THE ESTABLISHMENT OF THE C_3H INBRED STRAIN OF MICE FOR THE STUDY OF SPONTANEOUS CARCINOMA OF THE MAMMARY GLAND[1]

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Received May 24, 1935

In recent years many investigators have become interested in the use of inbred strains of mice for the study of spontaneous neoplasia. This advance has been encouraging to geneticists, who have insisted, with great reason, that intrinsic or genetic factors do play a very important role in the onset of spontaneous carcinoma of the female mammary gland. Such pioneer workers as LOEB, SLYE, LITTLE, LYNCH, and others have shown that by the proper manipulation of the process of heredity one is able to control to a very large degree the incidence of cancer in a group of mice.

The coal tar derivatives and other carcinogenic agents have given to investigators a means by which many types of neoplasia may be artificially induced at will in mice and other animals. Such divergent data, as the above, would tend to produce chaos, were it not for the significant findings obtained particularly by LYNCH (1925), by KREYBERG (1934), and by the members of the YORKSHIRE COUNCIL (1930, 31, 32, and 33). These investigators, by different means (LYNCH by hybridization, KREYBERG by use of distinctly genetic pure lines, and the Yorkshire workers by selection) have definitely shown that the response of the organism to carcinogenic materials is controlled by its own intrinsic constitution. In other words, the origin of cancer by the known carcinogenic agents is the response of the individual cell to an environmental "cancer-inciting" stimulus.

More recently, the purified hydro-carbons, such as 1-2-5-6 dibenzanthracene, benz-pyrene, etc., have been introduced into cancer research. Following the lead of the geneticists, investigators have used pure strains of mice in their sterol-painting and injection experiments. Among these may be mentioned ANDERVONT (1934) and BRANCH (1935).

In view of the difficulties of analysis, especially in this case of bringing together the contributions of divergent lines of investigations, it seems desirable at this time to present the genetic background of one strain of mice.

THE C_3H STRAIN

In 1920, the author obtained two mice from the laboratory of the department of genetics, CARNEGIE INSTITUTION OF WASHINGTON, Cold

[1] The continuation of this stock has been made possible by a grant from the JOSIAH MACY, JR., FOUNDATION.

Spring Harbor, New York. One of these (#1) was a male from the Little strain of dilute browns, the other mouse (#8) was obtained from the albino mice which Dr. H. J. BAGG had brought to the laboratory from Memorial Hospital, New York City. The past history of the Little dilute brown strain has been extensively described in the literature. The albino stock of BAGG had been imported by him from Chicago several years previously. The albino stock had been continued, up to that time, by BAGG by the non-introduction of any foreign blood. These two mice, #8 and #1, were mated during the summer of 1920. Among the numerous F_1 progeny produced by the above outcross was one female (#79) which proved to be a very interesting individual. Her history shall be briefly given. She was born December 14, 1920. Her breeding record is given in chart 1.

CHART 1.

This chart presents the breeding record of mouse #79 which was first mated to her own brother #77 and then to a son #352 obtained from the above cross.

LITTER SEQUENCE	MATED TO MALE	LITTER BORN	NUMBER IN LITTER	YOUNG RAISED
1	77	2–12–21	5	2
2	77	4–20–21	7	4
3	77	6– 8–21	7	5
4	77	7–22–21	10	8
5	77	9–12–21	6	4
6	352 (own son)	12–20–21	7	7
7	352 (own son)	1–29–22	8	6
8	352 (own son)	3– 9–22	?	Litter killed by mother

On the twenty-second of October, 1921, a nodule 0.8 cm. in diameter was noticed in the left axillary region (2nd gland). A second nodule 0.5 cm. in diameter was discovered in the right axillary region on the twenty-fifth of the same month. The mouse was operated on under ether anesthesia on the twenty-ninth of October. The original tumor was a compact adenocarcinoma. Since this was the fourth tumor to be discovered in an F_1 individual, the original tumor received the symbol F_1Da. The tumor was inoculated into several offsprings of mouse #79, and into other F_1 individuals. On the sixth of January, 1922, two other nodules had appeared in mouse #79. These were in the left (F_1Dc) and the right iliac (F_1Dd) regions respectively (the 4th nipple systems). By the twenty-fourth of January, 1922, the second or F_1Db tumor had reached a centimeter and a half in diameter. This was removed by operation on that date—and subsequently recurred in the scar tissue by the fourth of March of that year. This tumor was inoculated into several F_1 individuals and into close relatives of #79.

The third tumor (F_1Dc) in the left iliac region was also removed on the twenty-fourth of January. At that time it was a centimeter in diameter. A fifth tumor, F_1De, approximately a centimeter in diameter was noticed on the nape of the neck on the fourth of March, 1922. To the left of this nodule another one (F_1Df) occurred in the right axillary region. The mouse died on the seventeenth of March at which time four surface tumors were readily palpated. These were F_1De, F_1Df, F_1Dg, and a recurrence of F_1Db. Autopsy disclosed three metastatic nodules in the lungs. All other organs were apparently normal. Attempts to continue most of the tumors by transplantation led to indifferent success. Two tumors, F_1Da and F_1Dg, were continued by transplantation for several years. The data obtained with these tumors were reported in the Journal of Cancer Research, 1929.

The conclusion derived from this study was that the tumor tissue had deviated from the definitive somatic cell that gave rise to it by a process analogous to genetic mutation. The question whether these nodules were multiple primary or secondary nodules is of extreme genetic interest. In the transplantation work it was clearly demonstrated that distinctly physiological differences were encountered in these tumors derived from the same mouse. Thus, it is true that the same mouse may give rise to genetically different types of tumors at approximately the same time.

In addition to the possibility of working out genetically the fundamental differences of physiological behavior of these tumors by the process of transplantation, it occurred to the author that mouse #79 may be of interest in the study of the origin of spontaneous carcinomata. With this end in view, the progeny obtained from mouse #79 by the method illustrated in chart 2 have been kept normally in the laboratory until they either died or developed spontaneous carcinoma. In the early years I was interested not only in the spontaneous occurrence of carcinoma, but also in the working out of the genetics of transplantation of the two implants, F_1Da and F_1Dg. Thus, the author decided to produce, if possible, a strain of mice which had genetic constitutions similar to that of mouse #79. For this reason mouse #79 was mated to her own brother and then mated to her own son, #352, obtained by this cross. Among the progeny obtained, four females were mated to a single male (a brother). After several generations of transplantation the implants F_1Da and F_1Dg were lost. The interest than centered on the occurrence of spontaneous carcinoma of the female mammary gland. Hence, the line was continued primarily by a brother-to-sister mating. Two breaks have occurred in this system of inbreeding. Each one was necessitated by the loss of animals when the colony was transferred from one laboratory to another. The first break was the mating of mouse 11113 to her near relative 11242 shortly after the move to Ann Arbor, Michigan, and the second break the mating of mouse

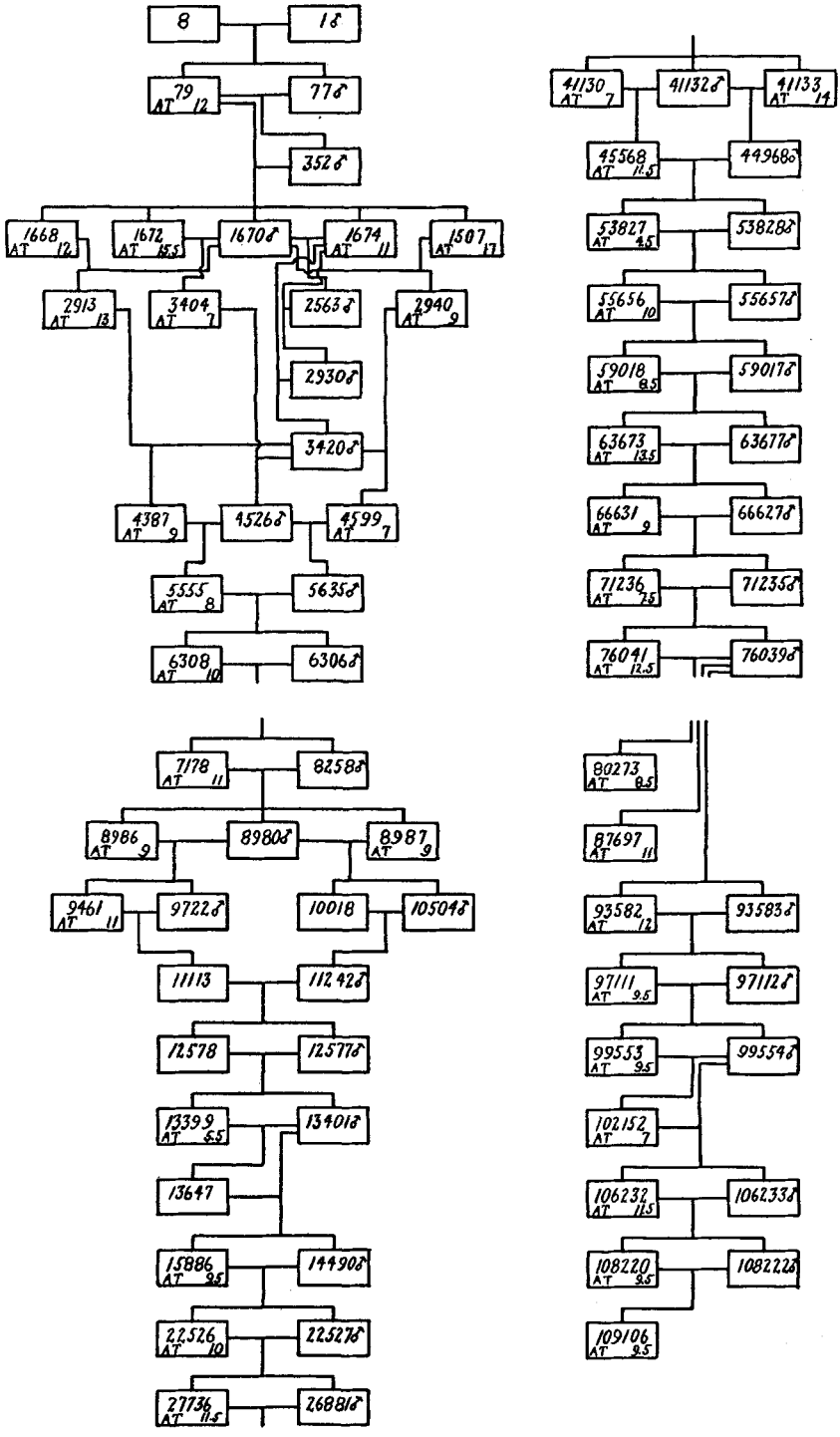

CHART 2

Males on the chart are indicated by the sign ♂. All other mice represented are females. The age at which spontaneous carcinoma of the mammary gland appeared is indicated below the serial number of the female. If the space is blank below the number of the female, the mouse died of some other cause than cancer. Only mice that are genetically related and which therefore constitute strain C_3H are included in this chart.

76039 not only to his own sister, but to the ensuing daughter and finally his own granddaughter necessitated by the move to Bar Harbor, Maine.

It will be noted that every female in direct descent for the last twenty-one generations has developed spontaneous carcinoma of the mammary gland. The several hundred descendents of mouse #79 in collateral lines, together with the numerous appearances of spontaneous carcinoma of the mammary gland in these mice, is not included in this report.

Of the forty-two female descendants of mouse #79, in this single line of descent, thirty-eight developed primary carcinoma of the mammary gland.

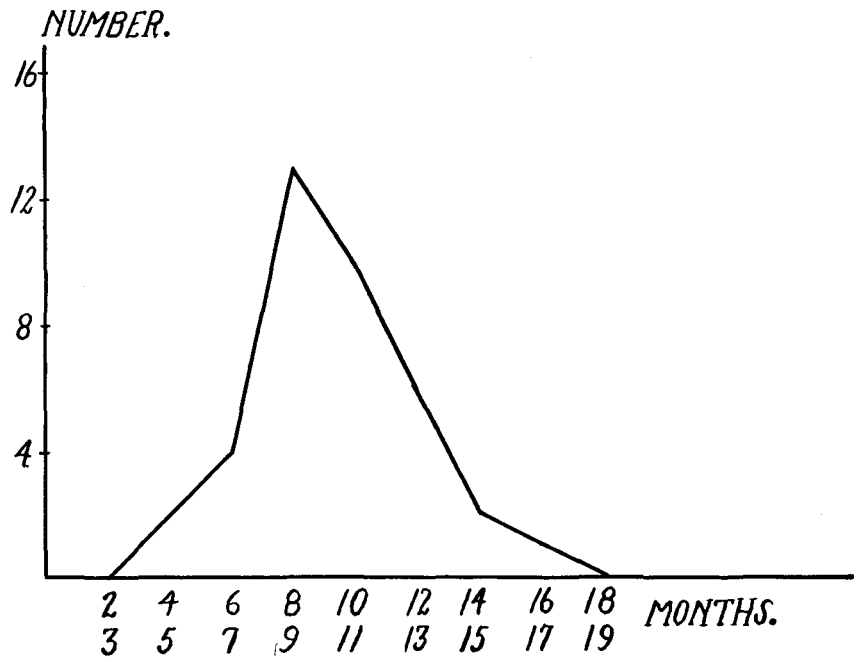

CHART 3

Age distribution of spontaneous carcinoma of the mammary gland in mice of the C_3H strain.

The age distribution of cancer is given in chart 3. The majority of the mice developed tumors between seven and ten months, with the mean between eight and nine months. This is earlier in life than data reported by SLYE (1927), MARSH (1929), and MURRAY (1934) on mammary gland carcinoma in other laboratories.

Derivation of Symbol C_3H

After a fair degree of inbreeding had been established, it seemed desirable to designate the various sub-strains thus obtained by letters of the alphabet. Consequently the descendents of mouse #5555 were designated

by the letter C. Two generations beyond, a further classification became desirable. Thus the descendents of mouse #8986 received the designation C_3H.

GENERAL DISCUSSION

The carcinogenic agents derived from coal tar produce several distinct types of neoplasia. The more frequent types are, however, epithelioma of the skin and sarcoma. Lynch has demonstrated that the response of the different organs of the body to neoplasia is apparently inherited independent of each other.

The purposes to which the inbred strains of mice, such as the above, can be utilized, are (1) the study of the incidence of spontaneous carcinoma, and (2) the study of the production of neoplasia by the recognized carcinogenic agents. The extent to which the above strain has been inbred is certainly sufficient to approach genetic homogeneity. In other words, biological variability has been reduced to a minimum.

The C_3H strain is to be considered a highly susceptible cancer family only so far as cancer of the female mammary gland is concerned. In regard to the tumors of the other organs it is still to be considered a resistant strain—since tumors other than those of the mammary gland have never been encountered in a period of fifteen years.

CONCLUSION

1. The lineal descendents of mouse #8986 constitute strain C_3H.
2. The C_3H strain, established by a system of pedigreed inbreeding over a period of fifteen years, is to be considered a pure line in regard to the occurrence of spontaneous carcinoma of the female mammary gland.

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