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A 'Transmissible Entity' in the Control of Cancer in Mice

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The paper is divided into three sections. Section 1 consists of an account of the historical development of a biological concept for cancer control in mice by the senior author. Section 2 presents new data on the effect of a liver-derived inhibitor of spontaneous tumors of mammary gland origin in mice in inducing a 'transmissible entity' that has been passed down through nine generations. The entity affects the biological characteristics of spontaneous tumors in mice and increases in potency in controlling cancer in mice of succeeding generations. The possibility of whether a genetic or a nongenetic transmission is involved in the passage of a 'transmissible entity' from generation to generation cannot be determined by the existing data. Section 3 lists the conclusions that have been reached concerning the biological and biochemical characteristics of the tumor inhibitor.

INTRODUCTION

It may be assumed, by some investigators, that by the use of about 3000 mice bearing spontaneous tumors of mammary gland origin (adenocarcinomata), as has been done in the present continuous experiment extending over 12 years, a final analysis of the data leading to final conclusions could be expected. In this case, however—that of the suppression of spontaneous tumors in mice—the problem is not so simple. The disease of cancer, even in mice belonging to highly inbred (homogeneous) strains, is extremely variable. Some of these variables, such as the presence of the virus of Bittner (MTV or the mother's milk agent), the estrogen hormone effect, and the genetic susceptibility and/or resistance complex, are known and at least partially analyzed. The present series of experiments dealing with the control of cancer with liver extracts from several species of animals has shown that

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there are many other variables, not fully known or appreciated and therefore not fully resolved.

With the discovery of these new variables in the control of cancer in mice, it becomes necessary to divide the orginal number of some 3000 cases of mice bearing spontaneous tumors into smaller and smaller classes in order to make continued progress toward the complete control of these spontaneous tumors possible.

A 'transmissible entity' in relation to the control of cancer apparently may be the 'missing link' in the chain of cause and effect as discussed in this paper, but even here time alone will tell.

Hence, the conclusion must be accepted that for the time being these studies on the control of cancer in mice by liver extracts, even though they belong to a continual sequence of observations leading to a progression of control of cancer, must be considered as progress reports. (The first step in the control of cancer is a suppression of its growth rate—and this observation has already been substantiated: Saunders, 1970.)

But the time element involved that has made the control of spontaneous tumors in mice at least partially controllable is far greater than the 12 years that the liver extracts of various species have been used. The sequence of events can be logically divided into four time periods (with some overlap) as follows:

Period 1. First was the production of an adequate supply of spontaneous tumors in experimental animals (1918–1930). When the present author became interested in cancer research, it was soon determined that spontaneous tumors in mice were so rare that a mouse so afflicted was worth $300. (This was the price Dr. G. H. A. Clowes paid for such mice when he was introducing biochemistry into cancer research in Buffalo, N.Y., from 1899 onward for several years: Clowes, 1936.)

Period 2. The production of an appropriate experimental animal for research was imperative since at that time there were no inbred strains of mice in existence. This need for science was accomplished by the process of genetic selection following an outcross (1921–1945). It has been maintained that 'the genetically homogeneous strains of mice constitute one of the greatest contributions of all times to medical research' (Heston, 1949).

The contributions of periods 1 and 2 of Strong above were then distributed to laboratories and investigators around the world. The evaluation of this good-will-to-science program should be reserved for some other time.

Period 3. The research program of the author for which the inbred mice and their spontaneous tumors were originally developed exclusively for the use of liver extracts in cancer control consumed the years 1918–1958. The original purpose was to investigate the cancer problem using genetics as the primary tool but incorporating other sciences as an adjunct to genetics, in the hope of ascertaining the fundamental nature of the cancer process.

In brief, the sequence of conclusions derived from this research program is as follows:

(a) 1922—'Race is the primary factor that determines whether or not a given individual shall or shall not grow the tumor mass progressively. Susceptibility and

non-susceptibility are manifestations of the genetic constitution of the individual' (Strong, 1922).

(b) 1924—'Our present data indicate that a particular host's reaction to a given neoplastic tissue is determined, to a great extent at least, by genetic factors introduced into the individual at the time of the formation of the zygote from which the individual developed' (Little and Strong, 1924).

As a result of this early interest in the cancer problem, and the reporting of data by many investigators, the author's concept on the nature of the cancer problem differs somewhat from others. One cannot ignore the host (and its genetic nature) in any aspect of cancer in any species including 'mice and men.' There is always a correlation or reverse correlation between the spontaneous tumor and either chemical or viral induction, even though the correlation may be rather subtle.

(c) 1938—'Two of my original inbred strains of mice were used. Mice of the CBA/ST give rise to spontaneous hepatomas, while mice of the A/ST strain never do. When 3-4-5-6 dibenzcarbazole was injected into mice of these two strains, hepatomas occurred early in CBA/ST mice but never in mice of the A/ST strain with the same amount of the "carcinogen" (carcinogenic in CBA/ST but not in A/ST?). All that the 3-4-5-6 dibenzcarbazole did was to hasten the onset of liver tumors that were already determined (genetically?) in the CBA/ST mice' (Strong and Smith, 1938). This principle has been greatly expanded and applied to lung tumors in mice (by Strong, Heston, Andervont, and others) and to other types of neoplasias by other investigators.

Sometimes this correlation between the spontaneous tumor and an induction system is reversed. For example, spontaneous mammary gland tumors occur in mice of cancer-susceptible strains (C_3H/ST breeders and virgin A/ST breeders only, etc.). But to get mammary gland tumors by chemical induction (for instance, methylcholanthrene) it is best to use a *cancer-resistant* mouse (such as JK/ST). In this case, the 'chemical carcinogen' effect obviously replaces the genetic susceptibility factor, etc.

(d) 1922–1958—That part of the original genetic concept that considered primarily an action–reaction system between a genetically determined host and a 'genetically' controlled tumor-cell (indicated first in 1922) (Strong, 1922) has continued to appeal to the present author most and is still, after 48 years, supermost in his mind (Strong, 1922, 1957*b*; Kaempffert, 1947). Incorporating some so-called new ideas, any deviation from normality consisting of (1) the introduction of a specific viral particle (either DNA with a peculiar coding system or RNA to interfere with normal transfer to the biological active protosynthetic processes in the cytoplasm) into the cell, or (2) the injection of a chemical carcinogen consisting, among other possibilities, of a source of energy when in combination with a genetic mechanism, or (3) even spontaneous genetic shifts which are known to occur in cancerous tissue would (Strong, 1926*a*,*b*), of necessity, change the equilibrium that normally exists between the host and the cells of the body. These genetic shifts, especially in association with an invading viral particle, are popular now in the 'new' science of

molecular biology but were amply considered by the older school of classical genetics.

(e) 1951—The conclusion was derived from an analysis of the data on chemical induction of tumors that the 'sum total of all cancers remain constant over a time period' (subsequent generations, etc.). Only the frequency of specific types of tumors changes by genetic selection (Strong and Sanghvi, 1951).

(f) 1957—The concept was published in *Science* that 'the various types of cancers which arise in the mammalian body have, at least, one characteristic in common. Cancer arises because the organism has lost control of a definitive part. Perhaps during the aging process, the mechanism that keeps the individual in equilibrium is disrupted, and as a result of this loss of control of all the parts, cancer of one or more elements is able to originate and to grow at the expense of the body' (Strong, 1957*a*).

This conclusion coupled with the observation published in 1951 that the 'sum total of all cancers remain constant over a time period' emphasizes the role of the host *in relation to all its parts* in the determination of some aspects of the characteristics of cancer. It is the author's candid opinion that if statistics continue to analyze any specific type of cancer against its absence rather than to analyze all cancer against no cancer, the role of the host, i.e., as part of the origin of the cancer process, will never be satisfactorily resolved. It is also true that if exclusive emphasis on the findings involving only the transplantable tumor is fostered, the cancer problem itself will never be resolved.

(g) 1927—Data were published that indicated a series of sudden changes in the physiological behavior of the cancer cell. This observation and others were the foundation stone for the study of histocompatibility (Strong, 1926*a*,*b*). This observation and those of Hauschka, Amos, and others indicate that the progression of the cancerous condition is a 'continuum of variability.' These observations lead to the conclusion that the process of controlled biological equilibrium is being disturbed continuously during the development and progression of the cancerous condition.

Therefore, from the survey of factual evidence (which could only be briefly outlined here) derived from many sources and obtained and verified by many investigators, the author is led to the conclusion that in order to control cancer, one must influence or control the mechanism or mechanisms within the body that is/are involved in the control of biological equilibrium.

(h) 1968—That part, at least, of the controlling mechanism regulating biological equilibrium in relation to cancer may be in the liver was indicated by a survey of the world literature as by many observations on the liver during many years of a cancer research program.

In part, the evidence for the development of the present concept of cancer control by liver extracts was assembled into a monograph from a series of lectures beginning with March 25, 1965, at Palomar College, San Marcos, California. The monograph was entitled *Biological Aspects of Cancer and Aging—Studies in Pure Line Mice* and was published by Pergamon Press in 1968 (Strong, 1968*a*).

The term 'in part' was used in the previous paragraph since some of the author's experimental evidence before publication and a survey of pertinent world literature were lost in transit to California from Roswell Park Memorial Institute in Springville, New York, in 1964 (Biological Station). This publication, therefore, was never intended for the final evaluation of the development of a concept of cancer control.

The essential feature, however, is not the establishment of every step in the development of a theory, but the demonstration of an established *fact* that by the injection of specially prepared liver extracts from several species of mammals, rodents, and fish, one can control or influence some of the biological characteristics of cancer such as (1) growth rate of cancer, (2) survival time of host, (3) percentage multiple primary tumors (hence the use of C_3H/ST mice), (4) percentage of regressions of spontaneous tumors, with or without recurrences, and (5) the percentage of metastases into the lung. A recent evaluation of the research program on cancer control in mice was contained in a letter (Saunders, 1970) dated April 28, 1970. Dr. Strong's 'original observation on the suppression of mammary cancer in C_3H/ST mice has been substantiated.'

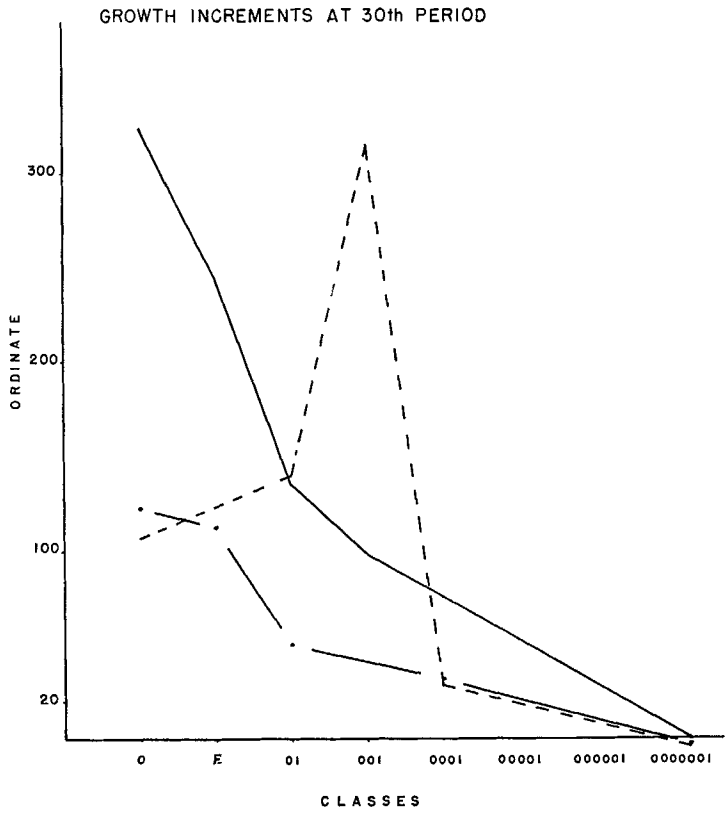

Fig. 1. This chart was drawn from the data on average increments of tumor growth (the ordinates) at the thirtieth observation period for three classes of tumor-bearing mice as follows: (1) the 'controls' (solid line), (2) the neomycin-treated mice (short-dash line), and (3) the mice receiving the tumor inhibitor intraperitoneally (dot-and-dash line). Generations of removal from a treated ancestor are given on the abscissa.

Period 4. During this period (1958–1970) spontaneous tumors in mice have been controlled by liver extracts.

NEW DATA

The new data obtained after the publication of the monograph mentioned previously and in eight subsequent publications (in period 4 cited above) are presented in Fig. 1. On the ordinate are averaged increments of tumor growth at the thirtieth period of observations for three classes of cancer probands as follows: (1) the 'controls' (207 mice—solid line; the term 'control' is used in this paper and elsewhere because from the new research the term 'control' has lost its significance—one must take into consideration the ancestry of a mouse in order to determine the biological characteristic of cancer); (2) tumor-bearing mice receiving neomycin in the drinking water (84 mice—short-dash line); and (3) tumor-bearing mice receiving the liver extract–tumor inhibitor (596 mice—dot-and-dash line). The data on the number of generations of mice in reference to a treated ancestor are given on the abscissa. The 0 indicates that there were no treated ancestors in the origin of the cancer proband (C_3H/ST mice are now at F_{141}); the E indicates that the cancer proband was born to or nursed by a mother which had a spontaneous tumor and was under treatment (intraperitoneally) with the liver emulsion containing a tumor inhibitor; the 0.01 indicates that the mother of a cancer proband was born to a female mouse with a spontaneous tumor which was also receiving the tumor inhibitor—i.e., the mother was not treated with the inhibitor. Successive generations indicate removal of a cancer proband from an ancestor with cancer receiving the tumor inhibitor.

DISCUSSION

New Data

It is obvious from an examination of Fig. 1 that all three curves reach a zero or negative value of tumor growth at approximately the same generation of removal from a treated ancestor (the 0.0000001 or 0^61 generation). The peak at the 0.001 generation for the neomycin-treated mice indicates a stimulation of tumor growth.

The second point of interest indicated by the receding curves is that there is a 'transmissible entity' in the determination of growth rate of tumors in mice and that this 'transmissible entity' increases in potency in controlling cancer in succeeding generations. This increase can be detected when the cancer proband is (1) continued as a 'control,' (2) receives only neomycin in the drinking water (with one exception in the 0.001 generation), or (3) receives intraperitoneal injections of the liver emulsion containing the tumor inhibitor.

The possibility of whether a genetic or non-genetic transmission is involved in the passage of a 'transmissible entity' from generation to generation cannot be determined by the existing data.

THE NATURE OF THE INHIBITOR OF SPONTANEOUS TUMORS IN MICE

Since the publications of this series of articles dealing with the control of cancer in mice are progress reports, the conclusions on some observations have had to be amplified. This applies particularly to the number of generations that the 'transmissible entity' has been detected.

CONCLUSIONS

1. The exposure of a mouse to an inhibitor of spontaneous tumors derived from liver extracts of various species *in utero* or by nursing a cancer proband receiving the inhibitor intraperitoneally alters the characteristics of cancer (growth rate and retrogressive potentialities) even though the neoplasm arises 8 or more months later in the offspring (Strong, 1969*a*).

2. The inhibitory effect on cancer has been passed through three generations of 'controls' and through five generations of the experimentals in which every cancer proband in the descent had been treated with the inhibitory (but not necessarily all the ancestors) (Strong, 1970*b*).

3. The further removed the cancer proband is from its tumor-inhibited–treated ancestor, the greater is the effect of the tumor inhibitor in the cancer proband. In other words, the effectiveness of the tumor inhibitor in suppressing the characteristics of cancer is increased through at least nine successive untreated generations of mice after it has once been introduced (present article). This conclusion is an expansion of conclusion 2 based upon additional data (Strong, 1970*b*).

4. Neomycin administered in the drinking water of a cancer proband will inactivate a tumor inhibitor administered intraperitoneally (Strong, 1968*b*). This result was obtained when the ancestry of the cancer proband had been ignored, although known. With the evidence obtained recently by the analysis of genetic heritage, conclusion 4 should therefore be revised. This has been done in conclusions 5, 6, and 7 as follows.

5. Neomycin when administered in the drinking water of a cancer proband obtained from a descent with no treated ancestors acts as an inhibitor of tumor growth in C_3H/ST and C_3HB/ST mice (Strong, in press).

6. Neomycin when administered in the drinking water of a cancerous mouse in which the grandmother had been injected with the inhibitor (while nursing the mouse which became the mother of the cancer proband) will inactivate or neutralize the inhibitory effect of a 'transmissible entity' originally conditioned by the intraperitoneal injection of the liver-derived tumor inhibitor. Compare with conclusion 4.

7. Neomycin administered in the drinking water of a tumor-bearing mouse whose great grandmother alone had received the tumor inhibitor will actually stimulate the growth rate of spontaneous adenocarcinomata of mammary gland origin in mice (Strong, in press). When the data of conclusions 5, 6, and 7 are all combined, thus ignoring genetic heritage, conclusion 4 is verified.

8. The injection of a liver-derived tumor inhibitor conditions or introduces a 'transmissible entity' in relation to the control of spontaneous tumor in mice; this conclusion amplifies conclusion 6 (present article).

9. This 'transmissible entity' increases in effectiveness in controlling cancer through succeeding generations of descent (present article).

10. Whether the 'transmissible entity' is genetic or not has as yet not been determined.

11. The inhibitor itself increases in effectiveness in suppressing spontaneous tumors up to 201–300 days of life (mouse liver alone tested), after which time the liver extracts (containing the tumor inhibitor) become less effective.

12. If two mice of a descent received the inhibitor (during tumor therapy), the effect of the inhibitor or its related 'transmissible entity' on cancer in the offspring is greater than when only one mouse of that descent had been so treated (in preparation).

13. The inhibitor of tumors in mice is heat sensitive (Strong, 1969*d*, 1970*a*). An emulsion containing the inhibitor and treated to 56C for 30 min (pasteurization) only once is less effective for the suppression of the growth capacity of spontaneous tumors than an emulsion not so treated (Strong, 1970*a*).

14. Two emulsions of common origin, one of which has been pasteurized (heated to 56C for 30 min), and the second not so heated, but both 'aged' under refrigeration, reach a maximal inhibitory action on cancer after a period of time of approximately the same magnitude; i.e., the difference between the two emulsions for their capacity to suppress tumor growth is in the early period of storage (Strong, 1970*a*).

15. Aging an emulsion containing the tumor inhibitor at room temperature produces a better inhibitor per unit of time than one aged in the refrigerator (Strong, 1969*d*).

16. An emulsion with the inhibitor aged for a period of time at room temperature and then placed under refrigeration has an intermediate effect upon the growth rate of cancer between room temperature aging and refrigeration (in preparation).

17. Heating the emulsion at 40C for 10 days weakens the activity of the tumor inhibitor.

18. Recovery of tumor inhibitory activity in the emulsion used in 17 takes place at a faster rate when it is kept at room temperature rather than under refrigeration (in preparation).

19. Heating an emulsion containing the tumor inhibitor at 45C for 14 days actually brings about the formation of some ingredient that will stimulate the growth of spontaneous tumors in mice (in preparation).

20. Partial recovery or loss of stimulation of tumor growth takes place in the emulsion prepared as described in 17 if kept at either room temperature or under refrigeration.

21. It is yet to be determined whether the emulsion prepared in 18 and kept in 19 (either room temperature or under refrigeration) will ever develop into an inhibitor of tumors.

22. Storage of an emulsion containing the tumor inhibitor under refrigeration increases its activity in suppressing the growth capacity of tumors (*Cytobios*, 1969).

23. This increase in inhibitory action on tumors mentioned in 21 may be cyclic in nature but with reduced cycles with advancing 'aging'. This observation, however, requires additional data (*Cytobios*, 1969).

24. There is no evidence of permanent 'decay' in the effectiveness of the inhibitor with long periods of storage under refrigeration. A maximal effectiveness is reached which is then retained. However, 'decay' may set in with longer storage periods than have been used to date. Also, keeping the emulsion for long times at room temperature may be conducive to 'decay' or even the formation of some toxicity (under experimentation).

25. A male mouse in the prime of life (between 201–300 days of life) is the best source of the tumor inhibitor so far determined.

26. Since a male mouse at 201–300 days of life is in 'biological equilibrium,' the concept is continued to be entertained that the origin of cancer in mice and its continuance may somehow or other be associated with a disturbance of 'biological equilibrium' as applied to the entire organism.

27. The second-best inhibitor of cancer in mice has been obtained from the liver of a male horned shark (*Heterodontus francisci*) (Strong, 1969*a*).

CONCLUSION

Considerable progress has been made in the control of cancer in mice. It would be a tragedy if not an outright disgrace to science to kill all the mice now (to phase out 'by 6/30/70') as suggested by one of the cancer funding organizations. The nature of the 'transmissible entity' (the missing link?) is quite unknown. Many other initial observations could also be made *only to the carefully controlled colony of experimental mice that are now available* in the laboratory in Sorrento Valley, San Diego, California. It would take someone at least 3 years to reproduce the experimental animals that are already available.

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