8492 C

Tumor Incidence in Reciprocal F_1 Hybrid Mice — A x D High Tumor Stocks.

JOHN J. BITTNER. (Introduced by C. C. Little.)

From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

With the increase in the number of inbred stocks of mice interesting and important data may be obtained in the study of the incidence of certain types of cancer by making reciprocal crosses. Previously all reports on the development of tumors in hybrid generations have been observations secured by crossing strains of animals which differ greatly in the percentage of animals showing growths. In this paper we wish to consider the spontaneous tumor incidence in mice of the first filial generation made by mating individuals of two strains in which mammary gland tumors frequently develop.

Stocks of Mice. (1) The "D" Strain. A sub-strain of the Little dilute brown race, previously described by Murray[9] was used as one parental race. (2) The "A" Strain. The other stock of animals employed was originally descended from the Bagg albino strain. Data on the tumor incidence has been reported by Strong[13] and Bittner.[1] A report comparing the breeding behavior and tumor incidence of the D and the A stocks is in press (Bittner and Murray).[2]

Females from each strain were used in breeding the first generation hybrids. When the A stock females were mated to D males, the hybrids were termed ADF_1; when the D females were crossed to A males the resulting generation mice were called DAF_1. All the F_1 individuals were employed as breeders in transplantation studies.

The number of animals dying non-cancerous or developing tumors are grouped in monthly age periods in Table I. The proportion of the total cancerous mice recorded in bimonthly periods is represented graphically in Fig. 1.

The A stock's tumor curve has a bimodal character if considered by monthly periods. This tendency is eliminated if the observations are grouped in bimonthly classes. The mode, in the latter recording, was during the 12-13 months period. For the ADF_1

9 Murray, W. S., *Am. J. Cancer*, 1934, **20**, 573.

13 Strong, L. C., *J. Heredity*, 1934, **25**, 119.

1 Bittner, J. J., *Am. J. Cancer*, 1935, **25**, 113.

2 Bittner, J. J., and Murray, W. S., in press.

TABLE I.

Age mo.	A Stock			ADF_1 Hybrids			D Stock			DAF_1 Hybrids		
	No. Non-Tumor	No. Tumor	% Tumor	No. Non-Tumor	No. Tumor	% Tumor	No. Non-Tumor	No. Tumor	% Tumor	No. Non-Tumor	No. Tumor	% Tumor
4.5	24	2	0.7	23	0	0.0	9	0	0.0	0	0	0.0
5.5	11	1	0.4	17	1	0.3	8	0	0.0	2	0	0.0
6.5	16	7	2.6	8	7	2.2	10	2	1.9	3	0	0.0
7.5	16	13	4.8	2	13	4.1	10	0	0.0	3	1	2.2
8.5	19	14	5.2	3	22	6.9	6	2	1.9	4	1	2.2
9.5	7	35	13.0	6	23	7.2	0	2	1.9	1	1	2.2
10.5	14	31	11.5	2	34	10.6	7	6	5.6	1	1	2.2
11.5	9	31	11.5	4	43	13.4	8	9	8.4	1	2	4.3
12.5	10	28	10.4	3	37	11.6	5	7	6.5	0	4	8.7
13.5	7	36	13.4	0	33	10.3	0	14	13.1	1	1	2.2
14.5	8	18	6.7	1	34	10.6	4	18	16.8	0	8	17.4
15.5	7	19	7.1	0	29	9.1	9	12	11.2	0	7	15.2
16.5	1	16	6.0	1	16	5.0	6	12	11.2	1	3	6.5
17.5	0	7	2.6	0	11	3.4	6	6	5.6	1	2	4.3
18.5	0	5	1.9	1	9	2.8	4	8	7.5	1	2	4.3
19.5	3	1	0.4	1	4	1.3	4	5	4.7	0	5	10.9
20.5	0	3	1.1	0	1	0.3	1	3	2.8	1	3	6.5
21.5	0	0	0.0	1	2	0.6	1	0	0.0	0	2	4.3
22.5	0	1	0.4	0	0	0.0	0	0	0.0	0	1	2.2
23.5	0	1	0.4	0	0	0.0	1	1	0.9	2	1	2.2
24.5				0	0	0.0	0	0	0.0	0	0	0.0
25.5				0	1	0.3	1	0	0.0	0	1	2.2
26.5										0	0	0.0
27.5										0	1	0.0
28.5										0	0	0.0
29.5										0	1	0.0
30.5										1	0	0.0
Total	152	269		73	320		100	107		23	46	

hybrid generation the largest number of tumors was recorded during the eleventh month. In the D stock and DAF_1 generation the modes were in the fourteenth and fifteenth months respectively.

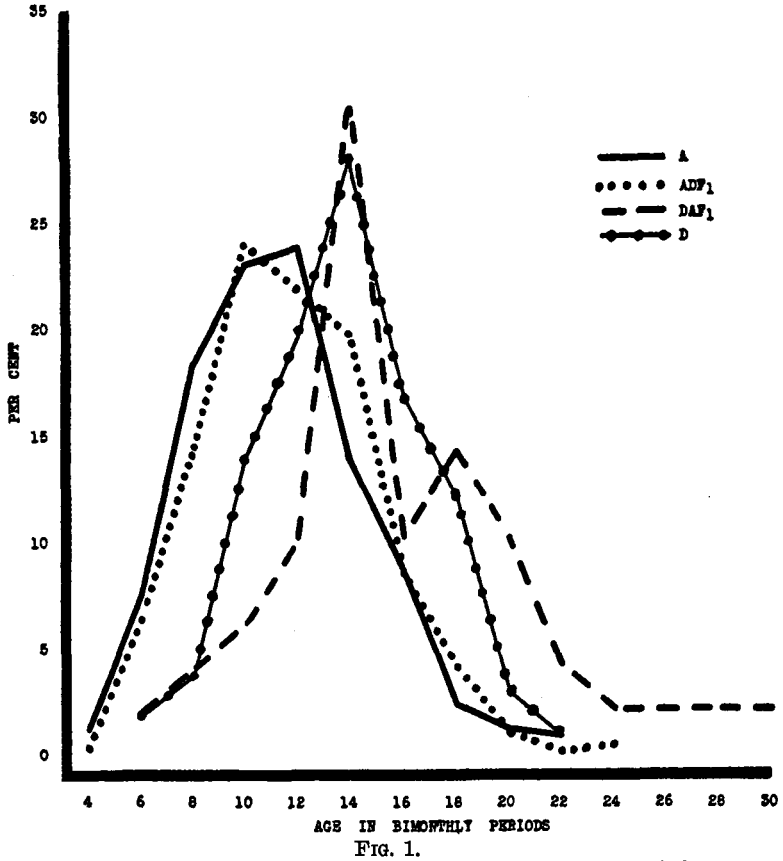

FIG. 1.
Percentage of all tumors developing in bimonthly age periods.

The mean age at observation of tumors in animals of the A and ADF_1 classes was approximately the same (12.3 and 12.1 months—Table II). Tumors of the D stock and DAF_1 generations developed at significantly later ages or 14.2 and 15.6 months respectively. The age difference between the D and DAF_1 mice is probably also of mathematical significance (1.40 ± 0.44).

The non-cancerous animals of the ADF_1 class died at an average age of 6.9 months as compared with 9.1 months for the A stock. The age at death for similar groups for the other parental strain and the DAF_1 hybrid group was considerably later, or 11 months.

More than 50% of all the animals belonging to the 2 parental

TABLE II.

Age at Observation of Tumors or at Non-cancerous Death; Means and Variations from Means for A and D Stocks and Their Reciprocal F_1 Hybrids.

Stock	No.	Mean Age mo.	Standard Deviation	Coefficient of Variation
A—Cancer	269	12.3±0.13	3.27±0.10	26.56±0.77
A—Non-Cancer	152	9.1±0.19	3.66±0.14	40.02±1.55
ADF_1—Cancer	320	12.1±0.12	3.18±0.08	26.15±0.70
ADF_1—Non-Cancer	73	6.9±0.30	3.81±0.21	55.00±3.07
D—Cancer	107	14.2±0.20	3.09±0.14	21.76±1.00
D—Non-Cancer	100	11.0±0.35	5.23±0.25	47.63±2.27
DAF_1—Cancer	46	15.6±0.39	3.91±0.28	25.04±1.76
DAF_1—Non-Cancer	23	11.8±1.00	6.81±0.68	57.81±8.49

strains which lived to be 4 months old developed mammary gland tumors (Fig. 2). For the A stock the proportion was 63.9%.

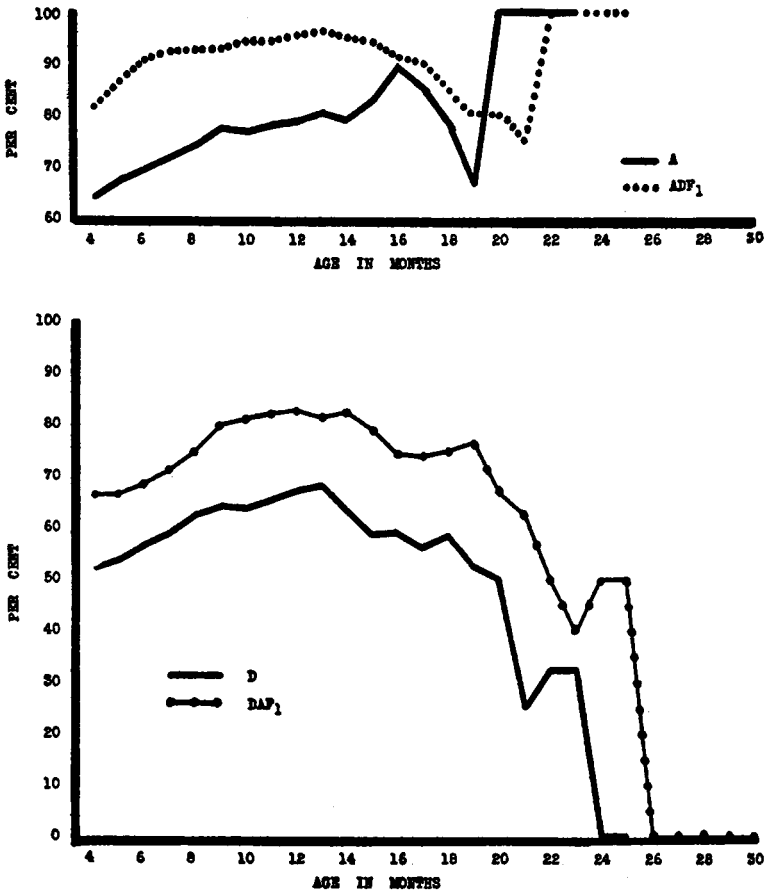

FIG. 2.

Percentage of mice living to each age period or longer which developed tumors.

This percentage increased with age until at the sixteenth month it was 89.5%. The percentage of cancerous animals in the D stock living at the beginning of the sixth month, or longer than the age of the youngest mouse to be observed with a tumor, was 56.3%. The percentage during the thirteenth month was 68.1%.

In the ADF_1 hybrid animals tumors developed in 86.5% of the animals which lived 5 months or longer. From the sixth through the seventeenth months the proportion was over 90%, reaching 96.6% at the thirteenth month. In the DAF_1 mice the first tumor was recorded during the seventh month and of the animals living, 71.9% were cancerous; by the fourteenth month, the proportion was 83.3%.

The differences between the percentages of animals to become cancerous in the various groups at the beginning of the fourth month were: A and ADF_1, $17.5\% \pm 2.1$; A and D, $12.2\% \pm 2.8$; A and DAF_1, $2.8\% \pm 4.1$; ADF_1 and D, 29.7 ± 2.6; ADF_1 and DAF_1, $14.7\% \pm 4.0$; and D and DAF_1, $15.0\% \pm 4.4$.

For the monthly periods corresponding most closely to the mean cancer ages for the strains, the percentages were: A stock, 79.0%; ADF_1 hybrids, 95.7%; D strain, 63.7%; and DAF_1 hybrids, 79.4%.

In addition to the mammary gland tumors which occurred only in females of the A stock, 4 breeding females had primary lung tumor at an average age of 20.8 months.[1] Twenty-three of 59 males of the A stock also had lung tumors. This type of cancer has never been observed in mice of either sex belonging to the D strain.

Not one of the ADF_1 hybrid females was observed which had only primary lung tumors. Two DAF_1 females were autopsied with primary lung tumors at 819 and 882 days. Two ADF_1 males, at 313 and 693 days, and one DAF_1 male, which was 775 days old when killed, also had this type of tumor. Females which had these tumors are not included in the mammary gland tumor data.

A few of the communications which have been published on the spontaneous tumor incidence observed in hybrids between high and low tumor lines are: Staff paper of the Jackson Memorial Laboratory,[12] Murray and Little,[10, 11] Korteweg[4] and Little.[5]

[12] Staff of Roscoe B. Jackson Memorial Laboratory, *Science*, 1933, **78**, 465.

[10] Murray, W. S., and Little, C. C., *Genetics*, 1935, **20**, 466.

[11] Murray, W. S., and Little, C. C., *Science*, 1935, **82**, 228.

[4] Korteweg, R., *Nederl. Tijdschrift voor Geneeskunde*, 1934, **78**, 240.

[5] Little, C. C., *J. Exp. Med.*, 1934, **59**, 229.

In the cross between the A and the D stocks, both of which were high mammary gland tumor lines, the mean tumor age in the subline of the dilute brown mice which were used was 1.9 months later than for the albino animals (7.9 x P.E.). In the F_1 animals derived from mating the A♀by D♂ the average tumor age was approximately the same as the A stock (1.1 x P.E.) For the DAF_1 or reciprocal hybrid cross, the average age at the recording of the growths was significantly later than in the A and ADF_1 classes (8.7 and 9.2 x P.E., respectively) and possibly also when compared with the D stock (3.2 x P.E.). Thus, it will be noted that the mean tumor ages in the hybrid generation mice resembled more closely those of the female parental stock.

Tumor incidence was measured by the percentage of animals living to the beginning of each monthly age period or longer which ultimately developed mammary gland tumors. Of the animals living to the beginning of the fourth month or longer, the proportion of which developed tumors was: A, 63.9%; ADF_1, 81.4%; D, 51.7%; and DAF_1, 66.7%. Comparisons between the parental strains and the hybrids show that the difference between the A and the ADF_1 percentages was 17.5% and the D and the DAF_1 15.0%. This relationship between the maternal parental stock and their F_1 hybrid generation is maintained for several months. For the monthly age period corresponding most closely to the mean ages of tumor development according to strains, the differences between the hybrid generations and their respective maternal parental strain were the same or 16.7%.

As the number of animals developing tumors in any stock is more or less dependent upon the number of individuals living to the cancer age, the higher incidence in the hybrid generations, as compared with the parental stocks, may possibly be due to heterosis. In the DAF_1 generation the average age at death of the cancerous as well as the non-cancerous mice was much later than for the ADF_1 individuals. If longevity has any effect on tumor incidence, the DAF_1 animals should have a larger proportion of cancerous animals than the ADF_1 mice, since the average tumor age apparently would not be affected. Such was not the case. Undoubtedly of more importance was the cancer susceptibility influence transmitted by the female parent to the hybrids in determining not only the proportion of these which developed mammary gland tumors but the average cancer age as well. Thus, these data show that extra-chromosomal influences are operative in crosses of tumor by tumor strains as well as in mating of tumor by non-tumor strains.

Lynch[6, 7, 8] has demonstrated that lung tumor susceptibility may be transmitted by mating males from a high lung tumor line to females from a low tumor line. Observations on a very small number of individuals considered above and unpublished data verify these findings. They may also indicate that lung tumor susceptibility may be transmitted by either parent.

Conclusions. 1. Reciprocal crosses between 2 inbred high tumor lines indicate that: (a) The mean mammary gland tumor age in F_1 breeding females is more nearly related to that of the maternal stock. (b) The proportion of animals developing tumors in the F_1 generation was considerably greater than in the maternal stocks. (c) The relative correlation of the tumor incidence between the maternal strain and the hybrid generation was approximately the same.

2. A small number of observations show that lung tumor susceptibility may possibly be transmitted by parents of either sex from the high lung tumor race.

8493 P

Mechanism of Methylene Blue in CO-Poisoning.

MATILDA MOLDENHAUER BROOKS.

From the University of California, Berkeley.

In order to see what effect methylene blue had upon the form of the hemoglobin in rabbits poisoned with CO, spectrophotometric analyses were made on blood at regular time intervals up to 20 minutes after removal of the animal from the gas chamber. Each rabbit was allowed to remain in an atmosphere of CO plus air, (% composition not determined) until it was unconscious and barely breathing, but not long enough to cause death. CO_2 was absorbed by soda lime. The animal was then taken out, a heart puncture made and 2 drops of blood immediately placed in a small tube filled to the brim with a measured amount of 0.4% NH_4OH. This was then tightly stoppered with paraffined corks excluding air, and shaken to cause complete hemolysis. A 0.03% methylene blue solution (Merck's medicinal) dissolved in 0.9% NaCl was then in-

[6] Lynch, C. J., *J. Exp. Med.*, 1924, **39**, 481.
[7] Lynch, C. J., *J. Exp. Med.*, 1926, **43**, 339.
[8] Lynch, C. J., *J. Exp. Med.*, 1931, **54**, 747.