The Origin of Some Inbred Mice*†

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The introduction of the use of inbred mice into cancer and other biological research has been a long and slow process. Even at the present time the application of this technic is not by any means universal. A glance at Hartwell's recent extensive survey of compounds which have been tested for carcinogenic activity (13) will show that, although the use of inbred mice is largely appreciated, considerable work is still being done on mice of unknown or uncertain origin. For example, in this monograph there are approximately 3,474 references to the use of animals in cancer research. Sixteen groups of animals are mentioned. There are 2,202 references to the use of mice (58.7 per cent of the grand total); 876 to rats; 339 to rabbits; 69 to guinea pigs; 412 to mice (many of the "heterozygous" mice used were obviously classified as follows: (a) 883 to mice whose origin is not stated; (b) 532 (24.1 per cent of the total use of mice) to the inbred strains developed by the present author, the origin of which will be discussed in this paper; (c) 454 to the use of "heterozygous" mice (many of the "heterozygous" mice used were obviously produced by hybridization from our inbred mice, but are not included, however, in the computed 24.1 per cent; and (d) 373 references to mice from all other sources. That is, when the source of mice is definitely stated (1,219 times) the use of the inbred strains of Strong was 40.4 per cent of the total use of mice (exclusive of derived heterozygous animals). This percentage would probably be even greater since many of the references to stocks are too vague for proper classification; e.g., (a) black agouti, (b) inbred, (c) cancer-resistant, (d) cancer-susceptible, and (e) inbred albino.

It is the conviction of many geneticists that the use of the inbred mouse in cancer research has made possible many contributions of a fundamental nature that would not have been made otherwise. Perhaps it would not be out of place to make the suggestion that within the near future all research on mice should be carried out on inbred animals or on hybrid mice of known (genetically controlled) origin where the degree of biological variability has been carefully controlled. A major step in this direction has been taken by investigators at the

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The use of the term "heterozygous" as applied to market or hybrid mice (not necessarily inbred) is greatly to be deplored. Heterozygous in genetics has the specific meaning of denoting that condition where the genetic determiners of heredity (genes) are in the heterozygous form. Since apparently several distinctive classes of "heterozygous" individuals are being used, it would be better to use either the nonspecific term "hybrid" or the more specific genetic terms of "F₁" or "backcross mice," etc. The relative values and proper use of inbred and heterozygous or hybrid mice has been very adequately discussed by Russell (19).

Rescoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, which has made available to others many of the inbred strains developed by them and from other sources. Their excellent book on the biology of the laboratory mouse (19) should fulfill a long felt need and should do much in emphasizing the desirability of using controlled mice in cancer and other biological research.

The application of genetic principles to cancer research by the author goes back nearly twenty-five years. The problems that confronted him first at that time were: (a) How could cancer research be placed on a quantitative basis, and (b) how would it be possible to apply genetic principles to a problem where so many unknown variables apparently were operative? The appreciation of the need of this type of quantitative biological research was perhaps even less then than it is now. In fact, a great approach to placing biological research on a quantitative basis had already been made by geneticists. A noteworthy attempt to cover adequately the application of quantitative methods in biology has been made by investigators at The Biological Laboratory, Cold Spring Harbor, Long Island. They have already conducted 9 annual Symposia on Quantitative Biology, beginning in 1933 (9).

A preliminary survey of the field in 1918 disclosed the fact that no inbred pedigreed mice fulfilled the requirements for quantitative or genetic research. Guinea pigs had been inbred by Dr. Wright in Washington; rats at the Wistar Institute by Dr. Helen Dean King. The nearest approach to an inbred stock of mice was the Bagg albino, where some brother-to-sister matings had been employed but where most of the stock had been carried on by pen matings—several females and males in one cage—which merely means that the stock was continued without introducing mice of a foreign source. In 1920, Bagg (2b) reported on the origin of the albino usually referred to as the Bagg albino. In this communication he referred to these mice as the white family in which 8 inbred generations had been recorded. Between 1918 and 1926 the author outcrossed many albino mice obtained from Bagg to mice showing the triple recessive characters: dilute, brown, and nonagouti. The F₁'s obtained were black agouti, brown agouti, black nonagouti, and brown nonagouti, which indicated that these albino mice were not homozygous at that time even for the common discernible color-producing genes.

Only 3 unpedigreed mice of the now well-known dba or dbr strain were then alive, sent by Dr. E. A. Wright of Boston to Dr. C. C. Little at Cold Spring Harbor, Long Island, following the depletion of the original pedigreed dilute brown stock by an epidemic of murine paratyphoid. It was necessary, therefore, to produce stocks of inbred mice as the first requisite for applying genetic principles to cancer research, thus placing it somewhat on a quantitative basis.

2 By the term "pedigreed" is meant that system of mating where all animals are given an individual identifying number early in life, and the complete vital records of each animal are individually entered in permanent form.
The origin of the A (21), C (22), C3H (23), CBA (24), JK (25), F (26), and NH (27) strains has been published in various periodicals. It is the purpose of this paper to bring together in one place these data, together with others that may lead to a better understanding of the origins and genetic relationships of these many inbred mice that are being used by investigators in various parts of the world. Among other advantages of this procedure would be: (a) avoidance of further confusion in the literature, and (b) aid to investigations of research where some degree of genetic relationship between individuals of the various stocks may have an influence on the results obtained.

Fig. 1 shows in condensed graphic form the origin of, and genetic relationships between, 11 of those pedigreed inbred strains which were developed between the years 1920 and 1927. Between 1920 and 1925, 4 unpedigreed pairs of mice from various sources were selected and started on a pedigreed in-breeding (primarily a brother-to-sister mating) regime, and thus were produced in the ensuing years the A, C, CBA, C3H, CHI, and C12I strains. Between 1925 and 1927, 12 additional unpedigreed pairs of mice were selected, primarily from the colony of Dr. W. E. Castle of Bussey Institution, Harvard University. It was impossible to continue all the original 16 selected strains, and only 5 (A, F, I, L, and N) are still in existence. These are the A, F, I, L, and N. The JK strain was produced by a cross between mice of the original J and K strains. Following a cross between mice of the A and D strains, 5 selected strains were developed, the criterion of choice being the age distribution of spontaneous tumors of the mammary gland occurring in female mice. Crosshatching of a strain indicates that it is no longer in existence. The circles indicate stocks that are not related to each other. All strains in squares are to some extent genetically related to all other strains in squares, although many of them have been separated from each other for many years. The small numbers from 1 to 12 indicate the number of generations after the establishment of the C strain before the various sublines were established. For example the C12I strain was established from mice of the 12th generation. The first 5 strains were named because their coat colors were known by significant letters as follows: A, albino; B, black-eye white; C, cinnamon; D, dilute brown; and E, extreme dilution.
existences. The others (those crosshatched in the chart) have all died out from one cause or another: (a) sterility and other defects occurring in the early generations of inbreeding, (b) the hazards involved in moving mice from one laboratory to another, (c) intercurrent and unexpected diseases, primarily at those times when new mice were brought into the laboratory, and (d) expense. No inbred strain has ever been deliberately discontinued. The crosshatched D stock was developed from the trio of unpedigreed dilute brown mice referred to above. They were continued at St. Stephen's College for several years and a few descendants were given to Dr. C. C. Little at Cold Spring Harbor. All the dba or dbrr strain are descended from this source. The original D strain established by the author in 1921 was continued at St. Stephen's College, Harvard University, University of Michigan, and Bar Harbor, Maine, but was lost shortly after the transfer of the mouse colony to New Haven. The unpedigreed A mice (one parent from Bagg albino, the other from an unpedigreed stock at the Carnegie Institute) were mated together and their descendants gave rise to the well-known A stock. As a distinguishing feature between mice of the Bagg albino and those of the A stock may be mentioned the fact that the incidence of spontaneous tumors of the mammary gland in the Bagg albino is extremely low, whereas in the A strain, which had been produced by selection toward a high incidence of spontaneous tumors of that gland, this particular neoplasm occurs frequently in breeder females. An individual of the A stock was crossed to a mouse of the D stock and produced mice Nos. 77 and 79. These 2 F1's were mated inter se and produced male No. 352. Male No. 352 was then backcrossed to his mother, F1 No. 79, and from this mating a large progeny was obtained, a few of which (1,668 female, 1,670 male, 1,672 male, 1,674 female, 1,507 female, and 1,510 female) are indicated in Fig. 2. This particular mouse (No. 79) has been described in more detail in another paper (28).

Originally the descendants of this mating were given the symbol HTF, signifying high tumor family, since numerous spontaneous tumors of mammary origin were found in them. It was soon apparent, however, that the biological variability indicated by analysis of the incidence of spontaneous tumors of the mammary gland (frequency or age distribution) warranted more than a single inbred line. Consequently sublines were established from time to time and continued by selection from that point onward. This was the origin of the C, C3H, CHI, CBA, and C12I inbred lines.

Thus was produced by genetic selection following hybridization a series of inbred sublines all related to each other to some extent at least, but differing among themselves in the incidence of spontaneous tumors of mammary origin. In sequence of cancer susceptibility these strains may be classified as follows: (a) C3H, (b) A, (c) C12I, (d) D, (e) C, (f) CHI, and (g) CBA. That is, selection following a cross between 2 original strains, A and D, with intermediate degrees of susceptibility to spontaneous cancer had given rise to divergent and extreme variants.

Fig. 2 presents the data on the serial number of the mice used in the production of these various sublines up to the point where the following strains were established: C, C3H, CHI, C12I, and CBA. Only those mice that were actually necessary for the production of the sublines are given. No mice in collateral lines are given in this chart.

Fig. 3 shows the pedigree of the first 53 generations of strain A; Fig. 4, similar data on the first generations of the C3H strain. The points at which the colony containing mice of these 2 strains was moved from one city to another are also given.

DISCUSSION

It is beyond the scope of the present paper to attempt a critical survey of the investigations that have been made possible in part by the use of these inbred strains of mice and their derived heterozygous individuals. Perhaps it would be of interest, however, to point out some of these contributions, especially in the field of cancer research. The original purposes for the establishment of these strains have been entirely fulfilled:

1. Both a statistical analysis of data obtained from the use of inbred mice and the practical attainment of reproducible results (i.e., the supply of adequate controls) have shown on many occasions that in these mice biological variability has been reduced to a minimum or, at least, has been kept in an almost static condition. These data demonstrate conclusively, therefore, that the host factor in cancer and other biological research has been placed on quantitative grounds.

2. The supply of these mice has further emphasized the point that the application of genetic principles has had a tremendous influence on many phases of cancer research. These contributions may be briefly mentioned: (a) The use of these mice (A x D outcross shown in Fig. 1, together with derived F1's, backcross and hybrid mice of the F2 to F4 generation obtained through selection) has demonstrated quite conclusively that the mechanism which determines susceptibility to the transplanted adenocarcinoma of the mammary gland is inherited according to mendelian principles. The final proof came with the establishment of mice whose response to the grafted tumor (susceptibility or resistance) was determined by one hereditary unit (gene) (29). (b) These
Fig. 2.—The actual interrelationships of individuals used in the development of the C, C3H, C121, CI21, and CBA inbred strains of mice from a cross between mice of the A and D strains. Only those mice used in the production of these sublines are given. No collateral lines are represented. The symbol HTF at the right of the chart denotes the single high tumor family referred to in the text, before the establishment of the 5 sublines C, C3H, C121, CI21, and CBA. The small numerals in the lower right hand corners of the squares denote the age in months at which spontaneous tumors of mammary origin were obtained. When a significant symbol indicating the subline, such as A, B, C, etc., is not given in the lower left hand corners of the squares, the symbol AT is used. For example AT 14 means that a spontaneous tumor was found at 14 months of age. F1DA and F1DG are the symbols given to 2 transplantable adenocarcinomas of the mammary gland which had originated spontaneously in mouse F1 No. 79 at 12 months of age (28).
inbred mice supplied a wealth of spontaneous tumors of many kinds, and it was soon possible to determine that many of these types occurred not only frequently but also in a uniform or predictable fashion. That is, within a narrow margin the frequency distribution of spontaneous tumors did not fluctuate from time to time (30). This observation applied to adenocarcinoma of the mammary gland, adenocarcinoma of the lung (31), and to myelogenous and lymphatic leukemia (14). In addition to these commonest types

FIG. 3.—The pedigree of the first 53 generations of the A strain, showing the lineal descent of a single pair of mice obtained in 1921. In nearly every case the mating has been by a brother to his own sister. The direct female line is on the left, the male line on the right, except in case of mice Nos. 13623, 13621, 13620, and 13622, where the mice on the left and right are females and the 2 intermediately placed animals are males. The age at which spontaneous carcinoma of the mammary gland occurred is stated below the serial numbers of the females; for instance, AT 15.0 means that a spontaneous tumor appeared at 15 months of age. Where the space below the number of a mouse is blank, the mouse in question died of some other cause than cancer. The collateral lines containing thousands of mice together with several hundred instances of spontaneous cancer are not included in this chart. The times at which the A stock of mice was moved from one city to another are also given.
Fig. 4.—The origin of the C3H strain. The first 58 pedigreed generations are given. The males are indicated by the sign ♂. All other mice are females. The age in months at which spontaneous carcinoma of the mammary gland appeared is indicated below the serial numbers of the females. If the space is blank below the number of the female, the mouse died of some cause other than cancer. The times at which the C3H strain of mice was moved from one city to another are also given.
of tumors, however, others such as hepatoma (32–34), carcinoma of the ovary (35), and local sarcoma of the uterus (36) have been of rather (requent occurrence (although at uncertain times or unpredictably) in individuals of the CBA, CBA, and CH1 strains respectively. (c) Another group of tumors also has occurred in mice of these inbred strains. These are, melanoma, small round cell sarcoma, lymphoblastoma (37), squamous cell carcinoma of anus, certain sporadic or isolated cases of adenocarcinoma of the mammary gland, adenocarcinoma of the duodenum, of the preputial gland (38), and of the cecum, and finally mixed cell tumors of the salivary glands (39). This group of tumors is of particular genetic interest, since they apparently arise sporadically and the mice that gave rise to them have been incapable of transmitting the tumor or susceptibility to these specific tumors to their descendants in direct line. They therefore have a nongenetic origin. (d) These mice have also provided control material for the analysis of that physiological state which is characterized by susceptibility or resistance to spontaneous tumors. These data have been adequately discussed at other times (10).

A. One other contribution of a genetic nature may be mentioned. The age distribution of spontaneous tumors of mammary origin in female mice of the various sublines mentioned in Fig. 1 and produced by selection has indicated that the difference between cancer and no cancer is probably only a quantitative (not a qualitative) difference (24). Since these mice were produced by the genetic principle of selection following a hybridization experiment and since, in this case, selection was effective in the establishment of statistically different sublines as far as the age distribution of spontaneous mammary cancer is concerned, the evidence is strong that genetic factors are involved in the origin of cancer of this gland.

The evaluation of genetic observations by other geneticists is complicated by the fact that mice from several sources have been employed. Of the two outstanding contributions to the genetics of the mammary gland in mice, one, that of Little with his collaborators at the Jackson Laboratory on extra-chromosomal inheritance (20), was made possible by observations on the dha (a derivative of the D stock referred to in Fig. 1) and the C57 (a valuable inbred stock developed by Little working in collaboration with H. J. Bagg at Cold Spring Harbor). The source of the other strains reported (20) is not clearly indicated. According to Bittner (3), however, the Z stock is the C3H; the X the CBA,--direct descendants of mice of the C3H and CBA strains--given to Bittner by the author in 1927 and 1928. The origin of the L strain is given in this paper. Thus of the 7 strains of mice used in the research on extra-chromosomal inheritance, 5 were from the inbred strains established by Strong. The other contribution, that of Bittner on the "milk" influence (47) has been developed in a somewhat similar manner. In the early research on the "milk influence, the C3H and A stocks (cancer-susceptible) were from the present author's source and the C57 (cancer-resistant) from the stock continued by Little.

The most fruitful field for the use of these inbred and their derived hybrid mice, aside from the genetic work outlined above, is perhaps in endocrinology. An analysis of these contributions has been made by Gardner (11). It is well, perhaps, to emphasize a few of those that have a particular genetic interest: (a) The incidence of carcinoma of mammary origin in male mice receiving estrogen parallels genetic susceptibility. This observation, originally made by Lacassagne (16) on inbred mice developed by Dobrovolskii-Zavaldskii, has been verified by many investigators and particularly by Gardner and others (11) on mice of the various strains referred to in Fig. 1. (b) Gardner has also shown that presumably the same hormone treatment in mice of various inbred strains is followed by varying responses of several tissues or organs. These responses are moderately uniform within mice of a given strain and suggest fairly well that the fixation of different genetic constitutional mice through hybridization and selection may be an important factor in the result obtained by the injection of a specific hormone. Gardner has also found specific types of tumors, other than those of mammary origin, occurring in mice of various strains treated with various hormones. Most of these growths occur also in the F1 as well as in the original strain, and may have a genetic basis. In the case of the adenoma of the pituitary gland the inheritance, although showing some dominance, may be of a complex nature. According to Gardner and Allen (12) carcinoma of the cervix may be obtained in female mice irrespective of genetic origin. Additional evidence, however, may indicate some genetic influence in this type of neoplasm also.

The field of the induction of tumors by carcinogens is beginning to show evidence that genetic factors influence the results obtained here also. Anderson (1) has demonstrated that the induction of carcinoma of the lung by a carcinogen parallels genetic susceptibility, thus verifying the conclusion of Lynch (17, 18) derived from the use of mice developed primarily by her. In fact, Anderson (1) maintains that there is a close parallelism between susceptibility to spontaneous lung tumors developed by genetic selection and susceptibility to the induction of tumors by the carcinogens. He further states that mice of the C3H strain (highly susceptible to spontaneous breast tumors) are more susceptible to the local subcutaneous induction of tumors with both 1,2,5,6-dibenzanthracene and methyl-cholangthrene than are mice of the Y strain (low susceptibility to the incidence of spontaneous tumors). Burdette and Strong (8) have obtained similar data using the C3H, CBA, CH1, NH, and JK strains.

Kirschbaum and his coauthors (15) have reported a similar situation in the induction of myelogenous and lymphatic leukemia in mice of the F strain (Fig. 11). These mice show a high spontaneous incidence of both types of leukemia as well as high susceptibility to the induction of both types of leukemia by a carcinogen. Strong (27) has recently reported that specific sublines of mice may be established following a hybridization and selection regime which give rise to specific types of tumors induced by the same carcinogen (spindle cell sarcoma, squamous cell carcinoma of skin, rhabdomyosarcoma) or to no tumor whatever. Strong and Williams (40) have also shown that carcinoma of the mammary gland may be induced by methyl-cholangthrene in a mouse belonging to a strain made negative to spontaneous tumors of that tissue by selective genetic principles. That is, the carcinogen may replace genetic susceptibility.

Genetic strains have been employed by several investigators for the induction of tumors by the carcinogens. Branch (7a) reported that he was able to get almost twice as many tumors in C3H (cancer-susceptible) as in strain A by the injection of dibenzanthracene, thus indicating that there is no correlation between susceptibility to spontaneous tumors of mammary origin and susceptibility to skin tumors. Kreyberg (15a), working on selected lines de-
developed by him, arrived at a general conclusion that spontaneous tumors of the breast and tar-induced tumors showed a definite segregation in different family lines—but an opposite distribution. Andervont, since 1934, has published extensively along similar lines. In one communication (2a) he reports observations on 8 established inbred strains as follows: C3H, C, C57, M, A, D, I, and Y. He concludes that "there are wide variations in susceptibility to both types of induced tumors. There is no correlation between the susceptibility to spontaneous mammary tumors and susceptibility to the induction of subcutaneous growths." Hence, "the conclusion may be drawn from the investigations that, up to the present time, a strain of mice has not been found which is resistant or susceptible to all types of tumor growth."

This challenge to genetics, expressed by Andervont, may be partly met after more of the inbred strains, many of which are mentioned in this paper, have been more fully investigated. If not, then the challenge should spur geneticists on, perhaps by a more critical application of the genetic principles of hybridization and selection, to the establishment of the ideal mouse, susceptible or resistant to all types of neoplasia.

SUMMARY

Data are presented showing the origin of 11 inbred strains of mice which are now being used in cancer and other biological research. Among these strains the A, C3H, CBA, and I are well known as indicated, partially, by the analysis of the use of mice in Hartwell's monograph on carcinogenesis (13). The others, the CHI, C121, C, JK, F, L, and N have also been inbred for at least 17 years. It is shown that the A, C3H, CBA, CHI, C121, and C are somewhat genetically related although they have been separated from each other by 20 years of inbreeding. The other strains are not related to any of these or to each other. A representative survey of the contributions that have been made possible, in part, by the use of these strains is also included.

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