STUDIES ON THE RELATION BETWEEN TUMOR SUSCEPTIBILITY AND HEREDITY

V. The Influence of Heredity upon the Incidence of Lung Tumors in Mice

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The existence of constitutional types which differ in their susceptibility to disease has long been recognized. Of recent years the subject has received special emphasis in the numerous investigations which have been carried on to determine whether such types are inherited. The differences may manifest themselves in the degree of resistance to bacterial disease, or may be apparent in the frequency of pathological conditions of non-bacterial origin. In either case the demonstration of the inheritence of distinct types may aid in isolating them and also contribute to the analysis of the phenomena of resistance and susceptibility.

A considerable number of data have been collected in our laboratory concerning the incidence of lung tumors in mice. It has already been shown (1) that strains of mice exist which differ significantly from each other in regard to the incidence of spontaneous lung tumors which occur in them. It has also been demonstrated that the same strains differ in the same general way in their liability to induced lung tumors. That the latter differences are inherited has been shown by appropriate crosses (2). The exact relation between induced and spontaneous tumors has not been determined. It is possible that they are identical as far as the nature of the susceptibility is concerned, but it is important to know whether data on spontaneous tumors in an experiment involving a cross between strains would parallel those yielded by induced tumors. Evidence bearing on this point is now at hand.

A cross was made between a low tumor strain and one which proved to be considerably higher. Only one male representing the high strain was used (No. 7001 in Fig. 1). It came from a stock that had been pen inbred for several years.

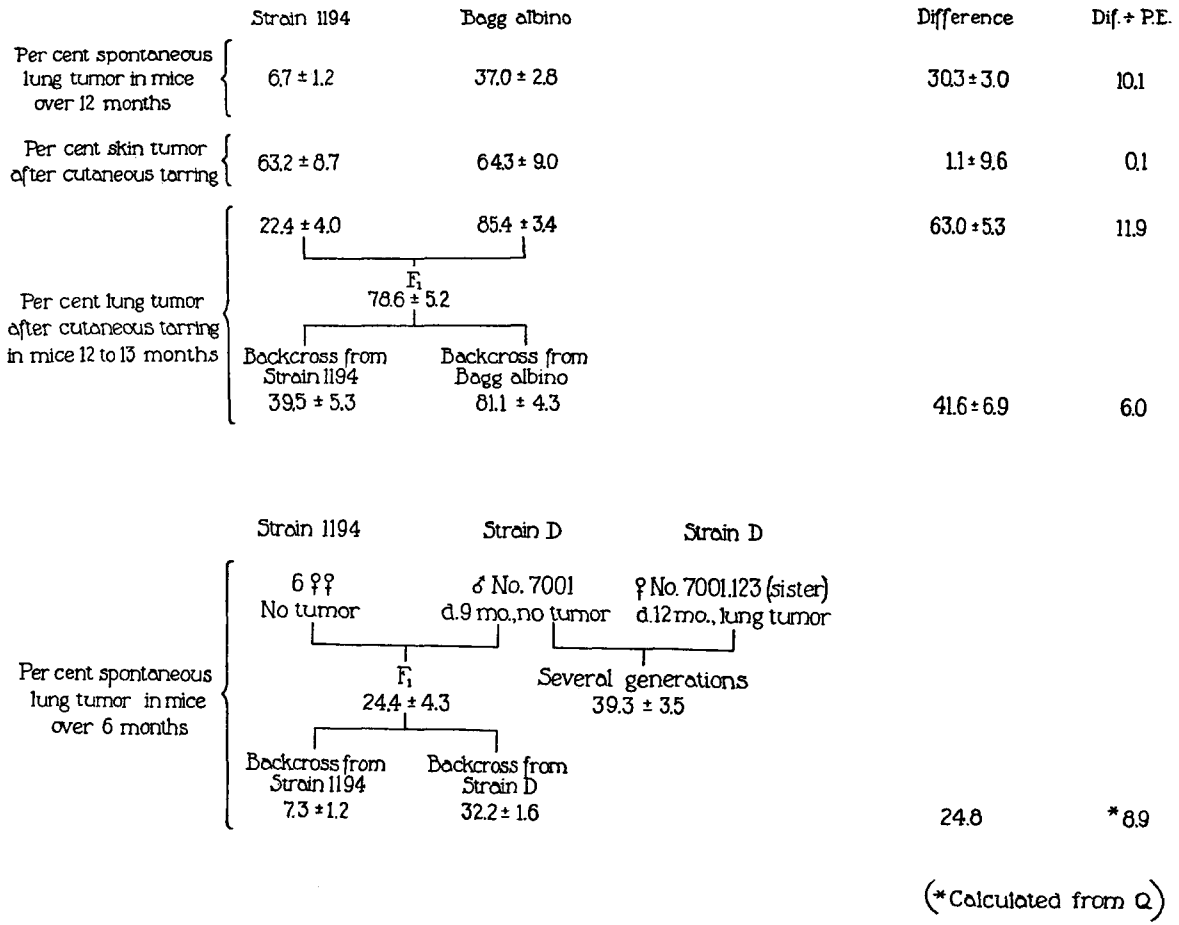
Strain 1194
Bagg albino
Difference
Dif. ÷ P.E.
Per cent spontaneous lung tumor in mice over 12 months
6.7 ± 1.2
37.0 ± 2.8
30.3 ± 3.0
10.1
Per cent skin tumor after cutaneous tarring
63.2 ± 8.7
64.3 ± 9.0
1.1 ± 9.6
0.1
Per cent lung tumor after cutaneous tarring in mice 12 to 13 months
22.4 ± 4.0
85.4 ± 3.4
63.0 ± 5.3
11.9
F1
78.6 ± 5.2
Backcross from Strain 1194
39.5 ± 5.3
Backcross from Bagg albino
81.1 ± 4.3
41.6 ± 6.9
6.0
Strain 1194
Strain D
Strain D
Per cent spontaneous lung tumor in mice over 6 months
6 ♀♀ No tumor
♂ No. 7001 d.9 mo., no tumor
♀ No. 7001.123 (sister) d.12 mo., lung tumor
F1
24.4 ± 4.3
Several generations
39.3 ± 3.5
Backcross from Strain 1194
7.3 ± 1.2
Backcross from Strain D
32.2 ± 1.6
24.8
*8.9
(*Calculated from Q)

FIG. 1

Cross between ♂ No. 7001 and 6 ♀♀ from Strain 1194

♂ parent		♀ parents	F_1 indicated by age in months at time of death.	
♂ No. 7001 9 mo.	×	♀ No. 7001.5 13 mo.	♂ 7 N.A. ♂ 8 ♀ 8 ♀ 9 M.met. ♂ 11 ♀ 13 ♂ 18 N.A. ♂ 19 ♀ 22 M. ♀ 26 N.A.	♂ 28 P.
"	×	♀ No. 7001.46 17 mo.	♀ 5 ♀ 6 ♀ 8 M. ♀ 8 M.met. ♀ 11 ♀ 16 ♂ 23 ♂ 31 ♂ Living	♂ 27 P. ♂ 28 P. ♂ 32 P.
"	×	♀ No. 7001.13 12 mo.	♀ 5 ♀ 7 M. ♀ 8 M. ♀ 8 ♀ 10 ♀ 11 ♂ 16 ♂ 24 ♂ 30	♂ 22 P.met ♂ 26 P.
"	×	♀ No. 7001.16 13 mo.	♂ 6 ♀ 7 M.met. ♀ 7 M.met. ♀ 7 ♀ 8 M.met. ♀ 8 M.met.	♀ 11 P. ♂ 26 P. ♂ 33 P. ♂ 35 P.
"	×	♀ No. 7001.2 12 mo.	♀ 10 M.met. ♀ 18 M. ♂ 21 ♂ 31	♂ 27 P.
"	×	♀ No. 7001.6 15 mo.	♂ 7 ♀ 7 ♀ 19 M.met. ♀ 22 M.met.	

Among 45 mice over 6 months old 11 (24.4%) have lung tumors
" 24 " " 12 " " 10 (41.6%) " " "
" 20 " " 18 " " 10 (50.0%) " " "

FIG. 2. M. = mammary tumor; met. = metastasis; N. A. = no autopsy; P. = pulmonary tumor.

Although it died at 9 months without having a tumor, mated with a sib which had a lung tumor at 12 months of age, it produced fourteen young, two, possibly three, of which had lung tumors, that is to say, lung tumors appeared in the first inbred generation. These descendants were backcrossed to the male parent and inbred in various ways. Among 89 offspring belonging to several generations, that lived more than 6 months, 39.3 per cent had lung tumors; of forty-four mice over 12 months of age, 52.2 per cent had tumors; and among twenty-five mice over 18 months, 64.0 per cent were tumorous. Other branches of this same stock (D) from which the male was selected have given a rate of 34 per cent among 222 mice over 6 months of age, so that it is apparent we are dealing with a strain fairly rich in pulmonary tumors. Male No. 7001 was crossed also with six females from Strain 1194. This strain has a lung tumor incidence of 6.7 per cent in mice over 1 year old, and the earliest age at which tumors appear is 18 months. The female parents and their offspring are charted in Fig. 2. The female parents died at various ages between 12 and 17 months—that is, below the usual tumor age in this strain. None had tumors of any sort. Every parent had amongst its immediate offspring one or more individuals with lung tumors, except No. 7001.6, which had only two daughters living well into tumor age, too small a number to be a test of her genetic constitution. This suggests, though it does not prove, that susceptibility may be dominant—or rather semidominant, since age and other influences must prevent the somatic expression of the tumor character in many individuals which genetically are tumor mice. There were in all forty-five F_1 mice that lived more than 6 months. Their lung tumor rate was 24.4 per cent. The twenty-four F_1 that lived more than a year had a rate of 41.6 per cent, and twenty mice that lived more than 18 months had a rate of 50 per cent. The numbers involved are small and these rates are not significantly different from those given by the descendants of male No. 7001 when inbred with a sib. The 14 mammary tumors which also occurred in this generation need not be considered here.

Males from the first filial generation were backcrossed to females of both parental strains. Forty-two backcross mothers were selected from Strain D. Of thirty-one that lived more than 6 months, 39 per cent had lung tumors—a rate like that of the inbred stock. From the low tumor stock, thirty-nine mice were used as the female parents. Only three lived more than 18 months and none more than 23 months. None had tumors.

The offspring from these crosses are charted in Fig. 3, which gives the distribution according to age at death of all mice that lived more than 6 months, except sixteen upon which no autopsy was obtained. Males and females of each group are represented separately. Among the males there were two doubtful cases of lung tumor, one at 18 and the other at 25 months. Both occurred in the backcross from the low tumor strain and in the totals were treated as tumor mice. Among the females the only complications as to diagnosis arose in connection with indi-

viduals that had tumors in the mammary gland. In such cases it was sometimes difficult to distinguish between primary lung tumors and metastases from mammary tumors. Among 331 females over 6 months of age 62 or 18.7 per cent had mammary tumors and twenty or 6.0 per cent had tumors which were diagnosed as primary both in the mammae and in the lung. In a total population of 622

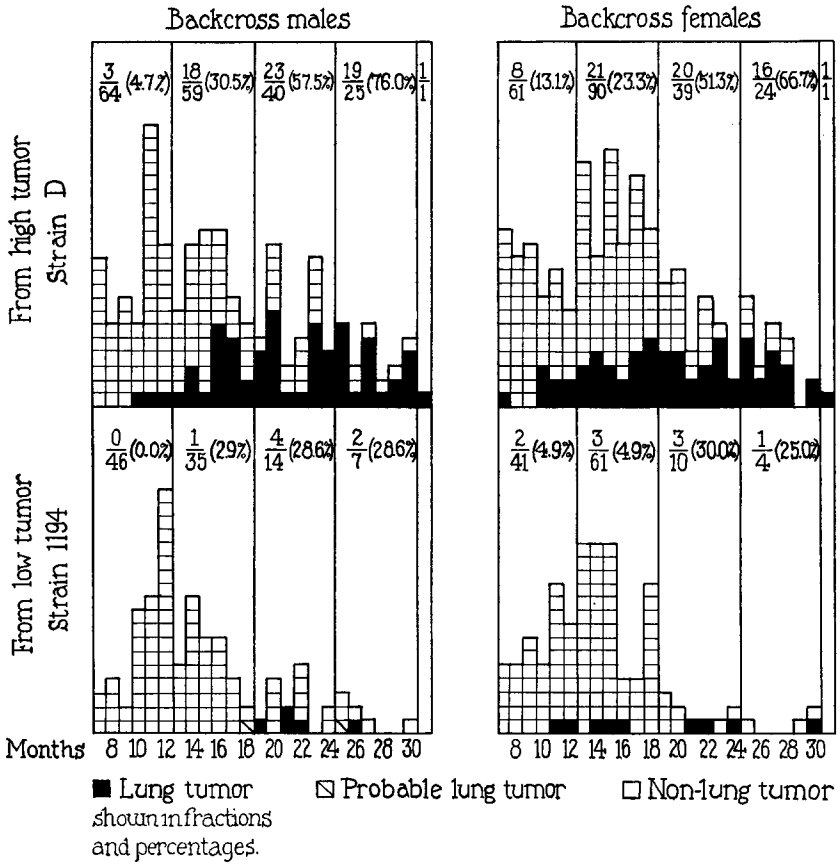

FIG. 3

backcross individuals there were two mice exhibiting epithelioma of the jaw, one being a female which had also a mammary tumor. There were four mice with sarcoma. Three of these sarcomas were subcutaneous. One of them occurred in a mouse which had a lung tumor, and another in an individual with both mammary and lung tumors. The fourth sarcoma probably had its primary site in the uterus with extensive metastases to the lungs, liver, kidney and adrenal gland.

No tumors except those in the lung are indicated on the chart. The individuals are grouped in age periods of 6 months.

If comparisons are made between corresponding age groups of the same sex (Table I) it is found that in every case the percentage of lung tumors is greater in the backcross from the high tumor strain than in that from the low strain. In the first age period, that between 7 and 12 months, in which the number of tumors is small, the difference in the percentages of the two backcross groups is, for the males, only 2.6

TABLE I

Comparison of the Backcross Groups—Males and Females Considered Separately

Sex	Age	Backcross from Strain D				Backcross from Strain 1194						
		Total mice	No. of mice with tumor	Per cent of mice with tumor	P.E. of per cent	Total mice	No. of mice with tumor	Per cent of mice with tumor	P.E. of per cent	Difference of percentages	P.E. of difference	Difference / P.E.
	mos.											
♂♂	7–12	64	3	4.7 ± 1.8		46	0	0.0 ± 0.0		4.7 ± 1.8		2.6
	13–18	59	18	30.5 ± 4.0		35	1	2.9 ± 1.9		27.7 ± 4.5		6.2
	19–24	40	23	57.5 ± 5.3		14	4	28.6 ± 8.1		28.9 ± 9.7		2.9
	25–31	26	20	76.9 ± 5.6		7	2	28.6 ± 11.5		48.4 ± 12.8		3.8
Total.....		189	64	33.9 ± 2.3		102	7	6.9 ± 1.7		27.0 ± 2.9		9.4
♀♀	7–12	61	8	13.1 ± 2.9		41	2	4.9 ± 2.3		8.3 ± 3.7		2.2
	13–18	90	21	23.3 ± 3.0		61	3	4.9 ± 1.9		18.4 ± 3.5		5.2
	19–24	39	20	51.3 ± 5.4		10	3	30.0 ± 9.8		21.3 ± 11.2		1.9
	25–31	25	17	68.0 ± 6.3		4	1	25.0 ± 14.6		43.0 ± 15.9		2.7
Total.....		215	66	30.7 ± 2.1		116	9	7.8 ± 1.7		22.9 ± 2.7		8.5

times its probable error—that is, merely possibly significant—and for the females it is only 2.2 times its probable error. For the period between 13 and 18 months the differences in the two backcrosses are clearly significant for both sexes. In the two later age periods the total numbers involved, especially in the backcross from the low tumor strain, were very small.

To compare the groups as a whole a method can be employed which has been described by Karl Pearson and J. F. Tocher (3). It was designed to give a more accurate comparison than that previously

possible between the death-rates of two groups of individuals in which the age distribution differed and in which death might be due to diseases which are a function of age. By this method the death rate is corrected by reduction to a "standard population" and a constant (Q) is found which expresses the ratio of the difference of corrected death-rates to the standard deviation of that difference. Since Q divided by 0.67449 is equal to the ratio of the difference of the percentages to the probable error of the difference, this constant may be used to make comparisons similar to those of the preceding paragraph. To test further whether the two systems of frequency are random samples of the same population the general χ_0^2 test of partial contingency also may be applied. Considering the males first we find in our present data that the crude tumor rate of the total population of males from the backcross to the high tumor strain is 33.9 $\pm$ 2.3 per cent and from the low strain 6.9 $\pm$ 1.7 per cent with a difference of 27.0 $\pm$ 2.9 per cent or 9.4 times its probable error. By applying the method of Pearson and Tocher to the groups of individuals in Table I the value of Q^2 is found to be 21.9054 and of Q, 4.683. Here Q divided by 0.67449 would be 6.9. While this figure is smaller than that obtained by the first method there is no doubt that the two populations are significantly different. In the partial contingency test the value of χ_0^2 proves to be 20.2484. Referring to Elderton's Table (4) we find by interpolation that P = 0.00045 for n' = five groups. Again the chances are tremendously against the probability that random sampling would account for the divergence found in the two populations. Similarly for the females, the crude tumor rates are 30.7 $\pm$ 2.1 per cent and 7.8 $\pm$ 1.7 per cent for the backcrosses to the high and low strains respectively. The difference is 22.9 $\pm$ 2.7 or 8.5 times its probable error. From the data in Table I, the value of Q^2 is found to be 15.2602 and the difference of the two groups according to their "corrected" tumor rates would be equivalent to 5.8 times the probable error. Furthermore, χ_0^2 is 14.1897 and P is 0.0068. Measured by these tests the backcross groups, both male and female, derived from the high tumor strain gave significantly higher tumor rates than did the corresponding backcross groups from the low tumor strain.

Thus far we have dealt with the sexes separately. In some of our previous data there have been indications that males are somewhat

more susceptible to lung tumors than females. A detailed analysis, however, of strictly comparable groups has not shown a significant difference. Despite this fact the possibility that sex may have a slight influence has not been precluded and a further investigation dealing with more critical evidence is under way. It may be of interest to examine the present data from this point of view. From Table II it will be seen that in the backcross from Strain D in three age periods the males have a higher tumor incidence, though none of them significantly so, and the total incidence is 3.2 per cent higher than that of the females. This figure is scarcely greater than its probable error (± 3.1 per cent). In the backcross from Strain 1194 in three age periods

TABLE II

Comparison of Males with Females in the Two Backcross Groups

Age	Backcross from Strain D			Backcross from Strain 1194		
	Difference of tumor percentages	P.E. of difference	Difference / P.E.	Difference of tumor percentages	P.E. of difference	Difference / P.E.
mos.						
7–12	−8.4	± 3.4	2.5	−4.9	± 2.3	2.1
13–18	7.2	± 5.0	1.4	−2.1	± 2.7	0.8
19–24	6.2	± 7.5	0.9	−1.4	± 12.7	0.1
25–31	8.9	± 8.4	1.1	3.6	± 18.6	0.2
Total.....	3.2	± 3.1	1.0	0.9	± 2.4	0.4

− sign indicates that the female showed the higher rate.

it is the females that have a higher tumor incidence and the total rate for the females is just slightly larger (0.9 ± 2.4 per cent) than that of the males. However, a greater difference between the sexes is brought out by the Pearson and Tocher method. Comparing the males in the backcross from Strain D with the females we find Q^2 to be 4.5306 and Q divided by 0.67449 to be 3.2. However, further calculation shows χ_0^2 to be 4.5063 and P to be 0.346. That is, according to the latter test the chances are about 35 in 100 that random sampling would account for the divergence noted in the two systems. The higher rate of the males cannot, therefore, be regarded as significant. In the other backcross groups (from Strain 1194) $Q^2 = 2.5792$ and Q divided

by 0.67449 is 2.4. For these data, χ_0^2 proves to be 2.5386 and P is 0.639. The higher rate of the females in this case is not significant.

More extensive data as to the effect of sex upon lung tumor incidence are being compiled. In the meantime, since no influence has been demonstrated, it may be permissible to class males and females together and compare the total populations in the two backcrosses. The combined data are listed in Table III and it will be noted that at every age period the differences are statistically significant. For a total of 404 mice obtained from backcrossing to the high tumor strain, the lung tumor incidence was 32.2 ± 1.6 while 218 individuals from the low tumor strain had a tumor rate of 7.3 ± 1.2. This gives a difference of 24.8 ± 2.0 per cent, over twelve times its probable error.

TABLE III

Comparison of the Backcross Groups—Males and Females Considered Together

Age	Backcross from Strain D				Backcross from Strain 1194						
	Total mice	No. of mice with tumor	Per cent of mice with tumor	P.E. of per cent	Total mice	No. of mice with tumor	Per cent of mice with tumor	P.E. of per cent	Difference of percentages	P.E. of difference	Difference / P.E.
mos.											
7–12	125	11	8.8 ± 1.7		87	2	2.3 ± 1.1		6.5 ±	2.0	3.2
13–18	149	39	26.2 ± 2.4		96	4	4.2 ± 1.4		22.0 ±	2.8	7.9
19–24	79	43	54.4 ± 3.8		24	7	29.2 ± 6.3		30.3 ±	7.3	3.5
25–31	51	37	72.5 ± 4.3		11	3	27.3 ± 9.1		45.3 ±	10.0	4.5
Total.	404	130	32.2 ± 1.6		218	16	7.3 ± 1.2		24.8 ±	2.0	12.6

If the method of Pearson and Tocher is again applied, the value of Q proves to be 6.009 from which we may deduce that the difference in the rates of the groups in question would be equivalent to 8.9 times the probable error. χ_0^2 is 33.3458. In Elderton's Table for $\chi_0^2 = 30$ we find P to be 0.000005. There is no question of the significance of the figure obtained here.

Table IV is appended in order to give another grouping of the same data which may be more convenient for comparison with previously published records of other strains. Here the differences in the tumor rates of the contrasted populations for mice over 6 months, 12 months and 18 months of age are undoubtedly significant.

Conclusions as to the number of genetic factors cannot be drawn with certainty. Several interpretations are possible. Although the female parents and the backcross mothers from Strain 1194 were without tumors, there is a possibility that growths might have developed if the females had lived longer. It is not yet clear just what the character is that is inherited. It may be that it is a type of constitution which has a tendency to react in a certain percentage of individuals in the production of neoplastic disease. According to this theory, tumor mice would not all be alike; there might be a gene corresponding to each of the various tumor percentages. This method of analysis has sometimes been applied to the tumor problem. It naturally results

TABLE IV

Comparison of the Backcross Groups—Males and Females Considered Together

Age	Backcross from Strain D				Backcross from Strain 1194						
	Total mice	No. of mice with tumor	Per cent of mice with tumor	P.E. of per cent	Total mice	No. of mice with tumor	Per cent of mice with tumor	P.E. of per cent	Difference of percentages	P.E. of difference	$\frac{\text{Difference}}{\text{P.E.}}$
Over 6 mos.	404	130	32.2 ± 1.6		218	16	7.3 ± 1.2		24.8 ± 2.0		12.6
Over 12 mos.	279	119	42.7 ± 2.0		131	14	10.7 ± 1.8		32.0 ± 2.7		11.8
Over 18 mos.	130	80	61.5 ± 2.9		35	10	28.6 ± 5.2		33.0 ± 5.9		5.6

in quite different conclusions from those obtained if either a single pair of factors with wide somatic fluctuations or multiple factors are considered as the heritable units.

From the pathological standpoint, differences in susceptibility have often been remarked. There is evidently an extensive series of conditions from highly susceptible to highly insusceptible. Animals which are classed as "tumor mice" may have nodules of very different size and malignancy. Individuals from the low tumor strain which are classified as "positive" may have but one nodule a fraction of a millimeter in diameter, while in mice of the high tumor strain all lobes of the lung may be peppered with nodules of various sizes. Occasionally the tumor mass may occupy the entire lobe. Histologi-

cally, lung tumors may be composed of alveolar, terminal bronchial or bronchial epithelial cells, occurring separately, in the same animal, or even in the same nodule. Sometimes the growths appear benign, but may show infiltration and metastasis to other organs. Nothing is known as to their rate of growth. A classification which would be satisfactory for genetic purposes has not been worked out. How much of this variation is controlled by heredity is not clear.

DISCUSSION

The results presented here may perhaps be more illuminating if viewed in connection with some of the observations previously made in this laboratory upon the inheritance of lung tumors in mice. As a fundamental consideration for this study, it must be remembered that, when mice are crossed, parents both of which are tumorous may produce offspring which develop tumors and others which do not though they live well into the tumor age; likewise, parents which live to an advanced age without exhibiting growths in the lung may number among their progeny both tumor and tumor-free mice. This phenomenon could be explained either by somatic variability or on the basis of several genetic factors (5).

Regardless of the number of hereditary units the fact of the somatic variability of the tumor character should be obvious although it has apparently been disregarded by many authors. An individual that dies without producing a tumor is not thereby proved to be "non-tumor" genotypically. Tumor incidence varies with age, with sex and with environmental conditions, of which a few are known and probably a larger number unknown. It is plain that an individual genetically susceptible (carrying the gene or genes for tumor) might never actually develop a growth if the requisite secondary influences were non-operative. This impossibility of classification by inspection is one of the great obstacles to the genetic study of cancer. Since accurate identification of the non-tumor individual is impossible it is customary to make comparisons between groups of individuals on the assumption that the unknown, secondary, non-hereditary factors operate equally in each and then to make corrections for age, sex and whatever environmental influences can be checked.

During the course of our investigations a number of such comparisons

have been made and some of the more important items are outlined in Fig. 1. A necessary preliminary to the work included an extensive survey of the tumor incidence and tumor age of various strains of mice. Two strains were discovered (1), one derived from Bagg albinos and an agouti strain, No. 1194, which have been demonstrated to differ significantly in their lung tumor rates. Among mice of all ages over 12 months Strain 1194 has 6.7 ± 1.2 per cent, and the Bagg albinos 37.0 ± 2.8 per cent of growths in the lung. The numbers are sufficient for the differences to be mathematically significant, thus showing that the hereditary tendencies of the two strains differ in respect to tumors of the lung. When individuals from these strains are housed together in the same cages they have the tumor rates characteristic of their respective families and show no evidence of contamination (unpublished data). Since it is known that tumors can be induced by chronic irritation, it becomes a matter of importance to note whether or not these two types of constitution hereditarily maintained in separate lines of descent react in the same manner to a carcinogenic agent. Groups of mice from the albinos and the agoutis have been tested by tar painting, the tar applications being made in the customary way in the interscapular area of the skin. The result of the experiment showed that tumors in the skin were produced in both groups at about the same rate and in the same percentage (64.3 ± 9.0 and 63.2 ± 8.7 per cent respectively). The numbers of mice in the experiment were small, but there was no indication that the difference in susceptibility which has been observed in regard to lung tissue applies also to the skin. From this it seemed that tumor susceptibilities might be specific. A further investigation of this possibility is being made at the present time. It was evident that instead of testing by the induction of tumors in the skin, a proper test of these two mouse strains would be one which would affect the tissue upon which the original observations were made. In the first experiment the albino strain had given a high percentage of lung tumors but it was not clear whether they were spontaneous or had been induced by the treatment. A more precise test was subsequently made possible through the discovery by Murphy and Sturm (7) that primary tumors could be induced in the lungs of mice by cutaneous tarring provided the areas of application were varied. This modified method was therefore

applied to the strains in question. When the animals were about 13 months of age they were killed and it was found that the tumor rate in each strain had increased. The low strain gave a tumor incidence after tarring of 22.4 ± 4.0 per cent while that of the high tumor strain had gone up to 85.4 ± 3.4 per cent. It is to be noted that in the groups upon which the rate of spontaneous tumors was calculated there were no lung tumors at all in mice of either strain under 15 months of age. The difference between the two strains, shown by the incidence of tar-induced tumors, was twice as great as that in the spontaneous lung tumor rates. In an attempt to discover whether these hereditary qualities could be followed when the strains were crossed certain individuals from the two strains had been mated before they were subjected to tarring. When the resulting F_1 mice were painted, the tumor rate remained high (78.6 ± 5.2 per cent). The F_1 were then backcrossed to the two original stocks, and the offspring were tarred. The backcross from the Bagg stock remained high (81.1 ± 4.3 per cent), whereas in the backcross to the low strain the rate dropped to about half (39.5 ± 5.3 per cent).

As we have seen, these results have been duplicated by an experiment with spontaneous tumors. A male (No. 7001), from a strain in which lung tumors are frequent, was crossed with females from the same low tumor strain as that used in the preceding experiment, and their F_1, when backcrossed to the parental stocks, gave high (32.2 ± 1.6 per cent) or low (7.3 ± 1.2 per cent) tumor rates according to which parental strain was used. The familial influence is again evident.

SUMMARY

A male mouse from a strain with a high incidence of spontaneous lung tumors was crossed with several females derived from a low tumor strain. The first generation of offspring were then backcrossed to individuals of the original strains. The resulting two groups of offspring differed significantly in the incidence of spontaneous tumors of the lung.

These facts are discussed in relation to others previously discovered.

It seems clear from the evidence presented that there are among mice constitutional types which differ in incidence of tumors of the

lung and that the differences are inherited. The number of genetic factors involved has not been determined. No influence of sex was apparent. The possibility of there being genetic factors which affect tumor age will be dealt with later.

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