent for the males. Though the difference is not significant, the higher value again occurs in the female. In the experiments reported here a very significant sex difference in recombination was found in the interval *H-2-T*. Approximately twice as many crossovers were produced by female BC mice (15.4 percent) as by males (8.25 percent).

(2) Of the various mapped intervals of the ninth chromosome (See table 1), the most accurately determined crossover percentage is that for *H-2* and *T*. There is fair agreement between the 6.0 percent found in the BC generation (table 6) and the 8.25 percent in the TC generation produced by male *T* noncrossovers (table 4, part 2). For *H-2* and *Fu* the map distance remains somewhat uncertain, but these loci are no doubt fairly close together. If *Fu* and *Ki* are alleles, we then have two estimates for the distance between *H-2* and *Fu*. For allele *Fu* the frequency of crossing over indicated in heterozygous males is 3.9 percent; whereas, for allele *Ki* the distance appeared shorter in two point crosses (1.02 percent). This may indicate that some of the *T* crossovers in the above three point cross were actually *FuT*, and, if so, would suggest a shorter interval between *H-2* and *Fu* and a longer interval between *Fu* and *T*.

The order of the loci established was *H-2 Fu T*. If information from three point crosses is pooled (from the above and from a cross performed by DUNN and CASPARI 1945), the only possible order for all four loci would be *H-2 Fu Ki T*. DUNN and CASPARI crossed *Ki T / Fu* $\times$ normal-tailed mice and obtained 167 progeny with abnormal tails and 18 with normal tails. Of 9 of these normal-tailed mice, 6 proved to be genetically $+++$. Assuming that these tests were adequate, *Ki* and *Fu* cannot be

alleles, and the gene order $Ki Fu T$ is practically eliminated as a possibility, since all normal-tailed mice would necessarily have been produced by double crossing over. They favored either the order (1) $Ki T Fu$ or (2) $Fu Ki T$. The first of these is eliminated by the above crosses with $H-2$.

(3) There was only one good case (σ^7278) suggesting a separation of the d and k components; however, mutation from $H-2^{dk}$ to $H-2^k$ may possibly have occurred. Mutations are not uncommon at the $H-2$ locus (BORGES and KVEDAR 1952; BORGES, KVEDAR and FOERSTER 1954; FOERSTER and BORGES 1954). It is indeed unfortunate that supporting serological tests were not performed on this mouse as well as on the exceptional fused animal ($\varphi 539$). Therefore, more conclusive evidence is needed for proof of the separation of the components. The detection of a genetic marker to the left of $H-2$ would considerably facilitate such proof.

Thus, whether the $H-2$ locus is composed of inseparable components, or of two or more pseudoalleles cannot be decided at present. Pseudoalleles are genes which are very closely linked, though separable, with similar phenotypic effect, and which characteristically exhibit certain patterns of physiological interaction (LEWIS 1951). These genes are usually associated with chromosomal "repeats" or sectional changes of the chromosome.

If such is the case with $H-2$, it will be particularly difficult to determine the rate of crossing over within the complex, since large numbers of mice would be required. Moreover, if $H-2$ has a high mutation rate, it will be even more difficult to decide between a true crossover and a mutation having occurred for any particular mouse in question.

SUMMARY

1. Genetically tested crossovers were found in the interval $H-2-Ki$ (or Fu^{Ki}). Recombination occurred in 2/196 mice (1.02 percent), all offspring of heterozygous males.

2. Crosses were set up to test the order of the loci Fu , T and $H-2$. By genetic tests, where offspring were inoculated with strain A tumor 15091a, 17/284 BC mice were proven crossovers. The pattern of response of the crossovers indicated that the order of the loci is $H-2 Fu T$. If Ki is a separate locus from Fu , then the only possible order for all four loci is $H-2 Fu Ki T$.

3. Statistically significant differences were found between TC groups produced by the T noncrossover class mated to the C57BL/6 strain due to the following factors:

a. Inoculation technique. False negatives were lower after December 1, 1952 than before. The cytosieve was used for carrying 15091a in the donor line in the latter period, the trocar in the earlier period.

b. Age of animals inoculated. False negatives were slightly higher in older animals than in younger animals when the age division was ± 2 months.

c. Sex difference in recombination in the interval $H-2-T$. After differences in false negatives were adjusted, recombination was found to be higher among offspring produced by heterozygous females than by heterozygous males. For males the crossover value was 8.25 percent, $N = 1381$; for females the value was 15.4 percent, $N = 1128$.

4. It has not been possible to determine whether the components at the *H-2* locus are separable. One crossover was found in which it appeared that the *d* component had been lost, the *k* component retained. However, the possibility of mutation from *H-2^{dk}* to *H-2^k* having occurred in this one case could not be ruled out.

ADDENDUM

GORER and co-workers (personal communication) have recently established two crossovers within the *H-2* locus by serological tests. Within this region there are possibly five loci, *DEFKC*, but their order is not yet certain. The first cross (*DE^d/D^bE*) resulted in a *D^bE^d* crossover; this mouse was homozygous for *F* but has not been typed for *C*. The second cross (*DKC/D^bkc*) gave a *D^bKC* crossover, the parents being homozygous for *E* and *F*. In the future, a revision in nomenclature for the "alleles" contained within the *H-2* region will undoubtedly be necessitated.

ACKNOWLEDGMENTS

I wish to extend my sincere gratitude to DR. GEORGE D. SNELL and to DR. SEWALL WRIGHT for their inspiration, guidance and criticism of the work reported. Credit is due DR. SEWALL WRIGHT for the mathematical method and derivation of formulae found in table 4.

LITERATURE CITED

- ALLEN, SALLY LYMAN, 1955 *H-2'*, a tenth allele at the histocompatibility-2 locus in the mouse as determined by tumor transplantation. (Cancer Research **15**: 315-319).
- BITTNER, J. J., 1935 A review of genetic studies on the transplantation of tumors. J. Genet. **31**: 471-487.
- BORGES, P. R. F., and B. J. KVEDAR, 1952 A mutation producing resistance to several transplantable neoplasms in the C57 Black strain of mice. Cancer Research **12**: 19-24.
- BORGES, P. R. F., B. J. KVEDAR, and G. E. FOERSTER, 1954 The development of an isogenic resistant subline of mice (C57BL/6-*H-2^d*) resistant to transplantable neoplasms indigenous to the C57BL strain. J. Nat. Cancer Inst. **15**: 341-346.
- CLOUDMAN, A. M., 1932 A comparative study of the transplantability of eight mammary gland tumors arising in inbred mice. Am. J. Cancer **16**: 568-630.
- DUNN, L. C., and E. CASPARI, 1945 A case of neighboring loci with similar effects. Genetics **30**: 543-568.
- DUNN, L. C., and S. GLUECKSOHN-WAELSCH, 1953 The failure of a t-allele (*t^s*) to suppress crossing over in the mouse. Genetics **38**: 512-517.
- 1954 A genetical study of the mutation 'fused' in the house mouse, with evidence concerning its allelism with a similar mutation 'kink'. J. Genet. **52**: 383-391.
- FISHER, R. A., 1921 On the mathematical foundations of theoretical statistics. Trans. Roy. Soc. London, A, **222**: 309-368.
- FOERSTER, G. E., and P. R. F. BORGES, 1954 A subline of mice (DBA/1B), derived from the DBA/1 strain, resistant to transplantable tumors indigenous to the DBA/1 strain of mice. Transplantation Bull. **1**: 200-201.
- GORER, P. A., 1936 The detection of antigenic differences in mouse erythrocytes by employment of immune sera. Brit. J. Exp. Pathol. **17**: 42-50.
- 1937 The genetic and antigenic basis of tumor transplantation. J. Pathol. Bacteriol. **44**: 691-697.
- GORER, P. A., SALLY LYMAN, and G. D. SNELL, 1948 Studies on the genetic and antigenic basis of tumour transplantation: Linkage between a histocompatibility gene and 'fused' in mice. Proc. Roy. Soc. London, B, **135**: 499-505.

- GORER, P. A., and Z. B. MIKULSKA, 1954 The antibody response to tumor inoculation. Improved methods of antibody detection. *Cancer Research* **14**: 651-655.
- GRÜNEBERG, H., 1952 The genetics of the mouse. The Hague: Martinus Nijhoff.
- HAUSCHKA, T. S., 1952 Immunologic aspects of cancer: A review. *Cancer Research* **12**: 615-633.
- HAUSCHKA, T. S., and A. LEVAN, 1953 Inverse relationship between chromosome ploidy and host-specificity of sixteen transplantable tumors. *Exp. Cell Research* **4**: 457-467.
- HOECKER, G., S. COUNCE, and P. M. SMITH, 1954 The antigens determined by the *H-2* locus: a Rhesus-like system in the mouse. *Proc. Nat. Acad. Sci. U. S.* **40**: 1040-1051.
- LEVAN, A., and T. S. HAUSCHKA, 1953 Endomitotic reduplication mechanisms in ascites tumors of the mouse. *J. Nat. Cancer Inst.* **14**: 1-44.
- LEWIS, E. B., 1951 Pseudoallelism and gene evolution. *Cold Spring Harbor Symposia Quant. Biol.* **16**: 159-174.
- LITTLE, C. C., 1941 The Genetics of Tumor Transplantation. *Biology of the Laboratory Mouse*. Edited by G. D. Snell. Philadelphia: The Blakiston Company.
- 1947 The genetics of cancer in mice. *Biol. Rev.* **22**: 315-343.
- SCHREK, R., 1936 A biological method for sterilizing contaminated transplantable rat tumors. *Am. J. Pathol.* **12**: 531-543.
- SNELL, G. D., 1948 Methods for the study of histocompatibility genes. *J. Genet.* **49**: 87-108.
- 1951a Five alleles at the histocompatibility-2 locus in the mouse as determined by tumor transplantation. *Cancer Research* **11**: 281-282.
- 1951b A fifth allele at the histocompatibility-2 locus of the mouse as determined by tumor transplantation. *J. Nat. Cancer Inst.* **11**: 1299-1305.
- 1952 Preliminary data on crossing over between *H-2* and *Fu*, *Ki* and *T* in the mouse. *Heredity* **6**: 247-254.
- 1953a A cytosieve permitting sterile preparation of suspensions of tumor cells for transplantation. *J. Nat. Cancer Inst.* **13**: 1511-1515.
- 1953b The genetics of transplantation. *J. Nat. Cancer Inst.* **14**: 691-700.
- 1953c Transplantable Tumors. *The Physiopathology of Cancer*. Edited by F. Homburger and W. H. Fishman. New York: Paul B. Hoeber, Inc.
- SNELL, G. D., P. R. F. BORGES, D. E. KELTON, and SALLY LYMAN ALLEN, 1952 Histocompatibility genes in the mouse; methods of study, and some results. *Genetics* **37**: 626-627.
- SNELL, G. D., and G. F. HIGGINS, 1951 Alleles at the histocompatibility-2 locus in the mouse as determined by tumor transplantation. *Genetics* **36**: 306-310.
- SNELL, G. D., E. RUSSELL, E. FEKETE, and P. SMITH, 1953 Resistance of various inbred strains of mice to tumor.homoiotransplants, and its relation to the *H-2* allele which they carry. *J. Nat. Cancer Inst.* **14**: 485-491.
- SNELL, G. D., P. SMITH, and F. GABRIELSON, 1953 Analysis of the histocompatibility-2 locus in the mouse. *J. Nat. Cancer Inst.* **14**: 457-480.