A STUDY OF INHERITANCE IN MICE WITH REFERENCE TO THEIR SUSCEPTIBILITY TO TRANSPLANTABLE TUMORS.*

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So far as can be ascertained, no systematic investigation has been made as to whether the susceptibility of mice to transplantable tumors is regularly transmitted from generation to generation, or whether it is inherited as a Mendelian character. It was the existence of a practically absolute difference in the susceptibility of common mice and Japanese waltzing mice to a tumor, which originated in a mouse of the latter variety, that led to the present study of the inheritance of this racial peculiarity.

Racial differences in the receptivity or susceptibility to transplantable tumors have been noted in mice by many investigators. Haaland[1] found that whereas a certain tumor grew on inoculation in nearly one hundred per cent of Berlin mice, it grew in only twenty-four per cent of Hamburg mice, and was practically innocuous to Christiania mice. He claims that susceptibility may vary also with change of location. Thus mice taken from Ehrlich's laboratory to Norway were found to be almost wholly insusceptible to a highly virulent sarcoma received directly from Ehrlich. He discusses the possible influence of change of climate and of variations of such conditions as heat, light, moisture, but concludes that diet is probably of greater importance. Jensen[2] has also observed distinct differences in the susceptibility of various stocks of rats to an inoculable sarcoma. A similar variation in the susceptibility of different stocks of rats to the Flexner-Jobling[3] tumor has recently been noted by Gay.[4] Loeb[5] found that a sarcoma which was readily propagated in white rats failed to grow when inoculated into colored and hybrid rats. He[6] also noted that a tumor which originated in a Japanese waltzing mouse grew on transplantation in nearly one hundred per cent of this variety of mice, but failed to

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develop in common mice. The insusceptibility of certain breeds of mice to the Jensen tumor and of common mice to a transplantable tumor of Japanese waltzing mice was mentioned in the Fourth Report of this Commission.[7] Such results have been obtained so frequently that it is evident that the tissues of certain races of mice furnish a more favorable soil for the growth of implanted tumor cells than do the tissues of other races.

Various attempts have been made to correlate differences in susceptibility with certain anatomical peculiarities such as pigmentation of fur. Cuénot and Mercier[8] of the University of Nancy have recently investigated this question. They call attention to the occurrence in mice of numerous mutations which have been already defined by them. At least one hundred and twenty-eight varieties of mice, of which the following are a few examples, wild gray, white, black, yellow, waltzing or non-waltzing, pied or uniform in color, eyes black or red, etc., differing one from another by at least one determinant of the germ plasm, are recognized. Having at their disposal an almost complete collection of the possible varieties of mice, they have tested their susceptibility to a tumor which grew on inoculation in nearly one hundred per cent of Parisian white mice. As the result of its first inoculation into the Nancy race of mice, this tumor grew in only 16.6 per cent, but, as the result of later transplants, tumors developed in sixty-five and sixty-eight per cent of the animals. Thus a distinct racial difference between the mice of Paris and the mice of Nancy was apparent at the outset, although the tumor subsequently became adapted to the alien race, as was shown by the high percentage of successful results with later transplants. After a systematic inoculation of many different varieties of mice in their possession none were found to be either absolutely or relatively refractory to the inoculable tumor. There is, therefore, no correlation between susceptibility and certain recognized inherited characters, such as those determining the coloration of the fur, of the eyes, etc. These authors state that the difference in

susceptibility between the mice of Paris and those of Nancy is, therefore, an invisible difference. The possible influence of diet is mentioned, but they think that the difference may be due to something of a more complicated nature.

The present investigation was undertaken in order to determine if the susceptibility to an inoculable tumor is transmitted in accordance with principles of heredity such as are embodied in Mendel's law. In the greater number of experiments an inoculable tumor which originally developed spontaneously in a Japanese waltzing mouse was used, although the Jensen and the Ehrlich Stamme II. tumors were also employed in certain instances in order to ascertain how generally applicable were the results obtained in experiments with the first named tumor. The waltzing mouse tumor was especially adapted for the study of the problem at hand on account of the uniformity of the results obtained from its inoculation into different varieties of mice. Thus from the first it grew in practically all Japanese waltzing mice inoculated, whereas repeated implantations in common tame mice were unsuccessful. The original tumor developed in a waltzing mouse which had been brought from Japan, but no difficulty was found in propagating it in Japanese waltzing mice reared in this country.

The histological structure of the tumor has already been described (see Fourth Report of the Cancer Commission of Harvard University, 1907, 49; Cf. Jour. Med. Research, 1907, XVII., 177). It resembles in most respects the tumor found by Loeb in a Japanese waltzing mouse. The epithelium in certain portions of the tumor is arranged in gland-like acini containing more or less colloid material; in other portions it is arranged in solid alveoli the central cells of which appear vacuolated from the presence of fatty material, so that there is considerable structural resemblance to the sebaceous and preputial glands; and a considerable part of the epithelium consists of flattened or spindle-shaped cells which are often intimately intermingled with connective tissue cells. The stroma is in places rather more cellular

than is commonly found in spontaneous carcinomata of mice.

The first transplants of the tumor grew very slowly, a phenomenon which has been repeatedly observed in the artificial propagation of mouse tumors. Only three mice survived long enough for the tumor to attain any considerable development. In one of these a portion of the tumor nodule consisted largely of gland-like structures, whereas the remainder consisted of atypical connective tissue (illustrated in preceding paper). This tumor had attained a size only slightly larger than a pea in the nine months following its implantation. Unfortunately none of this tumor was used for inoculation, the strain having been propagated from the other more rapidly growing tumors of this generation. The portion of the nodule consisting of connective tissue was definitely limited from the surrounding tissue, and appeared to be of the nature of a new formation rather than of the character of a reaction to the tumor epithelium. It was of course impossible to determine at so long a period after the inoculation whether the sarcomatous portion of the nodule was derived from the host or from the implanted tumor. The appearance of sarcoma in this strain is of interest since Loeb several years previous to this had observed the development of sarcoma in a similar tumor which he succeeded in propagating in Japanese waltzing mice. Apart from this single case, all other tumors of this strain which have been thus far examined were epithelial in character. More or less of the gland-like arrangement of the epithelium, such as occurred in parts of the original tumor, persisted in the first two generations, and epithelial cells which were undergoing a fatty metamorphosis were found in considerable numbers. Neither of these features appeared, however, in subsequent generations, but the tumor maintained a constant type, its only important structural feature consisting of the arrangement of the epithelium in solid masses or alveoli with a relatively small amount of stroma.

The growth of this tumor even after long continued

propagation is slow as compared with the Jensen tumor, and in several experiments the average weight of the tumors is only slightly in excess of .5 gram after five weeks' growth. If the tumor is allowed to continue its growth indefinitely, metastasis occurs in a large proportion of cases. Notwithstanding its slow growth this tumor has been found to be peculiarly adapted to the study of inheritance with reference to susceptibility. There is but slight tendency to necrosis, so that tumors of great size are often obtained in which there is little or no necrotic tissue. This makes possible a more uniform dosage of living tumor epithelium than can be obtained with tumors which are to a large extent necrotic. The inoculation results thus appear to be correspondingly more uniform than is the case with most of the transplantable tumors.

The characteristics of the Jensen and the Ehrlich St. II. tumors are well known. The Jensen tumor is a carcinoma the epithelium of which has an alveolar arrangement, whereas the Ehrlich tumor is an adenocarcinoma. Both originated in the subcutaneous tissue — presumably in the mammary gland — of the mouse.

In much of the investigation of the experimentally inoculable tumors there apparently has been too much dependence placed upon number and too little attention devoted to adequate control experiments, taking into account various conditions which may influence the nature of the results. The great difficulty lies in the impossibility of obtaining identical conditions in successive experiments. The influence of dosage on the result of the inoculation of tumor tissue has received considerable attention, and the age of the animals used has been taken into account by certain investigators.[14]

Apart from those instances in which marked racial differences have been met with, apparently but slight attention has been paid to the racial peculiarities of the animals employed in the propagation of tumors. When mice are obtained in large numbers from one or more dealers, variation in the nature of the stock used must be inevitable.

Variation in regard to susceptibility must always be taken into account in the determination of fluctuations in the growth of tumors. Thus, as I shall demonstrate later on, it is possible to produce by experimental breeding waltzing mice which are in every case insusceptible to a tumor that gives very nearly one hundred per cent of positive results in other waltzing mice. The alternation of such stocks would give fluctuations in infectivity from one hundred to no per cent.

In studying the differences in the susceptibility of various categories of mice it was necessary in each experiment to take into account certain known variable factors such as the dosage of tumor, the order of inoculation, and the age and general condition of the animals to be inoculated. In determining the influence of any one of these factors it is obviously impossible to make all associated conditions identical in the series to be compared. Recognizing, therefore, the inevitable variance of associated conditions, it is possible, however, either to make them approximately equivalent or to reverse the varying conditions in the course of successive experiments.

In all experiments the tumor tissue was implanted subcutaneously following the method employed by Bashford. The desired dose of living tumor was drawn into a trochar, which was inserted through the skin far back on the side of the animal and then thrust forward in the subcutaneous tissue toward the axilla, where its contents were deposited. The dose was estimated in each instance by the eye, so that there was necessarily more or less variation in the amounts received by different individuals. A dose considerably larger than that necessary to produce tumors in susceptible animals was inoculated in each case. The practice was followed of gently massaging the tissue about the site of inoculation in order to distribute the tumor tissue along the needle track so as to bring as much of it as possible into immediate relation with the tissues of the host. This was designed to increase the efficiency of the dose employed. In each experiment a single tumor was used to inoculate a comparative series of

mice. Since it often required two to three hours to inoculate the total number of mice in a given experiment, there was a possibility of deterioration on the part of the tumor during this time. On this account the series of mice known to be susceptible were usually inoculated last, while the series of mice whose susceptibility was to be tested were inoculated first.

With the exception of a small number of mice of which only the approximate age was known, the exact age was recorded for each series. For comparative series, mice of approximately equal age were used as far as possible. Since it has become a matter of experience that the results are more uniform as well as more often positive in young animals, the supposedly insusceptible series was usually given the benefit of any difference of age. In certain experiments comparative series of old and young mice of the same variety were inoculated for the purpose of demonstrating the influence of age. Other conditions, such as general nutrition, etc., will be discussed later in connection with the individual experiments.

The results obtained in the following experiment illustrate a relative difference in the susceptibility of two races of common mice to two inoculable tumors. In this and in following experiments temporary tumors which disappear spontaneously are not recorded in the tables, although their occurrence is usually mentioned elsewhere. One of the two lots of mice used was purchased from a Providence dealer; the other lot was taken from a family of mice which has been bred for some time in the laboratory, and which originated from a mouse obtained from Buffalo, N.Y., mated with one obtained from Cambridge.

EXPERIMENT A.

Jan. 31, 1909. Ten Providence mice and ten mice of Buffalo-Cambridge stock were inoculated subcutaneously on the right side with the Ehrlich St. II. tumor.

Feb. 1, 1909. The same mice and also two other Providence mice and two other Buffalo-Cambridge mice were inoculated on the left side with the Jensen tumor.

The Providence mice were young adults not over two or three months old,

while the Buffalo-Cambridge mice were all considerably older, the first five being six months and the remaining seven about four months old. All mice bearing tumors were killed on May 4, 1909. The results are tabulated below:

Variety of Mouse.	Results.		Remarks.
	Ehrlich Tumor.	Jensen Tumor.	
Providence No. 1.....	O	O	Died April 30.
" " 2.....	O	O	
" " 3.....	O	O	Tumors both wholly necrotic.
" " 4.....	O	O	
" " 5.....	+	O	Weight of tumor only .04.
" " 6.....	+	O	
" " 7.....	O	O	
" " 8.....	+	O	Jensen tumor necrotic.
" " 9.....	O	O	
" " 10.....	O	O	
" " 11.....	.....	O	
" " 12.....	.....	O	
Total positive.....	3 or 30%	O or O%	Tumors in 25% of entire series.
Buffalo-Cambridge No. 1..	O	+	
" " " 2..	O	+	
" " " 3..	O	O	Nephritis.
" " " 4..	O	O	
" " " 5..	+	+	
" " " 6..	+	+	
" " " 7..	+	O	
" " " 8..	+	+	
" " " 9..	+	O	
" " " 10..	O	O	
" " " 11..	.....	O	
" " " 12..	....	O	
Total positive.....	5 or 50%	5 or 41⅔%	Tumors in 58⅓% of entire series.

Combined weight of tumors in Providence series..... 13.845
Combined weight of tumors in Buffalo-Cambridge series 48.995

In addition to a difference in the degree of susceptibility shown by the two races of mice used in this experiment, a distinct difference in the infectivity is apparent in the two tumors used. The Jensen tumor failed to grow in the Providence mice, whereas the Ehrlich St. II. tumor grew in thirty per cent of those inoculated. In the other series there was an equal number of Jensen and Ehrlich tumors, and one or both developed in fifty-eight per cent of the mice.

The possibility of obtaining by selection a race of mice wholly immune to the inoculable tumors was considered. The mice which proved refractory in each experiment were used for breeding, and the offspring, together with control series of susceptible stocks of mice, have been inoculated from time to time. In Experiment B the Jensen tumor was inoculated three days previous to the Ehrlich tumor.

EXPERIMENT B.

July 3, 1908. Five offspring of the insusceptible Providence mice from Experiment A and five mice of mixed laboratory stock were inoculated on the left side with the Jensen tumor. The mice of both series were of equal age — sixty days.

July 6, 1908. The same mice were inoculated on the right side with the Ehrlich "Stamme II." tumor. The inoculations resulted as follows:

Variety.	Results.		Remarks.
	Ehrlich Tumor.	Jensen Tumor.	
Providence No. 1.....	+	+	
" " 2.....	O	O	
" " 3.....	O	O	
" " 4.....	O	O	
" " 5.....	+	+	
Total positive.....	2	2	One or both tumors in 40%.
Laboratory No. 1.....	O	+	Metastases in lungs.
" " 2.....	+	+	
" " 3.....	+	O	
" " 4.....	O	+	
" " 5.....	+	O	
Total positive.....	3	3	One or both tumors in 100%.

In this experiment the Providence race was shown to be less susceptible than a control series of mixed laboratory stock of equal age. An equal number of Jensen and of Ehrlich tumors developed.

EXPERIMENT C.

Jan. 26, 1909. Four offspring of the insusceptible Providence mice from Experiments A and B and four mice of Buffalo-Cambridge stock were inoculated with the Ehrlich St. II. tumor. Age of Providence mice, four months; of Buffalo-Cambridge mice, five months.

Variety.	Results.	Remarks.
Providence No. 1.....	O	
" " 2.....	O	
" " 3.....	O	
" " 4.....	O	Tumor sloughed off after long period of growth.
Total positive.....	O	Tumors in O%.
Buffalo-Cambridge No. 1	+	
" " " 2	+	
" " " 3	+	
" " " 4	+	
Total positive.....	4	Tumors in 100%.

Only one tumor was inoculated in this instance. The great difference in the results with the two races of mice are apparent. The breeding of the refractory animals in the more insusceptible race will be continued with the end in view of obtaining a race which is absolutely rather than relatively insusceptible. The view held by certain investigators that inbreeding increases susceptibility to tumors is not borne out by the results of this and subsequent experiments.

The susceptibility of Japanese waltzing mice to the same two tumors of the common mouse was also tested.

EXPERIMENT D.

Five Japanese waltzing mice of unknown age and ten young adult common mice were inoculated on April 22, 1908, with the Jensen tumor and on the following day with the Ehrlich St. II. tumor No. 1925. The results are as follows:

Variety of Mouse.	Results.		Remarks.
	Ehrlich Tumor.	Jensen Tumor.	
Japanese waltzing No. 1.	— ?	0	Died 12 days after inoculation.
" " " 2.	0	0	Other four under observation 60 days or longer.
" " " 3.	0	0	
" " " 4.	0	0	
" " " 5.	0	0	
Total positive	0	0	Tumors in 0%.
Common No. 1	+	+	
" " 2	+	+	
" " 3	+	+	
" " 4	+	+	
" " 5	+	+	
" " 6	+	+	
" " 7	+	0	
" " 8	+	+	
" " 9	+	+	
" " 10	+	+	
Total positive	10	9	One or both tumors in 100%.

The ten common mice were killed at periods ranging from nineteen to fifty-one days after inoculation.

Combined weight of Ehrlich tumors in ten common mice	23.880
Combined weight of Jensen tumors in same	15.270
Total	39.150

In the four waltzing mice which survived neither the Jensen nor the Ehrlich tumor developed. Tumors developed in all the common mice, the Ehrlich tumor in ten, the Jensen tumor in nine. From this experiment it might appear that the Japanese waltzing mouse is not susceptible to these tumors, but since the animals used were of unknown age,

but were apparently rather old, the evidence is not conclusive.

Further data concerning racial susceptibility to the same two inoculable tumors of the common mouse were obtained in Experiments E and F. The Jensen and the Ehrlich tumors were inoculated into small comparative series of common mice, Japanese waltzing mice, and hybrids* which had been obtained by cross-breeding these two varieties. Hybrids obtained from both Japanese waltzing and common mothers were used.

EXPERIMENT E.

April 25, 1908. Five Japanese waltzing, ten hybrids, and five common mice were inoculated subcutaneously with Jensen tumor No. 1917.

Variety of Mouse.	Age.	Result.	Remarks.
Common No. 1	1 month.	+	
" " 2	1 "	+	
" " 3	1 "	+	
" " 4	1 "	O	
" " 5	1 "	+	
Total positive	.....	4	Tumors in 80%.
Hybrids C♂. J♀ No. 1 ...	2 months.	O	
" " " " 2 ...	2 "	O	
" " " " 3 ...	2 "	O	
" " " " 4 ...	2 "	O	
" " " " 5 ...	2 "	O	
Total positive	.....	O	Tumors in O%.

*A full description of these hybrid mice will be given further on in this paper.

EXPERIMENT E. — *Continued.*

Variety of Mouse.	Age.	Result.	Remarks.
Hybrids J♂. C♀ No. 1 ...	1½ months.	O	
" " " " 2 ...	1½ "	O	
" " " " 3 ...	1½ "	O	
" " " " 4 ...	1½ "	O	
" " " " 5 ...	1½ "	O	
Total positive	.....	O	Tumors in O%.
Japanese waltzing No. 1 ...	2 months.	Dead 7	days after inoculation.
" " " 2 ...	2 "	" 19	" " "
" " " 3 ...	2 "	O	
" " " 4 ...	2 "	O	
" " " 5 ...	2 "	O	
Total positive	.....	O	Tumors in O%.

The Jensen tumor grew to large size in four (eighty per cent) of the five common mice, but failed to develop in both sorts of hybrids and in the waltzing mice which survived. The tumor used in this experiment was not considered especially favorable for inoculation, but it nevertheless grew in four of the five common mice. The tumors developed for a time in many of the hybrids and appeared as well-defined nodules ten days after the inoculation, but were, with one exception, absorbed during the following week. This temporary growth took place in two hybrids of a Japanese waltzing mother, and in all five hybrids of a common mother. A second inoculation of the three surviving waltzing mice and the ten hybrids, thirty-four days later, with the Jensen tumor resulted negatively in the waltzing mice, and in one tumor which grew to large size in one of the hybrids. Of the nine hybrids, in which the reinoculation of the Jensen tumor resulted negatively, seven were in a subsequent experiment inoculated with the Japanese waltzing mouse tumor with the result that this tumor developed in all.

The susceptibility of these varieties of mice to the Ehrlich tumor was also determined.

EXPERIMENT F.

April 25, 1908. Five Japanese waltzing mice, ten hybrids, and five common mice were inoculated subcutaneously with the Ehrlich St. II. tumor No. 1923.

Variety of Mouse.	Age.	Weight of Tumor after				Remarks.
		33 days.	37 days.	48 days.	65 days.	
Common No. 1	2 mos.	4.170				
" " 2	2 "	1.870				
" " 3	2 "	3.140				
" " 4	2 "	4.730				
" " 5	2 "	2.290				
Total weight	.....	16.200	.....	.....	.....	Tumors in 100%.
Hybrids C♂. J♀. No. 1....	1 mo.	0	0	0	0	
" " " " 2....	1 "	3.270				
" " " " 3....	1 "	Small.	.....	.....	2.800	
" " " " 4....	1 "	0	0	0	0	
" " " " 5....	1½ "	0	0	0	0	
Total weight	.....	3.270+	.....	.....	2.800	Tumors in 40%.
Hybrids J♂. C♀. No. 1....	1½ mo.	2.905				
" " " " 2....	1½ "	Small.	.....	.....	6.450	
" " " " 3 ...	1½ "	0	0	0	0	
" " " " 4....	1½ "	0	0	0	0	
" " " " 5....	1½ "	2.200				
Total weight.....	.....	5.105+	.....	.....	6.450	Tumors in 60%.
Japanese waltzing No. 1...	1 mo.	Died 11	days af	ter inoc	ulation.	Tumor living.
" " " 2...	1 "	Small.	.....	2.680		
" " " 3...	1 "	Small.	.....	.....	.950	
" " " 4...	1 "	Small.	.330			
" " " 5...	1 "	0	0	0	0	
Total weight.....	.....	.....	.330+	2.680+	.950	Tumors in 75%.

The five hybrids in which the Ehrlich tumor failed to grow were reinoculated thirty-three days later with the same tumor with the result that it failed to develop in four, and grew in one, which was inoculated at the same time with the Japanese waltzing mouse tumor.

Of the five common mice inoculated all (one hundred per cent) developed tumors. Tumors were present in all five hybrids of a Japanese mother at the end of a fortnight, but in three the tumors later disappeared, while in two (forty per cent) they developed to great size. Tumors likewise developed in all hybrids of a common mother: two disappeared spontaneously, two grew rapidly, and one remained stationary for a time, but later grew rapidly. Positive results were thus obtained in sixty per cent. Of the five Japanese waltzing mice, one died eleven days after inoculation and presented a small mass of living tumor. Three (seventy-five per cent) of the remaining four developed tumors which, however, varied greatly in size and were all much more slowly growing than the tumors in the control series of common mice.

From the data furnished by these experiments a number of conclusions may be drawn. Certain races of common mice show relative differences in susceptibility to the Jensen and to the Ehrlich tumors. The differences are maintained through successive generations, even though all the respective races are kept under essentially identical conditions, *i.e.*, in the same room and fed with the same food. The Ehrlich tumor is capable of growing in varieties of mice in which the Jensen tumor invariably fails. Thus the Jensen tumor failed to grow in both old and young waltzing mice, whereas the Ehrlich tumor grew in young mice of this variety. Japanese waltzing mice are thereby less susceptible than common mice to the inoculable tumors of the latter. Young hybrids of both common and Japanese waltzing mothers are in a certain number of cases susceptible to the Ehrlich tumor and in fewer cases susceptible to the Jensen tumor. These tumors attained slightly greater development in hybrids of a common mother than in hybrids of a Japanese waltzing mother, although the numbers used were too small to attach great importance to this point. With respect to the number of tumors which developed, young hybrids, however, appeared to be no more susceptible to the Ehrlich tumor than waltzing mice of similar age. Since in all these

experiments the difference in susceptibility of the various races of mice to the Jensen and the Ehrlich tumors are for the most part relative rather than absolute, it did not appear that these tumors were especially favorable for the study of the inheritance of susceptibility. The variability in the biological behavior of these tumors had to be always taken into consideration. More extensive experiments were planned with reference to susceptibility to the Japanese waltzing mouse tumor, which appeared to be especially adapted for such investigation.

Since this tumor has in every instance failed to develop when inoculated into common mice, the cross-breeding of Japanese waltzing and common mice was undertaken with the end in view of testing the susceptibility of the hybrids thus produced. With the exception of a few lots of animals which are in each case designated, all hybrids used in the two preceding and all subsequent experiments were derived from the reciprocal cross-breeding of four common albino mice, two of each sex, with four black and white Japanese waltzing mice, two of each sex. The four Japanese mice were presumed to be susceptible to the tumor, since up to that time it had developed in one hundred per cent of all such mice inoculated. The actual susceptibility of these four mice could not be tested, for the obvious reason that the development of the tumor would make the necessary amount of breeding from them impossible. The four common white mice were inoculated with negative result after a sufficient number of hybrids had been obtained. On account of their age at the time of this inoculation, the result of the inoculation is of less importance than the fact that the tumor has in every instance failed to grow in young mice of the same stock.

The offspring derived from the cross-breeding of common albino mice and black and white Japanese waltzing mice present features of considerable interest, in addition to certain recognized inherited characters such as the coat coloration, the waltzing habit, etc. Since most of the points have been covered in the investigations of Haacke,[9] von Guaita,[10]

Cuénot,[11] and others, such data will be but briefly considered here.

No distinctive difference was noted in the hybrids (F_1) derived from the mating of common males with Japanese waltzing females as compared with those derived from Japanese waltzing males and common females. The mice of this first filial generation (F_1) were invariably lively, vigorous mice, of rather wild or excitable disposition. They were considerably larger than the waltzing mice and somewhat smaller than the common mice from which they were derived. In none was there any tendency to waltz, so that the waltzing habit is to be considered a recessive character. With frequent handling much of the "wildness" of disposition disappeared, although it persisted in certain individuals who remained excitable and vicious when mature. In the mice of this generation either the entire coat or the greater part of it was pigmented. In those in which the pigmentation was incomplete there was a white streak on the forehead and on the belly. Thus, as has been noted by other investigators, the pigmented area is much greater in the hybrids than in the partially pigmented or spotted parent. Hybrids of the following coloration were derived from the several matings: gray, gray with white streak on forehead and belly, and black. One of the common females on being subsequently mated with a different Japanese male gave birth to two spotted black and white, two black, one gray, and three gray with white streak on forehead and belly, in a single litter of young.

In the second generation (F_2) differences of temperament were noted. In a single litter certain individuals were of gentle disposition, whereas others were wild and vicious. The waltzing character appeared again in this generation, but the number of mice which waltzed fell somewhat short of the Mendelian expectation. In the fifty-four mice of this generation inoculated, ten or 18.5 per cent waltzed, but waltzing mice were known to have previously died from several of the litters taken for these experiments. This generation presented greater variation in color and in color distribution than the preceding. In addition to gray, black, and these

colors combined with narrow streaks of white, there were spotted mice with a greater amount of white, pure albinos, and spotted brown and white with brown eyes. The color and the waltzing characters were inherited independently of one another, just as has been found concerning the inheritance of other Mendelian characters. Thus in this generation there were gray, gray and white, black, black and white, brown, and albino mice which waltzed. They also differed in other respects than in color from the pure bred Japanese waltzing mouse. The size attained by certain individuals greatly exceeded that of the latter variety, and approximated that of the common mouse. The wild disposition was often combined with the waltzing habit, so that when disturbed peculiar movements were performed, which were never observed in the true Japanese waltzing mouse. They often rolled over or turned somersaults in an effort to escape. Coördination developed late in many of the young which turned out to be waltzers. These, even when more than half grown propelled themselves by irregular convulsive movements, and, after they had learned to use their feet, they frequently fell or rolled completely over.

Although such waltzing mice may breed true with regard to the waltzing habit, they, nevertheless, differ essentially from the so-called Japanese waltzing variety from which they have originated. Thus purity may be attained with respect to a single character such as waltzing, but with the recognition of numerous other characters the chance of the reoccurrence of the combination of characters peculiar to a given variety or breed becomes progressively smaller.

In breeding for the third generation (F_3) the mating of the animals was purposely made promiscuous rather than selective. In the sixteen mice of this generation used for inoculation only one waltzed.

In the following experiments the susceptibility of the various series of mice to the inoculable tumor of the Japanese waltzing mouse was tested. The mice are numbered in the order of their inoculation, and are arranged in comparative series so that certain series of mice serve as controls to others, the susceptibility of which is to be tested. The

object of the first experiment was to determine if the hybrids (F) obtained by mating a common male with a Japanese waltzing female are susceptible. Since at this time the tumors were of small size, two tumors were required to inoculate the total number of mice. Series of hybrids, series of common mice, and series of Japanese waltzing mice were inoculated at the same time with the two tumors.

EXPERIMENT I.

March 17, 1908. Five common mice, five hybrids, and five Japanese waltzing mice, Nos. 1938 to 1952 inclusive, were inoculated subcutaneously in the region of the right axilla with tumor No. 1873, which had been growing seventy-seven days in a Japanese waltzing mouse.

March 18, 1908. Three similar series of mice, Nos. 1953 to 1968 inclusive, were inoculated in the same manner with tumor No. 1886, which had grown sixty-three days in a Japanese waltzing mouse.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
1938	Common.	45 days.	43 days after inoc.	0
1939	"	45 "	24 " " "	0
1940	"	80 "	Alive 3½ mos. " "	0
1941	"	80 "	" 3½ " " "	0
1942	"	80 "	" 3½ " " "	0
1943	Hybrid F (C♂.J♀).	5½ weeks.	77 days after inoc.	2.380
1944	" " "	5½ "	49 " " "	3.855
1945	" " "	5½ "	77 " " "	1.320
1946	" " "	5½ "	77 " " "	9.820
1947	" " "	5½ "	32 " " "	.977
1948	Japanese waltzing.	2 months.	41 days after inoc.	.134
1949	" "	2 "	78 " " "	.115
1950	" "	2 "	78 " " "	.930
1951	" "	2 "	55 " " "	.480
1952	" "	2 "	81 " " "	1.720
1953	Japanese waltzing.	2 months.	80 days after inoc.	2.900
1954	" "	2 "	77 " " "	.330
1955	" "	2 "	77 " " "	2.180
1956	" "	2 "	76 " " "	4.450
1957	" "	2 "	77 " " "	.160

EXPERIMENT I. — *Continued.*

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
1958.....	Hybrid F (C♂. J♀).	5½ weeks.	71 days after inoc.	3.750
1959.....	" " "	5½ "	76 " " "	2.150
1960.....	" " "	5½ "	80 " " "	3.840
1961.....	" " "	5½ "	49 " " "	1.570
1962.....	" " "	5½ "	80 " " "	1.370
1963.....	" " "	5½ "	76 " " "	1.230
1964.....	Common.	81 days.	Alive 10 weeks after inoc.	0
1965.....	"	81 "	" 10 " " "	0
1966.....	"	81 "	" 10 " " "	0
1967.....	"	81 "	" 10 " " "	0
1968.....	"	81 "	" 10 " " "	0

All of the eleven hybrids developed tumors as well as the control series of ten Japanese waltzing mice. The ten common mice inoculated at the same time, with the same material, developed no tumors. The tumors grew on the average more rapidly in the hybrids than in the Japanese waltzing mice. The hybrids were, however, younger than the Japanese waltzing mice. Subsequent experiments were planned so as to yield data concerning the influence of age, size, and body growth on the rate of tumor growth.

Having established the susceptibility of the hybrid offspring of common male and Japanese waltzing female mice, the next point to be determined was whether the hybrid offspring of Japanese waltzing male and common female mice are susceptible. A single tumor was inoculated into forty-seven mice arranged in comparative series. Ten mice of each of the series in which tumors developed were killed thirty-six days after the inoculation, and the tumors weighed in order to estimate the comparative rates of growth.

EXPERIMENT 2.

June 2, 1908. Tumor No. 1956, which growing in a Japanese waltzing mouse had attained the weight of 4.450 grams in seventy-six days, was inoculated into the following comparative series of first generation hybrids, Japanese waltzing mice, and common mice in the order of their enumeration.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2073.....	Hybrid F_1 (J♂. C♀)	5 weeks.	36 days after inoc.	1.850
2074.....	" " "	5 "	36 " " "	5.195
2075.....	" " "	5 "	36 " " "	3.315
2076.....	" " "	5 "	36 " " "	4.950
2077.....	" " "	5 "	36 " " "	2.790
2078.....	" " "	5 "	36 " " "	.300
2079.....	" " "	5 "	36 " " "	3.590
2080.....	" " "	5 "	36 " " "	2.060
2081.....	" " "	5 "	36 " " "	2.940
2082.....	" " "	12 "	36 " " "	1.045
Total..	10 Hybrids F_1 (J♂.C♀)	.....	.....	28.035
2084.....	Hybrid F_1 (C♂. J♀)	7 weeks.	36 days after inoc.	2.530
2085.....	" " "	7 "	36 " " "	1.120
2086. ...	" " "	7 "	36 " " "	1.160
2087.....	" " "	7 "	36 " " "	.390
2088.....	" " "	7 "	36 " " "	2.750
2089.....	" " "	7 "	36 " " "	2.300
2090.....	" " "	7 "	36 " " "	1.080
2091.....	" " "	7 "	36 " " "	2.910
2092.. ..	" " "	7 "	36 " " "	1.260
2093.....	" " "	7 "	36 " " "	.890
Total..	10 Hybrids F_1 (C♂. J♀)	.....	.....	16.390
2094.....	Common.	6 weeks.	18 days after inoc.	0
2095.....	"	6 "	Alive 2½ mos. " "	0
2096.....	"	6 "	" " " " "	0
2097.....	"	6 "	28 days " "	0
2098.....	"	6 "	18 " " "	0
2099.....	"	6 "	Alive 2½ mos. " "	0
2100.....	"	6 "	" 2½ " " "	0
2101.....	"	6 "	" 2½ " " "	0
2102.....	"	43 days.	" 2½ " " "	0
2103.....	"	43 "	" 2½ " " "	0
Total..	10 Common.	.....	.....	0

EXPERIMENT 2. — *Continued.*

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2104.....	Japanese waltzing.	5 weeks.	36 days after inoc.	.995
2105.....	" "	5 "	36 " " "	1.670
2106.....	" "	5 "	36 " " "	1.850
2107.. ..	" "	5 "	36 " " "	1.450
2108.. ..	" "	5 "	36 " " "	.900
2109.....	" "	5 "	36 " " "	2.550
2110.....	" "	5 "	36 " " "	1.240
2111.....	" "	5 "	36 " " "	.475
2112.....	" "	5 "	36 " " "	.720
2113.....	" "	5 "	36 " " "	.370
Total..	10 Japanese waltzing.	.....	.....	12.220
2114.....	Japanese waltzing.	5 weeks.	84 days after inoc.	4.670
2115.....	" "	5 "	21 " " "	1.050
2116.....	Young common.	12 days.	Alive 2½ mos. " "	0
2117....	" "	12 "	" 2½ " " "	0
2043....	Hybrids F_1 (J♂.C♀)	12 weeks.	27 days " "	.580
2083.....	" " "	12 "	91 " " "	11.110

Tumors developed in all hybrids derived from common females as well as in all hybrids derived from Japanese waltzing females. In a comparative series of ten common mice no tumors developed. The combined weight of the tumors in ten hybrids (J ♂ × C ♀) more than doubles that of the tumors in ten Japanese waltzing mice, although the two series are of the same age. The tumors also grew more rapidly in hybrids (C ♂ × J ♀) than in Japanese waltzing mice. The fact that the latter were the last to be inoculated may have influenced the results to some extent. The fact that both categories of hybrids proved susceptible to the tumor indicated that the susceptibility is transmitted by the male as well as by the female.

Since it was thought possible that the tumor might grow in very young common mice, this occasion was taken to test their susceptibility. Two young nursing mice, Nos. 2116 and 2117, aged twelve days, were inoculated, but the

tumor failed to grow in these as well as in older mice. Hybrid No. 2043, which had been inoculated with the Ehrlich tumor, was inoculated also with the Japanese tumor with the result that both tumors grew.

Further experiments were planned to determine if susceptibility to the tumor is transmitted in accordance with Mendel's law of heredity. On this hypothesis it would be necessary to consider susceptibility as a dominant character, since it is demonstrable in all the hybrid offspring. The hybrids of the first generation (F_1) were bred together, and the susceptibility of the second generation (F_2) was tested.

EXPERIMENT 3.

June 29, 1908. Tumor No. 2115, which had attained the weight of 1.050 grams twenty-one days after its implantation in a Japanese waltzing mouse, was inoculated into six hybrids of the second generation (F_2), seven Japanese waltzing mice, and seven common mice.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2125.....	Hybrid F_2.	7 weeks.	Alive 2½ mos. after inoc.	0
2126.....	" "	7 "	" 2½ " " "	0
2127.....	" "	7 "	" 2½ " " "	0
2128.....	" "	7 "	" 2½ " " "	0
2129.....	Japanese waltzing.	10 weeks.	36 days after inoc.	.430
2130.....	" "	10 "	36 " " "	.340
2131.....	" "	10 "	36 " " "	.290
2132.....	" "	10 "	36 " " "	.320
2133.....	" "	10 "	36 " " "	.520
2134.....	" "	10 "	36 " " "	.560
2135.....	" "	10 "	24 " " "	Not weighed.
2136.....	Hybrid F_2.	31 days.	Alive 2½ mos. after inoc.	0
2137.....	" "	31 "	" 2½ " " "	0
2138.....	Common.	29 days.	Alive 2½ mos. after inoc.	0
2139.....	"	29 "	" 2½ " " "	0
2140.....	"	29 "	" 2½ " " "	0
2141.....	"	29 "	" 2½ " " "	0
2142.....	"	29 "	" 2½ " " "	0
2143.....	"	29 "	" 2½ " " "	0
2144.....	"	29 "	" 2½ " " "	0

Of the series of six mice of the second generation (F_2) tumors developed in none. Tumors developed in all of a control series of seven Japanese waltzing mice, and in none of a series of seven common mice. In order to test in a thorough manner the susceptibility of generation F_2, further experiments of a similar nature were necessary to obtain the sufficient number of results.

EXPERIMENT 4.

July 23, 1908. Five hybrids of the second generation (F_2), five Japanese waltzing, and five hybrids of the first generation (F_1) were inoculated by the usual method with tumor No. 2135, which had grown twenty-four days in a Japanese waltzing mouse.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2162.....	Hybrid F_2.	45 days.	Alive 4 mos. after inoc.	0
2163.....	" "	45 "	" " " "	0
2164.....	" "	45 "	" " " "	0
2165.....	" "	45 "	" " " "	0
2166.....	" "	45 "	" " " "	0
Total ...	5 Hybrids F_2.	.....	.....	0
2167.....	Japanese waltzing.	45 days.	48 days after inoc.	.650
2168.....	" "	45 "	48 " " "	.420
2169.....	" "	45 "	48 " " "	.720
2170.....	" "	45 "	48 " " "	.590
2171.....	" "	45 "	48 " " "	.550
Total ...	5 Japanese waltzing	.....	.....	2.930
2172.....	Hybrid F_1.	14 weeks.	48 days after inoc.	.360
2173.....	" "	14 "	48 " " "	.570
2174.....	" "	14 "	48 " " "	1.690
2175.....	" "	18 "	48 " " "	.470
2176.....	" "	18 "	48 " " "	.350
Total ...	5 Hybrids F_1.	.....	.....	3.440

No tumors developed in five mice of generation F_2, whereas tumors developed in all mice of the control series of Japanese

waltzing mice and hybrids of generation F_1. The ages of generation F_2 and of the Japanese waltzing mice were in this instance identical. The mice of generation F_1 were considerably older.

In the following experiment it was necessary to employ three tumors in the inoculation of the thirty mice whose susceptibility was to be tested. Comparative series were in each instance inoculated with a single tumor. In order to determine the influence of age on the rate of tumor growth, series of young and old Japanese waltzing mice were inoculated. The susceptibility of the four common parents (P_1), and of generation F_1 hybrids, obtained by crossing Japanese waltzing mice with common mice of another stock, was tested. (The latter are indicated as "alien" in the table, Experiment 5.)

EXPERIMENT 5.

Aug. 4, 1908. Three tumors, Nos. 2129, 2132, 2133, which had been growing thirty-six days in Japanese waltzing mice and which weighed .430, .320, and .520 respectively, were used to inoculate comparative series of second generation (F_2) hybrids, Japanese waltzing mice, first generation (F_1) hybrids including four derived from an alien stock, and four common mice, the parents of the hybrids ordinarily used in these experiments. Tumor No. 2129 was inoculated into twenty mice, Nos. 2184 to 2203 inclusive; tumor No. 2132 into seven mice, Nos. 2204 to 2210 inclusive, and No. 2133 into eight mice, Nos. 2211 to 2218 inclusive.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2184.....	Hybrid F_2.	36 days.	Alive 5 mos. after inoc.	0
2185.....	" "	36 "	" 5 " " "	0
2186.....	" "	36 "	" 5 " " "	0
2187.....	" "	36 "	" 5 " " "	0
2188.....	" "	36 "	" 5 " " "	0
Total....	5 Hybrids F_2.	.....	.....	0
2189.....	Japanese waltzing.	5 weeks.	49 days after inoc.	.610
2190.....	" "	5 "	49 " " "	.350
2191.....	" "	5 "	49 " " "	.400
2192.....	" "	5 "	49 " " "	.540
2193.....	" "	56 days.	49 " " "	.130
Total ...	5 Japanese waltzing.	.....	....	2.030

EXPERIMENT 5. — *Continued.*

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2194	Hybrid F_1.	12 weeks.	29 days after inoc.	1.700 (estimated).
2195	" "	12 "	49 " " "	.050
2196	" "	12 "	49 " " "	3.360
2197	" "	12 "	49 " " "	.240
2198	" "	12 "	49 " " "	.770
Total ...	5 Hybrids F_1.	.....	....	6.120
2199	Japanese waltzing.	21 weeks.	49 days after inoc.	.510
2200	" "	Very old.	49 " " "	.450
2201	" "	" "	49 " " "	.100
2202	" "	5 months.	49 " " "	0
2203	" "	5 "	49 " " "	.370
Total ...	5 Japanese waltzing.	.....	.. ..	1.430
2204	Hybrid F_2.	35 days.	28 days after inoc.	0
2205	" "	35 "	28 " " "	0
2206	Japanese waltzing.	57 "	49 " " "	1.450
2207	" "	57 "	13 " " "	(Small tumor, not weighed.)
2208	Hybrid F_1.	54 "	49 " " "	1.540
2209	Japanese waltzing.	19 weeks.	49 " " "	.140
2210	" "	19 "	49 " " "	.115
2211	Hybrid F_1? (" alien ").	8 weeks.	49 days after inoc.	.780
2212	" " "	8 "	49 " " "	.780
2213	" " "	8 "	49 " " "	.410
2214	" " "	8 "	49 " " "	.790
2215	Common.	6 months.	Alive 3 mos. after inoc.	0
2216	"	6 "	" 3 " " "	0
2217	"	6 "	20 days " "	0
2218	"	6 "	34 " " "	0

Tumors developed in none of seven mice of generation F_2, in all six of control series of generation F_1 mice, in all seven of the young, and in six of the seven old Japanese waltzing mice inoculated. The failure of tumor to grow in one of the

old Japanese waltzing mice furnishes the first negative result thus far obtained with this variety of mice. In four hybrids F_1, from the cross-breeding of a Japanese waltzing mouse with a common mouse of an "alien" stock, tumors developed, but grew more slowly than in the hybrids F_1 from similar cross-breeding of Japanese waltzing mice with the common stock first employed. The inoculation of the four common mice previously used for cross-breeding resulted negatively.

EXPERIMENT 6.

Aug. 25, 1908. Tumor No. 2114, which had been growing eighty-four days in a Japanese waltzing mouse and weighed 4.670, was inoculated into comparative series of second generation hybrids (F_2), Japanese waltzing mice, and first generation hybrids (F_1), — in all fifty-one mice. The mice were inoculated in the order of their enumeration.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2224.....	Hybrid F_2.	53 days.	57 days after inoc.	0
2225.....	" "	53 "	69 " " "	0
2226.....	" "	53 "	Alive 8 mos. " "	0
2227.....	" "	53 "	" 8 " " "	0
2228.....	" "	53 "	" 8 " " "	0
2229.....	Japanese waltzing.	28 days.	36 days after inoc.	.270
2230	" "	28 "	21 " " "	.100
2231.....	" "	28 "	36 " " "	.330
2232.....	" "	28 "	27 " " "	.550
2233.....	" "	28 "	36 " " "	1.030
2234.....	Hybrid F_1.	22 weeks.	36 days after inoc.	.795
2235.....	" "	21 "	36 " " "	.930
2236.....	" "	21 "	36 " " "	.310
2237	" "	21 "	36 " " "	.710
2238.....	" "	21 "	36 " " "	1.450
2239.....	Hybrid F_2.	37 days.	35 days after inoc.	0
2240.....	" "	37 "	6 " " "	0
2241.....	" "	37 "	37 " " "	0
2242.....	" "	50 "	Alive 8 mos. " "	0
2243	" "	50 "	" 8 " " "	0
2244.....	" "	50 "	" 8 " " "	0
2245.....	" "	50 "	" 8 " " "	0

EXPERIMENT 6. — *Continued.*

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2246.....	Japanese waltzing.	26 days.	36 days after inoc.	1.500
2247.....	" "	26 "	36 " " "	.930
2248.....	" "	26 "	36 " " "	1.120
2249.....	" "	26 "	36 " " "	.400
2250.....	" "	26 "	36 " " "	.880
2251.....	" "	26 "	36 " " "	.655
2252.....	" "	46 "	36 " " "	.620
2253	Hybrid F_1.	34 days.	36 days after inoc.	.510
2254.....	" "	34 "	36 " " "	2.015
2255.....	" "	34 "	36 " " "	.465
2256.....	" "	34 "	36 " " "	1.270
2257.....	" "	34 "	36 " " "	.570
2258.....	" "	34 "	36 " " "	1.205
2259.....	" "	22 weeks.	36 " " "	.665
2260.....	Hybrid F_2.	38 days.	4 mos. after inoc.	0
2261.....	" "	50 "	Alive 8 mos. " "	0
2262.....	" "	50 "	70 days " "	0
2263....	" "	50 "	57 " " "	0
2264.....	" "	50 "	Alive 8 mos. " "	0
2265	Japanese waltzing.	46 days.	36 days after inoc.	.185
2266.....	" "	46 "	37 " " "	.400
2267.....	" "	22 weeks.	36 " " "	.125
2268.....	" "	22 "	36 " " "	.070
2269.....	" "	22 "	36 " " "	.160
2270.....	Hybrid F_1.	22 weeks.	36 days after inoc.	.165
2020.....	" "	24 "	36 " " "	.785
2022.....	" "	24 "	36 " " "	.850
2023.....	" "	24 "	36 " " "	.075
2024.....	" "	24 "	36 " " "	.220

The mice in which tumors developed (with the exception of three) were killed thirty-six days after inoculation.

Combined weight of tumors in seventeen Japanese waltzing mice 9.335

Combined weight of tumors in seventeen hybrids F_1 ..... 12.990

One of the mice of generation F_2 died six days after the inoculation, but in none of the sixteen which survived did the tumor continue to grow. In two of these, however, there was an appreciable temporary growth of the tumor, for a rounded nodule about eight millimeters in its greatest diameter developed in the three weeks following the inoculation. The nodule was subsequently rapidly absorbed in both cases. Tumors developed in all the seventeen generation F_1 mice, and in the seventeen Japanese waltzing mice of control series. In this experiment the rate of tumor growth in the young Japanese waltzing mice approximated that in old hybrids of generation F_1, and exceeds that of four others which had been previously twice inoculated with an inoculable tumor of the common mouse, so that it seems probable that in still older F_1 hybrids the rate of tumor growth would fall below that of a corresponding series of young Japanese waltzing mice. By the appropriate selection of lots of mice with reference to age as well as to variety, age, therefore, may be made to counterbalance the influence of racial susceptibility.

EXPERIMENT 7.

Sept. 9, 1908. Three small tumors, Nos. 2167, 2169, and 2171, which had attained in Japanese waltzing mice a weight of .650, .720, and .550 gram respectively in forty-eight days, were inoculated into comparative series of second generation (F_2) hybrids, Japanese waltzing mice, and first generation (F_1) hybrids — in all forty-seven mice. Six of the latter variety had previously been twice inoculated with either the Jensen or the Ehrlich tumor without result, and the other ten were derived from cross-breeding with an alien stock.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2274.....	Hybrid F_2.	26 days.	Alive 3 mos. after inoc.	0
2275.....	" "	26 "	" 3 " " "	0
2276.....	" "	26 "	" 3 " " "	0
2277.....	" "	26 "	" 3 " " "	0
2278.....	" "	26 "	" 3 " " "	0
2279.....	Japanese waltzing.	5 weeks.	Alive 4 mos. after inoc.	0
2280.....	" "	5 "	50 days after inoc.	.225
2281.....	" "	5 "	50 " " "	0
2282.....	" "	5 "	50 " " "	.350
2283.....	" "	5 "	50 " " "	1.340
2284.....	Hybrid F_1 ("alien").	11 weeks.	50 days after inoc.	1.040
2285.....	" " "	11 "	30 " " "	Small nodule.
2286....	" " "	11 "	Alive 7 mos. after inoc.	0
2287.....	" " "	11 "	40 days " "	0
2288.....	" " "	11 "	Alive 7 mos. " "	0
2289.....	Hybrid F_2.	36 days.	Alive 7 mos. after inoc.	0
2290.....	" "	36 "	" 7 " " "	0
2291.....	" "	36 "	54 days " "	0
2292.....	" "	34 "	Alive 7 mos. " "	0
2293.....	" "	34 "	" 7 " " "	0
2294.....	Japanese waltzing.	5 weeks.	50 days after inoc.	.600
2295.....	" "	5 "	50 " " "	.720
2296.....	" "	5 "	50 " " "	.520
2297.....	" "	5 "	50 " " "	.535
2298.....	" "	5 "	50 " " "	.065

EXPERIMENT 7. — *Continued.*

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2299.....	Hybrid F_1 ("alien").	11 weeks.	50 days after inoc.	.200
2300.....	" " "	11 "	50 " " "	.200
2301.....	" " "	11 "	50 " " "	0
2302.....	" " "	11 "	50 " " "	0
2026.....	" " "	28 "	51 " " "	.620
2303.....	Hybrid F_2.	9 weeks.	Alive 7 mos. after inoc.	0
2304.....	" "	9 "	" 7 " " "	0
2305.....	" "	58 days.	" 7 " " "	0
2306.....	" "	37 "	" 7 " " "	0
2307.....	" "	37 "	" 7 " " "	0
2308.....	" "	37 "	" 7 " " "	0
2309.....	Japanese waltzing.	5 weeks.	50 days after inoc.	.100
2310.....	" "	5 "	50 " " "	.610
2311.....	" "	5 "	50 " " "	.750
2312.....	" "	5 "	50 " " "	.180
2313.....	" "	5 "	50 " " "	.070
2314.....	" "	5 "	50 " " "	.270
2027.....	Hybrid F_1.	28 weeks.	50 days after inoc.	2.180
2029.....	" "	28 "	49 " " "	.380
2035.....	" "	22 "	51 " " "	.670
2038.....	" "	22 "	51 " " "	.190
2039.....	" "	28 "	51 " " "	.490

Comparison of growth of the tumor in the different categories of mice will be illustrated by a subsequent chart.

None of the sixteen mice of generation F_2 developed tumors. In sixteen Japanese waltzing mice there were tumors in fourteen and negative results in two. Tumors developed in five hybrids of generation F_1 of the usual stock, but the inoculation of nine hybrids of generation F_1 of an alien stock resulted in tumors in three and in a small nodule of tumor tissue in another at the time of its death thirty days after inoculation. A distinct difference is here

apparent in the susceptibility of different lots of hybrids of generation F_1, which is to be accounted for by their derivation from two unrelated stocks of common mice.

Since the tumor also failed to develop in two of the Japanese waltzing mice it is possible that its virulence was somewhat diminished. Before this explanation can be accepted, however, it is necessary to consider other factors. It is not improbable that the failure of the tumor to grow in a small percentage of the young Japs was due to general malnutrition. In seven of the series of sixteen the body weight diminished instead of increased and the gain in the other nine was slight. It appears that, on account of a misunderstanding, sufficient nourishment had not been provided these mice, for when killed they presented no evidence of disease, and being so young they should have shown a considerable increase in weight during the period of the experiment. Tumors in some cases develop notwithstanding loss of weight, as may be seen not infrequently in individual instances, but such a condition of malnutrition is to be considered less favorable to the development of the tumor. The low percentage of tumors in the alien hybrids may be attributed to a greater degree of insusceptibility on the part of the common parent or a less degree of susceptibility on the part of the Japanese waltzing parent. In addition to this the series of mice was slightly older than the series of alien hybrids in Experiment V., and, on account of the death of several from this cage, they evidently had some infectious disease, the nature of which was not determined.

In order to determine if by any possibility the susceptibility to the inoculable tumor of the Japanese waltzing mouse reappeared in the next generation (F_3), the following two experiments were performed. Comparative series of Japanese waltzing mice, and hybrids of generations F_1, F_2 and F_3 were inoculated.

EXPERIMENT 8.

Nov. 13, 1908. Tumor No. 2346, which weighed .920 gram after growing forty-three days in a Japanese waltzing mouse was used to inoculate several series of mice, including those of the third generation (F_3), with the following results:

Number.	Variety of Mouse.	Age.	Result of Inoculation.
2389	Hybrid F_3.	17 days.	O
2390	" "	17 "	O
2391	" "	17 "	O
2392	Hybrid F_2.	31 days.	O
2393	" "	31 "	O
2394	" "	31 "	O
2395	Hybrid F_1.	6½ months.	+
2396	" "	6½ "	+
2397	" "	53 days.	O
2398	Japanese waltzing.	30 days.	+
2399	" "	30 "	+
2400	" "	30 "	+
2401	Japanese waltzing.	2½ months.	+
2402	" "	2½ "	+
2403	" "	2½ "	+

None of the mice of generation F_2 or F_3 developed tumors. All the Japanese waltzing mice developed tumors, as well as two of the three mice of generation F_1. This hybrid in which the tumor failed to grow had for one parent one of the common females which had been used in previous breeding, but for its other parent it had a Japanese waltzing male which had not been bred from previously. The mother sickened soon after the young were born and only this one survived from the entire litter.

EXPERIMENT 9.

Dec. 31, 1908. Thirteen third generation (F_3) hybrids and fourteen waltzing mice were inoculated with tumor No. 2398, which weighed .650 gram after growing forty-eight days in a Japanese waltzing mouse.

Number.	Variety of Mouse.	Age.	Result of Inoculation.
2430	Hybrid F_3.	65 days.	O
2431	" "	65 "	O
2432	" "	63 "	O
2433	" "	63 "	O
2434	" "	60 "	O
2435	" "	60 "	O
2436	" "	38 "	O
2437	" "	38 "	O
2438	" "	38 "	O
2439	" "	23 "	O
2440	" "	23 "	O
2441	" "	23 "	O
2442	" "	23 "	O
2443	Japanese waltzing.	60 days.	+
2444	" "	60 "	+
2445	" "	60 "	+
2446	" "	60 "	+
2447	" "	60 "	+
2448	" "	60 "	+
2449	" "	60 "	+
2450	" "	60 "	+
2451	" "	60 "	+
2452	" "	60 "	+
2453	" "	60 "	+
2454	" "	60 "	+
2455	" "	4½ months.	+
2456	" "	4½ "	+

In Experiment 9, fourteen Japanese waltzing mice and thirteen mice of generation F_3 were inoculated with result that the tumor grew in all of the former and in none of the latter series.

The following experiment was performed for the purpose of determining the influence of dosage * in the transplantation of the Japanese waltzing mouse tumor. The results, although too meager to allow of generalization concerning dosage, nevertheless furnish additional data concerning racial susceptibility.

EXPERIMENT 10.

Oct. 1, 1908. A portion of tumor No. 2266 was weighed, and then divided as nearly as possible into two equal halves. One of these halves served as the largest dose; the other half was again divided into two equal portions, and one of these was taken as the next dose. In this manner series of doses were obtained, each approximately of one-half the weight of the one preceding. In each series the doses were inoculated in the order of their size, the smallest dose first. The tumor tissue was deposited as near as possible in the same situation in each mouse, and the same technical procedure was followed in each case. The mice were of the same age and of approximate weight, but the two series were comprised of more than a single litter. The mice were killed and the tumors weighed forty-three days after the inoculation.

	Series A					Series B				
Number of mouse	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347
Dose in milligrams	2.8	5.6	11.2	22.5	45.	3.4	6.8	13.7	27.5	55.
October 7										
October 14										
October 20										
November 3										
November 10										
November 13. Weight of tumor in milligrams	490	30	220	720	630	130	70	280	920	440

* The influence of dosage and of "concomitant immunization," a closely related factor, are taken up very thoroughly in the Third Report of the Imperial Cancer Research Fund, which appeared while the present investigation was in progress.

It is apparent from the accompanying chart that there is in these two series some correspondence between the amount of tumor tissue inoculated and the size of the resulting tumors at any given time. That is, if the series are divided arbitrarily, it will be seen that tumors of greater weight have been produced by the larger doses (*i.e.*, those on the right hand side of each of the two series shown in chart). It will be seen also that the correspondence of the size of the tumors to the size of doses used is rather more marked at the time of the earlier observations. As the tumors continue to develop discrepancies appear, which are to be explained only by differences of soil, as provided by the tissues of the different individuals of the series. In both series the smallest dose resulted ultimately in a larger tumor than did a dose of twice its size. Certain differences which are apparent for a time disappear later on. Thus it seems probable that, if these tumors were allowed to develop for a long time, the effect of dosage would be counterbalanced by individual variation in the soil furnished the tumor. On the other hand, it is not improbable that, if larger numbers of animals were used in experiments of this sort, the combined results would be influenced by dosage. If consideration is given to the percentage increment in weight as well as to the absolute increase in tumor tissue after implantation it will be observed that there is little correlation between dosage and growth. Thus in the first series the smallest dose produced in forty-three days a tumor one hundred and seventy-five times its weight, while the largest dose produced a tumor only fourteen times its weight. It does not hold throughout the series, however, that the coefficient of growth is greater with the doses of small size. Thus the second smallest dose of the same series produced a tumor of only five and one-third times its weight.

The ten Japanese waltzing mice inoculated in this experiment all developed tumors, although some received very minute and others relatively large doses of tumor tissue. Both large and small doses are thus found to be effective.

The object of the following experiment was to determine

if the growth of the tumor in hybrids of the first generation modified it in any way so that it would grow in subsequent generations.

EXPERIMENT 11.

Dec. 31, 1908. Eight hybrids of generation F_2, and two control Japanese waltzing mice were inoculated with tumor No. 2259, which had grown somewhat over four months in a first generation hybrid, having recurred after an attempt at removal.

Number.	Variety of Mouse.	Age.	Result.
2420	Hybrid F_2.	67 days.	o
2421	" "	67 "	o
2422	" "	67 "	o
2423	" "	40 "	o
2424	" "	40 "	o
2425	" "	40 "	o
2426	" "	40 "	o
2427	" "	40 "	o
2428	Japanese waltzing.	4 months.	+
2429	" "	" "	+

The tumor by growing for a period of time in mice of the first generation (F_1) is not so modified as to enable it to grow in mice of the second generation (F_2). The results are similar to those in the experiments in which the tumors grown in Japanese waltzing mice were used. In another instance the inoculation of common mice with a tumor which had grown in a first generation hybrid was unsuccessful.

The results of all these experiments, together with those of all other inoculations of the Japanese waltzing mouse tumor, are combined in the following table:

RESULTS OF ALL INOCULATIONS OF THE JAPANESE WALTZING MOUSE TUMOR.

	Common Mice.		Japanese Waltzing Mice.		Hybrids, Generation F_1.		Alien Hybrids, Generation F_1.		Hybrids, Generation F_2.		Hybrids, Generation F_3.	
	+	−	+	−	+	−	+	−	+	−	+	−
Transplant I..	.....	...	3									
" II..	.....	.....	(2).....		One died within a short time. One grew tumor on reinoculation.							
" III..	.....		(1).....		Grew tumor on reinoculation.							
" IV..	2		1									
" V..	7		4									
" VI..	.....		4									
" VII..	.....		3									
" VIII..	.....		6									
" IX..	.....	.....	8									
" X..	6		3	Inoculated with tumor from Hybrid F.								
Experiment 1..	10		10		11							
" 2..	12		12		22							
" 3..	7		7		.....	.....	.....		6			
" 4..	.....		5		5		.....		5			
" 5..	4		13	1	6		4		7		Failure in old Jap.	
" 6..	.....		17		17		.....		17			
" 7..	.....		14	2	6		4	5	16			
" 8..	.....		6		2	1	.....		3		3	
" 9..	.....		14		.....	.....	.....		.....		13	
" 10..	.....		10									
" 11..	.....		2	Inoculated from Hybrid F_1.					8			
Total.....		48−	142+	3−	69+	1−	8+	5−		54−		16−

The results may be summarized as follows: A racial peculiarity, *i.e.*, susceptibility to an inoculable tumor, appears in the first generation (F_1) obtained by cross-breeding a susceptible with an insusceptible race of mice. This peculiarity occurs in hybrids of both common and Japanese

waltzing mothers, so that it is transmitted by the male as well as by the female parent. In the next generation (F_2) of mice, produced by the breeding inter se of the hybrids (F_1), all were like common mice insusceptible. The appearance of a peculiarity in the first generation resulting from the cross-breeding of two different varieties of animals and its total disappearance in subsequent generations I am unable to accord with Mendel's law or any other principle of heredity as yet discovered. After having found the development of the tumor to be more rapid in the hybrids obtained by cross-breeding an insusceptible with a susceptible mouse than in their susceptible parent, we are then confronted with the almost incredible results in that these hybrids, of which very nearly one hundred per cent are proven susceptible to the tumor, when bred together produce offspring none of which (0 per cent) are susceptible. A certain proportion of the mice of the second and third filial generations manifest the waltzing character, but are nevertheless insusceptible. Thus we have the hybrids of the first generation, which from their appearance alone cannot be distinguished from common mice, susceptible to the tumor of the Japanese waltzing mouse, and waltzing mice of the second and third generations, which are all insusceptible.

On account of the peculiarity of these results, it is necessary to consider as far as possible all the conditions which could in any way be of influence. The method of inoculation employed has been described already, and the same method was used both in the series of mice to be tested and in the control series. Whether, by the inoculation of much smaller or of much larger doses, the tumor could be made to grow in mice of the second or third filial generations has not been ascertained. The comparative series of mice were reared in essentially the same environment, since they were kept in the same room, and were fed with food prepared in common for all. Before the experiments were ended bacterial infections were noted in certain individuals, but these occurred both in the mice which proved susceptible as well as in those which proved refractory. Change of conditions

dependent upon the lapse of time necessary in the breeding of the successive generations does not account for the difference in their susceptibility, for certain of the generations to be compared were reared and tested simultaneously. The mice of generation F_3 and a portion of those of generation F_2 were reared from parents which had already been inoculated. Other observations with other inoculable tumors indicate that the inoculation of the parent does not render subsequent offspring immune. In generation F_2 the susceptibility of fifty-four mice was tested. Of these, thirty-three were of uninoculated parents and twenty-one were of parents either one or both of which had been inoculated. Even, if the latter are excluded in the consideration of the result, the number left is sufficient to base conclusions upon. Thus, after close analysis, the almost absolute difference in the susceptibility of the different generations to the tumor must be considered as the result of cross-breeding a susceptible with a non-susceptible variety of mice rather than of adventitious influences.

That identical results might not be obtained if other stocks or varieties of mice were used is indicated by the partial rather than absolute susceptibility of the "alien" hybrids inoculated in Experiment 7.

The fact, however, that mice which show the greatest possible susceptibility to an inoculable tumor produce young which are in every case insusceptible indicates the necessity of taking the racial factor into account in practically all investigations of the inoculable tumors. The failure to recognize the influence of racial differences leads to erroneous conclusions with the use of large as well as small numbers of animals. Unless the susceptibility of the stock of mice employed has been previously ascertained by repeated inoculations each experiment must be carefully controlled, otherwise the results obtained are unreliable. The uncertainty of the character of mice purchased in the open market must always be taken into account. Certain investigators have controlled their experiments by dividing a lot of mice into two series, one designed to control the other. It is

obviously unsafe to compare results in different lots obtained from the dealers. It would appear that, in order to adequately control certain experiments, it would be necessary to take comparative series from the same litter of animals.

Results such as those already outlined have a direct bearing on the question of the so-called "Fluctuations" or "Rhythms" in the growth of the transplantable tumors of mice. The subject has been taken up at a considerable length both by Calkins and by Bashford and his collaborators. In that which has been rather loosely termed the "virulence" of tumors, Calkins[12] distinguishes two distinct factors, the property of the tumor which he terms "infectivity," as indicated by the percentage of the positive results following its inoculation, and the time factor (rate of growth) in the development of tumors. He criticises Bashford's previous work in that he has taken the "infectivity" of tumors as an index of their energy of growth. He endeavors to show that there is no correlation between the two. One of the two charts used by Calkins to illustrate this point shows a fairly uniform correlation of the percentage of successful results with the estimated rapidity of growth, so that his contention is not proven by the data which he collected. In the construction of his chart for the comparison of infectivity with the growth energy of tumors Calkins takes as a measure of the latter the average time in days required by the tumors of a given generation to kill the animals in which they are growing. It is obvious that the opportunity for error in such a method of estimating the energy of tumor growth is so great that the reliability of any conclusions which may be derived therefrom is to be questioned. Death may be the result of conditions either wholly independent of, or secondary to the growth of the tumors. Racial differences in the lots of mice used in the consecutive transplants are apparently not considered, and beyond stating that he is unable to account for rhythmic variation in the activity of tumors by the inoculation on different occasions of more or less susceptible animals, — *i.e.*, animals of different ages, — no further comment is made concerning this factor. From the study of growth energy estimated by

the periods required for the tumors to kill, Calkins concludes that there is no evidence of a rhythmic development such as that which is found in early embryonic cleavage or in free living organisms such as the protozoa, but that rhythms of tumor growth, if they occur, are probably to be found in the individual mouse. Variation in the infectivity of a given tumor he thinks is possibly due to the stimulus of a foreign organism, which may be either ultra-microscopic or unrecognizable, rather than to an inherent property of the tumor cells. He states that the spirochetes which are often found in mice with tumors may account for differences in the percentage of the successful results, possibly from the reason that they in some way prepare the soil for the tumor cells.

Bashford,[13] and later Bashford, Murray, Haaland, and Bowen,[14, 15] on the other hand, have taken the ground that the percentage of successful results attending the inoculation of a tumor furnishes an index of the energy of growth. They explain that this method of measurement is a purely arbitrary one, and that it was selected on account of its convenience of application. The fact is recognized that if a tumor should be obtained, the transplantation of which was uniformly successful (one hundred per cent), any fluctuations in the energy of growth could not then be estimated by the method adopted. Bashford, Murray, and Bowen, in taking up "The experimental analysis of the growth of cancer," consider the following factors which influence the rate and amount of tumor growth: race and age of the mice employed, site of implantation, dosage and method of inoculation, and variations in the character of the tumor. Apart from the matter of race, due attention appears to have been paid to the influence of these various factors. It is stated that mice of the same race were used, and also that varieties prized by mouse fanciers were avoided as unsuitable. Just what is indicated by "race" is not apparent. If it is meant that the mice used were similar in appearance or that they were obtained from one or from several dealers of a given locality, but little is signified by the term "race." In the present investigation, invisible racial differences are found to be of such

importance with reference to variations in the growth of tumors that the exact origin of all mice used in the successive transplants should be determined. The necessity of this is not overcome by the employment of large numbers.

The graphic method employed by Bashford and his collaborators, by which the percentage of successful results is depicted by a branching curve of the nature of a genealogical tree, is of considerable value in presenting the results of successive transplants. By the construction of what is termed the "main stem" of the curve through the selection in each generation of the highest percentage of successes obtained, it is shown that the highest percentage of successful results may steadily arise until a maximum is reached, and that this is often followed by a sudden drop. It seems not improbable that selection plays a part in the attainment of the high percentage of successes, although it is shown that descending branches may subsequently also arise to a high point. The fact that, in a large number of the generations of tumor depicted, a rise in the "implantability" of certain tumors is offset by a lowering of this in others is not in accordance with the hypothesis expressed by these investigators that the fluctuations in the activity of the tumor are due to some inherent property of the tumor tissue. Thus in the graphic records both ascending and descending branches are found in a given generation of a tumor, and only rarely are all the branches directed upward. They, however, would possibly explain such differences in the activity of the tumors of a given generation either by differences in the period of their development before inoculation or by variation in the cells of the tumor from which they were derived. It seems probable that such differences are to be explained in part at least by other factors, such as varying degrees of susceptibility in the lots of mice used.

The conclusions reached by these authors are that secondary biological alterations of the character of the inoculable tumor may accompany the adaptation of its cells to growth in a strange individual. The accelerated rate of growth in a propagated tumor may be a result of long continued

selection of the most rapidly growing tumor tissue. Environment is also to be considered from the fact that tumors growing in the mice of one country are transplanted with difficulty to the mice of a foreign country. Evidence is found, however, of periodic changes in the activity of tumor epithelium, and "that the duration of the alternating phases of increased and diminishing energy of growth is an inherent character of the parenchyma cells of the same order of permanence as the histological arrangement in which they grow."

Variation in the rate of growth of transplanted tumors must be accepted as an established fact. A marked difference in the rate of growth of the first as compared with that of subsequent generations of a transplanted tumor is found almost invariably. From the fact that the tumor, employed in the greater part of this investigation, usually grows in one hundred per cent of the Japanese waltzing mice inoculated, it is apparent that the number of successful results cannot be taken as an index of the rate of tumor growth, as Bashford and his collaborators have done. Although it is probably true that those inoculations which yield the highest percentage of successful results usually also yield the most rapid rate of tumor growth, it is questioned whether the ability of the tumor cells to adapt themselves to a new soil is to be taken as an accurate measure of their rate of growth. The method employed by Calkins of estimating the energy of growth by the time necessary for the tumor to kill the animal is also inaccurate.

On account of the necrosis of a variable proportion of the tumor tissue implanted, the amount of living tissue with which the tumor commences its development, which has been termed by Bashford, Murray, Haaland, and Bowen "the effective initial dose," cannot be accurately estimated.

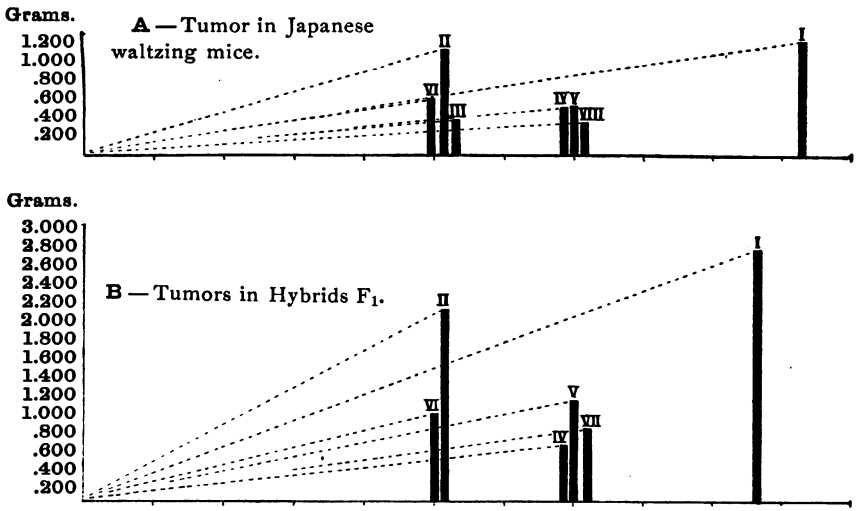
The above-named authors show that in certain strains of tumors the size attained is proportionate to the size of the doses employed. With other strains the reverse is found to be true, and small doses produce more tumors and larger tumors. The character of a tumor may vary, and, whereas

large doses give at one time a higher percentage of rapidly growing tumors, at another they result in a spontaneous absorption of the tumors which develop. Thus it would appear that the injection of equal doses in the successive transplants of a tumor does not assure the equality of the actual amounts of tissue with which the tumor commences. With many tumors, therefore, no more than approximate uniformity of dosage is possible. The results obtained in Experiment 9 indicate that dosage in the case of the relatively slow-growing, Japanese waltzing mouse tumor is of less importance than the character of the animal into which the tumor is inoculated. Approximately equal amounts of living tumor were, however, inoculated in all comparative series of mice. Since it is impossible to weigh the tumors at intervals during their growth the construction of a curve showing the percentage increments in weight is practically out of the question. They may be measured and charted by the graphic method frequently employed, but this method lacks accuracy, especially in tumors of small size, and in my own experience I have found that such estimates of the relative sizes of a series of tumors correspond only roughly with their actual weights. The various generations of a tumor may be weighed after certain definite periods of growth, and compared with the average weight attained by tumors of other generations. By this method the average daily increase in weight may be estimated for the tumor's development in a single animal or lot of animals with more accuracy than by any method which has been used.

In the accompanying chart (Text Fig. 1) the average weights attained by the tumors in seven consecutive transplants are illustrated by the heights of the black columns, and the time taken in development is shown by the position of the columns on the base line. It is at once apparent that the tumor in one transplant may attain in thirty-six days about three times the weight attained in fifty days in another transplant. It is to be noted that the average weight of the tumors is much higher in the hybrids than in the Japanese waltzing mice in each transplant, and also that variation

apparent in the former corresponds more or less closely with variations in the latter. The average daily increase in the weight of the tumors may be estimated by dividing the average weight by the number of days for which they were developing.

FIG. 1.



Average weights attained by the tumors of seven consecutive transplants indicated by heights of black columns. Time taken in development shown by position on base line, each division representing a period of seven days.

With regard to variations in the average increase in weight per day, there is in these generations of the tumor more or less correlation between hybrids and Japanese waltzing mice. On investigating the ages of the various lots of mice, it was found that with few exceptions the growth of the tumor was retarded when it was inoculated into older mice and accelerated when inoculated into younger mice. The influence of age is quite apparent when lots of different ages of the same variety are inoculated simultaneously (see Text Fig. 2). Haaland's observations that inoculable tumors develop with greater uniformity in the young than in the old are confirmed by these experiments. It cannot be assumed, however, that

differences in the rate of growth in the successive generations of the tumor are dependent solely upon differences in the age of the animals inoculated. Other factors, such as the condition of the tumor at the time of its inoculation, undoubtedly influence the results.

In this series of experiments, those generations of the tumor which showed exceptionally rapid growth resulted from the inoculation of tumors which had grown for a relatively long period of time (seventy-six days) in a single animal. Other generations produced by the inoculation of tumors which were taken after a short period of growth (twenty-one or twenty-four days) showed a much slower rate of growth. The influence of personal equation in the selection of the tumors to be inoculated has been already mentioned. At one time a rapidly growing tumor and at another time a tumor which had been growing slowly for a long time may suit the convenience of the investigator. There is ample evidence to show that the character of a tumor as regards its power of growth becomes modified by continuous transplantation. The differences in the growth of the first as compared with subsequent transplants have been repeatedly noted. This modification is probably due to changes in the tumor's environment rather than to an inherent tendency of its cells.

In addition to those conditions already discussed, such as the age of the inoculated animals, the method of inoculation, the condition of the tumor at the time of inoculation, etc., there are other varying conditions to which but little attention has been given. The periodical activities of certain tissues of the host in which the tumor grows may influence the growth of the latter. Haaland has noted the retarding influence of pregnancy on the growth of mouse tumors. The activities of the mammary gland and the ovary are periodical. Such conditions, however, affect individuals rather than lots of mice. The shedding of the fur is periodical in mice, and it may occur simultaneously in large numbers of mice. The manufacture of so great an amount of fur within so short a period of time is brought about by a sudden

growth of the epithelium of the hair sheaths, which probably surpasses in rapidity the growth of many of the malignant tumors. It is not improbable that the growth of the implanted tumors may be affected by such marked variation in the activity of tissues of the inoculated animal. Seasonal changes, with variations of temperature producing changes in the vascularity of the skin, and conditions influencing the general nutrition of the animals may be of great importance. In Experiment 7 the low rate of growth may be due largely to the fact that the animals were underfed. Haaland has suggested that changes in food, and possibly climate, resulting from the transportation of mice from Germany to Sweden may modify the susceptibility of mice, and it does not seem improbable that such changes might influence the rate of growth of tumors. The growth of tumors appears to be modified by associated bacterial infection, and, as Calkins suggests, it may possibly be influenced by an infection with an unrecognized organism.

Thus, although there is no difficulty in establishing the occurrence of variation in the rate of tumor growth, the dependence of this either wholly or in part upon an inherent property of the tumor cells does not appear to be proven. The environmental conditions influencing the growth of transplanted tumors are numerous, and it is obviously impossible to obtain identical conditions in successive experiments. Among those recognized factors which influence the growth of tumors, differences of race or, better, of blood relationship are of great importance. It has been demonstrated in the foregoing experiments that such inherited differences in susceptibility over and above all other varying conditions account for fluctuations in the growth of the tumor as great as those which are ordinarily found in successive generations. If the tumor which is growing in Japanese waltzing mice is inoculated into the first generation of hybrids, obtained by cross-breeding with insusceptible common mice, its growth is thereby greatly accelerated. If transferred back to waltzing mice its growth is retarded. The breeding together of highly susceptible parents does not ensure susceptible

offspring, for notwithstanding the great susceptiblity of the first generation of hybrids their offspring proved to be all insusceptible. Thus the rate of tumor growth may be varied at will. It is possible that, by appropriate selection of lots of mice with reference to age as well as variety, age could in some cases be made to counterbalance the influence of racial susceptibility.

This might also prove true of dosage, if it were possible to determine the most effective dose for each transplant. Slight variations such as occur in these latter factors in the foregoing experiments are insufficient to overcome the influence of race. Not only are racial differences important with regard to susceptibility, but it is also possible to go further, and account for much of the variation by individual or family differences. Thus it has been observed that the growth of the tumor is much more rapid in hybrids obtained from one pair than in those from another pair, although in both pairs the respective parents were taken from a single family of Japanese waltzing mice. Even with closely related mice, therefore, there is considerable variation with regard to the character of the soil furnished for the growth of the tumor.

Heretofore attention has been directed chiefly to variations in the growth of different generations of the tumor. The fact that the different generations are produced by the inoculation of different individual tumors, which have been more or less modified by peculiarities in the environment furnished by the tissues of the animal in which they have grown, should be taken into consideration. Not only is the character of the soil upon which tumors are implanted of importance, but also differences in the properties of the individual tumors inoculated. In the experiments here presented, comparative series of different varieties of mice were inoculated simultaneously with a single tumor. The constant differences found in the rate of tumor growth in respective series of mice cannot in this case be ascribed to any difference in the character of the tumor tissue inoculated.

The error involved in the comparison of the development of the tumor in different generations is thereby avoided.

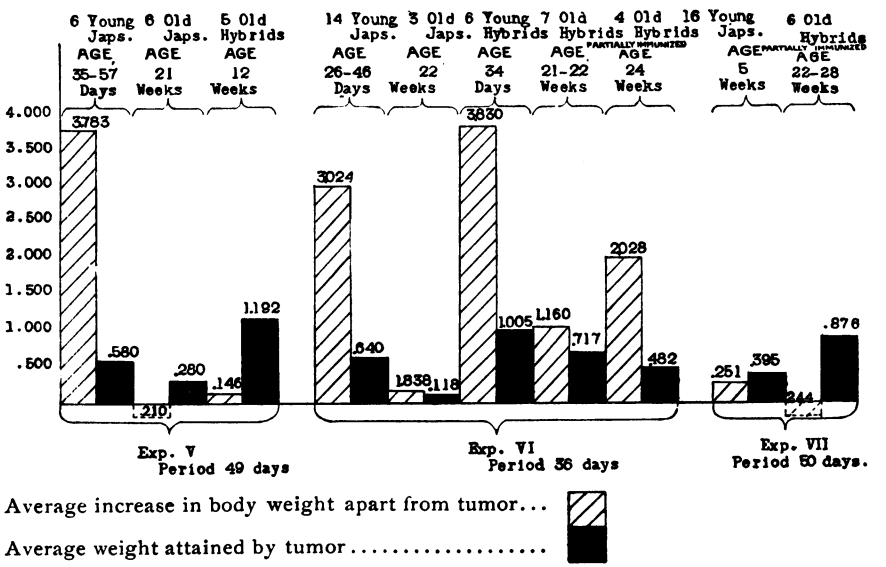
The unsuccessful inoculation of common mice with the Japanese waltzing mouse tumor affords no protection against subsequent inoculation with the Ehrlich tumor. Eight common mice, which in Experiment 1 had been inoculated with the Japanese tumor, were inoculated ten weeks later with the Ehrlich tumor, with the result that large rapidly-growing tumors developed in all. Likewise the inoculation or even the transient growth of the Jensen or of the Ehrlich tumor does not protect first generation hybrids against the Japanese waltzing mouse tumor. The latter tumor grew to large size in each of seven hybrids which had been twice inoculated (Experiments E and F) with either the Jensen or the Ehrlich tumor, although these tumors had grown appreciably after the first inoculation.

In an attempt to account for the rapid growth of tumors in the hybrids of the first generation, it was thought that perhaps the greater size of these mice or the rapidity of their growth might be of influence. The ultimate size attained by a mass of tumor tissue is obviously dependent to a large extent upon the size of the animal in which it grows. In each experiment the first generation hybrids weighed much more than the Japanese waltzing mice. If, however, mice of the same series or even of the same litter were compared, no correlation was found between the weight of the animal and the weight of the tumor. The larger tumors were as often found in the smaller as in the larger animals of a given litter. Thus in its early development at least the size of the tumor is in no wise dependent upon the relative size of the animal. Whether, in the latter period of their development, the tumors would grow more rapidly in large than in small animals was not ascertained.

It was thought possible that the more rapid growth of the first generation hybrids, as compared with Japanese waltzing mice of similar age, might account for the more rapid development of the tumors in the former. By weighing the mice

at the beginning and at the end of the experiment the increase in the weight of the body apart from the tumor was determined. The body growth is invariably greater in hybrids than in Japanese waltzing mice of similar age. If, however, practically full grown hybrids are compared with young rapidly growing Japanese waltzing mice the tumors nevertheless are on the average larger in the hybrids. In a given variety of mice the tumor develops more rapidly in those series in which the body growth is greatest, — that is in young animals, — but the rate of body growth cannot be taken as a basis upon which to explain the greater growth of the tumor in hybrids. Since it develops more rapidly in hybrids than in Japanese waltzing mice irrespective of increase in body weight, it appears that there is a qualitative rather than a quantitative difference in the soil furnished the tumor by these two varieties of mice. In the following chart the average weight attained by the tumors together with the average increase in body weight is compared in different series of mice in three experiments.

FIG. 2.



Average increase in body weight apart from tumor...

Average weight attained by tumor.....

It is not at all certain whether the rapidity of tumor growth in young animals is dependent upon the rate of body growth or upon other conditions associated with youth. It is not uncommon to get rapidly growing tumors in old individuals, although usually it is slower than in the young. Such individual variations may be acquired, therefore, during extra-uterine life. Tumors may continue to develop notwithstanding decrease in body weight. In one experiment five mice were inoculated at the same time with the same tumor, and tumors developed in all. One grew progressively more and more emaciated, and it was finally found that the animal had been gradually starving on account of a deformity of the teeth, which interfered with eating. The tumor of this mouse was much smaller than those of the other four, but it had continued to grow notwithstanding a loss of weight on the part of the rest of the body.

The histological character of the Japanese waltzing mouse tumor is not modified in any way by transplantation into first generation hybrids. The tumors in general show less necrosis, and the tissues surrounding the tumor are considerably more vascular in the hybrids than in Japanese waltzing mice, so that it seems not improbable that a more active vascular reaction on the part of the hybrid may account for the more rapid growth of the tumor in them.

A study of the histological changes concerned in the reaction of the non-susceptible mice of the second generation (F_2) has been made, but this subject will be taken up in a separate paper.

The results of this investigation have led to the following conclusions:

The successful transplantation of tumor tissue in mice is dependent upon three main factors, — the method of inoculation, the character of the individual tumor employed, and the nature of the soil upon which the tumor is implanted. All three are subject to great variation, as are also the numerous subsidiary conditions upon which they are based.

With regard to the nature of the soil or environment furnished the implanted tumor cells by the tissues of the

host, racial differences are especially important. Although it is possible that susceptibility to inoculable tumors may be affected at times by external conditions, the occurrence of distinct differences in the susceptibility of lots of mice bred and kept under identical conditions shows that susceptibility may be of the nature of a racial peculiarity. As such it is transmitted from generation to generation of imbred varieties or races of mice. The insusceptibility of a race of mice to a given tumor may be either relative or absolute, and relative racial insusceptibility may be augmented by the inbreeding of the more insusceptible individuals.

A tumor which originated in a Japanese waltzing mouse has been transplanted for many generations in mice of the same variety, but invariably fails to grow when inoculated into common mice. Attempts to inoculate Japanese waltzing mice with the Jensen tumor have been unsuccessful, and the Ehrlich "Stamme II." tumor in this variety develops more slowly and in fewer cases than in common mice.

The susceptibility of hybrids, resulting from the cross-breeding of common albinos with Japanese waltzing mice — each either relatively or absolutely insusceptible to the tumors of the other — has been ascertained.

The hybrids of the first generation (F_1) were found to be slightly more susceptible than Japanese waltzing mice, but less susceptible than common mice to the tumor of common mice. The Japanese waltzing mouse tumor, on the other hand, grows more readily in these hybrids than in Japanese waltzing mice. The offspring of such hybrids are found, however, to be absolutely insusceptible to this tumor, as are mice of the third filial generation. Susceptibility to an inoculable tumor is neither, therefore, inherited in accordance with Mendel's law, nor are the results obtained from cross-breeding explained by any other known principle of inheritance. The number and the uniform character of the results are regarded as adequate basis for this conclusion. Whether the facts presented concerning the elimination of a racial peculiarity as the result of cross-breeding will be found to

correspond with any generally applicable law remains to be answered by future observation.

There is no correlation between any of the visible racial characters and susceptibility to the inoculable tumors employed. Thus the hybrids of the first generation outwardly resemble common mice, but are nevertheless susceptible to the Japanese waltzing mouse tumor, whereas the waltzing mice which appear in subsequent generations are insusceptible to the same tumor.

It is possible that results of another sort may attend the cross-breeding of other varieties or breeds of mice, but from the data at hand it is apparent that the factor of race or, better, of blood relationship is of considerable importance in the investigation of transplantable tumors. In each of the foregoing experiments the racial element has outweighed all minor variations in associated conditions. It seems probable that even slight racial differences may account for a large proportion of the fluctuations frequently noted in the so-called "infectivity" of tumors and in the rate of tumor growth.

The methods heretofore proposed for the measurement of the rate of tumor growth are inaccurate, and for its estimation the exact weight or volume of the tumor as well as the period of growth must necessarily be ascertained.

Many of the variable conditions which possibly influence tumor growth have apparently not been investigated. The activities of certain of the tissues of the inoculated animals are periodical. An instance of this is to be seen in the activity of the hair follicles at the time of the shedding of the fur.

It is found that the size of an animal does not influence the rate of growth of a tumor, except possibly in its late development.

Tumors grow most rapidly in young animals in which the body growth is greatest. This does not explain the more rapid growth of the Japanese waltzing mouse tumor in hybrids, however, and the difference between the soil furnished by such hybrids and that furnished by Japanese

waltzing mice appears to be a qualitative rather than a quantitative one.

The histological character of the tumor is not modified in any respect by its implantation into hybrids, neither is it thereby adapted for growth in the mice of subsequent generations or in common mice.

REFERENCES.

1. Haaland, M. Beobachtungen über natürliche Geschwultzresistenz bei Mausen. Berlin klin. Wochenschr., 1907, xliv, 713.

2. Jensen, C. O. Uebertragbare Rattensarkome. Zeitschr. f. Krebsforschung, 1908, vii, 45.

3. Flexner and Jobling. Metaplasia and metastasis of a rat tumor. Proc Soc. Exp. Biol. and Med., 1907, v, 52.

4. Gay, F. P. A transmissible cancer of the rat considered from the standpoint of immunity. J. of Med. Research, 1909, xx, 175.

5. Loeb, L. On transplantation of tumors. J. of Med. Research, 1901, vi, 28.

6. Loeb, L. Ueber Entstehung eines Sarkoms nach Transplantation eines Adenocarcinoms einer japanischen Maus. Zeitschr. f. Krebsforschung, 1908, vii, 80.

7. Fourth Report of the Cancer Commission of Harvard University. J. of Med. Research, 1907, xvii, 137.

8. Cuénot, L., and Mercier, L. Études sur le cancer des souris. Ya-t-il un rapport entre les différentes mutations connues chez les souris et la réceptivité à la greffe? Compt. rend. Acad. d. Sc., 1908, cxlvii, 1003.

9. Haacke Ueber Wesen, Ursachen, und Vererbung von Albinismus, etc. Biol. Cblt. 1895, xv, 45.

10. von Guaita. Versuche mit Kreuzungen von verschiedenen Rassen der Hausmaus. Ber. Natur. f. Ges , 1898, x, 317.

11. Cuénot, L. L'hérédité de la pigmentation chez les souris. Arch. zoöl. exper , 1907, vi, 1.

12. Calkins, G. N. The so-called rhythms of growth energy in mouse cancer. J. Exper. Med., 1908, x, 283.

13. Bashford, E. F. Interpretation of the differences and variations in transplanted tumors of one organ. Second Report of Imperial Cancer Research Fund, 1905, 52.

14. Bashford, E. F., Murray, J. A., Haaland, M., and Bowen, W. H. General results of propagation of malignant new growths. Third Report of Imperial Cancer Research Fund, 1908, 262.

15. Bashford, E. F., Murray, J. A., and Bowen, W. H. The experimental analysis of the growth of cancer. Third Report of Imperial Cancer Research Fund, 1908, 284.