THE RELATION OF HEREDITY TO THE OCCURRENCE OF SPONTANEOUS LEUKEMIA, PSEUROLEUKEMIA, LYMPHOSARCOMA AND ALLIED DISEASES IN MICE: PRELIMINARY REPORT

STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE: 28TH REPORT

MAUD SLYE

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

The literature does not contain any definite studies in the relation of hereditary predisposition to the occurrence of spontaneous leukemic diseases, and therefore no accurate and detailed comparisons can be made between the results of this pioneer work and any similar studies.

Familial occurrence in human leukemia has often been noted, and numerous case reports can be found in the medical literature. For example, Schereschewsky (13) reports the cases of a brother and sister, aged sixty-one and fifty-four respectively, with chronic lymphatic leukemia. He refers to the report by Weiss of a family in which a brother and sister had chronic lymphatic leukemia and another brother had acute lymphatic leukemia; also a report by Rosenow of chronic lymphatic leukemia in a mother and son. Hirschfeld (4) cites an instance of a man, aged sixty-seven, with aleukemic leukemia, and a brother, aged seventy, with typical lymphatic leukemia. McGovran (8) reported three cases of leukemia in one family, one of them myelogenous and two lymphatic. Richards (10) recorded two cases of lymphatic disease in one family, one lymphosarcoma, the other lymphatic leukemia. There is also the family reported by Braun (1) in which two brothers and a sister, all in the forties, died within a period of six years of malignant lymphoma, pseudoleukemia, or sarcoma. The recorded cases of such familial occurrence, however, are so scanty

1 Read before The Society for Cancer Research, Chicago, April, 1928.

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as to suggest that they might represent mere coincidence. More striking is the report by Dameshek, Savitz, and Arbor (3) of identical male twins, fifty-six years old, who died only sixty-eight days apart of chronic lymphatic leukemia.

Of significance in regard to the relation of leukemia to other neoplastic conditions is a family, two members of which were recently examined post mortem by H. G. Wells. One, a man of sixty-one, died of classic lymphatic leukemia. His sister, who died seventeen months later, had exhibited numerous soft tumors in various bones, especially the skull and the left shoulder girdle, for five years before her death; the number of recognizable bone tumors being estimated at about thirty. These all disappeared completely under repeated roentgen-ray treatments. At autopsy there was found extensive invasion of the retroperitoneal lymph nodes by a growth of small round cells, resembling lymphosarcoma, which also infiltrated the kidney. No visible bone growths were present, even in the vicinity of the pathological fractures, which had healed under radiation. It was learned that another brother had died twelve years before, with a diagnosis of cancer of the stomach. An operation, gastro-enterostomy, had disclosed, as the operation record stated, "a saddle-shaped carcinomatous ulcer upon the lesser curvature of the stomach near the pyloric end. There is a large mass of firm glands in the gastro-hepatic omentum." No microscopic examination or post-mortem examination was made. Two sisters of this family were operated upon for uterine fibroids at the ages of thirty-five and fifty-five years respectively. There were no other known instances of neoplastic conditions in the immediate members of this family. The father had died at seventy-one, the mother at ninety-four, supposedly of pneumonia and myocarditis respectively, but post-mortem examination had not been made.

Although the data given in these reports of human leukemias are few, they are in agreement with the facts demonstrated in this laboratory: first, that the leukemic diseases have an hereditary basis and tend to run in families; second, that they tend to occur in cancer strains.

Some general observations have been made in regard to lymphoid hyperplasias in rodents. Murray (9), in his early observation on the relation of heredity to cancer occurrence in mice, noted eight cases described as lymphoma, all occurring in the descendants of two female mice with mammary gland carcinoma.
Bullock and Curtis (2), in their general discussion of tumors of the rat, state that thymus tumors in their stock, although one of their largest groups of tumors, occurred only in their Copenhagen strain of rats, which was a small strain. Here, in studies of both mouse and rat lymphoid hyperplasias, we find points in general confirmation of my own findings.

Throughout my studies in the incidence and inheritability of spontaneous neoplasms in mice, leukemic diseases have always been included. As early as the second report (15) mice with leukemic diseases were included, both in the charts and in the discussions, among those showing neoplasms; and they have continued to be so included from that date to the present time. In the third report (16) I made the following statement: "Throughout my entire work, the leukemias and pseudoleukemias have fallen almost wholly within proved cancer strains. Leukemic individuals have transmitted cancer with the same certainty as have cancerous individuals, in the cancer strains." In all percentages given of neoplastic diseases, the leukemias have consistently been included, and throughout the entire series of publications, statements such as that quoted above have been made. In the 16th report (18), which was a study of the relation of heredity to the occurrence of secondary neoplasms in analyzed strains of mice, I stated: "Leukemias and pseudoleukemias, occurring in the cancer strains, pick out predominantly the same organs for infiltration which show the primary and the secondary tumors of that strain." Again, in the 18th report (19), the following statement is made: "It is interesting to note that in the animals of this stock, chronic leukemia and pseudoleukemia (lymphatic leukemia, not lymphogranulomatosis) have occurred only in the cancer strains, and have behaved as if they were true neoplasms. . . . Chronic leukemia and pseudoleukemia are as much true neoplasms as are lymphosarcomas."

McDowell and Richter (7, 11, 12) have recently published their reports concerning hereditary susceptibility to inoculated leukemia. They conclude that susceptibility to inoculated leukemia is a dominant. They state that two different lines of inoculated leukemia give different proportions of susceptible mice. This finding suggests the possibility of pathologic differences between these two lines of leukemia or differences in technic. The writers do not report success in grafting myelogenous leukemia or pseudoleukemia. Their only reported successes were with lymphatic leukemias. Our own attempts to graft myelogenous leukemia
(few in number) were not successful. The possibility of pathologic differences in their two lines of leukemia is emphasized in their own findings by the fact that they found no leukemic changes in the blood of some of their animals counted as susceptible to leukemia. The success of their inoculation of lymphatic leukemia undoubtedly depends upon actually introducing into the host the lymphoid cells themselves, which would constitute a graft.

It is interesting to note the similarity between the McDowell-Richter results in grafting lymphatic leukemia, and the Tyzzer-Little results (6, 23) in grafting carcinomas. The latter studies have been discussed at length in previous reports in this series. Both the experiments of McDowell and Richter and those of Tyzzer and Little involve the grafting of cells from a neoplastic growth into a normal mouse. In both cases susceptibility to the graft is reported as dominant, and apparently in the opinion of both sets of workers there may be multiple factors or linked genes involved.

As I pointed out (20) in discussing the Tyzzer-Little results, susceptibility to grafted cancers has as its most important bases (1) the ability of the assaulted tissues to regenerate normally and quickly; (2) ability of the host to support both itself and the grafted growth. At that time I called attention to the fact that the fundamental basis of susceptibility to grafts—that is, the ability of the assaulted tissues to regenerate normally—is very different from the fundamental basis of susceptibility to spontaneous neoplasms; as the basis of susceptibility to spontaneous neoplasms is the tendency of the assaulted tissues to regenerate abnormally in the uncontrolled and undifferentiated method of the neoplasm. A grafted tumor or leukemia is in the nature of a "parasite" growing in a foreign host. It is the cells of the graft that proliferate, not the cells of the host. A spontaneous neoplasm is the result of the cell proliferation of the tissues of the animal itself, and is the outcome of its own metabolism. Grafted neoplasms, including leukemias, are then foreign cells introduced into normally regenerating hosts, and producing the neoplasm or leukemia by dividing there, not by causing division in the host cells. This is a very different matter from division in the cells of the host itself.

We would therefore logically expect that the inheritance behavior of these grafted neoplasms and leukemias (that is, the behavior of graft acceptance by normal mice) would be different
from the behavior of spontaneous neoplasms or leukemias, which are the result of abnormal proliferation in the tissues of the host itself. That is, normal proliferation is dominant over abnormal proliferation. All studies in grafted neoplasms published to date, as well as these studies in spontaneous cancers and leukemias, have shown this dominance of normal over abnormal proliferation.

These, then, are the facts that have, to date, uniformly been found in the studies of these two different types of neoplastic growths. It is interesting to find this consistently logical difference in the heredity behavior of spontaneous neoplasms, including leukemia, on the one hand, and of grafted neoplasms and leukemias on the other.

Krebs, Raasch-Nielsen, and Wagner (5) have recently published their report on the origin of these leukemic diseases. In explaining the leukemic infiltration in organs of animals with implants, they give four possibilities:

"(1) That the implanted cells are carried around in the organism and continue their growth in whatever locality they finally settle

"(2) That the tumor contains a microbe

"(3) That the tumor contains a toxic substance, produced by the implanted tissue, which, by spreading through the organism by way of the blood or lymph circuit, provokes the pathologic proliferation of the tissues

"(4) That in some animals the disease occurs spontaneously, independently of the implantation."

Against the first of the possibilities, they make the following points:

"(1) The peculiar manner in which the proliferation of the pathologic tissue takes place

"(2) The fact that, in some cases, enormous accumulations of round cells are found without it being possible to demonstrate the existence of a single white blood corpuscle in the vessels involved

"(3) The fact that round cell accumulations are not infrequently found surrounding even very thick-walled, to all appearances uninjured arteries

"(4) The predilection of the lymphoid proliferations for certain organs

"(5) The invariably symmetrical occurrence of round cell accumulations in double organs (kidneys and lungs)

"(6) The fact that the round cell accumulation very often is
found where metastases from malignant tumors rarely or never are seen, such as the renal pelvis and the spleen."

My own conclusions are in agreement with their first "possibility," namely that "the implanted cells are carried around in the organism, and continue their growth wherever they find lodgment." All of their objections to this explanation vanish, if we remember that it is not the cells of the host but only the engrafted cells that divide and proliferate wherever they lodge and find suitable conditions for growth.

Early in my work there began to appear instances of spontaneous leukemia, both myelogenous and lymphatic, and of the allied diseases, pseudoleukemia and lymphosarcoma. The first occurrence of pseudoleukemia was in female 5, which died March 24, 1911, of carcinoma of the mammary gland metastasizing in the lungs, and pseudoleukemia. She was a member of strain 190, which yielded many cases of sarcoma and of carcinoma, as well as instances of the leukemias, though neither of her parents had leukemic disease. Her mother, female 3, died of mixed sarcoma-carcinoma of the mammary gland, malignant adenoma of the liver, and sarcoma metastases in the kidneys. The ancestry behind female 3 is unknown to me, as she is the first member of the strain in my hands. The father of 5, male 360, died of uncertain causes without neoplasm, but he was a heterozygote, as his mother, 411, was non-cancerous and his father, 436, died of carcinoma of the lung. Cancerous female 5, therefore, was the daughter of cancerous mother 3 by heterozygous non-cancerous father 360.

The first occurrence of lymphatic leukemia was in female 6, a member of strain 143, which died March 25, 1911, of carcinoma of the mammary gland, carcinoma of the mediastinum, and leukemia.

The first occurrence of lymphosarcoma was in female 157 of strain 196, which died July 31, 1911, of mediastinal lymphosarcoma. Her mother, 94, died of puerperal infection without cancer or leukemic disease. Her father, 18, died of pseudoleukemia.

Following 157 was female 180 of strain 190, dying of pseudoleukemia. Strains 143, 190, and 196, of which all the foregoing mice were members, were all derived from strain 90.

The next occurrence of any leukemic disease was in female 246 of strain 60, which died September 1, 1911, of myelogenous
leukemia. Following her was female 293 with carcinoma of the mammary gland and lymphatic leukemia, dying September 14, 1911. This mouse was a member of origin strain 139, unrelated to any of the foregoing strains, which showed 100 per cent of neoplasms in all mice living to be more than seven months old. Neither of the parents of 293 had any leukemic disease. This strain 139 has repeatedly been reported and the charts published in this series.

Female 293 was the original female of both strains 146 and 164, parts of which are included in this preliminary report. I include in this paper parts only of these two strains, because in all crosses which I have made, I have tried to extract not only cancer lines and heterozygous lines, but also non-cancerous lines. Both strains 146 and 164 have yielded non-cancerous lines (lines which from their beginning have never to date produced any neoplasms, either malignant or benign, or any leukemic diseases of any sort). These non-cancerous lines have not been included in this report, as I herein seek to show the occurrence of leukemic diseases, and hence include only the lines which furnished them, namely the cancer lines. Both strains 146 and 164 are classic in my serial reports, and parts of each have been published repeatedly. Thus, very early in this work, all of the various leukemic diseases appeared frequently and in cancer strains only.

During the fourteen years between March 24, 1911, when the first case came to autopsy, and September 28, 1925, the date of the 50,000th autopsy in these studies, there occurred a total of 975 cases of leukemia, pseudoleukemia, and lymphosarcoma. This number does not include any cases of lymphogranulomatosis resembling Hodgkin's disease, nor any lymphoid hyperplasias of uncertain classification. Consideration and classification of these latter diseases in my stocks awaits a later date. I have not, up to the present, attempted to line up the leukemias between the 50,000th and 93,000th autopsies. This, also, I hope to do in due time.

Of the 975 cases which occurred during the first 50,000 autopsies, 499 were in females and 476 in males, this being almost an equal distribution between the sexes. There is, therefore, no possible sex limitation to be considered in the leukemic diseases, and each mouse can be classified by what he actually shows and not by conjecture. All of these 975 individuals were members of strains that showed other malignant diseases besides the leukemias;
and 149 of them had other malignancies in addition to their leukemic disease. Of this total of 975 cases,

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>1st 10,000 necropsies</td>
<td>202</td>
<td>2.02%</td>
</tr>
<tr>
<td>2nd</td>
<td>272</td>
<td>2.72%</td>
</tr>
<tr>
<td>3rd</td>
<td>233</td>
<td>2.33%</td>
</tr>
<tr>
<td>4th</td>
<td>152</td>
<td>1.52%</td>
</tr>
<tr>
<td>5th</td>
<td>116</td>
<td>1.16%</td>
</tr>
</tbody>
</table>

Total 975 or slightly under 2% of all deaths at all ages in the first 50,000 autopsies.

Throughout my experience there has never been a case of any of the leukemic diseases which has arisen in any family not showing other forms of malignancy. Possibly they could be so bred out as to show no other forms of malignancy.

On the other hand, there have been very many strains in which there has never been an occurrence of the leukemias. For example, in all the families of Japanese waltzers carried in this laboratory for the past twenty years there has never been a case. This includes the six origin strains of waltzers reported in my study of thyroid malignancies (22) and all their inter-hybridized derivatives. This means that no waltzer in my laboratory, no matter what his origin, has ever shown any of this category of diseases, not even a generalized lymphoid hyperplasia. No strain of house mice in my hands, and no tramp house mouse autopsied in this series, even those bred out for years in the laboratory (amounting to some hundreds of mice), has ever shown a leukemic disease. No Peromyscus (the wild whitefoot common all over the United States) of any species in this laboratory has shown a leukemia.

So far as my stock is concerned, this limits the occurrence of the leukemias to laboratory Mus musculus derivatives, and to strain 90 and its derivatives. The species origin of this strain 90, I do not know. My strain called 90 was derived from grey-white piebald, white-footed mice brought direct from Japan about 1908. Its origin in all probability was Mus musculus derivative, as it hybridizes most readily with all of these stocks, making very vigorous and fertile hybrid derivatives. It is a strain repeatedly reported in this series, highly cancerous, and one which has originated nearly all of the families showing internal malignancies in my stocks.

Even among Mus musculus derivatives, there are hundreds of
families in which no leukemia, pseudoleukemia, or lymphosarcoma has occurred.

From the foregoing data, a few facts appear with regard to the occurrence of these diseases:

1. All instances of leukemia, pseudoleukemia, and lymphosarcoma have been in families carrying other forms of malignancy. That is, these diseases have uniformly occurred in cancer strains only.

2. Whole races of mice persisting for years in the laboratory, such as *Mus musculus*, Japanese waltzers, and wild Peromyscus secured from many localities from the Atlantic to the Pacific coast, have been wholly free from these diseases. This includes all families of these stocks, both those that have had other malignant diseases and those that have been altogether free from malignancy.

3. All occurrence of these diseases, then, has been in a limited number of cancer strains of laboratory *Mus musculus* derivation. In my stocks these have not been diseases which have occurred sporadically in practically any strain.

These facts furnish valuable negative evidence for the inheritability of the tendency to leukemic diseases. These diseases have definitely been confined to certain cancer strains, and in my stocks have occurred nowhere else. Data for the negative evidence of the relation of heredity to these diseases are easy to be sure of, because nothing resembling these diseases has ever occurred in any of the stocks reported free from them.

On the positive side there is far greater chance for error in inclusion or exclusion. Pathologists do not agree in their diagnoses of these diseases. The criteria as to which cases are genuine leukemia and which are inflammatory conditions involving marked leukocytic invasion, are not as accurate as we could wish; especially when, as in this work, it has not been possible to study blood smears, and the diagnosis has been made from histological preparations. Also the criteria which distinguish pseudoleukemia from lymphosarcoma on the one side, from lymphatic leukemia on another, and from inflammatory changes which involve definite lymphoid hyperplasias, are still defective. Possibly the genetic test may ultimately be found valuable as a final one, and as a help in settling some of the disagreements in diagnosis.

This difficulty in being sure of the diagnosis has delayed my report regarding the relation of heredity to the occurrence of the
leukemic diseases, so that it is now over nineteen years since the first occurrence in my laboratory of any of these diseases.

The types of leukemic diseases found in the Slye stock have been ably discussed by Simonds (14), who made a special pathologic study of the cases occurring in the first 15,000 necropsies. It will therefore be unnecessary in this paper to discuss the histology of these diseases as they occur in my stocks.

**EXPLANATION OF CHARTS**

**CHART 1:** Chart 1 shows part of strain 146, Branch I, with ancestry. The parent female of this branch of the strain was 236, dying of uterine infection without tumor. The parent male of the branch was 2160, dying of an angiosarcoma of the mammary gland. The mother of these two mice was 529, with a mixed sarcoma-carcinoma of the mammary gland, with secondaries in the lungs. The father was 242, dying of uncertain causes without tumor; he was, however, hybrid non-cancerous. He was derived from female 1 with a carcinoma of the mammary gland. From such a mating, that is cancer × heterozygote, only two types would be derived, cancerous and hybrid non-cancerous. Female 236, therefore, was a hybrid non-cancerous mouse. Her grand-

1 Some of these charts have been published previously in this series of studies, and they will be published again; as it is necessary that every new criterion that arises be applied to these analyzed strains.
mother, previously referred to, 293, died of a carcinoma of the mammary gland and lymphatic leukemia. It was here that leukemia entered this family. Note the occurrence, in this branch of the strain, of the three types of malignancy bred in: unmixed sarcoma in female 3626, unmixed carcinoma in females 3194 and 4730, and general lymphosarcomatosis in addition to carcinoma of the mammary gland in female 3194. In the second generation, note the persistence of the three types of malignancy: pure sarcoma in male 7780 with a subcutaneous sarcoma, metastasizing in the lungs; pure carcinoma in females 8304 and 5418, and a lymphosarcoma of the thymus ¹ in male 5836.

**Resume of Chart 1:** From this mating of hybrid non-cancerous female 236, with cancerous male 2160 (both probably carrying leukemic disease potentially), there were derived three non-cancerous to three cancerous (one with leukemic disease), which would be the expectation for neoplasms as recessives. In the second generation, in each case, from the mating of a hybrid non-neoplastic mouse with a neoplastic mouse (one with general lymphosarcomatosis), there were again derived 50 per cent hybrid non-cancerous and 50 per cent cancerous individuals (including one lymphosarcoma). This again would be consistent with the expectation for neoplasms as recessive.

**Chart 2:** Chart 2 shows Branch III of this same strain 146. The parent female of this branch of the strain was 1145, dying with a carcinoma of the mammary gland. The parent male was 3426, dying of a carcinoma of the lungs. We have here an instance of double cancerous parentage, one parent having carcinoma of the mammary gland and the other carcinoma of the lungs. These two mice came, of course, from the same parentage and grandparentage as those shown for Branch I of this same strain, given in Chart 1; that is, parent female 529 with a mixed sarcoma-carcinoma of the mammary gland metastasizing in the lungs, grandmother 293 with leukemia and mammary gland carcinoma, and grandfather 274 with primary carcinoma of the lungs.

The resulting strain, from crossing carcinoma of the mammary gland by carcinoma of the lungs, was 100 per cent malignant in all mice that lived to be over seven months old. Note the occurrence here of pure carcinoma of the mammary gland, females

¹ The designation “lymphosarcoma of the thymus” in this paper refers to round-cell tumors arising in the upper mediastinum in a location suggesting thymus origin, but with recognition of the fact that by the time such tumors have reached lethal dimensions, their exact point of origin is obscured.
pure carcinoma of the lungs, female 3388; and the absence from this branch of the strain of pure sarcoma. Note the two cases of lymphosarcoma: female 4167 with lymphosarcoma of the entire subcutaneous tissues, the spleen and kidneys, and, in the second generation, female 4520 with lymphosarcoma in the inguinal subcutaneous tissues, the pelvis, and near the spine. There were also two cases of pseudoleukemia, female 3933 in the first filial generation, and female 5244 in the second.

Resumé of Chart 2: In this branch of the family from the mating of two cancerous parents, with cancer in different organs, 100 per cent of malignancy occurred. The leukemic disease introduced by grandparent 293 was followed by four cases of leukemic disease within the next two generations. Evidently 1145 and 3426 were heterozygous to leukemic disease.

Chart 2: Chart 3 shows the origin of strain 164 with part of the maternal ancestry. This strain was derived from the same original ancestry on the maternal side as strain 146, and the female parent, 1236, was a member of strain 146. Female 1236 died of a thrombosed auricle without tumor or leukemia. Her mother, 529, died of a mixed sarcoma-carcinoma of the mammary gland, with secondaries in the lungs; her maternal grandmother was 293 with carcinoma of the mammary gland and lymphatic leukemia. It was here that the leukemias entered this family. The parent male was a common house mouse that died of uncertain causes without tumor of any sort. He came of a strain that never showed neoplastic growth of any kind nor any leukemic
disease throughout its entire life in my laboratory. We have here a cross between a hybrid non-cancerous female (her mother being cancerous and her father hybrid non-cancerous) and a pure-bred non-cancerous male. The first hybrid generation showed no neoplasm and no leukemia, which would be the expectation from such ancestry as is here presented. This first hybrid generation has been bred out in four branches and has been reported previously in part.

In this chart I have indicated as pure non-cancerous 4921, 5652, 4214, and 5343, because they were respectively the parents of Branches I and II of this strain, neither of which ever showed a tumor of any sort. They have been charted previously in these studies (17, 21). Mice 4224, 6480, 4338, 4378 have been classified as heterozygous non-cancerous, because they were the parents of Branches III and IV respectively, both of which were cancerous lines. These branches also have been repeatedly represented by charts and description in these studies.

Resume of Chart 3: From the mating of non-cancer by heterozygote, F' was 100 per cent non-cancerous. This would be the expectation.

Chart 4: Chart 4 gives part of strain 164, Branch III, showing how lines A, B, and C were derived. Note the occurrence of thymus lymphosarcoma in male 3672, generation 2, line A, of this Branch III, where neither parent (4224 × 6480) had leukemic disease or any form of malignancy, but where leukemic disease had been introduced by the maternal grandmother four generations
back, female 293. Note also male 8276 with sarcoma of the mammary gland derived from his grandmother three generations back, 529. Both these occurrences illustrate how the tendency to malignancy can be carried potentially through one generation after another, by the right selective matings. I have, in previous reports, given many similar instances.

Resumé of Chart 4: From the mating of heterozygote by heterozygote (4224 × 6480), F' of Branch III showed six non-cancers : two cancers (perfect ratio) (g₂ of the entire strain).

**Chart 4**

**Part of Strain - 164 - Branch III**

<table>
<thead>
<tr>
<th>Ancestry</th>
<th>Carcinoma Mamm. gland, Leukemia</th>
<th>Carcinoma Lung</th>
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<tr>
<td>G Ge Parents</td>
<td>Sarcoma-Carcinoma Mamm. gland</td>
<td>Uncertain</td>
</tr>
<tr>
<td>Parents</td>
<td>Thrombosed Auricle</td>
<td>Uncertain</td>
</tr>
<tr>
<td>G I</td>
<td>Uncertain</td>
<td>Chronic Nephritis</td>
</tr>
<tr>
<td>G II LINE A</td>
<td>Lympho Sarcoma</td>
<td>Intest. Infect.</td>
</tr>
<tr>
<td>Thymus</td>
<td>Intest. Infect.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intest. Inf.</td>
<td>Intest. Inf.</td>
</tr>
<tr>
<td>87672</td>
<td>8419</td>
<td>9726</td>
</tr>
<tr>
<td>97923</td>
<td>85276</td>
<td>87125</td>
</tr>
<tr>
<td>G III</td>
<td>Intest. Infect.</td>
<td>Intest. Inf.</td>
</tr>
<tr>
<td>Uncert.</td>
<td>88321</td>
<td>88325</td>
</tr>
<tr>
<td>11053</td>
<td>8832</td>
<td>1037</td>
</tr>
</tbody>
</table>

F₂ (g₃ in chart) showed, from mating non-cancer × cancer, line A, 100 per cent non-cancerous heterozygotes (perfect ratio). In line B, non-cancer × heterozygote, 1 : 1 (perfect ratio). Line C, heterozygote × cancer, 1 : 1 (perfect ratio).

**Chart 5**: Chart 5 shows Branch IV of this same strain 164. The ancestry is shown in Chart 3. The parent female of this branch was 4338, which died of uterine infection, and the parent male was 4378, dying of pulmonary infection. These parents are shown in their origin family in Chart 3. We have here, in Branch IV, a mating of two hybrid non-cancerous mice that produced both tumorous and non-tumorous offspring. We know that they must have been hybrid, because they yielded in their immediate
offspring both tumorous and non-tumorous individuals. Only hybrids can produce both types. Recessives could produce only their type; dominants could produce only their own type; therefore, 4338 and 4378 must have been hybrids. They were non-cancerous; therefore, non-cancer must be the hybrid type. The hybrid form wears always the appearance of the dominant. Therefore, non-cancer must be the dominant form.

The first generation from this cross of two heterozygous mice produced 4 pure non-cancerous to 6 hybrids to 3 cancerous (or 10 non-cancer : 3 cancer), which is almost the perfect mendelian expectation if cancer were recessive. This first hybrid generation was bred out in four lines, A, B, C, D. Note that again some carcinoma and some sarcoma occurred in this strain. This is interesting as coming from grandparent female 529 with a mixed sarcoma-carcinoma of the mammary gland (shown in Chart 3). The fact that this female transmitted both pure carcinoma and pure sarcoma to some of her offspring in both these strains, as well as mixed tumors to some of them, is strong evidence that her tumor must have been a true mixed tumor.
Note male 5854 in the second generation, with a thymus lymphosarcoma. It took here four generations before the leukemic disease tendency, bred in with female 293, appeared in the resulting strain. This would seem impossible for a dominant. Note that 8522 × 10046, both with pseudoleukemia (g₄, line B, in the chart), gave 100 per cent leukemic disease in all mice living over seven months including lymphatic leukemia, pseudoleukemia, and lymphosarcoma.

Resume of Chart 5: From the mating of two heterozygous non-cancerous mice of F' (4338 × 4378), there were derived:

\[ F² : 10 \text{ non-cancers} : 3 \text{ cancers (or 4 : 6 : 3). Nearly perfect mendelian ratio.} \]

\[ F₆ : 10 \text{ non-cancers} : 3 \text{ cancers.} \]

The matings here were: 7468 × 6512 and 6629 × 7470.

(1) heterozygote × cancer, 2 heterozygotes : 1 cancer
(2) " " " " , 3 " " : 2 " 

Total, 5 " " : 3 " 

(3) non-cancer × non-cancer, 5 non-cancers (mating 6028 × 6057).

This is nearly perfect mendelian expectation.

\[ F₄ : 8 \text{ non-cancers} : 2 \text{ cancers.} \]

The matings here were: 6043 × 7848 and 7348 × 7414.

(1) heterozygote × cancer, 3 heterozygotes : 2 cancers.
(2) non-cancer × non-cancer, 5 non-cancers : 0 cancers.

Perfect mendelian ratios.

\[ F₆ : \text{Cancer} \times \text{cancer (leuk.)}, 0 \text{ non-cancers} : 3 \text{ cancers (8522 × 10046).} \]

Perfect ratio.

Note: The results in g₃ and g₄ of line B (matings 6475 × 5854 and 6750 × 4450) are not included in the total results of Chart 5, because only part of the offspring of these crosses is shown here, while the total result is covered by Chart 6. Line B is, however, included in part in this Chart 5, in order to indicate the uniformity of neoplastic production in lines A, B, and C, while line D produced only mice which were free from all neoplasms including the leukemias. F₆ of line C is not included in totals, as it is given only in part, in order to show the striking sequence of sarcoma in line C.
Chart 6: Chart 6 shows the breeding out of line B of Branch IV of this strain 164, from the 1st to the 5th generations. The parent female of line B was 6475 (F₂ of the strain), which died of chronic nephritis without cancer or leukemia. The parent male was 5854, with a highly malignant thymus lymphosarcoma. Note the occurrence in the third generation of mammary gland carcinoma (female 6750) derived from female 529 four generations back. It would seem impossible, if the carcinoma tendency were dominant, that carcinoma should not have appeared for four generations in any cross that could have been made of offspring from this cancerous female 529. This would be possible only with the cancer tendency as recessive, because then the hybrids could have carried malignancy potentially without frankly showing it. Were the cancer tendency dominant, it must have been frankly shown by those that carried it. In generation 4, note the first occurrences of pseudoleukemia, male 8522 and female 10046. The mating of 8522 and 10046 is shown in Chart 5; 10046 is here mated with 9708. Also note that male 5854, with lymphosarcoma, was followed by his grandson, 9808, with lymphosarcoma and leukemia.

At this time the leukemias were occurring very frequently in several branches of strain 164, and I was trying to secure double parentage of each of these diseases. This is a very difficult thing
to do, and it is only possible by breeding nearly everything out fully. By the time these diseases can be clinically diagnosed in mice so as to secure double parentage with the disease, the mice are too far advanced in their hyperplastic changes to have young. This is particularly true of the female. If she becomes impregnated after spontaneous leukemia is sufficiently advanced for certain diagnosis, she can rarely bring her young to birth alive and vigorous.

I do not recall that I have in more than two or three cases succeeded in getting vigorous young that lived to maturity, from a female mouse mated after I had been able to diagnose any of these leukemic diseases. In most cases where double leukemic parentage has been obtained, the crosses were made and the young already secured before either of the parents had the disease. This would show that the young did not contract the disease from the parents through contact, as they were separated from the parents a much longer time than the duration of these diseases in mice.

From the mating of leukemic female 10046 with hybrid non-cancerous male 9708, pseudoleukemia again occurred, male 10176. Note, also, female 13779 in this same generation, with carcinoma of the mammary gland. The result of this mating is: 4 non-cancer : 2 cancer. This same pseudoleukemic female, 10046, mated with pseudoleukemic male 8522 (Chart 5) gave 100 per cent leukemic disease.

_Resumé of Chart 6:_ From the mating of heterozygote × cancer (6475 × 5854), there were derived:

- $F' : 4$ heterozygotes : 1 cancer ($g_3$ in chart). Too few cancers.
- $F_2 : 4$ " : 3 " ($g_3$ in chart). Nearly perfect ratio.
- $F_3$ : from mating heterozygote × cancer (10046 × 9708).


4 heterozygotes : 2 cancers ($g_5$ in chart). Too few cancers.

_Chart 7:_ Chart 7 shows the continuation of this same line B, Branch IV, strain 164, from the 5th to the 10th generations. In the 7th generation double pseudoleukemic parentage had been obtained (18936 × 16782), and the resulting family showed 100 per cent pseudoleukemia, leukemia, and lymphosarcoma, in all mice that lived to be more than seven months old. Note the persistence of mammary gland carcinoma throughout this line B, Branch IV of the strain. Four of the mice with pseudoleukemia showed also carcinoma of the mammary gland. Male 26229 in the 9th generation, showed carcinoma of the jaw in addition to thymus lymphosarcoma.
In this family of thirteen members that showed 100 per cent pseudoleukemia, there was also over 38 per cent of carcinoma. The carcinoma tendency was evidently originally derived from grandparent female 529 (10 generations back) with a mixed sarcoma-carcinoma of the mammary gland. The absence of all unmixed sarcoma from line B of Branch IV is also striking, as in other branches of the same original derivation, many sarcomas arose, as for example in line C (Chart 5). Sarcoma was evidently also originally transmitted by female 529, with the mixed type of malignant growth, as this was the only sarcoma bred into the strain. Line B is one of the lines derived from female 529 with a mixed tumor, that showed the tendency to pure carcinoma, and no tendency to sarcoma or to mixed tumors. These facts are very strong evidence of the hereditary segregating out of the sarcoma tendency and the carcinoma tendency, that is, the segregating not only of the organ location of neoplasms, but also of the tissue location.

From matings in the 10th generation, I did not succeed in getting any young that lived to maturity, from inbreeding within this 100 per cent pseudoleukemic fraternity of line B. At this stage, fearing to lose the strain, I crossed in a member of the same generation from a different branch of the same line B. The resulting lines showed eight additional cases of pseudoleukemia within the next two generations, making a total of twenty-two
cases within six generations in this little line of the family. The fact that these eight additional cases arose demonstrates that not only did the members of this 100 per cent pseudoleukemic line produce the same disease when inbred, but they also transmitted it when outbred beyond the borders of the 100 per cent line.

Resume of Chart 7: From the mating of heterozygote × pseudoleukemia (10699 × 10176):

F′ : 4 heterozygotes : 3 cancers (g4 in chart). Nearly perfect.
F2 : 3 " : 3 " (g7 in chart). Perfect ratio.
F3 :
F4 : pseudoleukemia × pseudoleukemia : 100 per cent pseudoleukemia, leukemia and lymphosarcoma (g8, g9, g10 in chart). Perfect ratio.

Let us now omit carcinoma from the reckoning, and consider only the leukemias in Chart 7:

Heterozygous non-leukemic ♀ 10699 × leukemic ♂ 10176 (F1) gives: (F2) 5 non-leukemic : 2 leukemic. Nearly perfect ratio (too few leukemic).
Heterozygous ♂ 12905 × leukemic ♀ 15867 (F6) gives: (F7) 3 non-leukemic : 3 leukemic. Perfect ratio.
Total: heterozygotes × leukemias, 8 : 5. Nearly perfect ratio (too few leukemic).
Leukemia × leukemia (♀ 18936 × ♂ 16782) (F3) gives: (F4), (F5), (F6) three generations of 100 per cent leukemia. 0 non-leukemic : 11 leukemic. Perfect ratio.

Chart 8: Chart 8 lists the additional eight individuals with leukemic diseases which followed the outcross from the 100 per cent leukemic strain. Note how the tendency to carcinoma persists here, even in the late generations of the strain. Also note that sarcoma is still absent from this line.

Chart 9: Chart 9 shows Branch III, line A, of this same strain 164. The parent female, 8419, died of peritonitis without cancer or leukemia. The parent male, 3672, died of infiltrating thymus lymphosarcoma. Both of these mice are shown in the F2 of Chart 4, their original ancestry being the same as that of Branch IV, though the immediate parents of the branch are different. None of the immediate ten heterozygous offspring show cancer or
leukemic disease. However, from both the matings made of these F' heterozygotes, pseudoleukemia arose: males 5919 and 7292 in the 1st line, derived from 6254 × 6923, and in line 2, female 12605, daughter of 10597 × 8521. In the F₅ of this 2nd line, female 13978 had carcinoma of the mammary gland and pseudoleukemia.

**Resume of Chart 9:**

F': non-cancer × cancer: 10 heterozygous non-cancers: 0 cancers. Perfect ratio.

F₂: heterozygote × heterozygote: 10 non-cancers: 3 cancers (pseudoleukemia). Nearly perfect ratio (too few cancers).
Exactly the same figures obtain if we eliminate carcinoma and consider only leukemic diseases.

Let us consider for a moment what we find in the $F_2$ throughout these charts:

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<tbody>
<tr>
<td>Chart 4</td>
<td>5 N.C. : 0 Can.</td>
<td>1 : 1</td>
<td>4 : 0</td>
<td>1 : 1</td>
</tr>
<tr>
<td>Chart 5</td>
<td>5 N.C. : 0 Can.</td>
<td>1 : 1</td>
<td>4 : 0</td>
<td>10 : 6</td>
</tr>
</tbody>
</table>

These are all perfect ratios except that for heterozygote × cancer, where there are too few cancers.

**Chart 10**

<table>
<thead>
<tr>
<th>Chart No.</th>
<th>N.C. × N.C.</th>
<th>N.C. × Heterozygote</th>
<th>N.C. × Cancer</th>
<th>Heterozygote × Cancer</th>
<th>Cancer × Cancer</th>
</tr>
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</table>

From the various possible types of matings, the results are nearly perfect for cancer (including the leukemic diseases) as recessive.

In $F_2$ then, as well as in $F_2$, we find very close approximation to the mendelian expectation for cancer as recessive. While the numbers are small, they are illuminating. There is no difference apparent between the results in $F_2$ and $F_3$, when the same types of matings are made; and in N.C. × N.C., N.C. × Het. N.C., and N.C. × C. the ratios are perfect in $F_3$, as they were in $F_2$.

**Chart 10**: Chart 10 gives the totals resulting from all the matings included in the charts herein shown. These charts cover two branches each of strains 146 and 164, in which leukemic
RELATION OF HEREDITY TO LEUKEMIA

diseases occurred: Branches I and III of strain 146; Branches III and IV of strain 164. These two strains were derived from the same original source on the maternal side, namely female 293 with carcinoma of the mammary gland and lymphatic leukemia. It is interesting to see that this female introduced leukemic diseases into both branches of both these strains. The four branches whose origin was this female 293, include 135 mice. The ages were in every case over seven months, with one exception, male 1439, Chart 2, in the 100 per cent pseudoleukemia line. He was undoubtedly potentially leukemic, as he produced 100 per cent leukemic offspring in both crosses with neoplastic females 4167 and 4243.

All possible types of matings of cancer, including leukemias, were made. The results show almost perfect ratios for malignancy, including leukemias, as a simple mendelian recessive.

1. From mating non-cancer × non-cancer, 100 per cent non-cancer was obtained. 10 non-cancers : 0 cancer (perfect ratio).
2. From mating non-cancer × heterozygote, 100 per cent non-cancer was obtained. 8 non-cancers : 0 cancer (perfect ratio).
3. From mating heterozygote × heterozygote, 26 non-cancers : 8 cancers (nearly perfect ratio; too few cancers).
4. From mating non-cancer × cancer, 100 per cent non-cancer was obtained. 10 non-cancers : 0 cancer (perfect ratio).
5. From mating heterozygote × cancer, 30 non-cancers : 20 cancers were obtained (nearly perfect ratio; too few cancers).
6. From mating cancer × cancer (including the leukemias) 0 non-cancers : 21 cancers were obtained. 100 per cent cancer (perfect ratio).

Note that in the matings of heterozygote × heterozygote, and of heterozygote × cancer there are somewhat too few cancers for a perfect ratio. Too few cancers are always to be expected from such crosses, because apparently actual occurrence of cancer depends upon (1) hereditary tendency; (2) the external provocative factor operating upon susceptible tissues; and (3) a long life during which there is chance for such interrelation between heredity and external provocations.

The results in the strains covered in this report give perfect ratios from all crosses where these difficulties are not involved; that is, ratios are perfect in the crosses of non-cancer × non-cancer; non-cancer × heterozygote; non-cancer × cancer; and cancer × cancer.

I do not at this time suggest what the external provocative
factors may be in the incidence of the spontaneous leukemias. At present it is not possible either to disclaim or to identify them.

CONCLUSIONS

From the foregoing data and these charts, which are typical, as showing the incidence behavior of these diseases in my laboratory, certain facts seem to be demonstrated. These facts are as follows.

1. In my stocks the occurrence of every form of the leukemic diseases has been definitely confined to certain cancerous strains of *Mus musculus* derivation, during the nineteen years since the first appearance of this disease.

2. There have been very many strains in the laboratory completely free from any form of these diseases. Into these strains I have never bred any leukemia nor has it arisen sporadically in such strains.

3. This furnishes substantial negative evidence that the tendencies to these diseases and the absence of these diseases are subject to the control of heredity. There is no evidence as yet to identify any definite external provocative factor as another possible agent.

4. In my stocks, where any of the leukemic diseases have been bred in, these diseases have certainly appeared later in the strain, in what closely approximates the mendelian expectation for the type of mating made. This is true of the F<sub>3</sub>, F<sub>4</sub>, F<sub>5</sub>, etc., as well as for the F<sub>1</sub> and F<sub>2</sub>.

5. Where double leukemic parentage has been secured, it has been possible to derive strains showing 100 per cent of these diseases, in all mice living beyond seven months in every generation tested. This result has been obtained in spite of the very great difficulty involved in securing such a double leukemic parentage.

6. Where hybrid crosses have been made between leukemic diseases in one parent, and complete freedom from malignancy of any kind including leukemia in the other parent, leukemic diseases have appeared later in the strain in approximately the expectation for the type of mating made.

7. These facts form the positive evidence that heredity is a factor in the tendency to leukemic diseases.

8. Since it is possible very frequently to secure mice with leukemic diseases, where neither parent had these diseases or any other form of malignancy (some of these have been shown in the
charts presented), the tendency to spontaneous leukemic diseases cannot be a dominant. If the hereditary tendency to the disease were dominant, the tendency to its absence would be recessive, and two recessives (mice completely lacking the dominant) could not produce the dominant. Only the hybrid form can produce a form unlike itself. Hence these non-cancerous, non-leukemic pairs of parents that produce leukemic offspring must be hybrids. The hybrid wears always the appearance of the dominant. Therefore, the tendency to non-malignancy, non-leukemia, which is the normal condition, must be dominant.

9. The results obtained from the crosses herein reported, show almost perfect ratios for the tendency to malignancy, including leukemic diseases, as simple mendelian recessive, and the tendency to non-malignancy, including non-leukemia, as simple mendelian dominant.

10. Since in my stocks of mice the leukemic diseases very frequently occur in offspring, neither of whose parents had any of these diseases, the transmission factor cannot have been a contact transmission either intra- or extra-uterine. This furnishes evidence that these diseases are not contact infections.

11. Since in my stocks the leukemic diseases occur only in cancer strains, and strains free from other forms of malignancy have been wholly free from these diseases also, this is strong evidence in support of the view that the leukemias, pseudoleukemias, and lymphosarcomas are members of the neoplastic group.

12. Grafted leukemias, like other grafted neoplasms, involve proliferation of the implanted cells, not of the cells of the host. The function of the host is to support this graft, and we must therefore look for differences in the behavior of such normal hosts, and that of animals which themselves originate the neoplastic growth, whether it is carcinoma, sarcoma, or leukemia.

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