# The Transitory Nature of a Transmissible Entity Controlling the Growth of a Spontaneous Tumor in Mice

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Two hundred and sixty-three control mice of the $C_3$H/ST inbreds bearing spontaneous tumors of mammary gland origin have been studied in reference to the injection of a specially prepared liver extract into one or more of their ancestors. Of these, 24 mice were from the original inbred strain in which no liver extract had ever been injected. Two hundred and thirty-nine mice were among the descendants of a mouse that had received an intraperitoneal injection of a liver emulsion. These 239 mice were spread over 15 generations of lineal descent. All mice (263) of this experiment were kept as "controls" when their spontaneous tumors arose. The evidence obtained with the analysis of the growth rate of these tumors indicated the activity of a "transmissible entity" which increased in potency in suppressing these cancerous growths. The new evidence, obtained after six generations of lineal descent separation from the original injection of the liver extract, demonstrated quite convincingly that the amount of tumor inhibition became erratic and finally disappeared. The value of growth rate of tumors in mice of the 15th generation returned to the original rate obtained in mice of the 0 class in which there had been no treatment with liver extract in their ancestry.

In 1970, the appearance of a "transmissible entity" was reported in mice. This entity was involved in the growth rate and ultimate fate (complete regression or progressive growth) of a spontaneous tumor of mammary gland origin (Strong and Matsunaga, 1970). This entity was detected in mice of the $C_3$H/ST strain following the injection of a liver emulsion which had been proven to contain an inhibitor or inhibitors for the same type of spontaneous cancer. It was shown that the transmissible entity increased in potency in suppressing cancer in mice in successive generations of descent from a mouse (with a spontaneous tumor) that had originally received the liver extract (Strong and Matsunaga, 1970, 1971). In 1972, the suggestion was made that the transmissible entity is

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sensitive to change by either a) the injection of (an additional quantity of) a liver emulsion (even) after 12 generations from the injection of the same liver emulsion or b) time (Strong and Matsunaga, 1972).

At present, 15 generations of untreated mice of the $C_3H$/ST inbreds from a lineal descent, following the intraperitoneal injection of a liver emulsion, are now available for analysis in relation to the growth of a spontaneous tumor.

The purpose of the present paper is to report on the measurement of the growth capacity of spontaneous tumors in mice of $C_3H$/ST origin in which all mice over a 15 generation descent have been kept as "controls."

## MATERIALS AND METHODS

All mice used in this experiment received no experimental procedure. The only difference between individuals was the number of generations of lineal descent from a common ancestor that had been injected with an emulsion of liver following the appearance of a spontaneous tumor of mammary gland origin eleven years ago (at the Springville Laboratories of Roswell Park Memorial Institute, 1961).

The tumors were measured by vernier calipers in the two longest diameters, three times per week. The two measurements were multiplied together to estimate the relative size of the tumor masses over a period of time in which either progressive or retrogressive growth was indicated.

For purposes of analysis all tumor-bearing mice of any one generation of descent were grouped together, and the averages used. Successive classes of combined generations were also computed. They were, in sequence of descent, as follows: 1) The 0 class, in which there had been no injection of the liver extract into any individual of the cancer proband's ancestry (This is the original well-known $C_3H$/ST inbred that has been subjected to 148 generations of inbreeding over a 54-year period and their spontaneous tumors periodically measured.); 2) generations E, 01 and 02, in which E consisted of cancer probands that had been born to or nursed by a cancer-bearing mother receiving the liver emulsion and 01 and 02 with either one or two generations of descent free of the liver extract; 3) 03, 04, 05, and 06 with three, four, five, or six generations following the injection of the liver emulsion; 4) 07, 08, 09, and 010 for similar reasons; 5) 011, 012, 013, and 014; and 6) 015. It is anticipated that mice beyond 015 will eventually be added to class 6 when available.

## RESULTS

The results are given in Table I and Fig. 1. Table I presents the number of

TABLE I. Number of Tumor-Bearing Mice in Six Composite Classes from One Serial Descent

| Class | Generations | No. Mice |
|---|---|---|
| 1 | 0 | 24 |
| 2 | E 01 02 | 77 |
| 3 | 03 04 05 06 | 36 |
| 4 | 07 08 09 010 | 68 |
| 5 | 011 012 013 014 | 48 |
| 6 | 015 | 10 |
| **Total** | | **263** |

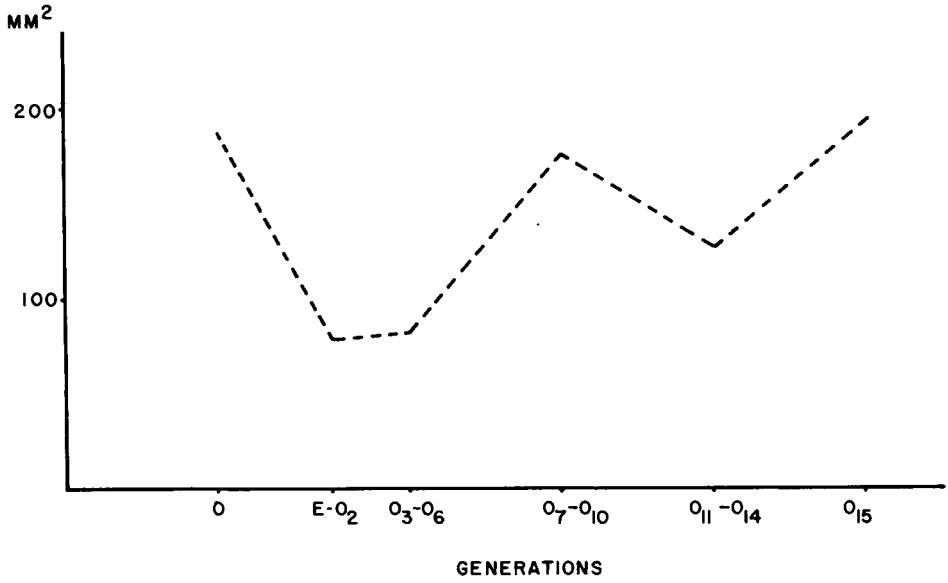


Fig. 1. Data on the average increments of tumor growth at the twenty-fifth period of observation. Classes 1–6 are spaced along the base line, while average increments of growth expressed in $mm^2$ are given on the vertical line.

tumor-bearing mice in each of six composite classes from one serial descent.

Figure 1 presents the data on analysis of growth of spontaneous tumors for mice of the six combined classes of descent from a single lineal descent. The six classes of cancer-bearing mice as spaced on the base line. The average increments of growth of tumors at the twenty-fifth period of observation (three per week) are given on the vertical line.

## DISCUSSION

Since all mice in this experiment were kept as "controls" it could be expected that a uniform growth rate of spontaneous tumors would be obtained. This expectancy is based on the fact that all mice belonged to a carefully restricted pedigreed system of exclusive brother-to-sister matings. This method of production of inbred mice has been rigidly followed for at least 148 generations of lineal descent from a single pair of mice. The expectancy of a uniform growth increment was not observed in this experiment as it had been found in mice of the same inbred strain before the liver emulsion had been introduced into the present laboratory of the senior investigator (Strong, 1964). Consequently, the conclusion may be entertained that some entity that influenced the growth and fate of spontaneous cancer in mice was either conditioned or induced as the result of the intraperitoneal injection of a specially prepared liver extract. This entity is transmissible from one generation to the next and it also seems reasonable to conclude that the entity is varying (being unstable) from time to time in relation to suppressing cancerous growth in mice.

Between classes 0 and 06, the transmissible entity appears to be increasing in its ability to suppress the growth of cancer. In fact, it almost appears that after increasing in potency the entity seems to become somewhat stabilized in tumor inhibition. But then (in 06 and 07) this suppressive tendency on tumor growth appears to wane. The waning process also appears to be variable, thus presenting the idea that the entity itself may be biologically unstable. The growth increment (and thus the rate) in mice of 015 is practically identical to the value that was determined in a mouse of the 0 class or the original $C_3H/ST$ mouse (Fig. 1). Thus it appears that the transmissible entity may have been "lost." But this conclusion may not be the final one. Since the entity has shown some tendency of being unstable in its ability to suppress a tumor growth and since nothing is known of a possible tumor-inhibiting entity (perhaps even a very weak one) in a mouse of the $C_3H/ST$ or any other strain before the introduction of the liver extract, caution is to be recognized. It is not safe to predicate a hypothetical condition that may have existed many years ago even more than trying to anticipate what will occur in 016 generations of untreated descent mice and beyond, especially in the time when biological instability seems to be involved. Could this instability of the controlling mechanism of growth (the transmissible entity) be correlated with the instability of the cancer process itself?

## CONCLUSIONS

The evidence presented here would indicate that an unstable biological

transmissible entity in controlling cancer in mice was "induced" or at least conditioned by a liver extract. Following several generations of descent in mice of the $C_3H/ST$ strain, the entity began losing or changing its characteristic of suppressing the growth of a spontaneous cancer. The results of this study lead to the following conclusions:

1. There is a biologically controlling mechanism for the growth of a spontaneous tumor of mammary gland origin in mice.

2. This entity is transmissible for several generations of a lineal descent from a liver-emulsion-treated mouse and appears to increase in potency in suppressing cancer for several generations of mice of untreated lineal descent.

3. Following a period when the effect on the suppression of tumor growth seems to be stabilized, a reverse trend of loss of inhibitory effect on cancer has been detected.

4. By 15 generations of lineal descent the transmissible entity appears to be either lost or at least inactivated in suppressing cancer.

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