THE RELATION OF HEREDITY TO SPONTANEOUS THYROID TUMORS IN MICE

STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE
25TH REPORT

MAUD SLYE

(From the Cancer Laboratory of the Otho S. A. Sprague Memorial Institute and the University of Chicago)

This intensive study of the occurrence of thyroid tumors was undertaken in order to demonstrate whether or not heredity bears the same relation to the tendencies to resistance and to susceptibility to these tumors which it has been found to bear to tumors in other organs (1–8).

No report has been found of any spontaneous thyroid neoplasms in mice except those from this laboratory (9). Moreover, very few malignant growths in the thyroid have been reported in other rodents; 5 in rats (10, 11, 12) and one in a coypu (a South American rodent) (13) being all that have been found in the literature. We are, therefore, dealing with a type of tumor relatively rare in all reports of rodents and one nowhere else reported in mice. The occurrence therefore, of malignant thyroid tumors in any considerable numbers within a given strain of mice deliberately bred for these tumors, could not logically be attributed to chance.

There have been to date in the entire stock of this laboratory twenty three mice that have shown enlargements of the thyroid gland. Of these, four have been simple colloid goiters, two have been papillary adenomas, and seventeen have been malignant growths. Some of these mice have died and the tissues have been examined since the publication of the pathological study of these thyroid growths. (Report 22 in this series.) This accounts for the difference in numbers.

Of these seventeen malignant growths, eleven occurred within one small strain of Japanese waltzing mice, strain J.D. 30–62–68,
intensively studied to determine how far selective breeding could control the occurrence of malignant thyroid tumors, and thus again to establish the relation of heredity to the tendencies to cancer resistance and cancer susceptibility. No simple goiters or adenomas of the thyroid have occurred in this strain.

During the past twenty years there have been used in these studies six wholly unrelated stocks of Japanese waltzing mice. The oldest of these, strain J.D. 1 and its derivatives, never produced a tumor of any kind either malignant or benign, during the fifteen years of its life in this laboratory. This strain had its origin in mice from the laboratory of Yerkes of Harvard.

Chart 1 shows a typical portion of strain J.D. 1. No member of this family has had any tumor.

The second stock, secured in the market in Chicago, strain J.D. 7 and its derivatives, also has been wholly free from tumors of every kind. The other four stocks were purchased from four different sources in New Orleans early in 1911. Two of these stocks have never shown any tumor. The other two New Orleans stocks have shown tumors: strain J.D. 16-45 which had a few scattering tumors mainly of the mammary gland tissues; and strain J.D. 30–62–68 which has shown eleven malignant thyroid tumors, and no other tumors of any kind.

The negative evidence here should be emphasized. Of six stocks of Japanese waltzers, comprising many hundreds of individuals in the main line and derivatives, in my hands during twenty years, four stocks have shown complete exemption from tumors of all sorts, even mammary gland tumors, although mammary gland cancer is frequently reported in other stocks of Japanese waltzing mice. The fifth stock has shown complete exemption from thyroid tumors, and the sixth stock, J.D. 30–62–68, has shown complete exemption from all tumors except those of the thyroid gland.

This evidence of exemption from cancer in whole stocks of animals is striking and most important. Outside the mouse stocks in this laboratory, none have been reported as producing
STRAIN J.P. 1

An analysed cancer-immune strain. No tumor has ever occurred in this strain.

Chart 1
any thyroid tumors whatever, either malignant or benign. Within this laboratory, after the first appearance of a thyroid cancer in a Japanese waltzer, all stocks of waltzers were extensively bred to see whether they would produce thyroid tumors. In five of these stocks it was never possible to secure even one such tumor. In the sixth stock, J.D. 30–62–68, where the analytic method was continuously directed toward securing thyroid tumors, eleven such tumors arose. This sixth stock was kept under conditions identical in every respect with those of the other five stocks.

These results strongly reemphasize that most hopeful outcome of these studies published in previous reports, that the tendency to resistance to cancer is hereditary.

The first occurrence of tumors of any kind in this strain (30–62–68) was in male 6219, shown in the 3d generation of chart 2. He died Dec. 29, 1913, at the age of 12 months, of bi-lateral chronic nephritis with coccidia in the kidneys. He showed also a malignant tumor of the thyroid gland which was mainly carcinomatous, but with areas resembling sarcoma (9). These tumors I shall for convenience designate 'sarcoma-carcinoma.' At that time, owing to budget limitations, only a small stock of Japanese waltzers (never exceeding thirty to fifty mice) was being carried. This number was distributed among six stocks, so that the number of mice carried in each stock was necessarily small. Accordingly it took nearly two years before the next thyroid tumor appeared, but it appeared in direct succession from male 6219. How many thyroid tumors might have developed if the strain could have been bred out completely it is impossible to say; but five generations later, that is in generation 8 of the strain, there appeared another sarcoma-carcinoma of the thyroid, female 16917 as shown in chart 3.

The succession of parents in direct descent to female 16917 from male 6219 is shown in chart 4.

On July 15, 1916, the next thyroid tumor in genetic succession in this strain appeared in female 14959, with bi-lateral sarcoma-carcinomas of the thyroid. This mouse was under my daily ob-
STRAIN J.D. 30-62-68
GENERATION 4 ~ GENERATION 8

PULMONARY INFECTION  CHRONIC NEPHritis

G4

8067 8 Mo.  8734 11 Mo.

G5

9322 8 Mo.  9860 10 Mo.  9410 10 Mo.  10803 12 Mo.  10312 10 Mo.

G6

9117 Intestinal Infection  11460 Intestinal Infection  11848 Chronic Nephritis  11574 Intestinal Infection  11674 Intestinal Infection

G7

11675 12 Mo.  11675 12 Mo.  11675 12 Mo.  12219 12 Mo.  12397 13 Mo.

G8

13510 10 Mo.  13522 10 Mo.  1697 23 Mo.  13288 10 Mo.  13752 11 Mo.

CHART 3
**STRAIN J.D. 30-62-66**

**GENERATION 10 - GENERATION 15**

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<th>G_12</th>
<th>G_13</th>
<th>G_14</th>
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**Typical Mendelian behaviour of the tendency to carcinoma of the thyroid, in cross between thyroid carcinomatous female and analysed immune male.**

**Chart 5**
SPONTANEOUS THYROID TUMORS IN MICE

servation until Oct. 30, 1916, when she died. See chart 5, generation 12. In this little family of forty members there occurred three malignant thyroid growths or 7.5 per cent of all deaths even including those which occurred in infancy.

Female 14959 died October 30, 1916, at the age of nine and one half months. She was the granddaughter of female 16917 (charts 3 and 4) and was dead before the appearance of thyroid tumor in her grandmother; thus showing that the susceptibility was in no way due to contact.

Female 16917 was born Aug. 9, 1915, and was never in contact with any of her grandchildren. Her tumor was first noted Mech. 15, 1917, four months before her death, and nearly five months after the death of her granddaughter 14959. She lived to be 23 months old.

Beginning with the appearance of thyroid tumors in female 14959, a more intensive study of this strain was carried on. At this time it seemed evident that susceptibility to malignant thyroid growths was being carried in Line II of this strain, as two of these tumors had occurred in direct descent, and therefore the attempt was made to see whether the mendelian method of inheritance applied.

The proof of the relation between heredity and the tendency to cancer immunity or susceptibility does not lie in securing the greatest possible number of cancers, as many seem to think, but rather, in establishing the fact that it is possible either deliberately to prevent or to occasion the occurrence of cancer, by selective breeding, where every other possible factor is under complete control. In order to do this, it is necessary to have strains of animals analyzed as to their individual cancer potentialities, and that live well into cancer age without intercurrent infections.

Female 16917, who later developed a mixed sarcoma-carcinoma of the thyroid, had been mated with non-tumorous male 13288, who died when about ten months old. None of their offspring showed any tumors. Two of them died, however, under six months, of intestinal infection. The others lived to be from seven to nine months old. Resistance to thyroid
carcinoma was here dominant over susceptibility to thyroid carcinoma.

Three pairs of these hybrids bore young, forming lines I, II, and III, shown in chart 5. No tumor of any sort has ever occurred in lines I or III, although they still persist in the laboratory, and the strain is now sixteen years old. This is another corroboration of the inheritability of cancer exemption. For sixteen years exemption from cancer has continued without a break in these families.

But in line II, from the mating of two hybrid carriers of the tendency to thyroid malignancy, female 13805 and male 13785, the next thyroid tumor occurred, in female 14959, with bilateral sarcoma-carcinomas of the thyroid. Note also the extracted cancer resistant line derived from this branch II.

Again, female 17394 of generation 13 (shown in chart 5), a hybrid-carrier daughter of female 14959 and male 17290, was mated with wholly unrelated male 13255. This male was an analyzed member of a Japanese waltzer strain wholly resistant to cancer of the thyroid and all other organs, strain J.D. 26-48. No tumor appeared in the first hybrid generation from this cross, generation 14 of the chart, although the members (being first generation hybrids) lived to advanced age for Japanese waltzers, that is from 20 to 27 months. The dominance of resistance over susceptibility to thyroid cancer is here again shown.

Two of these first generation hybrids were mated, female 22064 who died at 20 months of chronic nephritis and a hypertrophied heart, and male 23639 who lived to be 27 months old, dying of an unknown infection without cancer. Following this mating of these two hybrid carriers of cancer susceptibility, thyroid carcinoma occurred in the next generation in female 24843, who died when 13 months old. Thus there was again shown the typical mendelian behavior of the tendencies to resistance and to susceptibility to thyroid carcinoma.

Chart 6 shows the parental succession in direct descent of female 24843 from female 16917.

Again, female 24843 with carcinoma of the thyroid, was
CHRONIC NEPHRONAL INFECTION INTENTION INFECTION.

10 TO 15

GIO

SARC. CARC. THYROID INTEST. INFECT.

16917 X 13288

PULMONARY INFECT. INTESTINAL INFECT. CHRONIC NEPH INFECT.

13299 13805 13785 13254

INTESTINAL INFECT BI-LAT. SARC. CARC. THYROID UNKNOWN INFECT. PULMONARY INFECT.

14427 14959 17290 14389

UNCERTAIN CHRONIC NEPH. INTEST. INFECT.

19021 17394 X 13255

INTESTINAL INFECT CHRONIC NEPH. HYP. HEART UNKNOWN INFECT. WOUNDS

22845 22064 23639 22448

CHRONIC NEPH. CARCINOMA THYROID ACUTE AND CHRONIC NEPH WOUND CHRONIC NEPH.

21365 24843 23705 24383

Chart 6
STRAIN J.D. 44

Generation 15 - Generation 19

Carcinoma Thyroid

Acute and Chronic Nephritis

Puerperal Infection

Acute and Chronic Nephritis

Intestinal Infection

Chronic Nephritis

Chronic Nephritis

Intestinal Infection

Chronic Nephritis

Intestinal Infection

Chronic Nephritis

Intestinal Infection

Unknown Infection

Wounds

Intestinal Infection

Chronic Nephritis

Intestinal Infection

Chronic Nephritis

Intestinal Infection

Wounds

Extracted immune family derived from crossing thyroid carcinomatous female with immune male

Chart 7
mated with analyzed resistant male 23705 who died at nine months, of acute and chronic nephritis without tumor (shown in chart 7). In this branch, by the right selective mating of resistant members, no thyroid or any other tumors ever occurred. This again demonstrates the inheritability of cancer resistance, and the dominance of resistance to thyroid cancer over susceptibility; so that it is possible to derive a strain wholly free from cancer even where one parent is cancerous.

Whereas, by the crossing of the same carcinomatous female 24843 with analyzed immune male 24383 (shown in chart 8) by the right selective mating it was possible to secure three additional carcinomas and sarcoma-carcinomas of the thyroid gland within this little family. These were: (1) female 31909 of the 18th generation, eight and one-half months old, with a sarcoma-carcinoma of the thyroid like her progenitor 15 generations back, male 6219, and her progenitor 10 generations back, female 16917 (both shown in chart 4); (2) female 37841 (20th generation) dying at thirteen months with a carcinoma of the thyroid; and (3) her son male 39970 dying at ten months with a sarcoma-carcinoma of the thyroid.

In this part of the family, comprising 34 members, there occurred four cancers of the thyroid, or 11.7 per cent of all deaths, even including those which occurred in infancy. These four thyroid cancers occurred in almost perfect accordance with the expectation for a mendelian recessive. In the second hybrid generation (generation 17 in the chart) where an appearance of thyroid cancer might be expected, nearly all the members died under five months, so that the cancer potentiality of this generation was not perfectly tested, as thyroid cancer has not appeared under six months of age in any stock in this laboratory.

With the exception of generation 17, the occurrence of thyroid cancer shown in the chart is in classic form: namely, generation 18, showing the mating of cancerous female 31909 with resistant male 32556, produced only hybrid carriers, and no instance of malignancy; whereas from the mating of two of these hybrid carriers, in generation 19, thyroid cancer occurred in the 20th generation. Again, by the mating of a
cancerous female, 37841, with hybrid carrier male 35938, both of the 20th generation, thyroid cancer occurred in the 21st generation, in male 39970.

Chart 9 shows the succession of parents in direct descent from female 24843, through female 31909 and female 37841, to male 39970. The successive parents in this portion of the family, from generation 15 to generation 21, showed 28 per cent of thyroid malignancy.

Chart 10. Male 39970 (21st generation) with a sarcoma-carcinoma of the thyroid, was mated with hybrid-carrier female 39387 dying at nine months with chronic nephritis without cancer. By the right selective mating in each successive generation (after the 22d) of analyzed resistant individuals with hybrid carriers of susceptibility, all occurrence of cancer was deliberately held off for six more generations in each branch of the family, that is from the 22d to the 27th generation inclusive. In this 27th generation a double test was made to prove whether susceptibility to thyroid carcinoma had been carried through these six generations by this mating in each successive generation of analyzed resistant individuals with hybrid carriers.

In each branch of this 27th generation (shown in chart 10) two hybrid carriers of susceptibility to thyroid cancer were mated. Female 56881, dying when nine and one half months old of intestinal infection without cancer, was mated with male 54778 dying when six and one half months old with chronic nephritis without cancer. Three cases of thyroid malignancy occurred in the next generation (that is the 28th generation in the chart) from this mating; namely female 58774 dying at nine months with a sarcoma-carcinoma of the thyroid, female 58835 also dying at nine months with a carcinoma of the thyroid, and their sister still living, with a thyroid tumor well advanced at this writing.

While in the other branch (shown in chart 10) hybrid-carrier female 55601, 27th generation, dying at twelve months with chronic nephritis without cancer, was mated with hybrid-carrier male 55637 dying when twelve months old of intestinal infection without cancer. Their daughter still living at this writing, shows a thyroid tumor.
### Chart 10

<table>
<thead>
<tr>
<th>STRAIN J.D. 30 - 62 - 68</th>
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<tr>
<td>GENERATION 21 - GENERATION 20</td>
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<table>
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<th>Strain</th>
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<th>Status</th>
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<td>41977</td>
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<tr>
<td>6.27</td>
<td>Chronic Nephritis</td>
<td>61273</td>
<td>7.9 mo.</td>
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*Chronic Nephritis* | *Senile Atrophy* | *Acute Nephritis* | *Intestinal Infection* | *Unknown Infection* | *Living*
The other eight young in the 28th generation, offspring of female 56881 by male 54778, are omitted for lack of space. No one of them has developed cancer. This 28th generation shows to date, in the number of thyroid carcinomas, the perfect ratio for a mendelian recessive, that is three thyroid carcinomas to nine resistant. Some of these mice are still living, and the final ratio cannot be established until after necropsy of the entire generation. No other member of the 28th generation of the other branch shown in the chart has as yet developed cancer. Both these branches are under analytic study at the present time.

Résumé of Strain J.D. 30–62–68. (1) In a little family comprising 133 members in direct descent, deliberately bred through twenty-eight generations to see whether or not selective breeding could control the tendency to resistance and to susceptibility to thyroid cancer, there have occurred eleven malignant growths of the thyroid or 8.2 per cent of all deaths; thus showing that susceptibility to malignant thyroid growths is certainly carried in this strain.

(2) During these 28 generations there has never been a tumor of any other organ either malignant or benign, in any direct or indirect fraternity. Thus the strain has shown complete exemption from all neoplastic tendency except in the thyroid gland.

(3) From the mating of mice with thyroid carcinoma to resistant mice, no cancer has ever appeared in the first hybrid generation; thus showing that resistance to thyroid cancer is dominant over susceptibility.

(4) From mating two first generation hybrid carriers of malignant thyroid susceptibility, thyroid cancer has occurred in the next generation; thus showing the tendency to thyroid cancer to be a true mendelian recessive.

(5) By the continued mating of mice resistant to thyroid malignancy, with hybrid carriers, it has been possible to hold off the occurrence of malignancy as many generations as desired. This reemphasizes the fact of the relation of heredity to resistance and to susceptibility to thyroid cancer; and also reemphasizes the dominance of resistance over susceptibility.
After holding off the occurrence of thyroid cancer as long as desired, that is for six generations, by mating dominant resistant individuals with what were apparently hybrid carriers, in each successive generation, it was possible to occasion thyroid cancer again by mating two hybrid carriers, thus showing that the hereditary potentiality was carried through these six generations. This places beyond doubt the relation of heredity to the occurrence of resistance and susceptibility to thyroid malignancy; and it is in complete harmony with the results shown in all other tests of all other types and locations of tumors made in this laboratory for the past eighteen years.

To attribute this classic occurrence of a neoplasm so rare as thyroid cancer, in one strain of mice deliberately bred for that type of tumor, either to chance or to some assumed outside environmental influence playing only upon this one little family would be entirely unreasonable. There is no evidence whatever to support such an opinion.

As regards chance: these are the only mice ever reported with malignant thyroid growths among the thousands of mice used in medical research. It would strain credulity to assume that chance could place eleven of them in one small family. Moreover the rate of thyroid malignancy in human cancer is low. Thyroid cancers, according to our best knowledge, probably cause less than 1 per cent of all deaths from cancer. Whereas in this family deliberately bred for thyroid cancer, thyroid malignancy caused 100 per cent of the deaths from cancer.

In the human species, where chance breeding has full sway, thyroid cancer causes an exceedingly small fraction of 1 per cent of all deaths; whereas in this family of 133, where the breeding was carefully controlled in the study of thyroid cancer, it caused 8.2 per cent of all deaths at all ages even including infant deaths.

Most important of all as an argument against chance determining these eleven thyroid cancer deaths in a family of 133 members, is the fact that the occurrence of thyroid cancer was in perfect accord with the classic expectation for a hereditary mendelian recessive, and was evidently determined by selective breeding.
Environmental Conditions: The stocks of Japanese waltzers in my laboratory are peculiarly homogeneous in every detail of their handling and of the outside environmental influences which might affect them.

Housing: Because of the extreme delicacy of waltzing mice and their hypersusceptibility to infections, all waltzers are kept in wooden boxes 12 x 6 x 6 inches in size. These boxes have firmly fitted top covers of netted wire. They thus protect the mice from draughts and at the same time permit the entrance of light and air. All dancers are kept in the same room at the southwest corner of the laboratory which receives direct sunshine both from the south and the west. All boxes are so placed as to receive direct sunlight at some period of any day on which the sun shines, and no particular family has advantage or disadvantage in this respect.

Routine Protection against Infections: As a preventive of infections, the mice are changed into clean sterilized boxes twice a week, and the equipment of the boxes, of course is also changed. These mice pass under my personal observation daily and (except occasionally by my first assistant) are handled by no one else.

Diet and Water: They are watered once a day with water which is boiled and filtered and placed in sterile dishes. Their diet consists of thoroughly toasted fresh bread well moistened with doubly pasteurized whole milk; mixed birdseed (millet, hemp and canary); and timothy hay. No Japanese waltzer ever in my laboratory during the past twenty years, has had any other article of diet, or any variation in the routine. I personally feed and water these waltzing mice so that the routine of their care is not handled by dieners. All of these mice are fed from exactly the same general supply of food and water on each day. There is therefore no item of the placing of the cages or of the diet or water either in its content or its routine of supply, which could affect the incidence of cancer or any other disease.

These results sharply conflict with the conclusions of Burrows (14) who takes no account whatever of heredity as a causative
factor in cancer. He seems to think that the presence of what he calls "the archusia" or growth factor and identifies as vitamin B, together with the absence of "the ergusia" the growth-inhibiting factor, which he identifies as vitamin A, is the direct and universal cause of cancer growth. His conclusions are based upon studies of cancer tissue growing in vitro.

About 75,000 mice have been handled in my laboratory during the past twenty years. All have had diets identical in every item, with the exception of relatively few mice concerned in diet experiments. Seventeen have had thyroid cancers; over 74,975 have not had thyroid cancer. Of these, only six have ever shown even simple enlargement of the thyroid by retention of colloid. It is difficult to see what rôle vitamins A and B could have played as a causative factor determining either for or against thyroid cancer growth among these 75,000 mice. Certainly they cannot have played the exclusive rôle which Burrows assigns them.

The only rôle which could possibly be assigned to them would be an inability on the part of the mouse to use these two vitamins in the right proportion, although they were fed in the right proportion. This inability would have to be entirely local, since hundreds of mice in this laboratory with breast and other cancers have borne several litters of mice normal in every way, and suckled them to normal development; (15) thus proving that all functions in the mother were going on normally, even the function of normal secretion in those mammary tissues not involved in breast cancers. This inability would be of indefinite age occurrence, since cancer has occurred in my stock from age 12 days to age 5 years. But it would not be born with the mouse, since no mouse in this laboratory has ever had cancer at birth. Very few have developed cancer under eight months of age.

We would have to assume then a sharply localized inability to use vitamins A and B in the right proportion, although the animals were fed in the right proportion and all other functions apparently were normal. Such inability would be liable to arise at any age from twelve days to five years from an entirely
undetermined cause evidently resident in the constitution of the mouse but not born with it, and of such a nature as not even to affect breast secretions in general, where the mouse has a well developed breast cancer. This seems to be an unreasonable assumption.

I quote from Burrows as follows: "Cancer is a disease which must be prevalent in an undernourished race and one which suffers from substances and conditions capable of removing vitamin A from their tissues. It must disappear when the nutrition of this race is improved, they cease to be slaves of fashion, have protected themselves against improper drugs, abuses of certain trades and freed themselves from diseases such as syphilis, which cause undernutrition."

None of this applies in any degree whatever to the spontaneous cancers which have originated in this laboratory, numbering now between 5000 and 6000 and which must be accounted for by any correct theory of cancer. As I have repeatedly stated, the mice bearing these spontaneous tumors are among the largest and most perfect specimens in the laboratory. They are not the undernourished. They are quite obviously not the victims of any of the abuses enumerated by Burrows. They perform all their functions including the bearing and suckling of normal young, with a normal life span and normal reproductive potency. They do not in any respect fall within the category described by Burrows. I would suggest that there is no evidence to warrant assumptions of a complete analogy between tissue cultures of cancer, and spontaneous cancers arising and developing in a living organism all of whose functions are complexly and vitally interrelated. This complex interrelation must be taken into account in any theory of the nature of cancer. The controlling influence of heredity in determining the occurrence, type and location of cancer is indisputably proved by this study of the incidence of thyroid cancer. This fact also must be taken into consideration in any theory of the nature of cancer and of the stimulus for its growth.

Age: A glance at the charts will show that where the line is not being run out by inbreeding, these mice live to a ripe age.
for Japanese waltzers. The average age of the mice that have not shown cancer in this family is 10.6 months. This includes even those that died well under cancer age. Of those that lived to cancer age but did not have cancer, the average age was 11.3 months. The average age of those with thyroid cancer was 11.6 months. The average age therefore of those dying with thyroid cancer and those living to cancer age but resistant to malignancy, was almost identical. Age therefore is shown not to have been a factor in determining susceptibility to thyroid cancer in some of these mice, and exemption in others.

These thyroid tumors usually appear by the time the mouse is six or seven months old. Therefore the average age of 10.6 months for the entire group of non-tumorous mice, carries them well within the age of the usual origin of thyroid malignancy in the Japanese waltzers of my stocks.

Vermin: No waltzer in this stock has ever been found to have lice. If lice are present they are always evident at the ends of the hairs after the death of a mouse. As every mouse is autopsied by me, the presence of lice could not fail to be detected. The boxes which house the waltzers, and the table upon which they stand, are kept as free as possible from bedbugs, cockroaches and all other vermin. But when such vermin have appeared, they have in no wise selected the boxes in which have been housed the mice that have developed tumors. Indeed at the peak of incidence of these tumors, that is at about the present time, there are no vermin in the waltzer stocks. There have been, during the past four months, five thyroid cancers in this one family, J.D. 30–62–68; two of these mice with thyroid tumors are now living.

A glance at the charts will also show the narrow range of death causes in these Japanese waltzers thus protected from infections. Pulmonary infection and intestinal infection in the young, acute and chronic nephritis, senile changes and cancer in the older mice are almost the only causes of death ever occurring in these waltzers, and their death rate is very low. Frequently many days or even weeks pass without a death among the Japanese waltzers. There is thus an almost uniform
opportunity for every potentially cancerous waltzer to develop a cancer.

The actual incidence of thyroid cancer may therefore pretty safely be taken as the true possible incidence of thyroid cancer in all mice of this family concerned in the breeding, that lived to seven months or over, after the intensive study began in the 8th generation. There would, therefore, seem to be no item in the age, the environmental conditions, or the routine care of these mice, which could in any way explain the incidence of thyroid malignancy in strain J.D. 30–62–68. Whereas, the facts exactly parallel the required classic facts for hereditary control. By selective breeding, this little family of waltzers has carried susceptibility to thyroid cancer for fourteen years in my hands.

These malignant thyroid tumors grow rather slowly. The skin of the mouse is very loose over the entire ventral surface of the body and allows for a considerable extension of any subcutaneous growth. Even in the thyroid region there is room for considerable growth before the tumor becomes fatal; so that most of these mice with thyroid cancer live several months after the appearance of their tumors.

No evident systemic changes have accompanied any of these thyroid tumors, and death is usually sudden, with no preliminary illness. These mice with thyroid tumors often bear and nurse young up to the time of their death. Females 58774 and 58835 were nursing litters at the moment of their death. These young are now well-grown normal mice. In this respect also thyroid neoplasms in this stock are similar to other neoplasms under study in this laboratory; that is, they seem not to cause fatal systemic changes.

The thyroid tumors which have appeared in other stocks than the Japanese waltzers, were as follows: 2 adenomas in strain 148; 2 malignant growths and 1 simple goiter in strain 327 and derivatives; 2 malignant growths in strain 416; 3 goiters and 2 malignant growths in strain 90 derivatives. As strain 327 is a derivative of strain 148, 2 malignant growths, 2 adenomas and 1 goiter were derived from the same source.
As strains 416 and 90 are related, the remaining 4 malignant tumors and 3 goiters came from the same original source. In none of these strains was the selective breeding designed to test fully the occurrence of thyroid tumors, as it was in the Japanese waltzers. In strains 148, 90 and derivatives, the tests in the main were directed toward other types and locations of tumors.

All of the thyroid enlargements, then, including the malignant growths, the papillary adenomas and the simple goiters, have come from three strain sources: namely, strain 148, strain 90 and strain J.D. 30–62–68. In all the other hundreds of strains carried in this laboratory for twenty years, involving at least 50,000 mice, there has been complete exemption from all enlargements of the thyroid. In other mice used in medical research throughout the world, there has apparently been complete exemption from thyroid malignancy, as no case is anywhere reported. It is therefore impossible to deny the influence of heredity in determining the occurrence of thyroid cancers; especially with the careful control in every controllable feature, under which this study was carried on.

As has been emphasized in previous reports from this laboratory, there are apparently two factors necessary for the occurrence of cancer: first, hereditary predisposition, which has amply been demonstrated in this and previous studies; and second, chronic irritation or stimulation or trauma of the appropriate kind and degree, applied to the cancer susceptible tissues. This is a more difficult thing for ample demonstration, since complete biologic control is very difficult.

Ewing (16) in his study of human thyroid tumors makes no definite decision as to the nature of the irritation factor in human thyroid overgrowths. It is very difficult to assign this chronic irritation factor to anything definite in the cases of thyroid malignancy in my stock. None of the mice with thyroid overgrowth had been subjected to any gross lesion at any period of his life. In only two cases was there any evidence of the possibility of lesions of infective origin; male 38255, a member of strain 148, with an abscess at the base of the right
ear, and a colloid goiter on the left side; and Japanese waltzer female 37973 of J.D. 30–62–68, with an abscess of the left jaw and a carcinoma of the left thyroid. The thyroid carcinoma in this case, however, antedated the abscess on the jaw.

One case, female 20199 showing goiter, had also a carcinoma of the mammary tissues in the inguinal region, metastasizing in the lungs; and pseudoleukemia involving the lymph nodes of the neck. This mouse was a member of strain 148, not a Japanese waltzer. Although the irritation factor cannot be assigned exactly in these mice, it is of interest that the one strain showing a very high percentage of thyroid malignancy should be a strain of Japanese waltzers. All Japanese waltzers constantly hold the muzzle raised, so that the neck tissues are chronically in a somewhat stretched position. In addition to their upward stretching of the neck, there is also typical of Japanese waltzers, a constant vibratory motion of the head upon the throat, both up and down, and sideways. This might occasion chronic stimulations both physical and physiological of the thyroid glands. In this typical position and constant motion of the head, there might conceivably lie the chronic irritation or hyperstimulation factor in the causation of thyroid overgrowth in Japanese waltzers. This position and motion however, is typical of all Japanese waltzing mice. Only in those of hereditary predisposition has thyroid malignancy or even simple goiter occurred, and this in classic form.

Careful studies in the causative irritation factor in cancer are greatly needed. All such studies should however be carried on with animals analyzed as to their cancer potentiality, so that the hereditary factor is carefully controlled. With such animals, analyzed as to their cancer potentiality, and with the selection of animals whose hereditary tendencies are appropriate to the test, it should be possible to find the irritation causative factor in cancer. Some such studies are now under way in this laboratory.

SUMMARY

Among 61,700 necropsies performed upon this stock, comprising between 5,000 and 6,000 malignant neoplasms, there
have occurred 23 thyroid enlargements; 4 simple colloid goiters, 2 papillary adenomas, and 17 malignant growths.

Careful control was maintained of all outside environmental conditions, such as housing, exposure to sunlight, diet and water, age, vermin, etc. These environmental influences are thus eliminated as causative factors in the occurrence of thyroid cancer.

Among the hundreds of strains represented in these necropsies, only three have shown any thyroid enlargements of any kind whatever, viz. strains 148 and derivatives, 90 derivatives, and strain J.D. 30–62–68.

Eleven of these malignant thyroid growths have occurred in one small strain of Japanese waltzing mice, consisting of 133 members, strain J.D. 30–62–68, bred for thyroid malignancy. These eleven thyroid cancers have occurred in classic accordance with the laws of heredity for a mendelian recessive; thus heredity is shown to be an unquestionable factor in the occurrence of these thyroid cancers.

The tendency to resistance to thyroid cancer is shown to be dominant over the tendency to susceptibility, and the mendelian ratio is closely approximated. Thus the results of this study are in every respect in exact accord with all other studies of cancer heredity in this laboratory, which have concerned themselves with organs other than the thyroid.

Just as in human thyroid cancer, so also in mouse thyroid cancer, it is difficult to assign the irritation causative factor. One strain of Japanese waltzers, deliberately bred for thyroid cancer, has shown the highest percentage of thyroid cancer ever reported in any family of any species, that is 8.2 per cent of all deaths at all ages.

It is suggested that the constantly uplifted, somewhat strained position, and the vibratory motion of the head upon the throat, typical of all Japanese waltzers, may be the causative irritation factor in these mice, as this constant motion and tense position might reasonably be expected to stimulate hyperactivity both physical and physiological of the thyroid glands.
SPONTANEOUS THYROID TUMORS IN MICE

REFERENCES

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