FACTORS INFLUENCING THE INCIDENCE OF MAMMARY GLAND TUMORS IN AN INBRED STRAIN OF MICE

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During the last decade, there has been a growing disposition of clinicians, pathologists, and the medical profession generally, to view the problem of cancer from a biological standpoint. Correlated with this tendency there has been a marked increase in the attention experimental biologists have given the subject.

In attacking a problem of this nature experimentally, it is essential that the animals used shall measure up to certain requirements; viz.,

1. They should be a race the individuals of which are as much alike as possible.
2. A large percentage of individuals should develop cancer spontaneously. (Spontaneously is used here to differentiate between those cancers arising without mechanical stimuli and those arising from transplantation of tissue or other irritants).
3. The type or types of tumor arising should be as few and uniform as possible.

Fortunately, a race of mice was available to this laboratory which embodied all of these characteristics. Previous to the time when they were used in the study of cancer they had been intensively inbred (50–75 generations) in a study of the effects of brother to sister matings. They were then genetically homozygous, since this condition is practically reached in 18 to 20 generations when matings of this type are employed.

Previous to the advent of the idea of using them in the study of cançer, they had been used simply as an experiment in race purification; that is, that as many generations as possible should be produced. Because of this method of breeding few of the animals were kept until they reached the age of cancer.

It was noticed, however, that occasionally some of the older females of the breeding stocks developed adenocarcinomata of the mammary gland.

With the increased interest in the biological aspects of cancer, it was thought to be desirable to attempt the production of side lines in the stock which would develop cancer in a large percentage of individuals. This breeding process has been going on for a number of years with the result that the stock has become highly cancerous. Very opportunely it happens that these tumors are all of the adenocarcinomatous type, thus simplifying the problem.

The study from a genetic standpoint has been complicated by two major difficulties:

1. The impossibility of raising all of the young to an age when cancer might be expected to appear.
2. None of the normal breeding males have ever developed tumors.

CHART No. I

1528 × 1525
6013 × 6014
*9936 × 9769
12664 × 12662
†50968 × 50966
53602 × 53601*
54847 × 54799

♀ 56234	♀ 56235	♀ 56237	♀ 56238	♀ 56239
2	3	4	5	6

* Not sibling but closely related individual carrying tumor.
† Underline denotes tumor.

From Chart I it is seen that the mating 54847 × 54799 produced five females which were bred. All of them had tumors.

Examining the following seven generations it is seen (Table I) that the number of tumorous females varies from 73 to 100 per cent cancerous, if those females which died before reaching the lowerrange of standard deviation (Graph 2, 250 days) are not included. From the totals of this Table it appears that 82 per cent of the breeding females in this family are cancerous.

TABLE I

Table Showing Percentage of Cancer in Eight Generations of the Descendants of Mating 54847 × 54799

Line I

Generation	No. ♀s bred	Cancerous	Non-Cancerous	Died before Cancer Age	% Cancerous	% Cancerous Deducting those Dying before Cancer Age
1	5	5	0	0	100	100
2	33	25	4	4	75	86
3	61	37	7	17	60	84
4	69	44	12	13	63	78
5	72	53	8	11	73	86
6	65	33	12	20	50	73
7	22	13	1	8	59	92
8	4	1	0	3	25	100
Total	331	211	44	76	63	82

Computing the per cent of breeding females which became cancerous among the descendants of the five cancerous sisters of the F_1 generation of this line (Table II), it is found that with the exception of ♀ 56235 which had but two female descendants both of which were cancerous, these females produced lines of females 80 to 86 per cent of which were cancerous. This shows that there is very little, if any, variation in the substrains of this family.

TABLE II

Table Showing Similarity in Percentage of Females Cancerous in Substrains of Line I

F_1 Females Line I	Per Cent Cancerous	Per Cent Cancerous Deducting those Dying before Cancer Age	No. of Individuals
56234	65	81	88
56235	100	100	2
56237	61	81	93
56238	67	86	58
56239	58	80	85

DEGREE OF CANCER INCIDENCE IN THE DILUTE BROWN STOCK

Since the stock has been giving rise to more than an occasional tumor, 1363 females have been used as breeders. Of these animals 703, or 51 per cent, died of cancer. The remaining 660 died without developing tumors.

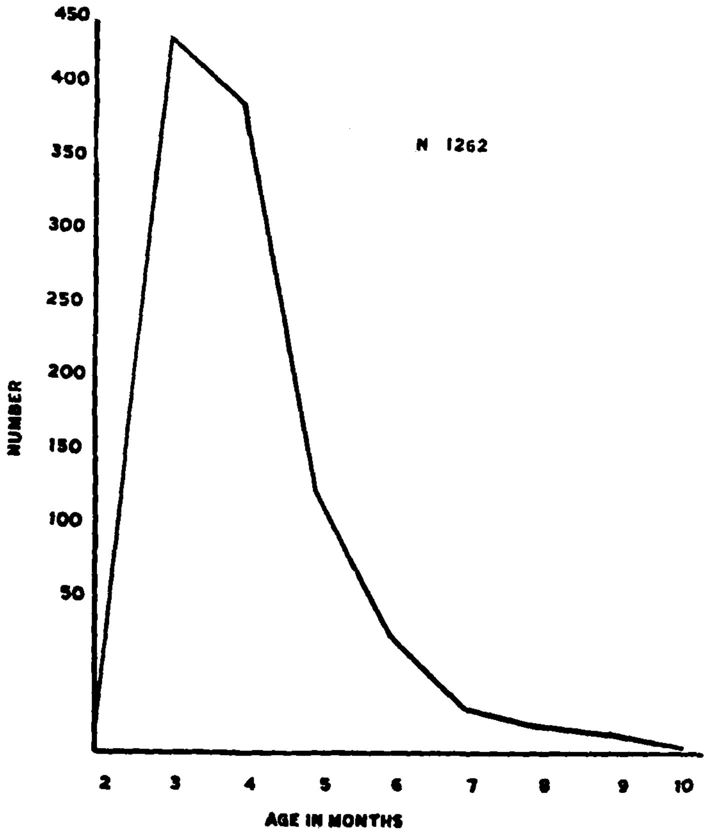
Examining Column 2 of Table III, it is seen that 50 of these 660 died before the age at which the very earliest tumors appeared. It is safe to say, however, that 51 per cent of this number, or 26, were potentially cancerous.

Column 6 of this same Table shows that .15 per cent of all the animals living to the age period (90–119) developed cancer during this month. Column 7, Row 4, shows that 53.54 per cent of all mice living to this age died of tumor; therefore, 53.54 per cent of 41, or 21.95 of those non-cancerous females which died in this period may be said to have been potentially cancerous. Following the same reasoning and comparing Columns 2, 8, and 9, it is found that while 703 were actually cancerous, 431 of the 660 (Column 1) were potentially of a like nature. Examining Column 5 of this same table it is found that the percentage of tumor occurrence among females which died during any age group starts at 4.65 per cent during the 90–119 day period, increases to 91.80 per cent during the 390–419 day period and then decreases to 0 during the 600–629 day period. From this it may be said (following the argument used above) that the true cancer rate for any period is the actual rate plus the potential rate. Thus (examining Columns 4, 9, and 10) it is seen that the true cancer rate in the various age groups starts at 51 per cent in the first month, increases to 98 per cent in the 390–419 day group and then gradually decreases. The fluctuation in the last two groups is probably due to the small numbers involved.

From this it is seen that the true tumor rate for the stock regardless of time of death is 83 per cent, but that if the mouse lives to be nine months old, this rises to 87 per cent, increasing to 98 per cent during the 15th month and then falling off slightly. From this it is seen that if a mouse lives to be 15 months of age without developing tumor, the probability that she will become tumorous decreases as she grows older.

Several investigators have reported, previously to this, that in high tumor stocks the incidence of tumor among non-breeding females is markedly less. This stock is no exception to this rule. Of 207 females raised under the same conditions as the breeding females except that after one month of age they were never in the same pen with a male, 24, or 11.5 per cent, developed tumors. In addition to this the mean age of tumor appearance was markedly greater; 15.8 months as compared with 10.5 months for the breeding females.

It is evident, from this, that the physiological and anatomical changes incidental to the bearing and nursing of young supply some of the irritating factors necessary for the appearance of tumors.

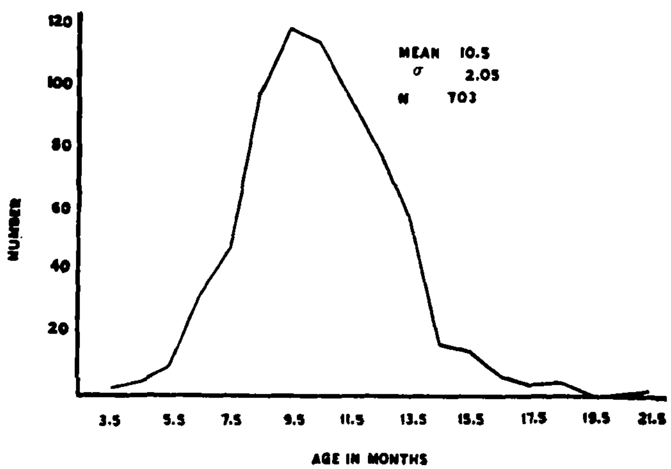


GRAPH NO. 1. CURVE SHOWING FREQUENCIES AGE AT FIRST LITTER.

There is then in the females of this stock a cancer potentiality of 11 per cent provided they live to twelve months of age. This potentiality rises to 96 per cent given the proper metabolic stimulants.

The purpose of the present paper is to attempt to determine what these stimulants or activators toward the cancerous condition may be.

Obviously the first difference between breeding and non-breeding females is the conception and birth of the first litter. Examining Graph No. 1 it is seen that in 1262 breeding females of this stock, the earliest age at which any of them give birth to their first litters is during the eighth week; that the great majority of them parture for the first time between the eighth and twentieth weeks; but that some may be fifty weeks or a year old before giving birth to their first litters. There is, then, a marked difference in this respect. Turning to Graph No. 2, or Column 3 of Table III, it is seen that the earliest tumors appear during the fourth month and the latest during the 21st month. Here there is another wide variable.



GRAPH NO. 2. CURVE SHOWING FREQUENCIES AGE AT APPEARANCE OF TUMORS.

Distributing these two dates (age at first litter and age at tumor appearance) in a correlation table, the coefficient of correlation is found to be + .1528 ± .0212 which is of little significance. The age at which the first litter is born is not, therefore, one of the prime factors.

If the breeding activity of the females of this race is examined it is found that they have from one to nine litters. Beginning

TABLE III

1	2	3	4	5	6	7	8	9	10
Age in Days	Number Dying Each Age Period		Those Dying Each Age Period	Per Cent Tumorous Individuals Dying in Each Age Period	Per Cent Tumorous Individuals Dying of Tumor Each Age Period	Per Cent Those Living which Finally Died of Tumor	Number Potentially Tumorous	Actually and Potentially Cancerous	True Tumor Rate in Per Cent
	Non-Tumorous	Tumorous							
1– 19	1		1			51.58	.51	.51	51
20– 59	8		8			51.58	4.13	4.13	51
60– 89	41		41			51.58	21.27	21.27	51
90–119	41	2	43	4.65	.15	53.54	21.95	23.95	55
120–149	73	4	77	5.48	.31	55.20	40.30	44.30	57
150–179	97	9	106	8.49	.75	58.42	56.67	65.67	61
180–209	82	32	114	28.07	2.94	63.29	51.90	83.90	73
210–239	75	47	122	38.52	4.83	67.42	50.56	97.56	79
240–269	76	98	174	56.32	12.02	71.56	54.39	152.39	87
270–299	59	119	178	66.85	17.58	75.48	44.53	163.53	91
300–329	49	114	163	69.94	22.85	78.56	38.49	152.49	93
330–359	19	99	118	83.90	29.46	82.74	15.72	114.72	97
360–389	22	78	100	78.00	35.78	82.11	18.06	96.06	96
390–419	5	56	61	91.80	47.46	85.59	4.28	60.28	98
420–449	4	16	20	80.00	28.07	78.94	3.16	19.16	95
450–479	2	14	16	87.50	37.84	78.38	1.57	15.57	97
480–509	2	6	8	75.00	28.57	71.43	1.43	7.43	92
510–539	1	3	4	75.00	23.08	69.23	.69	3.69	92
540–569	1	4	5	80.00	44.44	66.66	.66	4.66	93
570–599									
600–629	2		2	.00		50.00	1.00	1.00	50
630–659		2	2	100.00	100.00			2.00	100
	660	703	1363				431	1134	83

at an average of 111 days they continue for the first three litters with from 4.9 to 5.8 (Table IV Section 5) young in a litter. Of these young they nurse in the vicinity of 60 per cent to weaning age (one month). (Table IV Section 2). Between litters (Table IV, Section 1) they average around 55 days. This means that they are never inactive (on the average) sexually, since they are either bearing or nursing young all of the time.*

Examining the second section of Table IV it is seen that as the number of litters increases, the percentage of young nursed to weaning decreases (after the third litter).

From Table IV, Section 1, tumorous females, it is seen that

* Gestation period 21 days; young are allowed to nurse mother 30 days.

TABLE IV

Section		Age at 1st Litter	Days between Litters							
			1 and 2	2 and 3	3 and 4	4 and 5	5 and 6	6 and 7	7 and 8	8 and 9
1	Tumorous females	110	56	56	55	49	44	40	38	55
	Non-tumorous females	113	55	61	57	67	61	56		
	Per Cent of Young Weaned									
	Litter	1	2	3	4	5	6	7	8	9
2	Tumorous females	60	60	60	41	32	20	15	9	0
	Non-tumorous females	64	59	55	42	35	0	0		
	Per Cent of Young Born Dead									
3	Tumorous females	9	8	11	20	25	32	23	40	73
	Non-tumorous females	7	5	7	12	15	25	30		
	Per Cent Died Before Weaning									
4	Tumorous females	31	31	29	38	42	47	61	50	27
	Non-tumorous females	31	34	37	45	48	75	70		
	Average Number Young Per Litter									
5	Tumorous females	4.9	5.6	5.8	5.6	5.1	5.1	5.0	4.7	3.7
	Non-tumorous females	4.9	6.1	5.5	5.5	5.5	3.0	5.0		

generally speaking the resting period between litters decreases as the number of litters increases; that (Sections 3 and 4) there is a general tendency in both the born dead and died before weaning percentages to increase as the number of litters increases.

That part of this decrease in the rest period between litters is due to the method of breeding seems certain. When the breeding females become pregnant they are separated from the males and allowed to have their young in a small pen by themselves. Normally, they are left in the maternity pens until the young are weaned, a month after birth. If, however, their young are born dead or all die before the thirty day period, they are transferred to the breeding pen. That the resting period in such a circumstance is shorter is shown by the fact that in 333 litters (of females that later became cancerous) in which none of the young lived to weaning age, the period of rest before the next litter was 41 days; while in 1264 litters of these same females which nursed some of their young to weaning, the mothers rested 61 days before having their next litters. Data computed from the records of non-cancerous animals show approximately the same rest periods; 40 days after a litter which was not weaned, and 72 days after a litter which was weaned. These facts coupled with the finding that a larger percentage of the young are born dead or die before weaning in the later litters accounts for some of the divergence in rest periods as the mice grow older. (Table IV, Section 1).

It appears from this that the average female of this stock gives birth to her first litter at about 110 days of age; that this litter is slightly smaller than the succeeding four or five litters; that the second and third litters spaced at 50 day intervals are as healthy as the first but that with the shortening of the resting space in succeeding litters the ability of the female to bring forth and nurse to weaning healthy young diminishes until at the ninth litter all young born die before reaching weaning age.

Turning to Graph 2 which shows the distribution of the age at tumor of 703 breeding females, it is found that the mean age of cancer appearance among 703 breeding females is 10.5 months and that the standard deviation from this mean is 2.05 months.

From this it may be said that the great majority of the non-cancerous females which did not live to an age of 250 days were cancerous; but that breeding females living to this age or over must be reckoned as being non-cancerous. Of the 660 females dying before developing cancer (Column 2, Table III) 123 lived to be 250 days or over.

Comparing these two groups (cancerous females and non-cancerous females) it is seen (Table IV) that the ages at first litter are very nearly the same; that the females of each group resemble the other in their ability to raise their young to weaning age, and that the time interval between this and the second litter is approximately equal. Examining the table further, it is seen that the breeding behavior of the two groups for the first three litters is similar, but at the end of this time the resting period among the cancerous females begins to shorten, becoming less with each succeeding litter but the last; while among females of the non-tumorous group this resting period remains approximately the same.

Concurrent with this shortening of the resting period in the tumorous females there is a decreasing ability to raise young to weaning age. This same decrease appears among the females of the non-tumorous females but seems to progress more rapidly.

It appears then from these data that the stimulus giving rise to the tumors is not ordinarily given until the end of the third litter and that it manifests itself in a tendency to make the female become pregnant more quickly after parturition. It also appears that this upset is accompanied by physiological changes which make the nursing of young through a thirty-day period difficult. This latter change being present in non-tumorous mice, it is evident that it is not one of the prime causes of tumor.

Since this increase in mortality among the young mice increases as the mothers grow older it will be of interest to ascertain whether this increase is due to prenatal indisposition of the mother or whether it is primarily seated in the mammary glands and their failure to secrete.

If the former (prenatal indisposition) is responsible, the bulk of the increase may be looked for in Table IV, Section 3. From this table it is seen that the percentage of young dead at birth and before weaning remains constant for the first three litters, but that following the third litter both the percentages of young born dead and those dying before weaning increase rapidly. This tends to show that the upset is a general physiological one rather than that it is seated in any organ such as the ovary, uterus, or mammary gland.

Advanced cases of this upset are seen in those instances in which mice in which the cancerous growth is well developed* become pregnant and give birth to litters of young. These litters are often born dead and in those instances in which the young live, their span of life is rarely more than three or four days.

In those cases where the tumor is just beginning to manifest itself at the time of parturition many more young are brought forth alive and are not infrequently nursed by the cancerous mother to weaning age. Such behavior, however, is hard on the mother because she invariably becomes rapidly thin, coat rough, and shows all the signs of rapid aging. The tumor in such instances grows very rapidly.

It appears, then, that these tumors appearing as they do at a mean age of 10.5 months, are the result of an inherited predisposition to mammary gland carcinoma; that the secretions of the ovary and the changes accompanying oestrus is one stimulator; that the age at which the first litter is born is not significant; that the change bringing on the tumor does not ordinarily manifest itself until after the third or fourth litter; that following this period the future tumorous breed more rapidly but bring forth large percentages of their young dead and raise fewer young to weaning age.

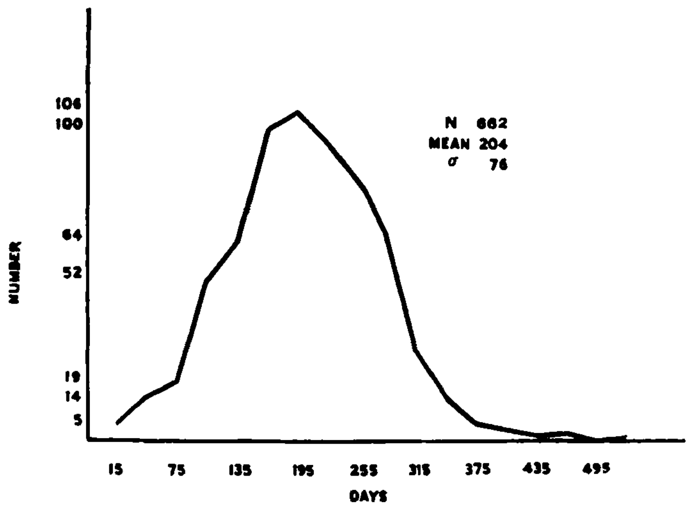
Examining Graph 3, which shows the distribution in 662 breeding females of the number of days after the first litter when they developed cancer, it is seen that the mean time elapsed between these two dates is 204 days ± a standard deviation of 76 days. From this and Graph 2 it is seen that the average tumorous females develop tumor between 254 and 376 days,

* 31 per cent of the cancerous females developed neoplasms before the birth of their last litters.

which is 128–280 days after the first litter, and that the average female has from three to seven litters during this period.

The average cancerous female then will have three characteristics:

1. She will develop cancer between the 254–376 day period.
2. She will develop cancer between 128–280 days after the birth of her first litter.
3. She will have from three to seven litters before tumor appears.



GRAPH NO. 3. CURVE SHOWING FREQUENCIES AGE AT APPEARANCE OF TUMORS.

Segregating these animals it is found that (Table V) 302 cancerous females fall within these three categories. Dividing these animals into groups according to the number of litters they had, it is found that the average age at first litter is greatest for those females which had three litters and that the age at first litter decreases as the number of litters born increases until it reaches a mean age at first litter of ninety days for those females having seven litters.

Correlated with this decrease, it is seen that the rest periods between the first and second, and second and third litters grow less as the number of ultimate litters increase.

From Table VII*a* it is seen that there is a correlation between the age at first litter and the fertility of the females, that while the ovary may function at the 70th day or earlier, the females which breed at an early age have not reached that degree of fer-

TABLE V

Number of Litters	Average Age at 1st Litter	Average Days between					
		1 and 2	2 and 3	3 and 4	4 and 5	5 and 6	6 and 7
3	112	63	70				
4	101	58	58	61			
5	95	47	52	55	53		
6	92	41	44	55	41	43	
7	90	38	39	41	41	46	45

$N = 302$

TABLE VI

Per Cent of Young Born Dead

Number of Litters	1	2	3	4	5	6	7
3	7	11	16				
4	9	7	14	20			
5	7	5	9	21	32		
6	8	8	8	19	20	26	
7	11	0	2	0	0	10	24

TABLE VII

Per Cent Died Before Weaning

Number of Litters	1	2	3	4	5	6	7
3	36	28	39				
4	36	33	36	40			
5	33	34	29	32	38		
6	46	35	17	42	52	54	
7	21	47	45	36	41	70	48

TABLE VIIa

No. of Litters	Average Age 1st Litters	Average Young per Litter						
		1	2	3	4	5	6	7
3	112	5.14	5.55	5.63				
4	101	5.47	6.00	5.93	5.27			
5	95	4.76	5.88	5.67	5.78	5.37		
6	92	4.54	5.82	5.42	5.65	4.97	4.91	
7	90	4.00	4.57	5.28	7.00	5.14	6.71	4.71

tility which characterizes those having their first litter at a later date. From this Table it appears that the highest degree of fertility at first litter is reached in those mice which have their first litters at approximately 100 days of age. It is also seen that the fertility increases with the subsequent second and third litters of those females having three and four litters, and in the subsequent second, third and fourth litters of those mice having more than four litters. It seems from this that the more vigorous females (as exemplified by their ability to have young at an early age and follow the first litter with from four to six more litters) attain their highest fertility more slowly than those animals which breed for the first time at an older age. With the exception of those females having three litters, the degree of fertility begins to decrease after the third in four litter females and after the fourth litter in females having more than four litters. This same tendency to have fewer young for the first and last litters is also shown by both the tumorous and non-tumorous females of Table IV. It seems from this that the ovaries of these mice give off ripe follicles at different rates during the beginning, the height and the ending of their breeding period; the last indication of sluggishness being a forerunner of the cancerous condition. That this sluggishness is a prerequisite of the tumorous condition seems unlikely inasmuch as the average litter sizes of the non-tumorous females in the several sections of Table IV parallel those of the tumorous females very closely.

That retarding of the activity of the ovary is followed or accompanied by general physiological sluggishness is indicated by the fact that along with it or following it there is an increasing inability to bring forth their young alive or to nurse a large percentage of the living young to the age of one month. Here then there are as forerunners of the cancer condition, a falling off of 16.5 per cent in 95 days in ovarian activity and a decrease of 20 to 25 per cent in physiological activity during the same period.

From Table IV it appears that the only striking difference between the tumorous and non-tumorous animals was the longer resting period between litters in the case of the latter. From Table V, however, it is seen that females having three, four or five litters average as long or longer resting periods as do the non-tumorous females. (Table IV, Section I.)

From a general survey of these data it may be said that the only indication toward the cancerous condition common to all these mice regardless of the degree of their sexual activity is a general slowing up of physiological processes previous to the development of the cancer cells.

IRRITATION OF NURSING

It has been shown by a number of investigators, that irritants of various kinds are agents in bringing on the cancerous state. One of the primary differences between breeding and virgin females is the fact that the breeding animals nurse their young. Among the females of the dilute brown stock, breeding mice may be divided into three categories: (1) those that nurse all of their young to weaning; (2) those that nurse a portion of the young of each litter to weaning; (3) those that are unable to nurse any of their young through a period of one month.

In the first of these classifications is found a condition in which the mammary glands function normally (as evidenced by the ability of the young to obtain sufficient food). In such a condition unless the litter is very large, the mammae show no ill effects of nursing, such as lesions, great length of nipple or inflammation.

In the second division, when for some reason the mother is unable to produce milk in sufficient quantity or of the proper quality to maintain all of the young, the signs of irritation are often present. These conditions are undoubtedly brought about by the efforts of the young to obtain food. Mechanical irritation of nursing is here at its maximum.

In the third division where the females are unable to raise any of their young, a condition of stasis of the ducts of the glands frequently exists. The young of such females, usually die at an early age, before becoming strong enough to irritate the nipples of the mother to any great degree.

It might be suspected from this that those females in class 1 showing a normal nursing condition are less subject to irritation than the females of the remaining two classes, and that the cancerous condition, if it is induced by irritation of nursing would appear at a later age in this class.

From Table X it is seen that in actuality there is very little difference in the age at which the females of these three classes produce their tumors; and that the ages at tumor all fall well within the standard of deviation from the mean age at tumor (Graph II); that the first and the third are very close to the mode and that the second is almost exactly upon the mean of this curve.

TABLE VIII

Per Cent Raised to Weaning

Number of Litters	1	2	3	4	5	6	7
3	57	60	44				
4	54	59	50	39			
5	59	61	61	46	29		
6	46	56	73	38	27	19	
7	67	53	51	63	58	19	27

TABLE IX

Per Cent Not Raised to Weaning

Number of Litters	1	2	3	4	5	6	7
3	43	39	55				
4	45	40	50	60			
5	40	39	38	53	70		
6	54	43	25	61	72	80	
7	32	47	47	36	41	80	72

TABLE X

	Average		
	Age at 1st Litter	Days between 1st Litter and Tumor	Age at Tumor
Nursed all young to Weaning	112	191	303
Nursed some young to Weaning	103	217	323
Nursed no young to Weaning	144	154	299

It appears from these data that the individuals making up these groups do not differ from each other to any marked degree, that these irritating factors all have the same effects or that they may have different effects working through the same time period with the same end result.

DISCUSSION

This study emphasizes the great diversity of conditions which may give rise to tumors. Here we started with a stock which might be said to be ideal in many ways. The individuals are as much alike as it is possible to make them, they live under identical environmental conditions and yet from birth they develop individuality in their behavior.

It seems that at the very outset the health of the mother introduces a variable, not only in her ability to nurse her young to strong healthy adults, but by her very ability to give birth to a larger or smaller number of young in her litters.

These two variables undoubtedly manifest themselves in the early or late maturity of the young as shown by the wide range over which the first litter is born. The age at first litter, while it shows no correlation with the age at which tumor appears, introduces another variable (degree of fertility) which apparently influences the whole breeding history of the individual.

Following this or concurrent with it, several other variables show their presence; (1) the normality or abnormality of the mammary gland; (2) the number of young among which the milk must be distributed (this is undoubtedly strongly linked with the amount of irritation or stasis present in the gland); (3) the period of rest between litters; (4) the great variety of sequences which are possible in the breeding life.

Also, it is evident from the data at hand that while all of these mice are potentially the same at birth as regards their ability to develop cancer, different individuals in the stock react differently to the same natural and environmental conditions. This is some times marked in sisters of the same litter, which occasionally have very similar histories until the time of tumor incidence in one; yet the other will go on producing and nursing young for several months before the cancerous condition arises.

It appears from these data that the circumstances necessary to initiate, i.e., to bring on the cancerous condition, must be studied in individuals and much more minutely than heretofore.