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What is a Control for Cancer in Mice?

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Summary. For over fifty years, the biological characteristics of spontaneous adenocarcinomata of C_3H/ST mice have been studied by the senior author. Two conclusions have been verified as follows (1) the growth capacity of these tumors over this extent of time has not significantly changed and (2) these tumors, except occassionally in very old mice do not regress under spontaneous conditions. About twelve years ago when liver extracts were used in an attempt to influence these spontaneous tumors, it was discovered that changes in the growth capacity of tumors in the controls were taking place. The problem was resolved when it was found that the controls which harbored a change in growth capacity of its "untreated" tumors had been born to a cancer proband which was either receiving the liver extract intraperitoneally or had actually a cancer proband under treatment in its immediate or remote ancestry.Thus for a mouse to be considered as a control for an experimental procedure on cancer the ancestry of the mouse must be taken into consideration.

The use of controls for an investigation of any problem is an essential feature of scientific method. Without the use of controls a final evaluation of any observation for experimentel procedure is, at least, in some doubt.

The study, therefore, of experimental cancer should be no exception to the rule.

During the past fifty years (1920–1970), spontaneous tumors of mammary gland origin have been occurring especially in mice of the well-known C_3H/ST and C_3HB/ST inbreds. The mice were kept in the various laboratories under very similar conditions in which the authors have worked – (1) Annandale, N. Y., (2) Ann Arbor, Mich., (3) Bar Harbor, Maine, (4) New Haven, Conn., (5) Springville, N. Y., (6) Del Mar, Ca., and (7) San Diego, Ca. The number of cases of cancer, at any one time, has been dependent, almost exclusively, upon the number of mice available, either as breeders or virgins. In all this time, studies on growth rates of tumors, survival time of the hosts, percentage number of regressions and other characteristics of spontaneous cancers have been made. The most significant conclusion that has been derived from the analysis of these extensive data has been that the growth capacity of these tumors over this extent of time has not significantly changed. Series of 1938 New Haven, Conn., 1960 Springville, N. Y., and 1965 Del Mar, Ca., have been compared for growth rate of tumors. These observations and derived conclusion were reported (Strong, 1968). The second conclusion is that these tumors, except in *occasionally* very old mice, do not regress under spontaneous conditions. The tumors grow progressively until the death of the host.

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It can be said, therefore, that normal female mice of either the C_3H/ST or the C_3HB/ST strains harboring a stabilized or uniformly growing cancer of mammary gland origin is a proper control to be used in comparison with any observed data derived from any experimental procedure.

It was found that sometime after the injection of specially prepared liver extracts were first used for the attempted control of spontaneous tumors in mice (1958), a disturbing observation on the growth rate of tumors in the so-called controls was made (Strong- 1969), It was concluded that "thus it is obvious from the results (obtained) that the exposure of the offspring by injecting the nursing mother with a specially prepared liver extract has affected: 1. the growth rate and 2. the percentage of complete regressions of spontaneous tumors of mammary gland origin that arises several months later in the offspring". This conclusion applies to both the controls and the experiment animals receiving the liver extract after cancer arises.

It is now known that this liver inhibitor effect on spontaneous cancer in mice is cumulative in succeeding generations and affects the so-called controls (no treatment of cancer proband) as well as the experimentals in which a liver extract was injected into the cancer proband (but also receiving neomycin in the drinking water; Strong, 1970).

The present report is a study of growth rates of spontaneous tumors of mammary gland origin in the untreated controls exclusively.

Methods

The methods of preparation of liver extracts and the use of mice with spontaneous tumors are the same as have been used in a series of publications. The essential features of methods were especially presented (Strong, 1969). They need not, therefore, be repeated in this paper.

Results and Discussion

The data on growth rates of spontaneous tumors of mice in successive generations derived from individuals of its ancestry treated, in the presence of a cancer, by specially prepared liver extracts are presented in Fig. 1. Mice (167) kept as untreated controls are on the solid line. The number of generations that a cancer proband is removed from a liver-extract-treated mouse bearing a spontaneous tumor is on the abscissa. The size of the tumors expressed as mm^2 of increments of growth at the 42nd observation period (3 times per week following the discovery of the tumor) are on the ordinate.

The symbol "0" indicates that there had been no individual in the ancestry of the cancer proband that had ever received the liver extract. "E" indicates that the cancer proband had been born to or nursed by a mother which had developed cancer of mammary gland origin and received a periodic injection of the liver extract (3 times per week). This is the same series as reported (Strong, 1969). The symbol "0.01" indicates that the mother of the cancer proband had been born to or nursed by a mouse with a spontaneous tumor and received the liver extract. The "0" in the symbol "0.01" indicates that the mother of the cancer proband, herself, had not received any liver extract up to the time that the eventual cancer proband had been weaned. The successive symbols of "0.001", "0.0001", etc. should be self-explanatory.

It is obvious from the inspection of Fig. 1 that the growth capacity of spontaneous tumors in mice kept as controls is being reduced in individuals belonging to successive generations of freedom from an injection of a liver extract into one of its female ancestors.

At the time this experiment had been performed mice of the C_3H/ST and C_3HB/ST strains had been inbred for, at least, 130 generations. These mice of the original C_3H/ST strain have never been outcrossed and consequently variables associated

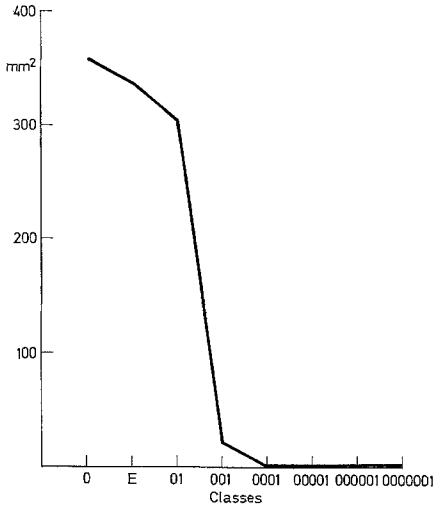

Fig. 1. Average growth increments of tumors at the 42nd observation period (3 per week) are given in mm^2 on the ordinate. The classes based on descent from a treated ancestor with cancer receiving the tumor inhibitor are on the abscissa

with this process must be eliminated as a possible cause of slower growth rates of spontaneous tumors and their complete suppression (100%) of tumor growth in mice kept as controls (no treatment) from the 0.001 class onward.

As a young man, following the production of the first adequate supply of spontaneous tumors in C_3H/ST mice, the senior author expressed the philosophy that he would like to destroy, before he retired, what he had created. The present data indicates that the goal of control of spontaneous tumors of mammary gland origin has been reached.

It is clear that the degree of inhibition of growth capacity of spontaneous tumors is increasing in control (untreated) mice following the remoteness of treatment in one of the ancestors of the cancer proband with the liver-derived-tumor inhibitor. The greatest change in the inhibition of growth rate of tumors takes place in mice of the 0.001 class (three generations removed from any therapeutic procedure).

All mice in this experiment were kept as controls (no treatment) as soon as their spontaneous tumors were evident. For a mouse to be called a control, however, in the sense of the original meaning of the word where there had been a

constant and stable growth rate of spontaneous tumors for approximately a fifty year period in one laboratory, it must now belong to the "0" class, where there has been no exposure of any individual of the cancer proband's ancestry to the liver extract containing the tumor inhibitor. For any other mouse bearing a spontaneous tumor to be considered a control (no treatment of cancer proband), one must take into consideration the ancestry of the cancer bearing individual. It is only by this procedure that the interpretation of a control for an experimental animal in relation to spontaneous tumors in mice can be established.

When this inclusion of the ancestry is done, it has been found that the growth rate of spontaneous tumors gradually diminishes in mice of successive generations of removal from a treated mouse in its ancestry until finally the control (no treatment of cancer proband) does not grow a spontaneous tumor but actually regresses its original small tumor without any treatment.

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