Further Investigations on the Origin of Tumors in Mice.*

VI. Internal secretion as a factor in the origin of tumors.

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On several previous occasions we have reported on experiments which had some bearing on the significance of internal secretion in tumor growth.

We found that an adenoma of the mammary gland of a rat after transplantation into the animal in which it originated began to grow at an extraordinary rate at the time when the animal was pregnant.[1] The transplant grew under the same conditions as the original adenoma and the normal mammary gland. The glandular structures, as well as the stroma, proliferated mitotically. After the conclusion of pregnancy the proliferation ceased, and even retrogressive changes occurred in a way similar to those in the normal gland in the period following parturition. The same hormone which during pregnancy causes the proliferation of the normal gland likewise called forth the growth of the adenoma, in the original tumor as well as in the transplant.

Yet there was some difference between the adenoma and the normal gland. The latter produced milk after the cessation of pregnancy; the adenoma did not. Evidently some changes had taken place in the structure of the adenoma which prevented this effect.

Later we showed that under the combined influence of the hormone of the corpus luteum and of mechanical stimuli, tumorlike new formations can be produced which have the structure of maternal placenta and which we designated as deciduomata.[2] They are of a temporary character.

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These experiments suggested to us the possibility that the hormone action in pregnancy might also cause the increased growth of the mammary carcinoma in mice, and we gave subsequent attention to this question. Especially did we request Miss A. E. C. Lathrop, who had bred the mice which we used in our experiment and had aided us throughout our experiments on heredity in cancer, to observe the growth of spontaneous cancers in mice that had become pregnant.[3] In no case could she observe an increased tumor growth under this condition. On the contrary the tumor growth often seemed to be retarded and in some cases to become again more rapid after labor; more recently a very striking retardation of tumor growth as a result of repeated pregnancies was reported by Maud Slye.[4]

This effect of pregnancy on the growth of spontaneous tumors in mice is in accordance with the influence which pregnancy exerts on transplanted tumors. The number of takes and the growth energy of the latter are markedly diminished (Haaland and others).

There are then exerted by pregnancy two opposing influences on tumors of the mammary gland. First the hormone favoring growth preponderates in the case of the normal gland and of the adenoma. In the case of carcinoma of the mouse, spontaneous or transplanted, the unfavorable influences of pregnancy alone are noticeable. These may be of an athreptic or toxic character. If any effect of the growth hormone is present in the case of the carcinomata of mice, it is very weak and therefore covered by the unfavorable influences. But in all probability the hormone is without effect in the case of carcinomata in which a growth stimulus residing within the cells is potent. With such a localized intrinsic growth stimulus active, the hormone is ineffective or only very weak in its effects.

We may then conclude that, while in adenomata or in temporary experimental tumors hormones directing growth are efficacious, they have lost this function in the case of carcinomata.

In agreement with these conclusions are some additional experimental results which have been obtained in the case of transplantable (homoio) carcinomata.

M. Goldzieher and E. Rosenthal[5] found that castration in mice does not influence the growth of homoio carcinomata.

According to Rohdenburg, Bullock and Johnson[6] removal of various glands either previous to or following the inoculation of a homoio tumor has only at best a slightly retarding influence on the growth of the tumors. The removal of glands previous to inoculation may increase the number of spontaneous recoveries.

These authors report, however, that subcutaneous injection of certain extracts of various tissues, especially of thymus gland, increases immunity and may cure cancer. Rohdenburg reports, furthermore, that in conjunction with Gwyer he has observed even the cure of certain human cancers as a result of the treatment of the patient with thymus extract, subcutaneously administered.

If these results should be substantiated by later investigators, this would indicate a certain effect of glands with internal secretion on tumor growth; it would, however, be an effect limiting growth. At present these results as to the effect of a constituent of the thymus are so isolated that we must defer judgment as to their significance. On the whole, we may then conclude that while in adenomata hormones regulating growth may at least in certain cases be efficacious, in the case of malignant tumors they are so no longer.

In the treatment of numerous cases of inoperable cancer of the breast, surgeons (Beatson, Alexis Thomson, H. Scott, F. Cahen)[7] extirpated the ovaries. It seems that in the large majority of cases no definite effect was obtained through this procedure. It is, however, possible that in some cases the progress of the disease was retarded. Whether a definite cure has ever been accomplished in this way is doubtful. Notwithstanding this lack of a marked effect of castration, Cahen expresses the belief that the ovary secretes substances which are necessary for the growth of carcinoma.

While the experimental evidence thus points to the conclusion that the internal secretion of the ovary has no noticeable influence on the growth of mammary cancer, after it has once been established, there remains the possibility that ovarian hormones may influence those tissue changes which lead to the production of mammary cancer. Removal of the ovary should, then, under certain conditions be able to prevent the occurrence of cancer of the breast, or at least diminish its occurrence in a noticeable way.

In case such an influence of internal secretion should exist it must be a specific one. There is no reason to assume that removal of an organ with internal secretion should influence the development of cancer in organs the growth of which is not affected by their internal secretion under normal conditions. And, indeed, the statistics of Sticker[8] have shown that in male cattle and horses castrated in early life, various kinds of cancers appeared just as frequently as among non-castrated animals.

We have approached this question experimentally.[9] We used for this purpose strains of mice in which we had previously followed the normal cancer rate and the cancer age through a number of generations, and in which this cancer rate and cancer age had been found approximately constant; we selected particularly the strains in which the normal incidence of cancer was higher than fifty per cent and which belonged to the first-age class.

We found that castration carried out during the first four or five months of life seemed to prevent cancer. We had previously observed that prevention of breeding likewise has some influence on the cancer incidence in mice, but to a much less extent than castration.[10] It remained for us (1) to confirm these results through further experiments, (2) to define in a more definite way the time limit at which castration is still effective, (3) to determine whether a graded relation exists between the age at which castration is carried out and the cancer rate or whether an " all or nothing " rule prevails and (4) to determine whether castration affects the

tumor rate of different strains of mice equally or whether its effect is limited to certain kinds of mice.

These questions we wish to answer in the following experiments.

In addition we continued our observations on the effect of breeding and non-breeding on the cancer rate, and furthermore we carried out the reverse experiments: we transplanted ovaries into castrated male mice in order to determine whether cancer of the mammary gland can be made to appear in such animals.

I. Tumor incidence and tumor age in castrated mice. — We shall first classify our observations according to the age at which castration had been carried out, and subsequently compare the results in the different strains. We shall include the data published in our former paper, because in several groups mice were still alive at the time of our earlier publication and the records were therefore not quite complete at that time.[11]

Without tumors.	*With tumors.*
(1) English, 3–6 months old when spayed.	
3:6–8 m., 2:8–10 m., 2:16–18 m., 5:18–20 m.	O
1:22–24 m., 1:24–26 m.	
14 (5 I, 2 II, 7 III)	O
(2) ($8\frac{1}{2}$+328) F_4 and F_5. About 5 months old when spayed.	O
1:13 m., 4:17–18 m. (3 still alive at conclusion of experiment.)	
5 (5 II)	O
(3) 782a F_4 (=$8\frac{1}{2}$+328), 3–4 months old when spayed.	O
2:8 m., 2:9 m., 1:$9\frac{1}{2}$ m., 1:$12\frac{1}{2}$ m., 1:$13\frac{1}{2}$ m., 1:$16\frac{1}{2}$ m.	
8 (5 I, 3 II)	
(4) 1158 F_5 (=[Waltzer+English]+ English [orange]), about 3–4 months old when spayed.	O
1:$8\frac{1}{2}$–$9\frac{1}{2}$ m., 2:11$\frac{1}{2}$–12$\frac{1}{2}$m., 2:12–13 m., 1:$12\frac{1}{2}$–$13\frac{1}{2}$ m., 3:$14\frac{1}{2}$–$16\frac{1}{2}$ m., 2:17–18 m.	
11 (3 I, 8 II)	

Without tumors.	*With tumors.*
(5) 1158 F_4 about 3–5 months old when spayed.	
1:9—11 m., 2:14½—16½ m.	1:9—11 m.
3 (1 I, 2 II)	1 (1 I)
(6) English, 4–6 months old when spayed.	
4: about 18 m., 9 alive: about 22 m.	1:13 m., 1-15 m., 1-16 m., 1:18 m.
13 (4 II, 9 III)	4 (4 II)
(7) English, 5–6 months old when spayed.	
2:8 m., 2:16 m., 1:18 m., 1:19 m., 3:23 m., 3:24 m., 1:26 m.	O
13 (2 I, 3 II, 8 III)	
(8) 782a F_6 and F_7, 6 months old when spayed.	
2:8—9 m., 1:10 m., 1:14 m., 1:17—18 m., 2:18—19 m.	1:11 m., 4:12 m.
7 (3 I, 2 II, 2 III)	5 (5 I)
(9) English, 6–7 months old when spayed.	
1:11 m., 1:14 m., 2:19 m., 2:21 m., 2:23 m.	1:14 m., 1:24 m.
8 (1 I, 1 II, 6 III)	2 (1 II, 1 III)
(10) 1162 F_6 (=8½+328) 6–7 months old when spayed.	
2:20—21 m.	1:12¼ m., 1:14 m., 1:15½ m. (ovaries left), 1:18 m. (ovaries left)
2 (2 III)	2 (2 II) (2 II with ovaries left)
(11) (8½+328) F_4 7–8 months old when spayed.	
1:11 m., 1:16 m.	1:9 m., 1:11 m., 2:12 m.
2 (1 I, 1 II)	4 (4 I)
(12) (8½+328) F_7, 7–8 months old when spayed.	
1:15 m., 1:19 m.	1:16½ m., 1:19½ m. (ovary left)
2 (1 II, 1 III)	1 (1 II) (1 III ovary left)
(13) 1157 F_6 (=8½+328) 8 months old when spayed.	
1:16 m., 1:19 m., 2:22 m.	1:14 m.
4 (1 II, 3 III)	1 (1 II)
(14) (8½+328) F_3 and 869a (=8½+328), 8½–10 months old when spayed.	
3 (3 II)	13 (4 I, 8 II, 1 III)
(15) (344 [English Sable]+German) F_7, 10½ months old when spayed.	
1:13½ m., 1:19½ m., 1:19½ m.	1:19½ m., 1:20 m.
3 (1 II, 2 III)	2 (2 III)

Our results are summarized as follows (T.o.c. signifies time of castration):

	Without tumors.	*With tumors.*
(1) English T.o.c. 3–6 months	14	0
(2) 8½+328 T.o.c. about 5 months	5	0
(3) 8½+328 T.o.c. 3–4 months	8	0
(4) (Waltzer+English)+English T.o.c. 3–4 m.	11	0
(5) (Waltzer+English)+English T.o.c. 3–5 m.	3	1
(6) English T.o.c. 4–6 months	13	4
(7) English T.o.c. 5–6 months	13	0
(8) 8½+328 T.o.c. 6 months	7	5
(9) English T.o.c. 6–7 months	8	2
(10) 8½+328 T.o.c. 6–7 months	2	2*
(11) 8½+328 T.o.c. 7–8 months	2	4
(12) 8½+328 T.o.c. 7–8 months	2	1†
(13) 8½+328 T.o.c. 8 months	4	1
(14) 8½+328 T.o.c. 8½–10 months	3	13
(15) 344+German T.o.c. 10½ months . . .	3	2

* 2 additional, incomplete castration.

† 1 additional, incomplete castration.

Altogether one hundred and thirty-three of those pedigreed mice which survived a sufficiently long period after castration to permit a definite conclusion are included in our records. There are in addition three mice which had been incompletely spayed; all of these three developed tumors. Thirty-five of the one hundred and thirty-three castrated mice developed tumors.

These figures as such have, however, little significance. In order to appreciate the results obtained, it will be necessary to consider the age at which the castration was carried out. Again the fact must be emphasized that our figures cannot claim absolute accuracy. In regard to a very small number of mice the records are not quite trustworthy; it is furthermore probable that in certain cases errors in the age of the animals amounting perhaps to four to six weeks have occurred. This latter factor may explain a few slight apparent discrepancies which are noted. But making allowance for these shortcomings, we do not doubt that in all essential respects our results are approximately accurate. We desire, however, to test our conclusions still further in future experiments. However, before interpreting our figures it will be necessary to give for comparison the records of non-castrated control mice of strains corresponding to those we used for castration.

Controls for the mice used in experiments 1, 6, 7 and 9 (English, and in particular 869a):

Without tumors.	*With tumors.*
869a and related families.	
44% (76% I, 18% II, 6% III)	56% (79% I, 21% II) I age class
English Sable.	
F_1–F_6 30% (65% I, 25% II, 10% III)	70% (66% I, 27% II, 7% III) I age class
F_4–F_7 33% (57% I, 25% II, 18% III)	67% (41% I, 53% II, 6% III)
Controls for mice used in Experiments 3, 8 and partly 14.	
782a = 8½ + 328.	
32% (71.5% I, 28.5% II)	68% (53.5% I, 46.5% II) I age class
39% (71% I, 24% II, 5% III)	61% (64% I, 36% II)
Controls for mice used in Experiments 2, 10, 11, 12, 13 and partly 14.	
8½ + 328.	
F_1–F_4 49% (70% I, 23% II, 7% III)	51% (65½% I, 31% II, 3½% III)
F_3–F_4 43% (60% I, 33% II, 7% III)	57% (56% I, 40% II, 4% III) I age class
Controls for mice used in Experiments 4 and 5.	
(*Waltzer* + *English*) + *English* (*orange*).	
I 20% (33% I, 67% II)	80% (67% I, 33% II)
II 33⅓% (90% I, 10% II)	66⅔% (75% I, 25% II) I age class
Controls for mice used in Experiment 15.	
344 + *German.*	
F_2–F_6 35½% (31% I, 69% II)	64½% (58½% I, 38% II, 3½% III) between I and II age class, but nearer the I age class

In comparing the tumor rate in castrated mice with that in controls, we come to the following conclusions:

(1) Five groups of mice (Nos. 1, 2, 3, 4 and 5) were castrated between the ages of three and six months; the large majority of the animals were probably spayed at the ages of three and four months, some at the age of five and very few at the age of six months. In four of these groups, comprising thirty-eight mice, no tumor occurred. In one additional small group, castrated between the ages of three and five months, one tumor occurred, presumably in a mouse castrated at the age of five months. There was in addition a group (No. 7) castrated at the age of five to six months, in which no tumor occurred. The large majority of the latter were probably five months old. Altogether we have therefore a record

of fifty-four mice almost all castrated between the ages of three and five months in which only one tumor developed. The composite record of groups Nos. 1, 2, 3, 4, 5 and 7 is:

Without tumors.	*With tumors.*
54 (16 I, 23 II, 15 III)	1
98.2% (29% I, 43% II, 28% III)	1.8%

The normal tumor rate among these mice varies between fifty-six per cent and eighty per cent. One half to three quarters of these tumors appear normally in the first age period, namely in animals between the ages of six or seven and twelve months. The number of castrated mice, fifty-four, is so great and the difference between the results obtained in castrated mice and in controls is so remarkable, that a coincident can be excluded. On the whole, the tumor rate is very constant in the controls of those strains which we have used, and the variations even in groups which are considerably smaller than fifty-four are not very considerable. If we consider the mice spayed between the age of four and seven months (Groups 6, 8, 9 and 10) we find thirty mice without tumors and thirteen with tumors. Among a group of forty-three mice there occurred therefore thirteen tumors. This corresponds to a tumor rate of thirty per cent, as compared with an average tumor rate of about sixty per cent in the controls.

The combined record of Nos. 6, 8, 9 and 10 is as follows.

Without tumors.	*With tumors.*
30 (4 I, 7 II, 19 III)	13 (5 I, 7 II, 1 III)
70% (13% I, 23% II, 64% III)	30% (38% I, 54% II, 8% III)

The composite record of the controls is approximately as follows:

Without tumors.	*With tumors.*
35% (64% I, 27% II, 9% III)	65% (56% I, 40% II, 4% III)

We may conclude that in mice the large majority of which had been spayed at the ages of six and seven months, but including a few mice castrated at the age of four to six months,

tumors occur, but at a diminished rate, and the tumors occur somewhat later than in controls.

If we combine in a third group the mice castrated at the age of seven to ten and a half months, we obtain the following figures:

Without tumors.	*With tumors.*
14 (1 I, 7 II, 6 III)	21 (8 I, 10 II, 3 III)
40% (7% I, 50% II, 43% III)	60% (38% I, 48% II, 14% III)

The record of the controls is approximately that of $8\frac{1}{2}$ + 328 F_3, F_4. (The record of 344 + German, used in Experiment 15, is very similar.)

Without tumors.	*With tumors.*
43% (60% I, 33% II, 7% III)	57% (56% I, 40% II, 4% III)

We may then draw the conclusion that castration carried out at the age of seven to ten and a half months does not alter the tumor rate in any marked way; there is a possibility that under those conditions the tumors appear possibly a little later than in controls. In addition it is of interest to recall that in each one of three mice belonging to a group which was castrated at the age of six or seven months, but in which the castration had been incomplete, a tumor appeared. These three may be added to those mice which serve as a control for the castrated mice.

Considering all these experiments we may conclude (1) that castration practiced at the age of three or four months completely or almost completely prevents the appearance of carcinoma of the breast in mice, (2) that in mice castrated at the age of five to seven months cancer appears, but that in all probability the tumor rate is lowered and furthermore the tumors appear later in life, and (3) that in mice castrated above the age of seven months the frequency with which tumors appear is not markedly affected, but that possibly the age at which the tumors appear is somewhat higher. This point needs, however, further investigations. The effect of castration is therefore not sudden but of a graded

character. We may furthermore conclude that the effect of castration is not limited to a certain strain of mice, but is equally demonstrable in different strains. There is another conclusion which we may draw from our figures: If we compare the age at which those castrated mice in which tumors do not appear die with the age of the control mice which remain free from tumors, we uniformly find the age of death of the castrated mice to be higher. The difference between the two classes is quite marked.

In the castrated mice which die without tumors we find in the various groups the following figures:

(*a*) 29% I, 43% II, 28% III
(*b*) 13% I, 23% II, 64% III
(*c*) 7% I, 50% II, 43% III

These figures may be compared with the age at which control mice die without tumors:

(*a*) 64% I, 27% II, 9% III
(*b*) 60% I, 33% II, 7% III

We see that there is a very considerable difference and that the castrated mice which remain free from tumors die at a considerably later period of life than normal control mice which remain free from tumors.

This result might be due to a direct effect of castration and non-breeding, or it might be due to the fact that those mice which under usual conditions are affected by tumors are the healthiest mice which, if prevented from becoming cancerous, would live longest. Without altogether excluding at the present time the latter interpretation, we may conclude that castration independent of its effect on the tumor rate prolongs the life of mice. We find that the mice, castrated at an age when castration no longer prevents the appearance of tumors, still reach a considerably higher age than the normal control mice and furthermore we have found previously that non-breeding, which has only a slight effect on the tumor rate, still prolongs the life of the non-tumor mice.

We may therefore conclude that castration prolongs the life of mice which die without tumors. The older age which

castrated mice reach should make them more liable to a higher tumor rate. We actually find the opposite to be the case. The tumor rate would therefore be still lower in castrated mice if they died at as early an age as non-castrated mice.

II. Tumor incidence and tumor age in mice which are prevented from breeding. — In former investigations we came to the conclusion that in mice which are kept from breeding the tumor incidence is somewhat diminished and the age at which the tumors appear somewhat increased, but that the result varied to some extent in different strains of mice and that on the whole the effect is very much less marked than in castrated mice.

We have since made the following additional experiments, which, while they are essentially confirmatory of our previous results, permit us to draw some additional conclusions.

Without tumors.	*With tumors.*
(1) English (non-breeding mice).	
10 (3 I, 1 II, 6 III)	3 (3 II)
77% (30% I, 10% II, 60% III)	23% (100% II)

The former record of English non-breeding mice is as follows:

Without tumors.	*With tumors.*
45⅓% (22% I, 50% II, 28% III)	54⅔% (33% I, 44% II, 33% III)

An average record of English breeding mice is as follows:

Without tumors.	*With tumors.*
32⅔% (66% I, 25% II, 9% III)	67⅓% (58⅓% I, 34⅔% II, 7% III)

The tumor incidence in the non-breeding mice is lower and the tumors appear later. The non-breeding, non-tumor mice reach a higher age than the breeding non-tumor mice.

Without tumors.	*With tumors.*
(2) $(8\frac{1}{2}+328)$ F_4	
8 (4 I, 2 II, 2 III)	7 (6 II, 1 III)
53% (50% I, 25% II, 25% III)	47% (85½% II, 14½% III)

The record of the breeding strain $8\frac{1}{2} + 328$ is as follows:

43% (60% I, 33% II, 7% III)	57% (56% I, 40% II, 4% III)

The tumors are less frequent than in the total of the $8\frac{1}{2}$ + 328 strain, but may be more frequent than in some isolated groups of $8\frac{1}{2}$ + 328; however, the tumors appear later than in the breeding groups; and the non-breeding mice reach a somewhat higher age.

Without tumors.	*With tumors.*
(3) London F_6 (non-breeding mice).	
18 (1 I, 7 II, 10 III)	1 (1 I)
$94\frac{2}{3}$% ($5\frac{1}{3}$% I, 39% II, $55\frac{1}{2}$% III)	$5\frac{1}{3}$% (100% I)
Controls: London breeding mice.	
(*a*) Older records:	
73% (41% I, 29% II, 30% III)	27% (27% I, 43% II, 30% III)
(*b*) Newer records:	
72% (31% I, 40% II, 29% III)	28% (40% I, 51% II, 9% III)
(*c*) London F_6 (breeding group):	
72% (31% I, 54% II, 15% III)	28% (40% I, 60% II)

Again the tumor rate is lower in the non-breeding mice; the mice which die without tumors reach a higher age in the non-breeding than in the breeding groups.

In confirmation of our previous results we may then conclude that in non-breeding mice the tumor rate is, on the whole, somewhat lower than in breeding mice, but that the difference between the tumor rate and tumor age of breeding mice and non-breeding mice is on the whole slight and not comparable to that observed in castrated mice. Non-breeding mice which die without becoming cancerous reach a higher age than non-cancerous breeding mice. The effect of castration on the duration of life is to a great extent or entirely due to the fact that these mice do not breed.

We have a few additional records of mice which were kept from breeding. The number of the mice is too small to serve for statistical purposes; the results however confirm our other results in so far as they show that in non-breeding mice tumors may appear even in strains which are normally as poor in tumors as the Creams, and again the records indicate that non-cancerous, non-breeding mice reach a higher age than breeding mice.

Without tumors.	*With tumors.*
(*a*) Cream (non-breeding mice).	
4 (1 II, 3 III)	2 (2 II)
67% (25% II, 75% III)	33% (100% II)
Breeding controls.	
92% (30½% I, 39% II, 30½% III)	8% (22% I, 33% II, 45% III)

Certain groups of breeding "Cream" show, however, a somewhat higher tumor rate.

(*b*) "November 8th" F_6 (=European (151)+II daughter of No. 10) (non-breeding).	
3 (1 I, 2 III)	3 (1 I, 2 III)
50% (33⅓% I, 66⅔% III)	50% (33⅓% I, 66⅔% III)
Old controls (breeding):	
35% (44% I, 47% II, 9% III)	65% (32% I, 54% II, 14% III)
New controls:	
63% (62% I, 23% II, 15% III)	37% (31% I, 41% II, 28% III)
(*c*) (344+German) F_6 (non-breeding):	
1 (1 II)	3 (3 II)
25% (100% II)	75% (100% II)
Controls:	
35½% (31% I, 69% II)	64½% (58½% I, 38% II, 3½% III)

While these results as far as they can be used statistically, confirm our previous results, there is one non-breeding group, which we have observed since our former publication, in which the tumor rate in non-breeding mice was higher than in breeding controls, namely "German + Carter." This is a group which also in another respect was exceptional; it being one of those strains, in which the tumor rate increased generally in the later generations in the course of continued inbreeding.[12]

In breeding mice (controls) the early records were as follows:

Without tumors.	*With tumors.*
91% (24% I, 27% II, 49% III)	9% (12½% I, 39% II, 48½% III)
The newer records:	
66½% (43% I, 29% II, 28% III)	33½% (43% I, 34% II, 23% III)
or a special group (794):	
64% (50% I, 31% II, 19% III)	36% (11% I, 44½% II, 44½% III)

In the non-breeding mice the records are as follows:

19 (3 I, 1 II, 15 III)	40 (6 I, 14 II, 20 III)
32% (16% I, 5% II, 79% III)	68% (15% I, 35% II, 50% III)

While again the non-tumor mice die at a much later period of life in the non-breeding group, the tumor rate is here higher than in the breeding mice.

This is perhaps only an apparent contradiction to the results which we obtained in the other groups; it may be due to the very late appearance of tumors in this group; the mice almost belong to the IV. age class. We found that a much larger number of non-breeding than of breeding mice reach the age above eighteen months. The chance for the non-breeding mice to develop tumors is thus increased. This may partly explain the difference in the results obtained in this group. Whether it explains the results entirely is doubtful, if we consider the fact that in the non-breeding mice the percentage of tumors appearing in the third age period is only slightly increased.

We must therefore add to our former results the additional conclusion that in one group (German + Carter) the tumor rate was higher in non-breeding than in breeding mice.

III. Transplantation of ovaries into male mice. — The preceding investigations establish the significance of the ovaries and of internal secretion in the origin of cancer in mice. They show that females can be made to behave like males as far as the tumor rate is concerned, provided the ovaries are extirpated at a certain period in life.

It was of interest to inquire whether the reverse procedure, namely, the transplantation of ovaries into a male, would lead to the development of tumors of the mammary gland, provided males are selected which belong to strains rich in tumors. We accordingly chose young male mice which belonged to strains in which females are affected by tumors at a rate exceeding fifty per cent and in which the large majority of tumors appear in the first age period, that is, at the age of twelve months or below. Strains $8\frac{1}{2}$ + 328, English, and English + European hybrids were used in the large majority of cases. Such male mice were castrated usually at an age varying between two and four months; about ten days to four weeks following the castration one or two

ovaries of a sister were transplanted subcutaneously into the castrated brother.

Nineteen male mice thus treated survived a sufficiently long period of time to permit inclusion in this series.

Thirteen of these mice died towards the end of the first age period, usually between the ninth and twelfth months. In five of these mice the transplanted ovaries were found; in eight they were not recovered at the time of the autopsy. Five mice died in the second age period (between the twelfth and eighteenth months). In one of these mice ovaries were found; in four the search for the ovaries yielded negative results. The last mouse was killed in the third age period; in this case the transplanted ovaries were found.

The microscopic examination of the recovered transplanted ovaries, which is not yet quite complete, showed so far that in some cases only interstitial gland survives, while in other cases follicles and eggs survive and new corpora lutea are formed.

In no case did cancer in the mammary gland develop in the castrated male as the result of the transplantation, although in females of the same strain, dying at the same age, the incidence of cancer would have been considerable. In one mouse [(344 + 328) + 437] which died towards the end of the first age period, a lymph tissue tumor, perhaps of thymus origin, was found in the anterior mediastinum pressing on the heart. The occurrence of this tumor in this mouse is probably not connected with transplantation of the ovaries.

We interpret these negative results as indicating that the transplanted ovaries are probably not able to call forth rhythmic growth changes in the mammary gland with the same energy as the normal ovaries and consequently cancer is not induced in such animals as the result of the experimental procedure.

SUMMARY AND CONCLUSIONS

1. A hormone given off by the ovary regulates those tissue changes which lead to the development of cancer of the breast in mice.

2. The influence of this hormone is a quantitatively graded one. If the quantity of this hormone which had a chance to act on the tissues exceeds a certain limit, cancer appears as frequently as in non-castrated controls (castration at the age of eight to ten and a half months); if an intermediate quantity of the hormone has been active, the cancer rate is noticeably diminished and the cancer seems to appear later in life (castration at the age of five to seven months); if the quantity of the hormone is restricted still further, cancer does not appear at all or it appears only exceptionally (castration at the age of three to five months).

3. After an intermediate and late castration, not only tumors may appear in the first age period, but tumors which in control mice appear in the third age period are not prevented from developing. This fact suggests the conclusion that the tissue changes which eventuate in the development of cancer, occur at a much earlier period in life, and that castration affects these primary tissue changes rather than the secondary transformation of these changes into fully developed carcinomatous growth.

4. The effect of castration on the tumor rate is not limited to one particular strain, but is effective in all the strains which have so far been tested.

5. Prevention of breeding in mice lowers the tumor rate and raises the tumor age only slightly; its effect is not comparable to that of an early castration. Even in mice in which normally the tumor rate is low and tumors appear late in life, prevention of breeding does not prevent the occurrence of cancer. We found one strain in which the tumor rate was higher in non-breeding than in breeding mice. In this strain tumors appear in breeding mice late in life. In all the other strains tested so far in which normally the tumors appear at an early period of life, prevention of breeding had the typical effect stated above.

6. Castrated as well as non-breeding mice, which are not cancerous, reach a higher age than normal female non-cancerous mice. The chance to develop cancer is therefore better in castrated and non-breeding mice than in control

mice on account of the greater number of mice reaching a higher age. We may conclude that if in castrated and non-breeding mice the age at which they die were the same as in controls, the reduction in the tumor incidence would even be more marked than it is actually found.

7. Transplantation of the ovaries of the sisters into young castrated male mice belonging to strains with a high tumor rate has so far not led to the development of tumors in the male mice. We interpret this fact as indicating that the transplanted ovaries do not function with the same completeness and in the same rhythm as normal ovaries in the female mouse.

8. It has of course been noted by many authors that animals of male sex are only very rarely affected by cancer of the breast. The significance of this fact has so far been somewhat uncertain. Equally uncertain is the interpretation of the fact that in certain species cancer of the mammary gland is frequent while it is rare in other species.

It might be due to structural differences between the male and female breast, it might be due to the absence of the effect of pregnancy or of nursing in the male gland, or it might be due to a direct influence of the ovarian hormone acting independently of the hormones which are active during pregnancy.

Our results enable us to conclude that in all probability the ovarian hormone and not the effect of pregnancy and nursing is directly responsible for the difference in the cancer rate in male and female mice. While the great rarity of cancer of the breast in male mice might have suggested the conclusion that internal secretion is of importance in the origin of cancer as a matter of fact such a conclusion had not been drawn by previous authors.

Our investigations furthermore show that the relationship between the origin of cancer and internal secretion is a quantitative one; and it will now be possible to analyze experimentally the conditions underlying the origin of cancer of the breast.

9. The effect of hormones on the development of cancer is a specific one; a hormone influences the development of cancer only in those organs to which under normal conditions

it has a specific relation. We may assume that connections similar to that between cancer of the breast and ovary exist also between cancer of the uterus and ovary and between cancer of the prostate and testicle, and perhaps between thyroid and cancer originating in bone. Presumably other similar relationships exist.

10. In certain cases at least internal secretion does exert an influence on the growth of adenomata, but it is without noticeable effect on the growth of carcinomata after they have been established. On the other hand internal secretion has been proven to have a definite effect on those tissue changes which lead to the development of cancer, and a prevention of the effect of this internal secretion at a sufficiently early period of life may prevent the development of cancer, while the elimination of such an internal secretion cannot possibly exert any marked effect on the growth of cancers after they have once developed, at least in the large majority of cases.

11. We see then that, in the main, three factors are active in the development of cancer: (*a*) heredity, (*b*) irritation (physical stimulation), (*c*) internal secretion (chemical stimulation).

Wherein the hereditary factors consist is not certain at present, but these factors seem amenable to further analysis.

Internal secretion seems to cause cancer only in coöperation with hereditary factors. On the other hand, hereditary factors need — at least in the case of certain cancers — the coöperation of hormones in a definite quantity, if cancer shall develop.

Physical stimulation is also under certain conditions associated with hereditary factors in the origin of cancer. On the other hand, the evidence points to the conclusion that if the physical stimulation is sufficiently strong cancer may develop without the coöperation of these hereditary factors.[13]

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3. We regret to announce that Miss A. E. C. Lathrop, who throughout a period of more than ten years collaborated with us in our work on heredity of cancer, died in the beginning of the year 1918. Adverse conditions having made it impossible for her to acquire that scientific knowledge which she had always desired, she aided us most conscientiously and with great understanding in our work until increasing weakness made a further continuance impossible.

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11. Towards the end of our new series through an inadvertence on the part of the attendant, some mice were mixed up. While the records are therefore not absolutely correct, the error thus incurred and similar possible errors in the first series are not of a nature to impair our main conclusions; they could at best necessitate only slight quantitative modifications.

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