OVARIAN SECRETION AND TUMOR INCIDENCE

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Since Johannsen (1903) (2) first expounded his theory of "pure lines" in genetic problems, investigators in all branches of this type of research have come to realize the importance of having stocks of plants or of animals, the genetic constitutions of the individual members of which are as homogeneous as it is possible to make them.

In plants the procedure followed in the establishing of such a homogeneous race is relatively simple, the ovum of the plant being fertilized by pollen from the same individual. In higher animals the process becomes more difficult since self-fertilization is not possible. The process followed by animal geneticists in the production of pure lines is known as inbreeding. The more common forms of this technic are: (1) the mating of brothers and sisters, (2) the back-crossing of the young animals to their fathers and mothers.

By the continuous use of these methods a line can be made practically homogeneous in from fifteen to twenty generations.

The race of mice which has been used in the present experiment is the result of such a homologizing process. Starting in 1909 with a single pair, this line has been developed by Dr. Little to the present time, by brother to sister matings for the most part, and occasionally a back-cross mating. In some of the later generations close cousins have been mated.[1]

Contrary to prevailing opinion in regard to inbreeding, such a process does not have a deteriorating effect upon the descendants of these animals, the colony today being as virile and vigorous as any of the less closely bred stocks in the laboratory.

[1] This stock because of its genetic uniformity is especially adapted to experiments involving the transplantation of tissues, etc., because the individual members are so nearly alike.

One of the most outstanding features of this inbreeding has been the appearance of a line within the stock which develops, spontaneously, adenocarcinoma of the mammary gland in 80 per cent of the normal breeding females which live to be of cancer age.

The inheritance of the tendency toward the development of these neoplasms in this strain of mice has been established elsewhere (Murray 1927) (5). It is sufficient for our present purpose

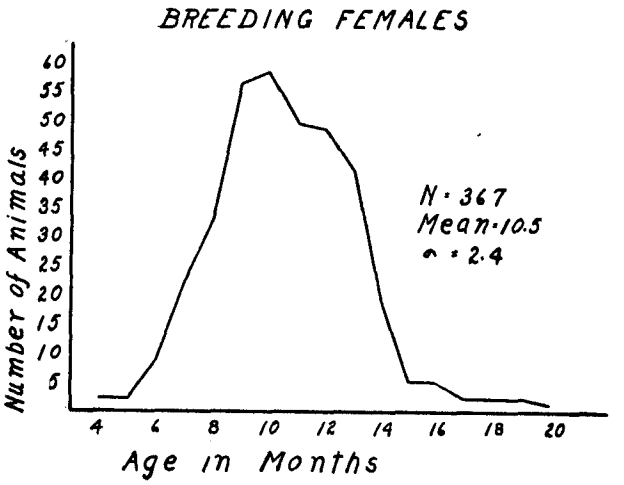

FIG. 1. THIS CURVE SHOWS THE RELATIVE FREQUENCIES WITH WHICH THE NEOPLASMS OCCUR DURING SUCCESSIVE THIRTY-DAY PERIODS.

to show (Fig. 1) that these growths appear in the region of the mammae of the females between the ages of four and twenty months, the mode being at ten months, the mean at ten and a half months, with a standard deviation from this mean of 2.4 months.

That there is a positive correlation between the time of appearance of these growths and the normal sex life of the animals has already been demonstrated (Murray 1927).

Inasmuch as the normal (breeding) males of this stock never develop these tumors, the question presents itself: Is the development of these growths correlated in any way with the secretions of the ovaries?

In 1916, Lathrop and Loeb (3), and later Loeb (1919) (4) published reports in which they quoted data which they interpreted as signifying that there is a positive correlation between the number of oestrus periods which a mouse has gone through and the time of life at which it develops tumor. Lathrop and Loeb presented data to show that females of a high tumor race, gonadectomized at a series of ages up to eight months, showed a progressive gain in tumor incidence as the age at castration increased. Loeb later supplemented his findings by increasing the number of mice involved.

Later, in 1927, Cori (1) reported that females of a high tumor stock, when castrated between 15 and 20 days of age, failed to develop tumors. He also substantiated Loeb's finding, that females spayed between two and six months of age develop tumors in a degree markedly less than in the control animals. Both Cori and Loeb attempted to prove conclusively that secretions of the ovary were involved in the appearance of these tumors by transplanting ovaries to castrated males of the high tumor lines. The failure of any of these feminized animals to grow the tumors was probably due to the fact that the stocks used were not very closely inbred.

Late in 1927 (6), the writer published a preliminary report upon work of a similar nature to show the mode of behavior and frequency of appearance of mammary carcinoma in the highly inbred strain of dilute brown mice developed by Little. The present paper is the presentation of these, plus additional data collected since the publication of the above mentioned report.

Having established the age at time of appearance and percentage of occurrence of neoplasms in the normal breeding females, an attempt was made to collect data by which the following questions might be answered.

1. What is the effect of non-breeding upon tumor incidence in this strain?

Two hundred seven females were separated at one month of age and allowed to grow old under the same laboratory conditions as the breeding females, with the exception that they were never, after weaning, in the same pen with males. Of these two

hundred and seven, 24 or 11.5 per cent developed tumor of the mammary glands (Table 1).

TABLE I

	Number of Animals	Number of Tumors	Percentage Tumorous	Earliest Tumor	Mean Age Appearance.	Latest Tumor
Normal Females.....	479	367	80	3	10.5	21
Non-Breeding Females.	207	24	11.5	12	15.8	17
Spayed Females.....	210	36	17.1	10	17.5	20
Castrated Males.....	241	0				
Transplant Males.....	210	15	7	8	14.4	20

In addition to the marked decrease in percentage of tumorous animals when compared with the breeding females (80 per cent tumorous), it was found that these animals were noticeably older when the tumors appeared, the mean age of cancer appearance being 15.8 months as compared with 10.5 months for the normal breeding animals.

It appears from this that virginity (*a*) reduces the percentage of appearance of cancer, and (*b*) postpones the date of cancer appearance for approximately five months.

2. What is the effect of complete gonadectomy upon tumor incidence and age of tumor appearance?

Two hundred and ten young females of this same stock were castrated at 4 to 6 weeks of age and allowed to grow old under the same conditions as were the virgin females. Of these two hundred and ten animals, 36 or 17.1 per cent developed tumors of the mammary gland. Like the virgin females the time of cancer appearance was markedly later than in the case of breeding females—in this case 17.5 months—or seven months later than in the control stock.

From these data, it appears that complete gonadectomy lowers the cancer incidence in a marked degree and also probably delays the age of tumor appearance later than either the breeding animals or the virgin females.

3. Since the males of this line of mice have never produced mammary tumors, the question arises: Does the testicular tissue secrete hormones which act as inhibitors upon the appearance of tumors?

Two hundred and forty-one males were therefore castrated at ages varying from four to six weeks and allowed to grow old. None of these developed tumors although they lived to be two years of age before being disposed of.

It must be concluded from this that something other than the absence of the testicular secretions is necessary before males in this line will develop neoplasms of the mammary gland.

4. Will castrated males to which a complete ovary has been transplanted produce mammary tumors?

Two hundred and ten males of this stock were castrated at ages which varied from four to six weeks and a whole ovary of a sister was transplanted subcutaneously in the abdominal region in the vicinity of the incision used for the extraction of the testes. *Of these two hundred and ten animals, 15 or 7.1 per cent have developed mammary gland tumors.*

The mean age at time of appearance of these tumors among the transplant males was 14.4 months. This is 1.4 months younger than the mean for the virgin females. From these figures it may be inferred that the successfully transplanted ovary in a castrated male behaves in a manner which is similar, if not equivalent, to the normal ovary in a virgin female.

That the remaining transplant males used in this experiment were also genetically potentially cancerous, inasmuch as they have the same heritage for the development of tumor, is shown by Table 3.

In Table 2 is arranged a record of the female relatives of the males which grew tumors. (In the table the numbers record in each case the age in days of the mouse when the cancer was first noticed.) From this table it will be seen that in all but two cases, the mothers of these animals were tumorous; while in the two cases reported as non-tumorous, the mothers died at an early age. The percentage of tumor is not so high among the grand and the great grandmothers, but it is apparent that with the exception of T ♂ 29789 and T ♂ 30862 the transplanted males developed tumor later in life than any of their female ancestors did.

In those cases where the transplant males had castrated sisters

which became cancerous, it is seen that the females developed their tumors at an age which is uniformly older than the ages at which their brothers developed tumors. If the transplant males are compared in this same way with their virgin sisters which developed tumor, it will be seen that in one case, T ♂ 30449, two of his virgin sisters developed tumors at the same age as he did himself. That there may be great variation in this finding is shown by the fact that the brother of T ♂ 30449, namely T ♂ 30454, developed his tumor fully three months later than his brother or either of his sisters.

TABLE 2

Table 2 shows a record of the appearance of mammary cancer in the female relatives of the tumorous males. The number in each case represents the age at which the animal developed the neoplasm.

Transplant Males which Had Tumor	Age when Castrated	Age at Tumor	Age when Mother Had Tumor	Age when Grandmother Had Tumor	Age when Great Grandmother Had Tumor	Age when Breeding Sister Had Tumor	Age when Castrated Sister Had Tumor	Age when Virgin Sisters Had Tumor
29675....	38	411	318	364				
29789....	30	244	274	365	330	256		
29974....	33	541	301				615	
						328		417
30449....	31	416	387	302	364	409	477	416
						328	615	417
30454....	31	507	387	302	364	409	477	416
				N.T.				
30670....	37	576	296	7 mo.	415			
			N.T.			379		
30689....	37	373	7 mo.	361	334	391		
30816....	35	470	324	253				
30862....	32	290	330			256		
				N.T.				
30925....	27	485	333	7 mo.	364			
				N.T.				
30927....	27	510	333	7 mo.	364			
						226		
30939....	26	475	277	371		390		
						226		
30940....	26	526	277	371		390		
30994....	51	500	370	259				
			N.T.					
31889....	32	382	7 mo.	354				

Comparison of Table 2 with Table 3 illustrates that the feminine ancestry of both the transplant males which developed

tumors and an equal number of animals from the same experiment which did not, had a high percentage of cancerous ancestors. In those instances where a mother or a grandmother is not recorded as tumorous, it will be seen that they died with very few exceptions at an age which was below the lower range of the standard deviation as shown for the normal breeding females (Fig. 1).

TABLE 3

Table 3 shows the incidence of cancer in the female relatives of 15 non-cancerous transplant males. The numbers indicate the age of appearance of cancer in each case.

Transplant Males with No Tumor	Age at Castration	Age at Last Examination	Age at Which Mother Had Tumor	Age at Which Grandmother Had Tumor	Age when Great Grandmother Had Tumor	Age when Breeding Sister Had Tumor	Age when Castrated Sister Had Tumor	Age when Virgin Sisters Had Tumor
29725....	34	646		374				
29779....	30	642	349	415				
29853....	34	603	276	364			579	
			N.T.					
29886....	30	601	9 mo.	212	240			439
						211		
29914....	35	600	261	371		308		
			N.T.					
30071....	35	590	8 mo.	246	240			
			N.T.					
30116....	31	587	12 mo.	246	240			
							519	
30163....	28	584	308	301	330		502	
						347		
30202....	34	583	255	415		278		
						296		
						552		
30415....	31	567	324	343	154	501		
								446
30432....	36	376	288	302	364			426
30482....	33	562	303	323	364		585	
30576....	58	580	256					
			N.T.					
30713....	32	547	11 mo.	301	330			
30771....	37	577	386	296	364			

From these data and from the findings of the previous investigations with the normal breeding females, it is apparent that one of the underlying causes of the occurrence of these neoplasms in the females of this strain of mice is very definitely linked with the ovary and the activity of its secretions. That the testes

play no prohibitive part in the absence of the tumors in the normal and castrated males seems well established.

Inasmuch as Cori found no tumors among the castrated females gonadectomized at from 15 to 20 days of age, and insofar as Loeb has found a diminishing prohibitive effect in the change of the age of cancer incidence and the frequency of cancer occurrence as the age at spaying increased, it appears that the short time which the castrated females in this experiment retained their ovaries over the time of those which Cori operated was sufficient to have stimulated the mammary tissues in sufficient amount to instigate the formation of tumors in a small percentage of animals. That the time necessary for this stimulus to take effect is greater than in the normal breeding animals seems to depend upon the absence of the other changes accompanying normal gestation, parturition, and lactation.[2]

It appears that in this highly inbred strain of mice, the presence of the ovary and its secretions are of primary importance in the raising of the physiological condition of these mice to the threshold of mammary carcinomas. That other factors also play a part in the speed and frequency with which this threshold is reached seems also to be well established.

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[2] That none of these tumors among the castrated females is attributable to secretion produced by regenerated ovarian tissue seems certain. All of the animals were autopsied at death and any suspicious tissues were examined histologically. In no instance was ovarian tissue found.