The Inheritance of Susceptibility to Tumors Induced in Mice

II. Tumors Induced by Methylcholanthrene in the Progeny of C3H and JK Mice*

Walter J. Burdette, M.D.**

(From the Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

(Received for publication January 4, 1943)

Various inbred strains of mice show pronounced differences in susceptibility to carcinogenic agents. Previous experiments (2) with CBA, C3H, CHI, NH, and JK mice revealed that C3H and JK mice were respectively most and least susceptible to tumors induced by subcutaneous injection of methylcholanthrene. The F_1 progeny of the C3H and JK strains have been used in additional investigation of the inheritance of this susceptibility. The experimental conditions present when the parental strains were studied have been duplicated as nearly as possible. Use of F_1 animals makes it possible to investigate sex linkage and extrachromosomal influences, as well as to ascertain induction time, survival time, and types of tumors induced.

METHODS

The reciprocal crosses of C3H and JK parents were made and the data classified accordingly (Table I). The F_1 mice were not allowed to breed and at 60 days of age received a subcutaneous injection of 1 mgm. of methylcholanthrene [1] in 0.1 cc. of sesame oil. As in the work with the parental strains, a diet of nurishmix and lettuce was used. The temperature of the room was maintained at 70° F. and the humidity at 50 to 60 per cent. The animals were examined for tumors at intervals not greater than 14 days. Sections of the tumors from one group of mice were fixed in Bouin's fluid and stained in hematoxylin and eosin. Another group was allowed to live so that the survival times might be determined. The curves of induction time

TABLE I: INDUCTION AND SURVIVAL TIMES FOR F_1 (C3H × JK) MICE INJECTED WITH METHYLCHOLANTHRENE

Hybrids	Number of mice injected	Tumors	Died without tumor after first tumor appeared	Percentage dying without tumors	Induction time in days: Mean	Induction time in days: Median	Survival time: Number	Survival time: Mean days
Males	88	86 *	1	1.2	83.6	84	58	50.5 ± 1.2
F_1 (C3H × JK)	187	185		0.6	86.4			51.6 ± 1.0
Females	99	99	0	0.0	87.2	85	53	52.8 ± 1.3
Total	187	185	1				111	
Males	36	35	1		83.6			
F_1 (C3H♀ × JK♂)	90	89			87.8	85		
Females	54	54	0		90.6			
Males	52	51	0		83.4			
F_1 (JK♀ × C3H♂)	97	96			83.2	84		
Females	45	45	0		52.8			

* One male alive without a tumor 488 days after injection.

* This investigation was aided by grants to Dr. L. C. Strong from The Jane Coffin Childs Memorial Fund for Medical Research and from The Anna Fuller Fund.

** Department of Surgery, The Johns Hopkins Hospital, Baltimore, Md.

[1] A preparation of methylcholanthrene other than that injected into the parents was used. However, Strong (4) duplicated the results reported for C3H mice (2) with the second lot of carcinogen. It is therefore unlikely that the difference in potency, if any, was appreciable.

for the F_1 were plotted on the same scale and in the same manner as for the parental strains (2).

RESULTS

Induction time is defined as the period from the injection of carcinogen to the appearance of a growing tumor. Survival time is the interval from the detection of a growing neoplasm to the death of the animal. The curves showing the induction times for

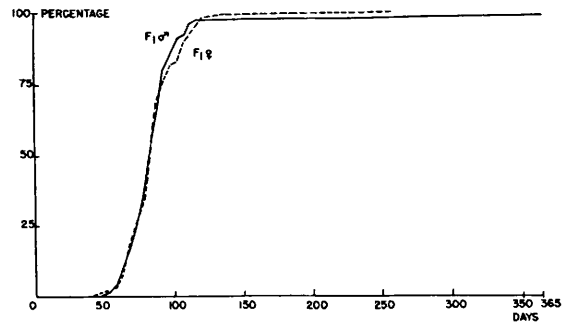

FIG. 1.—Time from injection of methylcholanthrene to appearance of tumors in F_1 males and females.

males and females (Fig. 1) are strikingly similar. There is no indication, therefore, that there is a sex difference. The median induction times are 84 and 85 days for the males and females respectively (Table I). The average survival times of the mice that developed tumors are 50.5 days for the males and 52.8 days for the females, both of which are longer than the average period of survival for the parental strains.

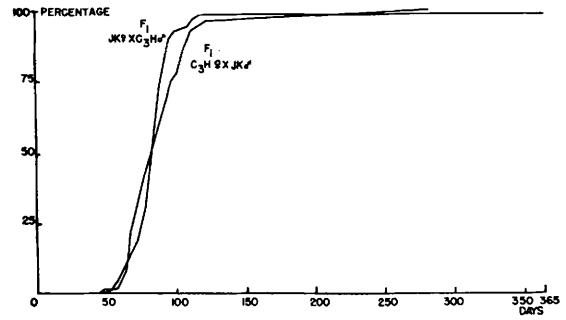

FIG. 2.—Time from injection of methylcholanthrene to appearance of tumors in the progeny of the reciprocal cross of C3H and JK mice.

The data are subdivided in order to compare the reciprocal crosses (Table I). The median induction time of the progeny of C3H females and JK males is 85 days. For the progeny of JK females and C3H males it is 84 days. The curves of induction time for the reciprocal crosses show little difference between the two groups (Fig. 2).

The curve for the F_1 values falls between the curves for the C3H and JK mice (2) and is nearer the values for the C3H than for the JK mice (Fig. 3). Superimposition of the curves plotted for the males and females of the three types of animals shows overlapping of the curves for F_1 and C3H mice only after 110 days, a value beyond the median and the mean induction times for both C3H and F_1 animals. At the present time one F_1 male has survived 488 days after injection of the carcinogen without developing a tumor.

The tumors of 50 mice were sectioned. Thirty-one (62 per cent) were spindle cell sarcomas. Five (10 per cent) were rhabdomyosarcomas, and an equal number were epidermoid carcinomas. The average induction time for spindle cell sarcoma was 90.0 days. That for rhabdomyosarcoma and epidermoid carcinoma was 78.0 days. Five mixed tumors with elements of spindle cell sarcoma and rhabdomyosarcoma appeared with average induction time of 88.0 days. Other mixed tumors found were 3 carcinoma-and-spindle cell sarcomas and one carcinoma-spindle cell sarcoma-and-rhabdomyosarcoma. Only serial sections could eliminate the possibility that some of the tumors classified as being composed of one neoplastic tissue might be

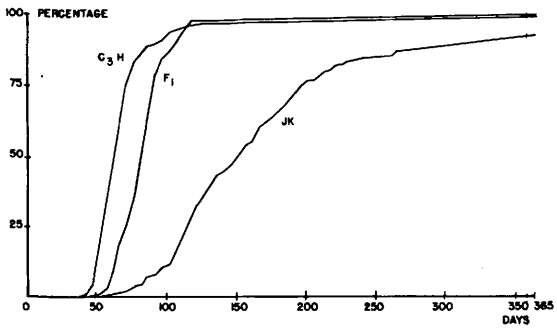

FIG. 3.—Curves of induction time for tumors in C3H and JK mice and their progeny.

mixed. There were lung tumors in 3 females and mesenteric metastases in 1 female and 1 male among 50 mice examined. Two F_1 females had mammary adenocarcinomas (5) as well as tumors at the locus of injection of methylcholanthrene.

DISCUSSION

Andervont (1) mated C3H mice with I and Y mice and found the susceptibility of the progeny to induced tumors intermediate to that of both parents. There was no evidence for extrachromosomal influence. He concluded that if there were genetic factors involved they were probably multiple. In this investigation an attempt has been made to answer several questions about the propagation of tumor susceptibility by similar methods.

As has been pointed out, the median and average induction times of the F_1 are intermediate to similar values for the parental strains. Although the curve of induction time is nearer that of the C3H than of the JK strain, the predominant type of tumor in the F_1 is spindle cell sarcoma, the same as for the JK

strain. The survival time of each class of mice is different from that of the other two, the survival time of the F_1 being greater than that of either parental strain. The longer life of tumor-bearing animals in the F_1 is probably a reflection of heterosis.

The results obtained by injecting mice of the parental strains with carcinogen (2) indicate that genetic factors for susceptibility are involved. The number and kind of genes acting are of interest. If there were only dominant genes responsible for the greater susceptibility of C3H mice, the F_1 animals should present the same curves, the same mean, and the same median as the C3H parents. This is not true (Table I and Fig. 3). If there were only recessive genes involved, the F_1 mice should have the same susceptibility as the JK parents. This also is not true. Genes for tumor susceptibility common to both strains may be detected only by outcrossing. Since the F_1 is intermediate in susceptibility, there is at least one dominant gene for tumor susceptibility in C3H mice. If it is assumed that genes are responsible and dominance is complete, the data indicate the probability that there are two or more genes responsible for tumor susceptibility and that all are not dominant and all are not recessive.

It is of interest to know whether any genes for susceptibility are linked with the sex chromosome. In C3H, JK, and F_1 mice there is no indication that susceptibility to induced tumors is sex limited in nature. In none of the data for the progeny of the reciprocal (C3H × JK) crosses is there evidence for sex linkage or a sex difference. Were there a sex linked gene or genes, there should be a difference in the susceptibility of the progeny in each reciprocal cross. The presence on the sex chromosome of a dominant gene or a dominant and also a recessive gene for susceptibility should result in F_1 females being more susceptible than F_1 males in the cross of C3H males and JK females. There is no evidence for this. Recessive sex linked genes should result in F_1 males being more susceptible than F_1 females in the cross of C3H females and JK males. This was not found to be true. Then there is no reason to believe that genes for susceptibility to induced tumors are located on the x-chromosome. The larger dose of carcinogen used may mask lesser contributors to the susceptibility effect.

It was desirable to determine whether, as in spontaneous mammary cancer (3), there is a milk influence involved in susceptibility to induced tumors. It is convenient to study this in F_1 mice, which are similar except that males of the reciprocal cross differ in having sex chromosomes derived from different parental strains. Since sex linkage should merely accentuate differences in the progeny of reciprocal crosses, no error is introduced by failure to detect a gene for tumor susceptibility on the allosomes. If there were a milk or other nonchromosomal influence, a difference in the F_1 of the respective reciprocal crosses would be expected. Since the C3H strain is the more susceptible, the reciprocal cross in which the C3H females were used should be the one in which a lesser induction time would be expected. There is no such indication either in the values of the means of the reciprocal crosses (Table I) or in a curve plotted for the induction time in these animals (Fig. 2). This is true not only when each of the two groups is considered as a whole, but also when the induction times for males and females in each cross are averaged. The difference between the lowest and highest mean in the group of males and females of the reciprocal crosses is 7.8 days.

The two mammary tumors that were found probably appeared so early as a result of the influence of methylcholanthrene, as Strong and Smith (5) have reported.

SUMMARY

1. Progeny of C3H and JK mice had average and median appearance times for tumors induced by methylcholanthrene intermediate to those of the parental strains.

2. The survival time of F_1 mice with tumors was longer than that of either parental strain.

3. The predominant type of tumor as judged by single microscopic sections was the same as that of the JK parents.

4. No evidence for linkage of susceptibility factors to the x-chromosome or for the presence of extrachromosomal influence was found.

5. The results obtained with the mice studied are compatible with the existence of more than one gene for susceptibility to induced tumors, at least one of which is dominant and at least one of which is recessive.

REFERENCES

1. Andervont, H. B. The Incidence of Induced Subcutaneous and Pulmonary Tumors and Spontaneous Mammary Tumors in Hybrid Mice. Pub. Health Rep., **53**:1665-1671. 1938.
2. Burdette, W. J., and Strong, L. C. The Inheritance of Susceptibility to Tumors Induced in Mice. I. Tumors Induced by Methylcholanthrene in Five Inbred Strains of Mice. Cancer Research, **3**:13-20. 1943.
3. Bittner, J. J. Breast Cancer in Mice as Influenced by Nursing. J. Nat. Cancer Inst., **1**:155-168. 1940.
4. Strong, L. C. Personal communication.
5. Strong, L. C., and Smith, G. M. The Local Induction of Carcinoma of the Mammary Gland by Methylcholanthrene. Yale J. Biol. & Med., **11**:589-592. 1939.