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Imperial Cancer Research Fund, London

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SECTION III

GENERAL PATHOLOGY AND PATHOLOGICAL ANATOMY

INDEPENDENT PAPER

STUDIES ON THE HEREDITY OF CANCER IN MICE

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THE extent to which the presence of cancer in individuals of one generation may be associated with an increased incidence of the disease in the next has been the subject of discussion for many years. The practical difficulties of the problem, especially the impossibility of obtaining data with reference to cancer in man embracing a succession of generations, complete in a pathological-anatomical sense as well as actuarially, have driven investigators to the collection of data in the short-lived mouse. Apart from isolated observations of the frequent occurrence of malignant new growths in small families of mice, deliberate attempts to gather observations in numbers adequate to the purpose have only been made by Tyzzer [3] and Bashford and Murray.[1] Tyzzer's results have been summarized in the account of the observations on the material of the Imperial Cancer Research Fund by Murray [2] in the 'Fourth Scientific Report' and need not be dealt with again, as the principles involved are the same as in the latter, more abundant, material.

The method consists in breeding large numbers of mice from animals suffering from cancer, crossing their offspring in the most varied manner and recording all the facts as to age, sex, and cause of death, in the animals so obtained throughout a period of years. From the accumulated data it is possible to construct complete pedigrees of all the animals, showing the frequency and position of malignant new growths in the ancestry of each. It is then easy to distribute the card-index into groups of any selected character, chosen so as to avoid the statistical difficulties which arise from the variations in the frequency of cancer with the sex and age of the individuals enumerated.

It was found that in mice the female sex was very much more liable to develop cancer than the male, the numbers available from the latter being too small to repay statistical investigation. So far as they are of interest, the observations on cancer in males will be dealt with

separately by a consideration of family pedigrees, and the main study will be concentrated, as before, on the observations of the relative frequency of cancer in female mice in the ancestors of which cancer has occurred recently or in remote generations.

In the mouse, as in the human subject, cancer arises with greater frequency in the higher age-periods, and although more than 2,000 mice have been bred in the laboratory for these observations during the past six years, no case of malignant new growth has been discovered in a female mouse less than six months old. The restriction of the inquiry to animals aged six months or more has reduced the numbers available to 800 females, among which 136 had developed malignant new growths up to October 24, 1912.

Relation to Age

These cancer cases are not distributed equally over the months from the sixth to the thirtieth, beyond which few mice survive. As the following table (Table I) shows, the differences in numbers of cases of cancer observed at each successive period of three months are considerable.

TABLE I

Age-period in Months	6–10	–13	–16	–19	–22	–25	*Over* 25
Cases of cancer . .	13	24	36	34	12	12	5

It might appear from such a table that in the mouse the majority of the cases of cancer occurred in the first eighteen months of life and particularly from the sixth to the nineteenth month, and that thereafter the disease was rarer. Such a statement would be entirely misleading and conceals important information, since no account is taken of the number of animals in which these cases have arisen. It is as if one should state that there are more cases of cancer in a year in London than in Dover, ignoring the enormous discrepancy in population. It is necessary therefore to state in some way the number of animals from which these cancer cases are drawn, and two methods of enumeration have been adopted. The first, and less reliable, consists in assuming that throughout the period of observation the other causes of death have been operating uniformly at each of the successive periods of life. We can then state the total mortality at each age-period and find what proportion the cancer deaths bear to the total mortality of animals of the same age. The second and more accurate method requires that the cancer deaths (or cases) be reckoned on the number of animals living, of equal age. This is the method adopted in human mortality statistics, the proportional numbers for each year being known as annual death-rates. An attempt will be made in the present paper to adopt this method, with modifications made necessary by the small numbers available, to the deaths of mice from cancer.

The first method, that of estimating the cancer fraction of the total mortality, was adopted in the earlier accounts of these observations[1,2] and also by Tyzzer.[3] As the breeding of fresh mice suffered no interruption, a date was fixed up to which the enumeration was to be carried. All the animals which had died from causes other than cancer were counted, and those whose ages at death differed by less than three months were added together, commencing with animals which were at least six months old at death. A difficulty arose in deciding the age-periods to which the cancer cases should be ascribed. Ordinarily cases of spontaneous cancer in the mouse terminate fatally within four to six weeks after the discovery of the tumour if left to themselves. If the tumours be extirpated death may be postponed for several months. On the other hand, the animal may die under the anæsthetic or be sacrificed early for other reasons. Hence the age at death of cancer mice bore a highly variable relation to the age of onset of the disease, and it was decided to adopt the age of onset as determining the age-period to which the animals should be ascribed, rather than the age at death. As some of the cancer cases were discovered just before the day selected for the enumeration, the mice living at the time were also counted and added to the totals of dead mice in the age-periods embracing their ages on the ' census ' day.

TABLE II

Age-period in Months	6–10	–13	–16	–19	–22	–25	over 25	Totals.
No tumour								
Dead . .	165	126	75	74	59	56	43	598
Living . .	15	16	12	17	5	1	0	66
Tumour mice .	13	24	36	34	12	12	5	136
Total . .	193	166	123	125	76	69	48	800
Percentage . .	6·7	14·5	29·3	27·2	15·8	17·4	10·4	16·6

(The first age-period is of 4 months' duration, 6–10 months. This gives a more uniform distribution at the higher age-periods than is obtained with the periods 6–9–12, &c.)

With all this additional information added, the first table takes the form seen in Table II. The discrepancy between the first age-period and the middle ones is now greater, and in particular the two highest age-periods are found to contain so few animals that their small number of cancer cases constitute a higher proportion of the total deaths at these ages than do the more numerous cancer cases at the first age-period. Another way of stating the results of this table is to say that one out of every six animals reaching the age of six months ultimately dies of cancer, while of mice dying after reaching the age of sixteen months, one in every five falls a victim.

The successive estimations of this kind which have already been made during the past four years have given similar results, and there is so far a justification of the fundamental assumption of a fair uniformity, in the operation of the other causes of death. The segregation of the

animals into groups, in which the ages do not differ by more than three months, is seen to be adequate to avoid the inclusion in one group of animals differing markedly in their liability to cancer. It will be maintained in all the subsequent tables attempting an analysis of the effects of ancestry on the liability to cancer.

Influence of Ancestry

From the register kept of births and parentage of mice born in the laboratory pedigrees have been constructed as in the two examples in Figs. 1 *a*, 1 *b*. The sex is shown by the usual signs ♂ and ♀ and the index number is inscribed within the sign. The dates of birth and of death are entered and the age stated in months and days. The ancestors, commencing with the mother and father, are set out reading from left to right, the ancestors which developed cancer being distinguished by the laboratory number of the tumour in red inside the sex symbol also in red. (The red lines are replaced by dotted lines in the figures.) When such a mouse of the breeding experiments develops a tumour, the laboratory tumour number is entered on the corner of the card, as a fraction with denominator zero, a convention adopted to run in series with the nomenclature used for transplanted tumours, as explained in other papers from the Imperial Cancer Research Fund.

When the pedigree cards were arranged in groups according to the most recent cancerous ancestor it was found that the majority of the cases of spontaneous cancer fell in the first two groups, namely, those with the mother cancerous and those with one or both grandmothers cancerous. The cards were in consequence distributed in two groups, the first including all the mice of which the mother, one or both grandmothers, or any combination of these ancestors had developed spontaneous cancer. The remaining mice formed the second group. The first group will be designated mice of recent cancerous ancestry, and the second, mice of remote cancerous ancestry. As is shown by Table III

TABLE III

October 24, 1912. Female mice of recent cancerous ancestry (mother, one or both grandmothers, or all three, cancerous). Ages at death or discovery of tumour.

Months	6–10	–13	–16	–19	–22	–25	*Over* 25	*Total.*
No tumour								
Dead . .	95	83	43	40	33	29	27	350
Living .	6	7	7	7	1	0	0	28
Tumour mice								
Mamma .	7	14	24	21	7	8	1	82
Other organs	1 } 8	3 } 17	4 } 28	5 } 26	2 } 9	0 } 8	1 } 2	16 } 98
Total . .	109	107	78	73	43	37	29	476
No. of cancer cases per 100 deaths from all causes .	7·3	15·9	35·9	35·6	20·9	21·6	6·9	20·6

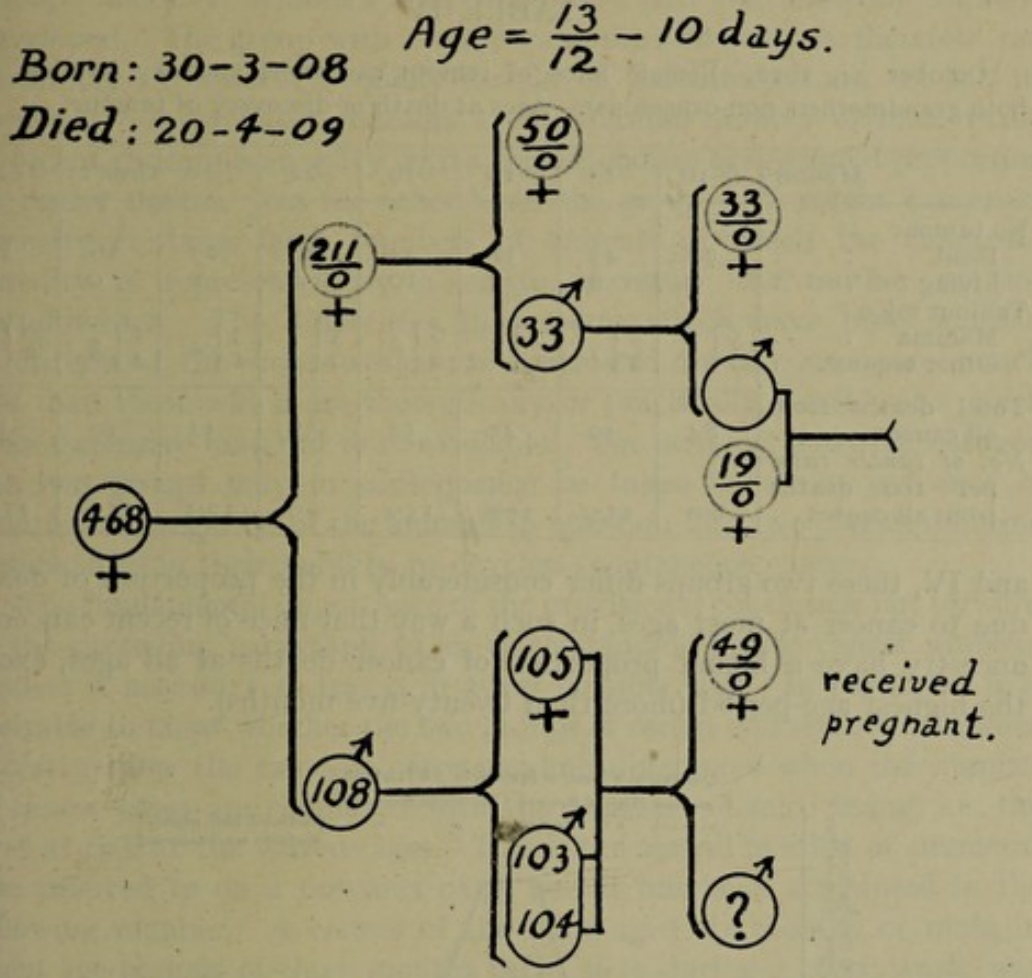

FIG. 1 *a*. Pedigree card of a female mouse of recent cancerous ancestry which died aged 13 months without developing cancer.

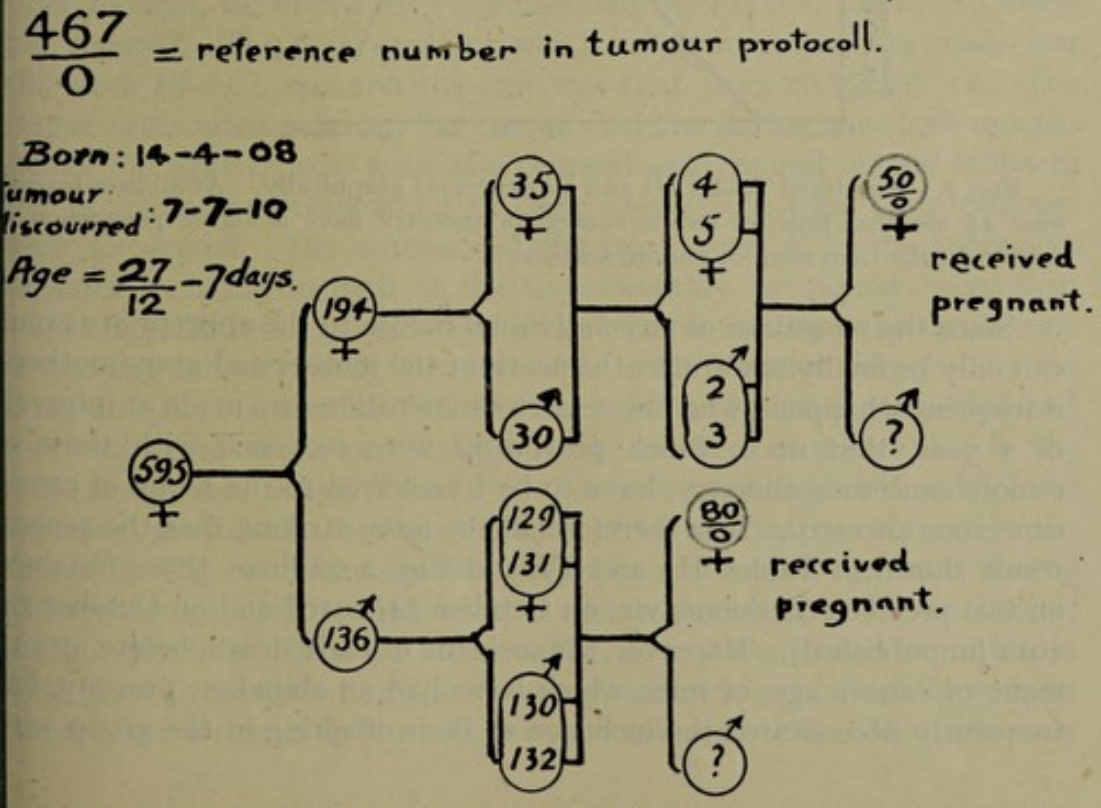

FIG. 1 *b*. Pedigree card of a female mouse of remote cancerous ancestry which developed cancer at the end of 27 months.

TABLE IV

October 24, 1912. Female mice of remote cancerous ancestry (mother and both grandmothers non-cancerous). Ages at death or discovery of tumour.

Months	6–10	–13	–16	–19	–22	–25	*Over 25*	*Total.*
No tumour								
Dead . .	70	43	32	34	26	27	16	248
Living . .	9	9	5	10	4	1	0	38
Tumour mice								
Mamma . .	5 } 5	5 } 7	7 } 8	6 } 8	3 } 3	3 } 4	1 } 3	30 } 38
Other organs .	0 }	2 }	1 }	2 }	0 }	1 }	2 }	8 }
Total deaths from all causes . .	84	59	45	52	33	32	19	324
No. of cancer cases per 100 deaths from all causes .	6·0	11·9	17·8	15·4	9·1	12·5	15·8	11·4

and IV, these two groups differ considerably in the proportion of deaths due to cancer at most ages, in such a way that mice of recent cancerous ancestry have a higher proportion of cancer deaths at all ages, except the highest age-period (more than twenty-five months).

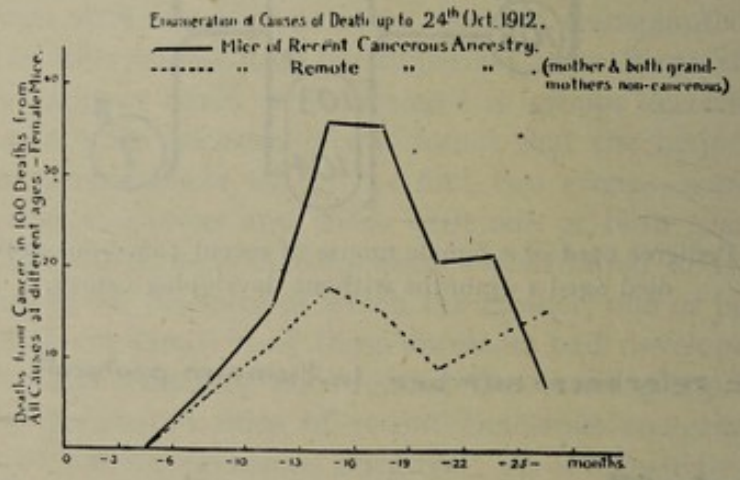

FIG. 2. Results of Tables III and IV expressed graphically. At all ages (except over 25 months) mice of recent cancerous ancestry have a higher proportion of cancer deaths than mice of remote ancestry.

Since the relegation of any individual mouse to the appropriate group can only be finally made after the death of the mother and grandmothers, it frequently happens, when successive enumerations are made at intervals of a year, that mice, which previously were reckoned with those of remote cancerous ancestry, have to be transferred to the group of recent cancerous ancestry. It is therefore all the more striking that the general result shown in Tables III and IV and Fig. 2 confirms those obtained on two previous occasions, viz. on October 24, 1910[2] and on October 24, 1911 (unpublished). Moreover, the accident of early death, before attainment of cancer age, of mice which have had an abundant progeny, has frequently necessitated the inclusion of their offspring in the group with

remote ancestry, although they may have had the tendency strongly developed. The group with remote cancerous ancestry is therefore not invariably of necessity equally devoid of hereditary taint in all its members. It probably contains a considerable number of mice really of recent cancerous ancestry with a corresponding exaggerated proportion of cancer deaths. On the other hand the group with recent cancerous ancestry contains large numbers of animals in which the cancerous heredity is represented by a single ancestor, the mother or one grandmother. The differences in ancestry which have been adopted as criteria of the groups which are contrasted are in consequence much less than those which are theoretically or practically possible if a much more extensive material were available. The striking disparity between the two groups may in consequence be taken as strong evidence of a natural segregation of the animals in question into two classes differing significantly in their liability to develop spontaneous cancer.

The fundamental importance of the provisional conclusion put forward in the preceding paragraph to an understanding of the cancer problem renders it necessary to test it in every possible way. In particular it is desirable to know whether the two groups of recent and remote cancerous ancestry show the same or corresponding differences when the number of cancer cases are compared with the numbers of mice living, i.e. the lives at risk at the various ages. This is the second method of enumeration referred to on a previous page, and it has been attempted in the following manner. A census of the mice aged six months or more in seven age-periods of three months each, alive during a given week, was made, keeping the recent and remote cancerous stocks in separate tables. Three months later the process was repeated, and so on at intervals of three months, till fifteen such censuses had been taken, embracing forty-five months. The first week for which an enumeration was made was the week 18–24 i. 09, and the last was that from 18–24 vii. 12. The cancer cases arising during the census week, or during the three months before the next census, were also counted and entered on the tables in the age-periods corresponding to their ages at the time their tumours were discovered. The numbers of the mice recorded in the successive censuses as living in each of the three-monthly age-periods were then added together, giving a measure of the relative numbers of mice in each of the age-periods during the forty-five months under review. In the same way the cancer cases were added together for each age-period and the total expressed as percentages of the numbers of mice living in the same age-period, Tables V and VI. The results of Tables V and VI are expressed graphically in Fig. 3 and afford an instructive contrast to the curves in Fig. 2. Whereas the proportion of cancer to total deaths suffered an important diminution in the second half of life in Fig. 2, the maximum is distinctly later in Fig. 3, and the curves resemble in their general configuration the curves of age-incidence which have been constructed for man in the national mortality records. The difference

TABLES V AND VI

FEMALE MICE OF RECENT AND REMOTE CANCEROUS ANCESTRY, LIVING AT 7 3-MONTHLY AGE-PERIODS DURING THE WEEKS STATED, FROM 18–1–09 TO 24–7–12

	Remote Cancerous Ancestry.								*Recent Cancerous Ancestry.*							
Months	6–	9–	12–	15–	18–	21–	24–	*Total.*	*Total.*	6–	9–	12–	15–	18–	21–	24–
18–24 i. 1909	26	26	15	0	0	0	0	67	126	51	46	22	4	3	0	0
18–24 iv. 1909	38	20	23	11	0	0	0	92	125	32	40	35	16	2	0	0
vii. 1909	41	29	18	19	9	0	0	116	141	32	30	34	30	14	1	0
x. 1909	28	29	22	15	15	4	0	113	132	37	23	17	24	21	10	0
i. 1910	9	27	22	19	11	5	2	95	106	14	31	15	13	15	12	6
iv. 1910	2	8	27	16	14	5	5	77	84	8	13	27	8	9	9	10
vii. 1910	0	1	5	20	10	8	3	47	61	10	7	12	17	6	3	6
x. 1910	9	0	1	3	15	7	7	42	48	10	7	6	7	13	4	1
i. 1911	5	5	0	1	2	9	4	26	41	2	10	6	6	4	10	3
iv. 1911	4	5	5	0	1	0	4	19	45	13	5	9	5	4	3	6
vii. 1911	10	4	4	4	0	1	2	25	66	33	12	4	7	3	2	5
x. 1911	28	7	3	1	0	0	0	39	58	16	21	8	2	4	3	4
i. 1912	14	18	6	3	0	0	0	41	59	29	11	11	2	1	2	3
iv. 1912	15	11	11	6	0	0	0	43	55	10	27	8	6	2	1	1
vii. 1912	3	10	10	10	2	0	0	35	38	7	10	16	4	0	1	0
Total	232	200	172	128	79	39	27			304	293	230	151	101	61	45
Cancer cases discovered at each age-period from 18 i. 1909 to 17 x. 1912	2	8	3	13	1	6	3			4	12	27	23	14	7	6
Cancer cases per 100 living at each age-period	0·9	4·0	1·7	10·2	1·3	15·4	11·1			1·3	4·1	11·7	15·2	13·9	11·5	13·3

between the percentages of cancer cases in the groups of recent and remote ancestry is again apparent. The irregularities in the curve for the remote cancerous ancestry are obviously due to the irregular distribution of the small number of cancer cases among the relatively short age-periods chosen.

It will have been noticed that the relative numbers living in the successive age-periods are not the same in the two groups, and it may be asked whether the differences in age-constitution are not in themselves sufficient to account for the differences in the numbers of cases of cancer in the two groups. The method adopted in constructing Fig. 3 by

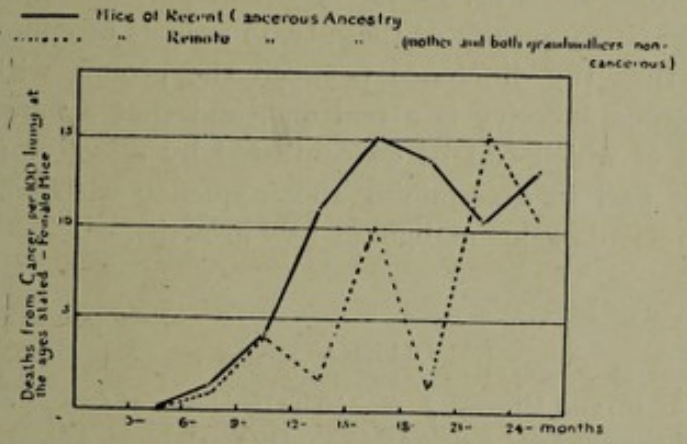

FIG. 3. Deaths per 100 living at the ages stated of female mice of recent and remote cancerous ancestry.

reckoning the cancer cases which occur in 100 mice living at each age-period in the two groups excludes this source of fallacy, but it is useful to determine the relative numbers of cancer cases in the two groups if the proportions in the various age-periods had been the same in both. This can be done by correcting the crude figures of Tables V and VI to a common age-constitution, the most obvious being the average of both groups together, reduced to 1,000 of all ages as in Table VII.

TABLE VII

18–i–09 to 17–x–12. Female mice living at each of the seven three-monthly age-periods named and the corresponding numbers in each age-period in 1,000 of all ages.

Months	6–	9–	12–	15–	18–	21–	24–	*Total.*
	536	493	402	279	180	100	72	=2,062 all ages.
Corresponding to	260	239	195	135	87	49	35	=1,000 all ages.

The numbers of cancer cases to be ascribed to each age-period in the two groups must now be increased or diminished in proportion, according as the actual numbers living in those groups fall short of or exceed the numbers in the average 1,000 of all ages. When this has been done the cancer numbers of Tables V and VI are changed into those combined together in Table VIII.

TABLE VIII

Months	6–	9–	12–	15–	18–	21–	24–	*Total in 1,000 female mice of all ages.*
Recent cancerous ancestry . . .	3·4	9·8	22·9	20·6	12·1	5·6	4·7	79·1
Remote cancerous ancestry . . .	2·2	9·6	3·4	13·7	1·1	7·5	3·9	41·4

Here again, as in the first method of enumeration, the cancer cases are nearly twice as numerous in the group of recent, as in the group of remote, cancerous ancestry.

The confirmatory results of these two methods of enumeration leave little doubt that the difference between the groups with recent and remote cancerous ancestry is a real one, and that a hereditary factor, the influence of which can be concentrated by selective mating, plays an important part in determining the frequency with which stocks of mice develop spontaneous malignant new growths.

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