STUDIES ON THE RELATION BETWEEN TUMOR SUSCEPTIBILITY AND HEREDITY.

IV. The Inheritance of Susceptibility to Tar-Induced Tumors in the Lungs of Mice.

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Previous communications from this laboratory have provided evidence in favor of the theory that susceptibility to tumors of the mammary gland (1) and tumors of the lung (2) is inherited. This evidence is based upon observations of spontaneous tumors. The discovery and perfection of methods of inducing tumors of various kinds have demonstrated the important rôle played by chronic irritation in the origin of neoplasms. It is conceivable that the so called "spontaneous" tumors also are the result of chronic irritation and that they seem to be spontaneous simply because the chronic irritant is unknown to the observer. This is a possible point of view though by no means one necessarily consequent upon the facts at hand. Whatever the relationship between the two kinds of tumors will ultimately prove to be, it has seemed pertinent to investigate the relation between chronic irritation and a demonstrated hereditary susceptibility.

The material used for this purpose consists of two strains of mice chosen from among a number which have been under observation in this laboratory for some years. They are an agouti strain, No. 1194, and the Bagg albinos. A somewhat detailed account of their history and numbers has already been published (2). The Bagg strain has a low rate of mammary gland tumors but a higher rate of lung tumors, while the opposite condition obtains in Strain 1194, which has a higher rate of mammary gland tumors than the preceding group but a low rate of tumors of the lung. The lung tumor rates have been shown (2) to be significantly different in the two stocks. Among 208 mice belonging to Strain 1194 which lived to be at least 12 months old, 6.73 per cent $\pm$ 1.17 had primary neoplasms in the lung. In the Bagg strain 37.04 per cent $\pm$ 2.80, in a total of 135 mice, had

tumors. The difference between the rates is 30.31 ± 3.04 and is undoubtedly significant. A few tumors of other types also have been found, and Strain 1194 produces a small number of epitheliomas of the jaw, but neither strain has developed tumors elsewhere in the skin.

An initial attempt in the investigation of the problem was made by testing the two strains in regard to their reaction to tar painting on the skin (3). The well known method of applying tar extract periodically to the skin between the shoulders was used, and in many cases carcinomas resulted. The percentage of tumors and the rates at which they appeared were practically the same in the two groups. Although the strains differed in respect to their spontaneous tumor rates, they showed no difference in their response to tar painting on the skin. It was pointed out at the time that the spontaneous tumors originated in one type of epithelium, *i.e.*, of the mammary gland (the lung tumor rates had not yet been demonstrated), while the tar painting test was applied to a different tissue—epithelium of the back. It was obvious that it would be a more interesting experiment to attempt to induce tumors in a tissue in respect to which the stock of mice already differed spontaneously. Since then the differences in lung tumor rates have been demonstrated, and the discovery by Murphy and Sturm (4) of a method of inducing tumors in the lung by tar painting presented the possibility of making the desired test.

Experiment 1.—30 agouti mice, 18 males and 12 females, between 2 and 3 months old, were selected from Strain 1194, and an equal number, 19 males and 11 females, from the Bagg albinos. The tar used was similar to that formerly employed, a residue obtained after distillation at 300°C., from which acids, bases, and phenols had been removed, and which was then dissolved in benzene. The novelty of the method lies in the loci which are subjected to treatment. The applications, instead of being made always on one spot, are distributed to 12 different regions of the body—on the dorsal and ventral surfaces, axilla, groin, neck, etc. The painting is done three times weekly, and four applications are made on each area, so that the treatment lasts approximately 4 months. After an interval of 6 more months, or when the animals are 12 to 13 months old, they are killed.

After such treatment very few mice develop skin tumors. Apparently no single region in the skin is irritated sufficiently to undergo neoplastic changes, but for some reason, as yet undetermined, a certain percentage of individuals will present primary tumors in the

lung. Since mice are subject to "spontaneous" tumors in the lung, it is necessary to differentiate between the spontaneous and tar-

Agouti mice from strain with low incidence of spontaneous lung tumors

Number	Sex	Length of life after tarring						Lesions		
		Jan	Feb	Mar	Apr	May	June	Skin	Lung - gross	Lung-microscopic
1	♀						†	—	—	–
2	♂						†	—	—	–
3	♂			†				—	—	–
4	♂						†	—	1 nod. in 1 lobe	+
5	♂		†					—	—	–
6	♀						†	—	—	–
7	♀		†					—	—	–
8	♀						†	—	Min. nod. in 1 lobe	+
9	♀			†				—	—	–
10	♀						†	—	Min. nod. in 1 lobe	–
11	♂		†					—	—	–
12	♂						†	—	—	–
13	♂		†					—	—	–
14	♂	†						—	—	–
15	♂						†	—	Min. nod. in 1 lobe	+
16	♀					†		—	—	–
17	♀	†						—	—	–
18	♂						†	Epithelioma	—	–
19	♀						†	—	Min. nod. in 1 lobe	–
20	♂	†						—	—	–
21	♂						†	Epithelioma	Min. nod. in 1 lobe	+
22	♂						†	—	2 • • 2 •	+?
23	♂			†				—	—	–
24	♂						†	—	—	–
25	♂						†	—	—	–
26	♂					†		—	—	–
27	♀			†				—	—	–
28	♀	†						—	—	–
29	♀		†					—	—	–
30	♂						†	—	Min. nod. in 1 lobe	+

Agouti mice surviving 6 months after tarring { 5 positive; 10 negative; 1 doubtful }

TEXT-FIG. 1.

induced growths. This is done by checking the age of the animals. As a rule, lung tumors do not develop spontaneously until later in

life. In our stocks 8 months is the earliest age at which such growths have been noted, and then in only 1 to 3 per cent of the individuals.

Albino mice from strain with high incidence of spontaneous lung tumors

Number	Sex	Length of life after tarring						Lesions		
		Jan	Feb	Mar	Apr	May	June	Skin	Lung-gross	Lung-microscopic
31	♂						†	—	Many nods in 4 lobes	+
32	♂						†	—	" " "5 "	+
33	♂						†	—	" " "4 "	+
34	♂						†	—	" " "5 "	+
35	♂						†	Hyperplasia	" " "3 "	+
36	♂				†			—	2 " "2 "	−
37	♂					†		—	—	−
38	♂				†			—	—	−
39	♂				†			Epithelioma	—	−
40	♂						†	—	Many nods in 3 lobes	+
41	♂						†	—	" " "4 "	+
42	♂						†	—	" " "4 "	+
43	♂						†	—	" " "5 "	+
44	♂						†	—	2 " "2 "	+
45	♂			†				—	Many " "3 "	+
46	♂			†				—	2 " "2 "	−
47	♂			†				—	Many " "2 "	−
48	♂			†				—	—	−
49	♂						†	—	Many nods in 4 lobes	+
50	♀						†	—	" " "4 "	+
51	♀						†	—	2 " "2 "	+
52	♀						†	—	Many " "5 "	+
53	♀						†	—	" " "5 "	+
54	♀						†	—	" " "4 "	+
55	♀						†	—	" " "4 "	+
56	♀						†	—	" " "5 "	+
57	♀						†	—	" " "4 "	+
58	♀						†	—	" " "4 "	+
59	♀						†	—	" " "3 "	+
60	♀						†	—	" " "4 "	+

Albino mice surviving 6 months after tarring { 22 positive, 0 negative, 0 doubtful }

TEXT-FIG. 2.

The youngest mouse in the Bagg strain in which a lung tumor has been found was 15 months old, and in the agoutis, 18 months. A

marked lowering of the tumor age or increase in tumor rate among mice which have been tarred in the way described may fairly be considered a result of the treatment.

Text-figs. 1 and 2 give the results of the experiment. Almost half of the agoutis died before the expiration of the 6 month interval. In the skin of 2 of the mice neoplastic changes had occurred; both animals proved to have carcinomas. At autopsy minute lesions or nodules were found in the lungs of 8 individuals, usually in only one lobe of the lung. Microscopic examination, however, revealed that only 5 mice had lung tumors, 1 was doubtful, and 10 were negative.

Of the albinos, a larger number lived to the end of the experiment. 1 that died the 4th month after tarring was stopped had an epithelioma on the back. 1 that died in the 3rd month after tarring had a primary lung tumor. All of the 22 mice that were killed after 6 months presented primary tumors of the lung, making a total of 23 positive and none negative. The large majority of them were multiple.

In this experiment the two strains gave different percentages of lung tumors after the tar treatment. Strain 1194, which "spontaneously" produces 6.73 per cent of lung tumors, produced 37.5 per cent when painted with tar. The rate of spontaneous lung tumors in the Bagg strain is 37.04 per cent, but when painted the incidence increased to 100 per cent. In both strains the rate increased, but the rate of the agouti stock did not increase sufficiently to overtake that of the albinos. A difference still exists between them.

The numbers of mice employed were not large, and it was, of course, desirable to repeat the experiment. The results, however, so strongly supported the conception of an hereditary difference in constitution in the animals of the two families that an experiment to test the point was carried out. If an hereditary difference persists even after tarring, then it should be possible to cross the two stocks and with the first filial generation thus obtained make backcrosses to the two original strains and demonstrate differences in the individuals of the backcross generation. In anticipation of the result actually obtained, mice in the first experiment had been crossed before the outcome of the experiment was known, and in the succeeding generation backcrosses were made after the discontinuance of tarring. For each generation a group from each of the original stocks was run at the

same time as controls. The controls are of the same nature as the original experiment and will be summarized after they have been described in detail.

F_1 from high tumor strain (Bagg albino) X low tumor strain (No. 1194)

Number	Sex	Length of life after tarring						Lesions		
		Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Skin	Lung — gross	Lung-microscopic
201	♂						†		1 lesion in 1 lobe	+
202	"						†		1 " " 1 "	+
203	"						†		2 " " 1 "	+
204	"						†		2 " " 2 "	+
205	"						†		3 " " 3 "	+
206	"	Died during tarring							0 " " 0 "	
207	"						†		3 " " 3 "	+
208	"						†		1 " " 1 "	−
209	"									
210	♀									
211	"						Living			
212	"									
213	"									
214	♂						†		8 " " 3 "	+
215	"						†		3 " " 3 "	+
216	"						†		0 " " 0 "	−
217	♀						†		4 " " 3 "	+
218	"						†		4 " " 3 "	+
219	"						†		3 " " 2 "	+
220	"						†		1 " " 1 "	+
221	♂						†		0 " " 0 "	−
222	"						†		1 " " 1 "	+
223	"						†		3 " " 2 "	+?
224	"						†		2 " " 2 "	+
225	"						†		0 " " 0 "	−
226	"						†		2 " " 2 "	+
227	"						†		2 " " 2 "	+
228	"						†		2 " " 2 "	+
229	"						†		5 " " 3 "	+
230	♀						†		5 " " 3 "	+
231	♂						†		2 " " 2 "	+
232	"						†		2 " " 2 "	+
233	♀						†		1 " " 1 "	?
234	"				†				0 " " 0 "	0
235	"						†		3 " " 2 "	+

22 positive - 79%
4 negative - 14%
2 doubtful - 7%

TEXT-FIG. 3

Experiment 2.—Before the conclusion of the tar painting in Experiment 1, crosses between the two strains were made in which all of the females were used and all of the males with the exception of 2 agouti males, Nos. 21 and 24, and 7 albino males, Nos. 43 to 49 inclusive. Many of the crosses, however, were un-

productive of offspring either through the sterility of the parents or through destruction of the young. 13 of the matings were successful. In 6 instances the male parent was from the albino or high tumor strain, and in 7 cases from the agouti or low tumor strain. A few of the next generation were too sickly to be used, but 35 of the F_1 generation were selected to be tested by the same method of tarring as were their parents. The results are shown in Text-fig. 3. The painting was begun when the mice were 2 to 3 months old, as in the previous experiment.

Agouti mice from strain with low incidence of spontaneous lung tumors

Number	Sex	Length of life after tarring						Lesions		
		Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Glands	Lung – gross	Lung-microscopic
251	♂	†						—	—	—
252	♀						†	—	Many nods in 4 lobes	—
253	♀						†	Mammary gland tumor	—	—
254	♀					†		—	—	—
255	♀						†	—	1 nod in 1 lobe	+
256	♂			†				—	No autopsy	
257	♂						†	—	1 nod in 1 lobe	—
258	♀						†	—	—	—
259	♂				†			—	—	—
260	♂					†		—	—	—
261	♀						†	—	—	—
262	♀						†	—	—	—
263	♂						†	—	—	—
264	♀						†	—	1 nod in 1 lobe	+
265	♀						†	—	1 " " 1 "	—
266	♂					†		—	—	—
267	♂						†	—	—	—
268	♀						†	—	—	—
269	♂	†						—	No autopsy	
270	♀						†	—	—	—

2 positive
11 negative
0 doubtful

TEXT-FIG. 4.

The mortality was very low. 1 male, No. 206, died before the tarring was completed, and 1 female, No. 234, died 3 months after the treatment was over. Neither had a tumor of any kind. 1 male, No. 209, and 4 females, Nos. 210 to 213 inclusive, were not killed with the others. They came from parents both of which had lung tumors and were kept for breeding purposes. In two other matings the parents were positive and the three offspring (Nos. 229, 230, 231) also were positive. In two matings the parents were both negative but they had not lived

the allotted period. Of their offspring four (Nos. 202, 203, 204, 205) were positive and one (No. 206) died during treatment. The rest of the F_1 came from parents one of which had shown a lung tumor in the first experiment and the other had not. There were 28 in all of which 21 were males. At autopsy numerous lesions were discovered in the lung, and upon microscopical examination 22 mice were found to have primary tumors, 4 mice were negative, and 2 were doubtful; that is, 79 per cent had undoubted tumors. All of the negative animals were males.

As controls for this experiment, groups of mice from each of the two original families were submitted to the same experimental treatment. The first group

Albino mice from strain with high incidence of spontaneous lung tumors

Number	Sex	Length of life after tarring						Lesions		
		Aug	Sept	Oct	Nov	Dec	Jan	Skin	Lung - gross	Lung-microscopic
236	♂						†	—	Many nods in 5 lobes	+
237	♂						†	—	1	?
238	♀						†	—	3	+
239	♂						†	—	—	−
240	♂					†		—	—	−
241	♂						†	—	Many nods in 4 lobes	+
242	♂						†	—	1	+
243	♂				†			—	—	−
244	♀	†						—	Many nods in 3 lobes	−
245	♀	Died during tarring						—	—	−
246	♂						†	—	Many nods in 3 lobes	+
247	♂						†	—	3	+
248	♀						†	—	5	+
249	♂						†	—	2	+
250	♂						†	—	—	−

8 positive
2 negative
1 doubtful

TEXT-FIG. 5.

comprised 20 agouti mice from the low tumor Strain 1194, and the second group consisted of 15 mice taken from the Bagg albino stock. Again the age factor was checked, only mice of 2 to 3 months being used.

Text-figs. 4 and 5 present the results. 13 of the agoutis and 11 albinos lived to practically the end of the experiment. (A few are included which died 2 days to 2 weeks before the end.) A colored female, No. 253, had a mammary gland tumor. There were no lesions in the skin. Of the agoutis, 2 were positive and 11 negative, while among the albinos 8 were positive, 2 negative, and 1 doubtful.

Backcross from F_1 (Bagg albino x No. 1194) X Bagg albino (high tumor strain)

Number	Sex	Color	Length of life after tarring						Lesions			
			Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Skin	Lung — gross		Lung-microscopic
340	♂	Agouti	—	—	—	—	—	†		∞ lesions in 4 lobes		+
341	"	Cin.	Died during tarring							0	0	
342	"	Albino	—	—	—	—	—	†		0 "	0 "	−
343	"	"	—	—	—	—	—	†		2 "	2 "	+
344	"	"	—	—	—	—	—	†		∞ "	5 "	+
345	"	"	—	—	—	—	—	†		4 "	4 "	+
346	"	"	—	—	—	—	—	†		∞ "	3 "	+
347	♀	"	—	—	—	—	—	†		4 "	4 "	?
348	"	"	—	—	—	—	—	†		∞ "	3 "	+?
349	"	"	—	—	—	—	—	†		3 "	3 "	+
350	"	"	—	—	—	—	—	†		∞ "	5 "	+
351	"	"	—	—	—	—	—	†		0 "	0 "	−
352	♂	"	—	—	—	—	—	†		3 "	2 "	+
353	"	Agouti	—	—	—	—	—	†		2 "	2 "	+
354	"	Albino	—	—	—	—	—	†		5 "	4 "	+
355	"	"	Died during tarring							0	0	
356	♀	Agouti	—	—	—	—	—	†		3 "	3 "	+
357	"	Albino	—	—	—	—	—	†		6 "	3 "	+
358	"	"	—	—	—	—	—	†		2 "	2 "	+
359	"	Agouti	—	—	—	—	—	†		4 "	2 "	+
360	"	"	Died during tarring									
361	"	"	—	—	—	—	—	†		4 "	3 "	+
362	"	Albino	—	—	—	—	—	†		0 "	0 "	−
363	"	"	—	—	—	—	—	†		6 "	3 "	+
364	"	"	—	—	—	—	—	†		3 "	3 "	+
365	"	"	†							0 "	0 "	−
366	♂	"	—	—	—	—	—	†		∞ "	4 "	+
367	"	"	—	—	—	—	†			0 "	0 "	−
368	♀	Agouti	—	—	—	—	—	†		∞ "	4 "	+
369	"	"	—	—	—	—	—	†		∞ "	5 "	+
370	"	Albino	—	—	—	—	—	†		∞ "	3 "	+
371	"	Black	—	—	—	—	—	†		4 "	3 "	+
372	"	Agouti	—	—	—	—	—	†		3 "	3 "	+
373	"	"	—	—	—	—	—	†		2 "	2 "	−?
374	"	Albino	—	—	—	—	—	†		5 "	3 "	+
375	♂	Agouti	—	—	—	—	—	†		3 "	2 "	+
376	"	Black	—	—	—	—	—	†		6 "	4 "	+
377	"	Albino	—	—	—	—	—	†		∞ "	4 "	+
378	"	"	—	—	—	—	—	†		∞ "	3 "	+
379	♀	Agouti	—	—	—	—	—	†		∞ "	5 "	+
380	"	Albino	—	—	—	—	—	†		1 "	1 "	−
381	"	"	—	—	—	—	—	†		2 "	2 "	+

	♂♂ Pos.	♂♂ Neg.	♀♀ Pos.	♀♀ Neg.	Doubtful
Colored	4	0	8	0	1
Albino	9	1	9	3	2
Total	13	1	17	3	3

30 positive - 81%
4 negative - 11%
3 doubtful - 8%

TEXT-FIG. 6.

Backcross from F_1 (Bagg albino x No. 1194) X No. 1194 (low tumor strain)

Number	Sex	Length of life after tarring						Lesions		
		Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Skin	Lung—gross	Lung-microscopic
301	♂	—	—	—	—	—	—†		3 lesions in 2 lobes	+
302	"	—	—	—	—	—	—†		0 " " 0 "	—
303	"	—	—	—	—	—	—†		2 " " 1 "	+
304	♀	—	—	—	—	—	—†		5 " " 2 "	+
305	"	—	—	—	—	—	—†		0 " " 0 "	—
306	"	—	—	—	—	—	—†		5 " " 2 "	+
307	"	—	—	—	—	—	—†		0 " " 0 "	—
308	"	—	—	—	—	—	—†		1 " " 1 "	—
309	♂	—	—	—	—	—	—†		0 " " 0 "	—
310	"	—	—	—	—	—	—†		∞ " " 3 "	+
311	"	—	—	—	—	—	—†		2 " " 2 "	—
312	♀	—	—	—	—	—	—†		2 " " 2 "	—
313	"	—	—	—	—	—	—†		1 " " 1 "	+
314	"	—	—	—	—	—	—†		1 " " 1 "	—
315	"	—	—	—	—	—	—†		3 " " 3 "	—
316	"	—	—	—	—	—	—†		1 " " 1 "	—
317	"	—	—	—	—	—	—†		2 " " 2 "	—
318	"	—	—	—	—	—	—†		0 " " 0 "	—
319	"	—	—	—	—	—	—†		0 " " 0 "	—
320	"	—	—	—	—	—	—†		2 " " 2 "	+
321	"	—	—	—	—	—	—†		2 " " 2 "	—
322	♂	—	—	—	—	—	—†		0 " " 0 "	—
323	"	—	—	—	—	—	—†		1 " " 1 "	—
324	"	—	—	†					0 " " 0 "	—
325	"	—	—	—	—	—	—†		1 " " 1 "	—
326	"	—	—	—	—	—	—†		2 " " 2 "	+
327	"	—	—	—	—	—	—†		1 " " 1 "	+
328	"	—	—	—	—	—	—†		1 " " 1 "	+
329	♀	—	—	—	—	—	—†		1 " " 1 "	+
330	"	—	—	—	—	—	—†		3 " " 2 "	—
331	"	—	—	—	—	—	—†		1 " " 1 "	+
332	♂	—	—	—	—	—	—†		0 " " 0 "	—
333	"	—	—	—	—	—	—†		2 " " 2 "	+
334	"	—	—	—	—	—	—†		1 " " 1 "	—
335	"	—	—	—	—	—	—†		0 " " 0 "	—
336	"	—	—	—	—	—	—†		∞ " " 2 "	+
337	"	—	—	—	—	—	—†		0 " " 0 "	—
338	"	—	—	—	—	—	—†		1 " " 1 "	—
339	"	—	—	—	—	—	—†		1 " " 1 "	+

♂♂		♀♀	
Pos.	Neg.	Pos.	Neg.
9	11	6	12

15 positive = 39%
23 negative = 61%

TEXT-FIG. 7.

In this experiment we may note that the cross between the two stocks gives a high percentage of tumors. Among the controls painted at the same time, the individuals from the high tumor strain showed a greater number of pulmonary tumors than did those from the low tumor strain—just as they had in the first experiment. Among the affected mice, the number of nodules and the number of lobes involved were greater in those from the high tumor stock than in the low.

Agouti mice from strain with low incidence of spontaneous lung tumors

Number	Sex	Length of life after tarring: Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Lesions: Skin	Lung – gross	Lung-microscopic
382	♂						†	—	—	–
383	♀						†	—	1 nod. in 1 lobe	–
384	♀						†	—	2 " " 2 "	+
385	♀						†	—	2 " " 2 "	+
386	♂						†	—	1 " " 1 "	–
387	♂						†	—	1 " " 1 "	–
388	♀						†	—	1 " " 1 "	–
389	♀						†	—	—	–
390	♀						†	—	—	–
391	♂						†	—	2 nods. in 2 lobes	–
392	♂						†	—	—	–
393	♀						†	—	1 nod. in 1 lobe	–
394	♂						†	Hyperplasia	1 " " 1 "	–
395	♂						†	—	—	–
396	♂						†	—	1 nod. in 1 lobe	+
397	♀						†	—	—	–
398	♂						†	—	—	–
399	♀						†	—	1 nod. in 1 lobe	–
400	♀						†	—	—	–
401	♀						†	—	—	–

3 positive
17 negative
0 doubtful

TEXT-FIG. 8.

Experiment 3.—Four groups of mice at the age of 2 to 3 months were tarred as in the preceding experiment. Two series were mice obtained from backcrosses between the first filial generation and the two original strains, and two series were mice from the original strains and were used as controls.

Group 1. Backcross from the High Tumor Strain.—These mice were obtained by crossing sons belonging to the first filial generation with the Bagg albinos

or high tumor strain. Each male was mated with several females. In a few cases one of the females used was the maternal parent from Experiment 1, but no young suitable for the experiment were obtained from them. The mothers of the offspring used were from stock. They had not been treated by tar painting, so that it is not known how many or which individuals would have been susceptible to tar-induced growths in the lung. 42 offspring were obtained, and the results of tarring are shown in Text-fig. 6. 3 mice died during tarring, and 1 in the 1st and 1 in the 5th month after tarring. At autopsy it was

Albino mice from strain with high incidence of spontaneous lung tumors

Number	Sex	Length of life after tarring						Lesions		
		Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Skin	Lung-gross	Lung-microscopic
402	♂			†				—	—	–
403	♂	†						—	—	–
404	♂						†	—	Many nods. in 5 lobes	+
405	♀						†	—	" " 5 "	+
406	♀	Died during tarring						—	No autopsy	
407	♂						†	—	Many nods. in 4 lobes	+
408	♂						†	—	" " 2 "	+
409	♂		†					—	1 " 1 "	+
410	♂						†	—	Many " 3 "	+
411	♂						†	—	" " 2 "	+
412	♂						†	—	1 " 1 "	–
413	♂						†	—	1 " 3 "	+
414	♂					†		—	—	–
415	♂			†				—	—	–
416	♂						†	—	2 nods. in 2 lobes	+
417	♂						†	—	3 " 3 "	+
418	♂						†	—	3 " 3 "	–
419	♂						†	—	—	–
420	♂						†	—	2 nods. in 2 lobes	–

10 positive
4 negative
0 doubtful

TEXT-FIG. 9.

found that the lesions in the affected individuals were very numerous. Among 14 males, all but 1 were positive, while among 23 females, 17 showed tumors of the lung, 3 were without them, and 3 were doubtful. If the two groups are taken together and the 3 doubtful cases classed as negative, the ratio is 30 with tumors and 7 without, or 81.1 per cent tumor mice. This rate is about the same as that of the F_1 and of the albino stock.

Group 2. Backcross from the Low Tumor Strain.—The same F_1 males which were backcrossed to the albino stock were also mated with females from Strain 1194, the low tumor strain. 39 offspring resulted and all were agouti as expected. 1 died the 3rd month after tarring. The ratio (Text-fig. 7) for the males was 9 with tumors to 11 without tumor, for the females, 6 positive to 12 negative. The two classes taken together give 15 positive to 23 negative, or 39.47 per cent. This is only about half the number of tumors found in the F_1 or the backcross group from the high tumor strain.

Group 3. Control from Low Tumor Strain.—20 control mice (9 males and 11 females) were selected from Strain 1194, Text-fig. 8. None died during the treatment. When killed, 1 showed an epithelial proliferation on the back of the neck at one of the painted areas, but it had not progressed far enough to be called an epithelioma. Only 3 of the animals produced tumors in the lung; the other 17 were negative.

Group 4. Control from the High Tumor Strain.—The control from the albino group consisted of 19 mice, of which 17 were males, Text-fig. 9. 6 died before the

	Strain 1194			Bagg albino		
	Tumor	Non-tumor	Doubtful	Tumor	Non-tumor	Doubtful
Exp. 1	5	10	1	23	0	0
" 2	2	11	0	8	2	1
" 3	3	17	0	10	4	0
Total	10	38	1	41	6	1

Strain 1194 11 tumor : 38 non-tumor = 22.45% ± 4.02
Albino 41 " : 7 " " = 85.42% ± 3.43
Difference = 62.97% ± 5.29

TEXT-FIG. 10.

end of the experimental period. The lesions in the lung were more numerous than in the preceding group, both as to number of individuals and number of lobes affected. Tumors were found in 9 of the 13 survivors. 1 of the mice dying only 2 months after the discontinuance of the tar treatment had a lung tumor, so that the ratio is 10 to 4. In this case also the albino mice from the high tumor strain give a greater percentage of tar tumors than do the agoutis from the low tumor strain.

In the first experiment samples of mice from two strains which differ in their spontaneous tumor rates were contrasted when subjected to a method of tarring which produces lung tumors. The control groups in Experiments 2 and 3 were repetitions of the same test, so that the three experiments may be compared from that point

of view. In each of the three trials, the colored (low tumor strain) and the albino (high tumor strain) groups differed from each other in their reaction, Text-fig. 10. During the series of experiments the percentage of tumors decreased in the case of the agouti strain but increased in the albinos; however, the percentage was always lower in the agoutis and higher among the albinos. The direction of the difference between the two was constant. Combining the figures for all of the trials gives a ratio of 11 tumors to 38 non-tumors for Strain 1194, or 22.45 per cent $\pm$ 4.02. For the Bagg albinos the ratio is 41 tumors to 7 non-tumors, or 85.42 per cent tumors $\pm$ 3.43. The difference between the per cents is 62.97 $\pm$ 5.29. Since the difference is more than eleven times its probable error, it is undoubtedly significant.

Since a distinct difference in the two families is, therefore, shown, it is interesting to note the results of their crossing. Experiment 2 (Text-fig. 3) shows that the F_1 generation when tarred, as were the parents, gives 79 per cent of tumors. This is almost as high a rate as that given by the more susceptible parent strain—the Bagg albinos, which gave 85 per cent of lung tumors. When the F_1 were backcrossed to the high tumor strain, a high rate (81 per cent) was again shown, but when backcrossed to the low tumor strain, Strain 1194, the rate dropped about half, 39 per cent. It seems evident that the percentage of tumors to be obtained in the lungs of mice after tarring depends upon the strain of mice employed and may be controlled by backcrossing the hybrids to one or the other of the strains used.

The data are insufficient to make possible an accurate determination of the number of hereditary factors involved. From the high tumor rate given by the first filial generation it would seem that at least one factor for susceptibility is dominant or partially so. On the basis of the "dominant hypothesis," however, we might expect a higher percentage than 39 in the backcross. Further generations will settle the question.

In solving the problem of the genes involved, help may possibly be obtained from a more detailed study of the histology of the tumors. The tumors do not all present the same picture. The majority are formed of cuboidal cells, somewhat resembling the epithelium of the terminal bronchi. Others are composed of columnar cells, similar to the cells of bronchial epithelium, which, supported by

strands of connective tissue, thrust out finger-like processes. They are comparable to the papillary cyst-adenomas described by Tyzzer. There are many gradations between the types. Some tumors have not the appearance of being malignant, while others show examples of mitosis and definite infiltration. In one case the cells were elongated almost to a spindle shape and were quite unlike either of the types already mentioned. So far a satisfactory classification of the types which would permit identifying them as different genetic classes has not been worked out. This must remain a problem for future investigation.

Another point to be considered is the adequacy of this method of tarring as a test of susceptibility. A slightly different procedure; such as a longer period of tarring, more frequent applications, a longer period between tarring and autopsy, or even a different tar preparation, might yield a higher percentage of tumors. It is possible that the albino strain which gives positive results in 85 per cent of the cases may really be a 100 per cent strain genetically but not show it because of the ineffectiveness of the method employed. In that case the agoutis also might give a higher per cent than 22. Variations in procedure have not been tested. At present we can merely state the results obtained by the method described.

SUMMARY AND CONCLUSIONS.

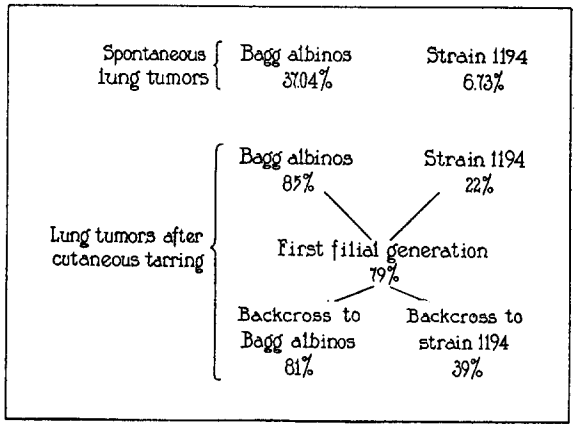
Evidence has previously been submitted in favor of the theory that susceptibility to spontaneous tumors of various types is inherited. The question arose whether susceptibility to tumors induced by tar could be shown to be hereditary by experimenting with the same strains of mice which had already been shown to differ significantly in respect to their spontaneous tumor rates.

Two strains of mice were selected for observation. One strain, the Bagg albinos, has a low rate of mammary gland tumors but a higher rate of spontaneous lung tumors. The other strain, No. 1194, agouti, has a higher rate of mammary gland tumors but a lower rate of tumors of the lung. A previous test showed no difference in the percentages of skin tumors which arose after tar painting.

It has already been shown that the difference in the lung tumor rates

is mathematically significant. When the two stocks are treated with tar by applying the irritant, not in the same spot, but on different areas successively, the percentage of lung tumors is increased in each stock, Text-fig. 11. The rate of the Bagg albinos increased from 37.04 to 85 per cent, that of Strain 1194 from 6.73 to 22 per cent. But a difference between them is still maintained, and this difference also is significant mathematically.

When the two strains are crossed and the offspring subjected to the tar treatment, the latter give a high percentage of lung tumors—79 per cent in 28 individuals—about the same as the parental high tumor strain. When the F_1 sons are backcrossed to the original



TEXT-FIG. 11.

stocks, the cross to the high tumor strain maintains the high tumor rate, 81 per cent in 37 mice, while in the cross to the low tumor strain the percentage drops to 39 per cent in 38 mice. This result indicates that susceptibility to tar-induced tumors in the lung is hereditary. The number of factors concerned has not yet been ascertained. Possibly one or more of them is dominant.

In general, the conception that susceptibility to pulmonary tumors is hereditary seems to be upheld by the fact that the two strains of mice described differ conspicuously in respect to spontaneous tumor rates under ordinary laboratory conditions; the strains differ also

under experimental conditions, as described in this report; and when crossed, their offspring by suitable backcrosses, will again show significant differences.

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