FURTHER INVESTIGATIONS ON THE ORIGIN OF TUMORS IN MICE.

V. The Tumor Rate in Hybrid Strains.

By A. E. C. LATHROP and LEO LOEB, M.D.

(From the Department of Comparative Pathology of Washington University Medical School, St. Louis.)

(Received for publication, April 29, 1918.)

In former investigations we have tested the cancer rate and cancer age in hybrid strains.[1] In some crosses the cancer rate in both parents was similar; in these cases the offspring followed the cancer rate and age of the parents. In other cases there was a marked difference in the cancer rate and cancer age of both parents. In such crosses we found in the majority of cases either an intermediate result or dominance of the parent with the higher tumor rate. Our results are therefore incompatible with the assumption that has been expressed that in crosses the parent with the lower cancer rate dominates. We found it desirable to continue our observations, especially with a view of increasing the number of crosses in which the cancer rate and cancer age of both parents showed a marked difference. For this purpose we had at our disposal a number of suitable strains or substrains whose cancer rate and age had been carefully followed through a number of years.

We made the following crosses:

1. Silver + English (Offspring of 177a); Strain a.

The two English females died without tumors 7 and 12 months old.

Without tumors.	With tumors.
$F_1 - F_3$ 8 (5 I 1 II 2 III)	14 (6 I 8 II)
36 % (63% I 12% II 25% III)	64 (43% I 57% II)

[1] Lathrop, A. E. C., and Loeb, Leo, *J. Exp. Med.*, 1915, xxii, 713.

2. Silver + English (Sable F_5); Strain c.

The two Sable females that entered into the cross with Silver died without tumor in the first and third age period respectively.

	Without tumors.	With tumors.
F_1	5 (3 I 1 II 1 III)	2 (1 II 1 III)
F_2	4 (3 II 1 III)	7 (3 I 4 II)
$F_1 + F_2$	9 (3 I 4 II 2 III)	9 (4 I 5 II)
	50% (33% I 45% II 22% III)	50% (44% I 56% II)

The combination of Silver and English is of particular interest in view of the great difference in tumor rate between these two strains. The rate of the English varies between 55 and 80% and that of Silver between 7 and 10%. The tumors appear early in both kinds. There attaches an additional interest to this combination because of the fact that the Silver represented originally a substrain of the English. In both crosses a similar result was obtained. The higher tumor rate of the mothers (English) prevailed over the low tumor rate of the (Silver) father. The tumors belong like those of the parents to the first age class. It is improbable that in this case in both hybrid strains an accidental selection of Silver individuals with a higher tumor rate took place, considering the uniformity in the low tumor rate of the Silver throughout several generations.

It is, furthermore, of interest to note that all the female English which entered into these strains died without tumors; but belonging to a strain rich in tumors they nevertheless carried within them the potentiality of a high tumor rate. The realization of this potentiality depends on more or less accidental (external) circumstances. While the English were prolific, but delicate, the Silver were very poor breeders. Both kinds of hybrids were prolific and usually vigorous. In this case, then, high tumor rate of the mother strain was linked with a considerable prolificity; both these characteristics were dominant over the opposite characters of the father strain.

London + Silver.

One London male was mated to three Silver females. Later the same London male was given to Cream females. Four generations were observed. The records of the generations agreed well with each other.

	Without tumors.	With tumors.
Total:	154 (34 I 89 II 31 III)	22 (4 I 11 II 7 III)
	88% (22% I 58% II 20% III)	12% (18% I 50% II 32% III)

The records of the parent strains are as follows:

London (old):		II age class.
	73% (41% I 29% II 30% III)	27% (27% I 43% II 30% III)
London (new):		I age class.
	72% (31% I 40% II 29% III)	28% (40% I 51% II 9% III)
Silver (old):		I age class.
	93% (41% I 45% II 49% III)	7% (50% I 50% II)
Silver (new):		
	90% (33% I 56% II 11% III)	10% (100%II)

In the cross the tumor rate is somewhat better than that of Silver. However, the rate is nearer that of the Silver (mother) than of the London (father). The tumors appear somewhat later than in either parent.

We may conclude that the result is intermediate, but perhaps somewhat nearer the mother strain which in this case has a lower rate. It is a valuable confirmation of our conclusion that the high tumor rate of the cross Silver + English was not due to an accidental selection of Silver mice with higher tumor rate. Mated with the London strain the Silver tumors remain true to their low tumor rate.

If we split off from larger strains definite families which preserve the characteristics of the larger strains and later hybridize the families, the tumor rate and tumor age of the offspring remain in accord with the tumor rate and age of the main strain. Such hybridizations may serve as controls to cases in which the tumor rates of both parents differ. We cite two examples:

A son of Tumor Mouse 344 (English Sable) was mated to a daughter of 328 (English Sable). This same son of 344 was later used for hybridization with German, European + English, 8½, Black Cream, and Cream. Mated to a sister this son of 344 had offspring with a very high tumor rate.

344 + 328

Without tumors.	With tumors.
F_1 F_3 F_4 1 (1 I)	6 (6 I).
14½% (100% I)	85½% (100% I)

(344 F_1 + 328 F_1) F_2 + 437 F_2 (437 = English Sable)

Without tumors.	With tumors.
F_1 — F_5 6 (3 I 3 II)	21 (15 I 6 II)
22% (50% I 50% II)	78% (71% I 29% II)

In both groups the typical English tumor rate and tumor age were obtained.

8½ + 328

Four daughters of 328 (English Sable) were crossed with an 8½ F_4 male.

Without tumors.	With tumors
F_4 62 (45 I 14 II 3 III)	84 (53 I 29 II 2 III)
42½% (72% I 23% II 5% III)	57½% (63% I 34.6% II 2.4% III)

The tumors belong to the first age class. 8½ had a low tumor rate of 17 %; the tumors belonged to the II age class. In the cross the higher tumor rate and the earlier tumor class of the mother prevailed. These records agree with the records of a similar cross published in our preceding paper.[1] Here the tumor rate was 61 % and the tumors belonged likewise to the first age class. We

see then that in different crosses between 8½ and English Sable the higher tumor rate and the earlier tumor age of the mother strain prevailed.

It was of interest to attempt the reciprocal cross, in which the father was English (344) and the mother belonged to the 8½ strain. Two crosses were made. First 344 (English Sable) was mated to an 8½ F_4 female and after 8½ F_4 had died, 344 was crossed with a young 8½ F_6 female.

English (344) + 8½ F_4.

Without tumors.	With tumors.
$F_1 - F_4$ 83 (31 I 25 II 27 III)	34 (11 I 16 II 7 III)
71% (37% I 30% II 33% III)	29% (32% I 47% II 21% III)

The tumor rate is intermediate, but approaches more closely the low rate of 8½ than the high rate of English. The tumor age is also intermediate between that of 8½ (II age class) and English (I age class).

English Sable (344) + 8½ F_6.

Without tumors.	With tumors.
$F_1 - F_3$ 33 (9 I 12 II 12 III)	5 (3 I 1 II 1 III)
87% (27% I 36½% II 36½% III)	13% (60% I 20% II 20% III)

In this the 8½ tumor rate dominates still more completely than in the former cross. The tumors appear early. The tumor age is apparently influenced by the English father. However, the number of tumor mice is so small in this cross that little value can be attached to the exact determination of the age class.

We see then that while in a cross of 8½ male with English Sable female, the English female dominates, in the reciprocal cross the 8½ female dominates either completely or almost so. As far as the tumor age is concerned, the English influence is still noticeable, but not entirely dominant. We must conclude that the English 344 father was less able to determine the tumor rate and tumor age of the offspring than various English females.

English Sable (344 F_1) + German.

The same 344 male which had been used in the former records was crossed with two rather old German F_4 females.

Without tumors.	With tumors.
$F_2 - F_6$ 16 (5 I 11 II)	29 (17 I 11 II 1 III)
35% (31% I 69% II)	64½% (58½% I 38% II 3½% III)

The tumors stand between the I and II age class, but are much nearer the I age class. The tumor rate of German varies between 50 and 31%. The tumor age varies between the II and I class. In this cross the difference between the two parents is, therefore, not very marked; on the whole the offspring seem to follow more closely the tumor rate of the father. 344 + German were very good breeders and a fairly strong, healthy strain. In former crosses between

English Tan and German, the tumor rates were 49 and 43 %. The tumors belonged to the first age class. We see, therefore, that the crosses between two high tumor strains again show a high tumor rate.

English Sable 344 + (European + English, Daughter of Tumor Mouse 146) = 344 + 146.

The female parent strain had formerly a tumor rate of 28 % (intermediate generations), but in the third generation the tumor rate was as low as 9 %. The tumors belonged to the II age class. The more recent records, which are, however, based on a smaller number of mice, show a tumor rate of 42 %, which is similar to the tumor rate in the first generations. The tumors appear late. The figures for the cross 344 + 146 are as follows:

Without tumors.	With tumors.
$F_1 - F_2$ 20 (3 I 9 II 8 III)	1 (1 II)
95% (15% I 45% II 40% III)	5% (100% II)

The tumor rate is very low. Evidently the low rate of the mother strain dominates. In this case the mother belonged probably to a substrain with a tumor rate lower than that of the average of the strain. The only tumor which was observed in this cross appeared in the II age period. In this cross the effect of the European strain dominates, therefore, over the English strain.

English Sable + (English Silver + I Daughter of No. 10 Nov. 3 Strain).

In this case a high tumor strain (male) (55 to 80 % I age class) was crossed with a medium tumor strain (female) (36 % II age class). The offspring follows the high tumor rate of the father. The old and new records combined give the following figures:

Without tumors.	With tumors
8 (6 I 2 II)	15 (6 I 9 II)
35% (75% I 25% II)	65% (40% I 60% II)

The tumors belong to the first age class. In this cross two high tumor strains enter into combination with a low tumor strain.

English Sable + European Hybrid F_3—(175).

European Hybrid F_3 is (European + No. 10 Nov. 3) F_1. European Hybrids had a tumor rate of 72 % and belonged to the II age class. There were, therefore, two high tumor strains combined in this case.

Without tumors.	With tumors.
$F_1 - F_4$ 10 (7 I 2 II 1 III)	31 (22 I 8 II 1 III)
24½% (70% I 20% II 10% III)	75½% (71% I 26% II 3% III)

The high tumor rate of both parents and the early age class of the father strain prevailed.

A second cross of the same kind in which, however, different individuals were used, was produced.

English Sable (482) + Daughter of 392 (May 1911 Group of European Hybrids).

The daughter of 392 later developed a tumor:

	Without tumors.	With tumors.
$F_1 - F_6$	46 (27 I 15 II 4 III)	38 (17 I 18 II 3 III)
	55% (58% I 33% II 9% III)	45% (42% I 50% II 8% III)

While the tumor rate in the offspring is high, it is lower than in the preceding cross which was of the same kind. The tumors belong here to the II age class of the mother strain. The fourth generation of the cross had in this case a low tumor rate. While certain variations in the tumor rate and tumor age in crosses of the same strains, but into which different individuals enter, do therefore, occur, these variations were in this case not very considerable.

Summary.—(1) Crosses between two substrains which have the same tumor rate and tumor age as the original strain from which they were split off, give the same tumor rate and age as those of the original strain.

(2) Crosses between different strains both of high tumor rate, have high tumor rate; in our case the tumor ages differed and the earlier tumor age of the English strain was dominant.

(3) Two crosses between Silver and English Sable which were both substrains of the English family, but differed markedly from each other in their tumor rate, had the high tumor rate of the mother.

(4) In crosses between English and $8\frac{1}{2}$, the English tumor rate and age prevailed, if the English was used as mother strain, while in two reciprocal crosses the tumor rate was the low rate of the $8\frac{1}{2}$ strain or it approached it. In the tumor age some influence of the English strain was apparently noticeable, although the tumor age was also influenced by the $8\frac{1}{2}$. Whether in this case the individual character of the English father (344) was responsible for the result, we cannot definitely decide at the present time.

(5) In a cross of the same English male (344) with a (European + English), the low tumor rate of the mother prevailed, while in two

crosses in which other English males were mated to females of high and medium tumor strains the English age prevailed and the tumor rate was high.

Crosses between English and Cream.

Crosses between English and Cream are of particular interest in view of the great difference in the tumor rate and tumor age in these two strains. We had previously reported on the following two crosses of this kind.[1]

English Tan (121) + 3 Cream:	42% II age class.
" Sable male (No. 4,444) + Cream:	No tumors.

In one cross an intermediate result was obtained, in the other the Cream strain dominated. It seemed desirable to add to these experiments a number of further crosses between English and Cream.

English Sable (344) + Black Cream.

The 344 substrain of English Sable was the offspring of a son and a daughter of Tumor Mouse 344 (English Sable). This substrain had a very high tumor rate. One of the males of this substrain was mated to a Black Cream female, the tumor rate of which varied between 2 and 10 %, and the tumor age between the II and IV age class.

Without tumors.	With tumors.
$F_1 - F_4$ 71 (36 I 18 II 17 III)	21 (8 I 9 II 4 III)
77% (51% I 25% II 24% III)	23% (39% I 41½% II 19½% III)

The tumor age stands between the I and II age class, but is nearer the II age class. This cross was, therefore, in tumor rate and tumor age intermediate between English and Cream, but somewhat nearer Cream.

(a) (344 + Black Cream) F_1 + Cream (White).

A male of the first generation of the former strain was mated to a Cream female (of white color).

Without tumors.	With tumors.
F_1 F_3 F_4 11 (3 I 2 II 6 III)	0
100% (27% I 18% II 55% III)	0%

This cross was three-fourths Cream and one-fourth English. The Cream prevailed. Tumors did not develop.

(b) English (344) + Cream.

Two males of the 344 English Sable family which differed from those used in the previous cross were mated to Cream females, not identical with the ones which entered into the previous cross.

Without tumors.	With tumors.
130 (61 I 43 II 26 III)	13 (6 I 5 II 2 III)
91% (47% I 33% II 20% III)	9% (47% I 38% II 15% III)
Almost I age class.	

The tumor rate is very low, almost corresponding to that of Cream, but the tumors appear earlier and in this respect perhaps the influence of the English father is noticeable.

English Sable (4,444) + Cream.

A part of this record was reported previously.

Two English Sable males were mated to nine Cream females which had been kept without breeding until they were 9 months old. Most of the daughters of the English father with English Sable females had an offspring with a high tumor rate. A daughter of this English Sable male and English female was mated to ($8\frac{1}{2}$) and the cross showed a high tumor rate.

We have, therefore, no reason to assume that this male did not possess potentiality to a high tumor rate.

These hybrids of English Sable (4,444) + Cream had formerly no tumors.

Without tumors.	With tumors.
$F_2 - F_5$ 170 (44 I 54 II 72 III)	5 (2 I 2 II 1 III)
97.2% (26% I 32% II 42% III)	2.8% (40% I 40% II 20% III)

The Cream again prevailed in this cross. The few tumors which were found appeared relatively early.

Black Cream + English White.

One Black Cream male was mated to ten English females, including 344 F_4, 437 F_4, English Sable F_5.

Without tumors.	With tumors.
$F_1 - F_3$ 86 (33 I 23 II 30 III)	57 (19 I 29 II 9 III)
61% (38% I 27% II 35% III)	39% ($33\frac{1}{3}$% I $50\frac{2}{3}$% II 16% III)

The tumor rate is intermediate between English and Cream and the tumor age stands between the I and II age class. In this cross the influence of the English strain is noticeable to a higher degree than in the majority of the other crosses between English and Cream.

Cream + English (Oct. 1913).

A Black Cream male was mated to a group of English females of the Oct. 1913 generations.

Without tumors.	With tumors.
$F_1 - F_4$ 84 (29 I 27 II 28 III)	25 (6 I 10 II 9 III)
77% (35% I 32% II 33% III)	23% (24% I 40% II 36% III)

The cross is intermediate between English and Cream, but somewhat nearer Cream. The tumor age stands between the II and III age classes. In the age class also the influence of the Cream is marked.

White Cream + White English.

An albino Cream male, son of a Black Cream, was mated to a White English female, the offspring of Tumor Mouse 437 and another tumor mouse belonging to the same group as 437.

Without tumors.	With tumors.
$F_1 - F_3$ 19 (8 I 7 II 4 III)	8 (5 I 2 II 1 III)
71% (42% I 37% II 21% III)	29% (62½ I 25% II 12½% III)

The tumor rate is intermediate between English and Cream. The tumors appeared as early as in the English.

Cream + English (Descendants of English Sable F_1 1,031).

Without tumors.	With tumors.
$F_1 - F_3$ 18 (6 I 7 II 5 III)	8 (1 I 5 II 2 III)
69% (33⅓% I 38⅔% II 28% III)	31% (12½% I 62½% II 25% III)

The tumor rate is intermediate, but the tumor age is that of the Cream; it stands between the III and IV age classes.

One substrain of Cream was Cream Y. Its record was as follows:

Without tumors.	With tumors.
$F_1 - F_4$ 14 (6 I 5 II 3 III)	Tumors were not observed in this substrain.

English Sable + Cream Y.

One English Sable F_4 male was mated to three Cream Y F_1 females.

Without tumors.	With tumors.
32 (14 I 14 II 4 III)	36 (11 I 22 II 3 III)
47% (43¾% I 43¾% II 12½% III)	53% (30⅔% I 61⅓% II 8% III)

The tumor rate is a little lower than that of the English. The tumor age stands between the I and II age classes, but is nearer the II age class, as far as the first and second age periods are concerned. In this instance, then, the result is intermediate between English and Cream in tumor rate as well as in tumor age, but with great preponderance of the English father.

693 + Cream.

693 was 121 (English Tan) + Cream. This male was mated to four Cream sisters which died without tumors. The tumor rate of the offspring of 693 was 42%; the tumors belonged to the II age class.

Without tumors.	With tumors.
$F_1 F_2$ 5 (4 I 1 II)	2 (2 I)
71½% (80% I 20% II)	28½% (100% I)

The number of mice in this strain is small, but the results indicate that the tumor rate will be intermediate between that of the father and mother strains. The tumor rate is certainly higher and the tumors appear earlier than in the Cream.

693 (=121 (English Tan) + Cream) + 773 (= 8½ + 328 English Sable).

Previous to the mating with the Cream females, 693, which was (121 + Cream F_3) F_1, had been mated to one 8½ + 428 F_1 female.

Without tumors.	With tumors.
14 (10 I 4 II)	21 (14 I 7 II)
40% (71½% I 28½% II)	60% (66⅔% I 33⅓% II)

Tumor rate and tumor age of this strain are that of the English. The father strain had a tumor rate of 42% and belonged to the II age class; the mother strain had a tumor rate of 57½% and belonged to the I age class. The English component dominated in the mother strain. The mother strain and with it the English tumor rate and age class dominated in the complex hybrids.

We may arrange these hybrids in two classes; in the first one the father of the hybrids was English and the mother Cream, while in the second class, the reverse conditions prevailed.

Father: English, Mother: Cream.

English Tan (121) + 3 Cream. 42% II age class. I (nearer English).
" Sable (4,444) + " 0–2.8% C (somewhat earlier tumors).
" " (344) + Black Cream. 236 between I and II age classes. I
(*a*) 344 + Black Cream F_1 + Cream White. C.
(*b*) 344 + Cream. 9% (almost I age class). C (almost I age class).
English Sable + Cream Y. 53 II age class. I (nearly English).
Composite Strains:
693 + Cream (= English Tan + Cream) + Cream. I.
693 + 773 (= 8½ + 328). 60% English prevails through mother strain. E.

Father: Cream, Mother: English.

Black Cream + English White	39% I–II age class.	I (English preponderant).
Cream + English (Oct. 1913)	23% II–III " "	I (Cream ").
White Cream + White English	29% I age class.	I (English tumor age).
Cream + English (1,031)	31% III–IV age class.	I (Cream " ").

I = Intermediate; E = English; C = Cream.

In four strains the father was Cream and the mother English. These four crosses were all intermediate, the tumor rate varying between 23 and 39%. The tumor age varied independently of the tumor rate; but the number of tumors being relatively small, not too much importance must be attached to variations in the tumor age. In the reciprocal crosses there are six strains. In three of those the tumor rate and tumor age were intermediate, but in two of these three the hybrids were nearer the English than the Cream. In the three other strains the hybrids followed the tumor rate of the Cream; in two of these, however, the tumors appeared earlier than in the Cream.

There are in addition two composite strains. In the first of these an English + Cream Hybrid with intermediate tumor rate was mated to a Cream. The admixture of the Cream reduced the tumor rate of the hybrid still farther. In the second composite hybrid the same hybrid strain was crossed with a hybrid strain with high tumor rate in which the English prevailed. In this the high tumor rate of the mother dominated.

Summary.—If a strain with a very high tumor rate (English) is crossed with a strain with a very low tumor rate (Cream), the hybrids show in seven out of ten instances an intermediate tumor rate. In three instances (50%) in which the mother strain was Cream, the tumor rate of the Cream prevailed, while this was found in no cross in which the mother was English and the father Cream. This again suggests the possibility that in certain instances the tumor rate of the mother strain may be of greater importance in determining the tumor rate than that of the father. Through further gradual addition of Cream to such hybrids the tumor rate was made to approach still more closely that of the Cream.

We see then that if we cross two strains with pronounced opposite tendencies, the result is in the majority of cases intermediate. While on the whole there is noticeable a certain correspondence between tumor rate and tumor age of these hybrids, certain independent variations of both seem to occur.

We shall now discuss crosses between Cream and 198 + (8 + German) F_4, between Cream and No. 10 Nov. 3 strain, and between Cream and European.

Cream + (198 + German Hybrid F_4) = 198 = English.

German Hybrid F_4 = 8 + German F_4. Two Black Cream males were crossed with two females. The record of these seven females was as follows:

Without tumors.	With tumors.
4 (3 II 1 III)	3 (2 I 1 II)

The old tumor rate of the mother strain was 63% (I age class), the later records showed a tumor rate of 43% (between I and II age classes).

The records of the hybrids are as follows:

Without tumors.	With tumors.
$F_1 - F_3$ 22 (5 I 15 II 2 III)	11 (5 I 6 II)
66⅔% (23% I 68% II 9% III)	33⅓% (45% I 55% II)

I age class: The tumor rate is intermediate, but nearer to the mother strain than to the Cream. The age class is that of the mother strain.

Cream + No. 10 (Nov. 3).

No. 10 (Nov. 3) = 151 (European + I daughter of No. 10 (Nov. 3)).

In the former cross of this kind the records were as follows:

Without tumors.	With tumors.
Former records:	
$F_1 - F_4$ 64% (16% I 29% II 55% III)	36% (6% I 37% II 57% III)
IV age class.	
$F_4 - F_6$, later records:	
74% (28% I 31% II 41% III)	26% (33% I 50% II 17% III)
I age class.	

The results were intermediate.

Two new crosses of the same kind were made in which different individuals were used.

(*a*) *White Cream + 697.*

White Cream was mated to three daughters of Tumor Mouse 697. No. 697 belonged to the No. 10 Nov. 3 strain. The records of these three females was as follows:

Without tumors	With tumors.
1 I 1 II	1 II

The cross behaved as follows:

Without tumors.	With tumors.
$F_1 - F_5$ 116 (47 I 41 II 28 III)	12 (2 I 4 II 6 III)
90½% (41% I 35% II 24% III)	9½% (16½% I 33½% II 50% III)
About IV age class.	

In this cross the Cream was dominant.

(b) White Cream + No. 10 (Nov. 3).

A White Cream male was mated to six No. 10 (Nov. 3) F_5 females which were offspring of the July, 1912, group. This is a cross which differs from the preceding one. The record of the original six females was as follows:

Without tumors.	With tumors.
2 I 1 II	2 I 1 II

The figures for the cross:

Without tumors.	With tumors.
98 (36 I 32 II 30 III)	51 (12 I 20 II 19 III)
66% (36½% I 32½% II 31% III)	34% (23% I 39% II 38% III)

The age class is between II and III.

The result in this hybrid is intermediate, just as it was in the previous crosses between Cream and No. 10 Nov. 3.

Cream + European.

(a) In one of our previous papers we reported on a cross between a Cream male and a European F_2 female which later became Tumor Mouse 428. All three daughters, which represented a cross between Cream and European, later developed tumors. In this case evidently the higher tumor rate of the mother strain was dominant. These daughters which later developed tumors were again mated to their Cream father.

Cream + (Cream + European 428) F_1.

Without tumors.	With tumors.
$F_1 - F_7$ 10 (3 I 6 II 1 III)	1 (1 II)
90% (30% I 60% II 10% III)	10% (100% II)

This cross resembles the Cream. Evidently the Cream father had a tendency to low tumor rate and the higher tumor rate of the Cream + European had been due to the influence of the European strain in the cross.

We produced a second hybrid strain between Cream male and European female; male Black Cream baby mice had been nursed by a tumor mouse of the G strain; one of these male Creams was mated to a European female. The offspring were very wild.

Without tumors.	With tumors.
$F_1 - F_4$ 70 (26 I 34 II 10 III)	34 (8 I 17 II 9 III)
68% (37% I 49% II 14% III)	32% (24% I 49% II 27% III)

The age class is between II and III, but nearer III.

In this case again the higher tumor rate of the European mother strain is dominant. In the tumor age there is apparently some influence of the Cream father strain noticeable.

We also made the reciprocal cross:

European + Cream.

A European F_7 male was mated to four Black Cream females. The four Black Cream females died without tumors, one in the I age period and three in the III age period. They behaved, therefore, like typical Creams.

The hybrids which were wild, similar to the Cream + European hybrids, had the following tumor rate:

Without tumors.	With tumors.
$F_1 - F_3$ 18 (10 I 5 II 3 III)	7 (1 I 3 II 3 III)
72% (55% I 28% II 17% III)	28% (14% I 43% II 43% III)

Again the higher tumor rate of the European is dominant over that of the Cream. The rate is similar to that of the reciprocal cross: Cream + European. The tumors belong, however, to the IV age class. Here the influence of the Cream is noticeable.

Summary.—We see, then, that in the crosses Cream + European, as well as in the reciprocal cross European + Cream, the higher tumor rate of the European dominates over the lower rate of the Cream. The tumor age is apparently influenced by the Cream component. If an additional Cream component is added to such a strain the tumor rate is lowered almost to the level of the Cream. In the cross between Cream + (198 + German Hybrid F_4) the result is intermediate; but on the whole the tumor rate is nearer that of the mother strain. The age class is that of the mother strain. In previous crosses between Cream and No. 10 (Nov. 3) the tumor rate had been intermediate. In a new cross of the same character in which new individuals entered, the tumor age was again intermediate and similar to the tumor rate of the earlier cross. The tumor age also was intermediate. In a second cross, however, the Cream tumor rate and tumor age were dominant. It is possible that in this case the three females which were sisters happened to transfer a tendency to a lower tumor rate.

German Hybrids.

We have previously reported on 8 + German Hybrids. The tumor rate in the old records is 41 per cent (II age class); in the intermediate and new records the tumor rate is 20 per cent (23 per cent), and the tumors appear somewhat earlier than those in the II age class. In one family of the later generations the tumor rate remained 43 per cent.

German + 8.

We now made the reciprocal cross: German + 8. One German male (about F_4) was mated to two No. 8 F_{12} females. These females died without tumors at the age of 12 months.

Without tumors.	With tumors.
$F_1 - F_4$ 112 (42 I 48 II 22 III)	0
100% (38% I 42% II 20% III)	0%

While in the 8 + German, the tumor rate of the German had been dominant, or at least very influential, we find in this strain a dominance of the No. 8. In the twelfth generation the tumor rate of the No. 8 had become very low (10%). We find here another instance in which apparently the mother strain is more influential in determining the tumor rate than the father strain.

Summary.—In the cross German + 8, we find dominance of the low tumor rate of the mother strain, while in the reciprocal cross 8 + German we find the higher tumor rate of the mother strain prevailing. In the two other crosses the higher tumor rates of the mother strains were dominant; the tumor age corresponded to that of the parents.

Waltzer Hybrids.

A series of hybridizations between waltzing mice, among which cancers are rare, and other strains, especially English, was carried out. In some crosses between Waltzer and English the high tumor rate of the English is almost dominant, while in the others the tumor rate is probably intermediate. The tumors always belong to the first age class, but they appear somewhat earlier in crosses which have a high tumor rate than in the others. In the crosses in which the English strain was almost dominant, further introduction of English made the record typically that of the English strain; while with the other hybrids, in which the combination with the Waltzer had considerably lowered the tumor rate of the English, the further intro-

duction of English did not raise the tumor rate of the crosses to a point where it was similar to that of the English; in this case the lower tumor rate of the father prevailed.

The crosses between Waltzer and Carter, Waltzer and No. 8, and (Waltzer + English) + (Cream + 10) either follow the tumor rate of both parents, or the higher tumor rate of the mother strain prevails.

Vermont Wild + (English) F_3.

A wild mouse captured in northern Vermont was mated to six English Sable (F_3) females, daughters of the Aug., 1911, group to which Tumor Mouse 437 belonged. These six English Sable mice died without tumors, two at the age of 7 months, two at the age of 10 months, one at the age of 13 months, one at the age of 14 months.

Without tumors.	With tumors.
$F_1 - F_4$ 241 (100 I 82 II 53 III)	138 (32 I 57 II 49 III)
63½ % (44% I 34% II 22% III)	36½% (23% I 41% II 36% III)

The tumor age is between the II and III age classes. The four generations behaved in a similar way.

While we do not know the tumor rate of the Vermont Wild mouse, it is at least probable that the tumor rate of these wild mice is low, perhaps comparable with that of the Cream. We would then have to conclude that the tumor rate of the hybrids is intermediate between that of both parent strains. The influence of the Vermont father strain is expressed in the relatively late tumor age, which differs so markedly from that of the English.

In a former similar experiment[1] we reported on the hybridization between a Michigan Wild mouse and English 101. In this case the tumor rate and tumor age of the crosses were approximately those of the English mother strain. We may conclude that in crosses between wild gray mice as the fathers and white mice as mothers, the result is probably either intermediate or the higher tumor rate and tumor age of the mother strain are dominant.

Parent with Higher Tumor Rate Is Dominant.

♀ indicates dominance of the mother strain.
♂ indicates dominance of the father strain.
The Roman figures indicate the age class of the offspring.
! indicates that importance can be attached to the results.
New records:
(*a*) Silver + English ♀ I !
(*b*) Silver + English ♀ I !
8½ + 328 ♀ I !
344 (English Sable) + German ♂.

English Sable + Cream Y ! ♂ (slightly below the English record, intermediate age class), also in intermediate record.
English Sable + (English Silver + I daughter of No. 10 Nov. 3 strain) ♂. The age class of the father dominates!
693 (English Tan + Cream) + 773 ($8\frac{1}{2}$ + 328) ♀. I age class !
Cream + (198 + German Hybrid F_4) ♀ (approaching intermediate) higher age class !
Cream + European ♀ (*a*) R !
Cream + European ♀ (*b*). Influence of Cream on tumor age R !
European + Cream ♂. Age class of Cream R !
8 + German ♀ (or intermediate) R !
(German + Carter) + (8 + German) ♀ (not much difference between the two parent strains).
(German + Carter) + (198 + German Hybrid F_4) F_3 (almost English tumor rate) ♀.
Waltzer + English (orange) (*a*) ♀. Age class of English.
(Waltzer + English (orange)) + English (orange) (*c*) ♀.

Complete English Tumor Rate and Age; Parent Strains with Similar Rate!

(Waltzer + English (orange)) + English Sable (*d*) ♀. English tumor age.
Walter + Carter ♀.
Waltzer + 8 ♀.
Old records:
Michigan Wild + English 101 ♀. English tumor rate.
English Sable (198) + ((103 + European) F_1 + III daughter of No. 10) ♂. Late tumors.
European 151 + I daughter of No. 10 ♀ !
European 151 + II daughter of No. 10 ♀ !
English + (8 + German) ♂ !
$8\frac{1}{2}$ + II daughter of No. 10 (not quite dominant) ♀ ! later decreasing tumor rate. Late tumors.
English Sable + (Silver + I daughter of No. 10) ♂ !
$8\frac{1}{2}$ + English Sable ♀. Early tumors !
English + (8 + German) ♂ !

Intermediate Tumor Rate.

New records:
344 (English Sable) +$8\frac{1}{2}$ F_4. Intermediate age class!
English Tan (121) + 3 Cream. Intermediate age class !
English Sable (344) + Black Cream. Intermediate age class !
English Sable + Cream Y. Intermediate age class (approaching tumor rate of English)!

693 (English Tan + Cream) + Cream !
Black Cream + English White. Intermediate age class !
Cream + English (Oct. 1913). Intermediate age class !
White Cream + White English. English age class !
Cream + English (1,031). Cream age class !
Cream + No. 10 (Nov. 3). Early tumors !
Cream + (198 + German Hybrid F_4) ♀. Early tumors (also in higher tumor rate records) !
White Cream + No. 10 (Nov. 3) (*b*). Intermediate tumor age.
Waltzer + English (white) (*b*). English age class.
Vermont Wild + English F_3 ♀. Late tumors.

Old records:

101 + (European + 103). Late tumors.
European + English Tan. Relatively late tumors!
Silver + I daughter of No. 10. Recently the tumor rate apparently increased and the tumor rate of No. 10 became almost dominating.
No. 8 + German.
Cream + No. 10 (Nov. 3) !

Parent with Lower Tumor Rate Is Dominant.

New records:

344 (English Sable) + $8\frac{1}{2}$ F_6 ♀. Earlier tumor age!
344 (English Sable) + 146 (European + English). The tumor rate in this cross was even lower than that of the mother strain ♀.
English Sable (4,444) + Cream ♀. Age class intermediate!
(*b*) 344 + Cream ♀. Age class intermediate!
(344 + Black Cream F_1) + Cream White ♀ !
White Cream + 697 (No. 10 Nov. 3) (*a*) ♂. Cream age class.
Cream + (Cream + European 428) F_1 ♂ !
German + 8 ♀ R !
(Waltzer + English (white)) + English (*e*) ♂. English tumor age !

Old records:

London + (European + 103) F_3 ♀. Late tumor age.
German + 108 ♀ !

DISCUSSION.

In discussing these results we shall refer for comparison to some similar crosses we obtained in our previous hybridizations.

1. In crosses in which a male with a low, and a female with a high tumor rate are mated (Silver + English), the high tumor rate of the mother strain prevailed. In Silver + 10 the tumor rate was formerly

intermediate, but it increased more recently so that the higher rate of the mother became almost dominant. In English Sable + (Silver + I daughter of No. 10) the high tumor rate of the English prevailed throughout. We find, therefore, that in crosses between Silver, which is a low tumor rate strain, and high tumor rate strains in the majority of instances, the crosses follow the high tumor rate strains of the mother, or the results are intermediate. In these crosses the mother strain was the one with the higher tumor rate. In a cross of Silver with a strain of medium tumor rate (London) in which the Silver was the mother strain, the crosses showed an intermediate tumor rate, which, however, approached the Silver more closely than the London rate. There is, therefore, perhaps some indication in this case that the mother strain is more potent than the father strain. Individuals which themselves die without tumor, but belong to a strain with high tumor rate, may transmit to the offspring the tendency to a high tumor rate.

2. We may consider as control experiments those in which the tumor rates of both father and mother strains were similar to each other (high, medium, or low). In these cases the tumor rate of the offspring remained unchanged and resembled that of both parents.

3. While in two crosses, $8\frac{1}{2}$ + 328 (English Sable), the high tumor rate of the English dominated, and the influence of the mother is also noticeable in the tumor age, in the reciprocal crosses the tumor rate and tumor age of the $8\frac{1}{2}$ mother were much more influential. Some influence of the English in tumor rate (in one of the strains) and tumor age is, however, still noticeable. Again we have some indication that the mother strain is more influential than the father in determining the tumor rate. In a previous cross, $8\frac{1}{2}$ + II daughter of No. 10, the higher tumor rate of the mother strain prevailed; but later the tumor rate declined, in accordance with the decline in the tumor rate in No. 8 and $8\frac{1}{2}$.

4. The German Hybrids do not give decisive results, perhaps owing to the fact that the tumor rate of the German strain varied between high and medium and that this strain happened to be crossed with other strains of high or medium tumor rate.

In English Sable + German the higher tumor rate of the father probably prevailed, but in the tumor age an influence of the mother was noticeable.

Crosses No. 8 + German were at first intermediate, tending towards the higher tumor rate of the mother. Later the tumor rate fell in accordance with the fall in the tumor rate of No. 8. In a cross English + (8 + German) the higher tumor rate and the tumor age of the father prevailed. In the German + Carter the lower tumor rate of the mother strain prevailed. In German + 108 the lower tumor rate of the mother strain was probably more influential. When a German father was crossed with a lower tumor strain, the latter seemed to prevail. In two complex crosses in which German entered, the higher strain of the mother dominated.

5. While in 8 + German the higher tumor rate of the German was almost dominant, in the reciprocal cross the low rate of No. 8 prevailed. We find here another instance of the apparently greater influence of the mother strain in determining the tumor rate in the offspring.

6. While if we use different individuals as representatives of strains which are being hybridized, certain variations may occur in tumor rate and tumor age as indicated in two crosses between English Sable and European Hybrids, essentially the result was of a similar character in both cases in regard to tumor rate. The tumor age, however, followed in one instance the father, in the other the mother; and as in so many other cases a certain correspondence between tumor age and tumor rate was discernible.

7. In the crosses between English Sable and Cream the results were in all probability not due to accidental selection of unusual individuals. Experiments in which several individuals of a certain kind were used agreed with others in which only one male entered in the cross. Furthermore, the later records of English Sable 4,444 + Cream showed the same results as the earlier ones. The crosses between English and Cream are especially significant, because both parent strains differ markedly in their tumor rate and age. Both these strains have been carefully observed through a number of generations and their tumor rate and age were found constant in those substrains wh ch were used for our experiments.

Seven out of ten crosses showed an intermediate tumor rate; in two of these the rate was nearer English than Cream, although the mother was Cream. In three instances in which the Cream was the

mother strain, the tumor rate of the Cream prevailed, while this did not occur in any of the reciprocal crosses. This again suggests the possibility that in certain cases the character of the mother strain may be of greater importance in determining the tumor rate than that of the father.

It is, furthermore, of interest that we find in the tumor rate of these crosses a range of variations from that approaching the tumor rate of the English to that of the Cream. Through a gradual further addition of Cream to such hybrids the tumor rate is made to approach still more closely that of the Cream. The tumor age also is mostly intermediate between that of the English and Cream, but may vary independently of tumor rate either in one direction or the other.

8. In crosses between Cream and 198 + German Hybrid F_4, in which the Cream was the father, the high tumor rate and the early tumor age of the mother strain were more potent than the tumor age and rate of the Cream, while of three crosses between Cream and No. 10, two gave an intermediate tumor rate and in one instance the low tumor rate of the Cream was dominant. In the latter case the tumor age of the Cream was likewise dominant, while in the former crosses the tumor age was intermediate.

We see then (1) that a certain correspondence exists between the tumor age and the tumor rate of the various crosses, the lower tumor rate being associated with later tumors, and (2) that crosses between the same strains in which different individuals are used, may show certain variations in tumor rate and tumor age among each other. In two crosses between Cream and European, as well as in one reciprocal cross between European and Cream, the European component influenced the tumor rate to a marked degree and was probably dominant. Cream, however, exerted a certain influence on the tumor age. In the reciprocal cross European + Cream, in which the Cream females behaved like typical Creams, the European again greatly influenced the tumor rate, although the tumor age was that of the Cream. As in the preceding cross, we see a certain dissociation between tumor age and tumor rate in the hybrids. The reciprocal crosses gave similar results. The combination of Cream + European female with a Cream male led to a close approximation of the Cream records.

If we now consider all the hybrids in which Cream entered, we find the following results:

Cream: intermediate results 12 (1 of these contained 2 Cream components). Omitting the latter hybrids, intermediate results 11. In 8 of these the Cream was the father and in 4 crosses Cream was the mother.

Cream prevailed: 5 (2 of these contained 2 Cream components). Omitting the latter, Cream dominated in 3 cases. In 3 of the 5 crosses the Cream was mother and in 2 it was father.

The strain associated with Cream was dominant: 5.

Two of these stood between this group and the intermediate and were also included among the intermediate. Excluding these two crosses, three kinds of hybrids belong to this group. It is possible that these strains will have to be classed as intermediate and that European was not completely dominant in these cases.

In three of these the Cream was father and in two the Cream was mother. We see, then, that in a large majority Cream crossed with a high tumor rate strain produces crosses with an intermediate tumor rate. In a few instances the Cream is dominant; and in a few cases the other strain with the higher tumor rate may be dominant.

9. The crosses in which Waltzer constitutes one of the parent strains suffer from lack of definite knowledge as to the exact tumor rate of the Waltzer strain. We know that tumors of the breast occur in waltzing mice; but as far as we were able to ascertain, tumors among the strains of waltzing mice which we used for hybridization were rare and presumably did not greatly differ from the tumor rate of the Cream. While their tumor rate may have been somewhat higher than that of the Cream they certainly did not represent a high tumor rate strain. Crossed with an English (orange) a tumor rate and tumor age appeared which approached those of the English mother. When these crosses were again hybridized with various English females, complete tumor rate and tumor age of the English mother were obtained. On the other hand, a cross between a male Waltzer and another English female, which herself developed a tumor, gave much lower tumor rate. It was probably intermediate between that of both parents. In a further cross between these latter hybrids and English the lower tumor rate of the father strain prevailed, but the tumors belonged to the first age class. We see then that, in this case, the Waltzer crossed with English imposed ts tumor rate with such an intensity that this

tumor rate held its own in further hybridizations of the cross with English. In a similar way in crosses in which (European + 103) entered, this hybrid strain had a general tendency to transmit lateness of the tumors to the offspring. In this instance the influence of the Waltzer in the cross which served as the father strain was so marked that it dominated in a second hybridization with an English; we see, therefore, that the lower tumor rate of the father may prevail even against the high tumor rate of an English mother. But in all these hybridizations the tumor age was very early and was probably impressed upon the offspring by the English component. In the crosses between Waltzer + Carter and Waltzer + 8 the higher tumor rate of the mother strain was in all probability dominant.

10. The same reservation which we made in the case of the Waltzer applies also in the case of the Vermont Wild mouse which was mated to an English female. We may assume that tumors are rare and appear late in this wild strain and that the Vermont strain probably resembles the Cream mice. The hybrids probably presented an intermediate tumor rate; the tumors were relatively late. We can be certain that neither the English tumor rate nor age dominated. In a previous experiment, however, in which a Michigan Wild mouse was mated to an English female, the English tumor rate and age prevailed. In accordance with what we have observed in the other cases, we may interpret these results as indicating that if we cross a high tumor rate strain with a low tumor rate strain, the results may sometimes be intermediate, but that at other times the high tumor rate strain may dominate.

Effect of Sex of Parents on the Tumor Rate of the Hybrids.

The parent with higher tumor rate is dominant in 24 strains.
The mother strain dominates in 17 strains.
The father strain dominates in 7 strains.
The tumor rate is intermediate in 19 strains.

Parent with lower tumor rate is dominant in 10 strains.
The mother strain dominates in 8 strains.
The father strain dominates in 2 strains.
Only in approximately 18 per cent of the strains does the lower tumor rate prevail.
Altogether in 25 crosses the mother strain dominated, and in 9 crosses the father strain dominated.
In 19 strains the result was intermediate.

We may therefore conclude that both mother strain and father strain may prevail; but the mother strain dominated in a much larger number of our cases than the father strain. This fact, taken with several observations in which in reciprocal crosses the hybrids followed the tumor rate of the mother strain, suggests that the mother strain may possibly be of greater significance in determining the tumor rate in cancer of the mammary gland than the father strain. While the tumor rate is certainly not altogether a sex-linked character, there may perhaps exist a partial linking of the determiners of the tumor rate with the sex determinant.

SUMMARY AND CONCLUSIONS.

1. In order to test our previous conclusions concerning the tumor rate in hybrid strains, we carried out additional hybridizations. For this we selected strains which differed markedly in their tumor rate and which had been followed through a number of generations and found constant in their behavior.

Hybridizations between strains or families of a similar (either high, medium, or low) tumor rate served as control experiments. In these cases the offspring showed a tumor rate similar to that of the parents.

2. In selecting for hybridization various groups of Cream mice representing a very low tumor strain and English Sable mice representing a high tumor strain, we obtained in the majority of cases hybrid strains with a tumor rate intermediate between that of both parent strains. In a few instances it approached somewhat the high tumor strain of the English, and in a few others the low tumor rate of the Cream.

3. In several hybrids between the high tumor strain English and the low tumor strain Silver, the latter of which was split off from the English, the high tumor rate of the English prevailed. A number of mice which served for hybridization were followed throughout their life and found to behave typically as to tumor rate.

4. If we omit the strains in which both parents had a similar tumor rate, we found the higher tumor rate to be dominant in twenty-four hybrids. In seventeen of these the mother strain dominated, and in seven the father strain. In nineteen hybrid strains the tumor

rate was intermediate. In ten strains the lower tumor rate dominated. In eight of these the mother strain prevailed and in two the father strain. The low tumor rate was, therefore, dominant in approximately 18 per cent of the strains.

5. There does not seem to be a fixed rule as to dominance in the tumor rate. In a considerable number of cases, and especially in well analyzed cases, the result was intermediate.

6. Altogether in twenty-five of our hybrid strains the mother strain, and in nine strains the father strain prevailed. In nineteen strains the result was intermediate. We conclude that both father and mother strain may dominate and that the tumor rate is not in the strict sense of the term a sex-linked character. However, the fact that the mother strain prevailed in a much larger number of our cases than the father strain, and that several times (but not in all cases), in reciprocal crosses, the hybrids followed the tumor rate of the mother strain, suggests the possibility that as far as the hereditary transmission of mammary cancer in mice is concerned, the mother may be more potent than the father, and that perhaps under certain quantitatively varying conditions the mother strain may dominate over the father strain. This statement is merely a tentative conclusion at the present time and needs further investigation.

7. The results of these investigations confirm our previous conclusion that in the majority of the crosses which we observed, the cancer rate is either intermediate between those of father and mother strain, or that it follows the tumor rate of the parent with the higher rate and only in a relatively small number of instances the cancer rate follows that of the parent strain with the lower tumor rate. On the whole, the heredity of cancer rate and cancer age follows the blending type of hereditary transmission.

8. While there is a distinct relation between high tumor rate and early cancer age, our observations make it probable that cancer rate and cancer age are to some extent independent of each other.

9. On the whole the different generations, including F_1 and F_2 of the various hybrid strains, showed a concordant tumor rate and tumor age.

10. If we consider, then, our results as a whole, we may conclude that in crossing strains which differ in their tumor rate no rule of domi-

nance which applies equally to all cases seems to exist. In a certain number of crosses the results are undoubtedly intermediate. In these instances the tumor rate and to some extent also the tumor age behaves in a manner similar to characters which differ in father and mother merely in quantity as in the length of organs. From such intermediate results all kinds of gradations exist, leading on one side to dominance of the strain with the higher tumor rate and on the other side to dominance of the strain with the lower tumor rate. However, in our experiments dominance of the strains with the higher tumor rate greatly predominated over the opposite extreme.

11. Our results on the whole are, therefore, in certain respects comparable with the inheritance of sex which Goldschmidt studied in hybrid strains of the gypsy mother. Here also all gradations from the male to the female were observed in the offspring. Goldschmidt assumes that in different hybrids there are created different quantities of certain substances which like enzymes determine according to their quantity the velocity of chemical reactions and the amount of certain substances produced. The latter determine in the hybrids the quantitative variations in the character which is analyzed. According to Goldschmidt, multiple allelomorphs, which in our experiments seem to determine the heredity of spontaneous cancer, depend upon differences in the quantity of a substance present in the different individuals or varieties. In whatever way we may conceive of the character of multiple allelomorphs, our results make it probable that multiple factors are involved in the heredity of cancer in mice.