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The Stimulation and Inhibition of the Growth Capacities of Spontaneous Tumors of Mammary Gland Origin in Mice (Adenocarcinomata)

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Following incubation at 40 C for 10 days, a liver emulsion was divided into two moieties. One (REF) was aged in the refrigerator; the other (ART) was aged at room temperature. With each moiety there were obtained three reactions in regard to the growth capacity of spontaneous tumors. These observations were in sequence (1) a stimulation of growth rate of tumors, (2) no effect on growth capacity of tumors, and finally (3) a pronounced inhibition of tumor growth. Since three of the entities contained in the liver emulsion have been determined by Dr. Arnold Mittleman of Roswell Park Memorial Institute, Buffalo, New York, a brief discussion of these facts is included. These observations emphasize very significantly the role of temperature control and aging of the tumor inhibitor in the development of cancer suppression in spontaneous neoplasms in mice.

INTRODUCTION

The inhibition of growth rates of spontaneous mammary gland adenocarcinomata in mice by a specially prepared liver extract in distilled water has been reported in a series of papers since 1968 (Strong, 1968, 1969*a*,*b*,*c*,*d*,*e*, 1970*a*,*b*,*c*; Strong and Matsunaga, 1970*a*,*b*, 1971*a*). The suppression of growth of cancer has been shown not to be uniform over a period of time, since cycles or rhythms of activity of a possible tumor inhibitor have been the constant rule. The tumor inhibitor has been 'constant in being inconstant.' The degree of inhibition of spontaneous tumors has usually fluctuated between 100 percent of growth suppression to none (i.e., to the same growth rate as the controls). Occasionally, however, the growth

This experiment has been made possible by contributions of private individuals in the United States. Many of the contributions have been given by friends and relatives as memorials to those who have died of cancer.

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rate of the tumor receiving the liver extract appeared to be higher than in the controls, thus indicating a stimulation of growth. Since this enhanced growth rate of tumors has been relatively rare and transient, little significance has, in the past, been emphasized.

Recently, the stimulation of growth rates of tumors has been so pronounced and extended over a period of time that it now becomes important to emphasize a stimulated growth of a tumor and the conditions underlying its appearance, continuance, and/or disappearance.

The purposes of the present paper, therefore, are as follows: (1) to present data indicating that the same liver preparation can serve either as a stimulator or as an inhibitor of spontaneous tumors in mice, (2) to indicate that this change of effect on the growth capacity of a spontaneous tumor can be brought about by heat and storage over a period of time undoubtedly of some constituent contained in a liver preparation known to be made up of a mixture of distinct chemical entities, (3) to report on the determination of some biochemical units contained in the liver extract, as reported by Dr. Arnold Mittelman, Program Director of the General Clinical Research Center of Roswell Park Memorial Institute of Buffalo, New York, and (4) to discuss, briefly, the possible relationship between the known entities of the liver emulsion and the facts that have been established on the stimulation and inhibition of spontaneous tumors of mammary gland origin in mice.

MATERIALS AND METHODS

The same techniques of preparation of liver extracts as have been used for the series of 12 papers referred to previously have been used for this report (Strong, 1968, 1969*a*,*b*,*c*,*d*,*e*, 1970*a*,*b*,*c*,; Strong and Matsunaga, 1970*a*,*b*, 1971*a*). The only difference now was that the liver extract, after pasteurization at 56 C for 30 min, was incubated at 40 C for 10 days. When this last procedure of incubation was completed, the liver emulsion was divided into two moieties on 10/27/69. One moiety was then stored in the refrigerator (−2.2 C) and was given the symbol REF; the other moiety was placed at room temperature (22.2–28.9 C) and received the symbol ART. On 7/23/70 the part that had been kept at room temperature was also placed in the refrigerator.

Since the aging of the liver emulsion has been shown to have an effect upon the biological activity of the tumor inhibitor (Strong, 1969*b*), the following schedule of starting successive series of four mice each bearing spontaneous tumors of mammary gland origin with a regime of receiving the tumor inhibitor by intraperitoneal injections for the moiety (ART) aged at room temperature was as follows.

Series 1 was begun on 12/26/69—age of liver extract since the addition of distilled water was therefore 70 days; series 2 on 1/28/70—age of material 103 days; series 3 on 2/20/70 at 120 days; series 4 on 4/7/70 at 172 days; series 5 on 5/4/70 at 199 days; series 6 on 6/1/70 at 227 days; series 7 on 7/6/70 at 262 days; and series 8 on 8/27/70 at 314 days.

A similar series at dated times was also done with the liver emulsion aged in the refrigerator (REF).

The source of mice bearing spontaneous tumors of mammary gland origin was the same as that reported in the previous papers dealing with the inhibitor. These were the well-known C_3H/ST and C_3HB/ST inbreds.

The biochemical analysis of the liver emulsion of Bull II 456, the same material that was used in this experiment, was done by Dr. Arnold Mittelman.

The results are presented in two figures.

RESULTS

Figure 1 contains the data of growth rate of spontaneous tumors in mice for (1) the controls on the solid line and (2) the eight series of mice, with the same histological type of tumors as the controls, that received periodic injections of a liver emulsion obtained from Bull II 456 (7 years of age) and incubated at 40 C for 10 days following pasteurization at 56 C for 30 min. These data were obtained with the ART material. Successive series of 1–2, 3–4, 5–6, and 7–8 are added together or combined for the purpose of presentation. Average sizes of the tumors expressed in square millimeters are given on the ordinate; the successive observation periods 5, 10, 15, to 50 (three periods per week), are given on the abscissa. It is seen from an inspection of Fig. 1 that in combined series 1–2 and 3–4 there was a stimulation of spontaneous tumor growth, whereas in series 5–6 and 7–8 there was obviously obtained an inhibition of spontaneous tumor growth.

Figure 2 presents the data obtained in the REF series on the effect of the liver emulsion which had been kept in the refrigerator following incubation at 40 C for

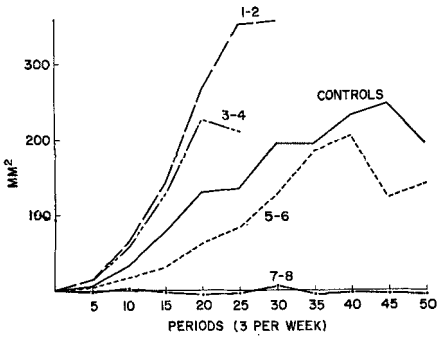

Fig. 1. Data on growth rate of spontaneous tumors for the ART experiment. Controls on solid line, series 1–2 on long dashed line, series 3–4 on short and long dashed line, series 5–6 on short dashed line, and series 7–8 on dotted and long dashed line. Ordinate shows size of tumors in square millimeters; abscissa shows successive determinations of tumor size at observation periods 5, 10, 15, etc. (three periods per week).

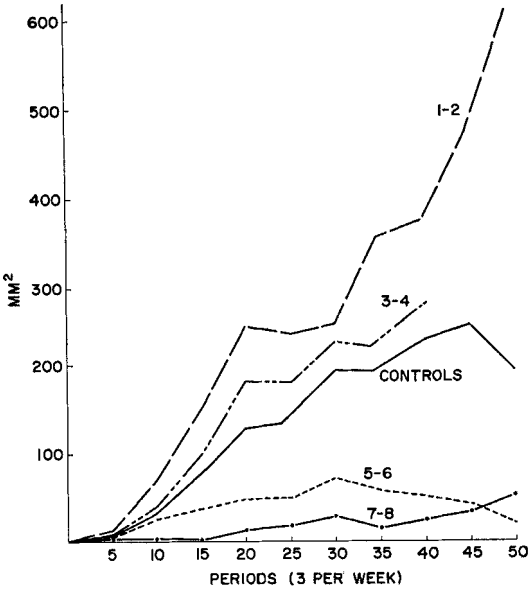

Fig. 2. Data on growth rate of spontaneous tumors for the REF experiment. Controls on solid line, series 1–2 on long dashed line, series 3–4 on short and long dashed line, series 5–6 on short dashed line, and series 7–8 on dotted and long dashed line. Ordinate shows size of tumors in square millimeters; abscissa shows successive determinations of tumor size observation periods at 5, 10, 15, etc. (three periods per week).

10 days. The data on the growth rate of tumors in the controls (the same as used in Fig. 1 for the ART series) are on the solid line. Successive series 1–2 (long dashed line), 3–4 (long and short dashed line), 5–6 (short dashed line), and 7–8 (solid dotted and long dashed line) are also indicated for comparative analysis.

Figures 1 and 2 are very similar, and the same conclusions can be derived from the study of either figure.

In series 7–8 of Fig. 1 there was a complete suppression of growth of all spontaneous tumors (eight mice). Except for one point (observation period 30) there were obtained values that indicated a regression of all tumors between observation periods 15 and 50 (i.e., 100 percent regressions of all tumors).

DISCUSSION

It was hoped that by the biochemical analysis of the liver preparation a better understanding of the known biological characteristics of the tumor inhibitor would ensue. So far, this possibility has not materialized. It is true that only a preliminary attempt has been made to analyze the liver emulsion known to contain an inhibitor of cancer in mice. The biochemical work has been done by Dr. Arnold Mittleman. Following several progress reports which have been quoted in a previous paper

(Strong and Matsunaga, 1971*b*), Dr. Mittelman reports that among the entities contained in the liver emulsion of Bull II 456 (the same preparation used in the present investigation of stimulation and inhibition of cancer growth in mice) there have been identified a 'small quantity of adenosine, a larger quantity of N^6-methyladenosine, and 5-methylcytidine. I will shortly send you the mole percent of these various constituents of Bull II 456.'

Now, among the several known biological characteristics of the inhibitor of cancer in mice, a few items stand out as requiring some eventual explanation, presumably by the analysis of a known constituent or constituents of the liver emulsion. These are briefly (1) that the tumor inhibitor is heat sensitive (Strong, 1969*e*, 1970*a*); (2) that the tumor inhibitor increases in potency in suppressing the growth capacity of spontaneous tumors in mice by aging (Strong, 1969*b*), this being brought about or conditioned in the liver emulsion by being stored in either the refrigerator (–2.2 C) or at room temperature (22.2–28.9 C); (3) that the tumor inhibitor's activity in suppressing spontaneous tumors is cyclical or rhythmical over a period of time (Strong and Matsunaga, 1971*a*); (4) that apparently the same entity which can exert an influence on the suppression of the growth capacity of a spontaneous tumor in mice can be converted into a stimulator of cancer by incubation at 40 C for 10 days (present data); (5) that the stimulator of cancer mentioned in (4) above can be reconverted into an inhibitor of the growth capacity of spontaneous tumors by storage either in the refrigerator (–2.2 C) or at room temperature (22.2–28.9 C); and (6) that the injection of a biochemical entity that has a pronounced effect upon the growth capacity of cancer (both stimulation and inhibition) is capable of 'inducing' or conditioning a 'transmissible entity' that has been proven to be involved in the biological or genetic mechanism for the control of spontaneous cancer in mice and is transmitted with increased potency in mice in suppressing cancer for several generations of untreated descent (Strong and Matsunaga, 1970*a*).

Obviously, it would be desirable to find some entity in the liver emulsion that undergoes periodical changes that may explain the variable characteristics of the tumor inhibitor.

Unfortunately, the pioneer or preliminary biochemical research of Dr. Mittelman and his associates has not solved the problem completely, even though the groundwork has apparently been established and may lead to the final answer.

One of the extenuating or complicating circumstances that is now obvious but that was not realized earlier must be reported. The schedule of using successive series of tumor-bearing mice for the same liver preparation following incubation and storage (Bull II 456) has been presented in this paper. It was found that three general observations (observed in sequence of time) on the growth of spontaneous tumors were made as follows: (1) there was an initial period of stimulation of tumor growth that was obtained between 12/26/69 to 4/4/70 (series 1–2 and 3–4); (2) there was no effect of the liver emulsion on the growth capacity of spontaneous tumors about 4/25/70 by the use of the same liver preparation, i.e., the tumors with the liver injections grew at the same rate as the controls; and (3) there was

observed the inhibition of tumor growth between 5/11/70 and 8/27/70 (series 5–6 and 7–8). The maximal effect of the suppression of tumor growth was obtained in series 7–8, indicated in Fig. 1, at which time the liver emulsion used was 267 and 314 days of age with storage at room temperature, respectively (ART).

Unfortunately, or perhaps fortunately, a sample of the liver emulsion of Bull II 456 that gave the data referred to above on the effects of tumor growth was given to Dr. Mittelman on 4/25/70, or at the time that the emulsion had no or little effect upon the growth capacity of tumors. This observation was not known at the time the liver emulsion was given to Dr. Mittleman.

What is imperatively needed, therefore, is the periodic testing of the liver emulsion for biochemical changes over a period of time while the actual inhibitor or inhibitors of cancer in mice is/are being converted from a stimulator of cancer to an inhibitor or vice versa.

Bearing in mind also that the liver emulsion conditions or activates a 'transmissible entity' following intraperitoneal injections into one or more ancestors of the cancer proband, the nature of this possible 'mutagen' must eventually be known. If the materials or possible changes in the materials responsible for this biological effect on the resistant controlling mechanism or mechanisms for spontaneous tumors in mice prove to be a nucleoside or a mixture of nucleosides or some chemically related entity or entities, then rapid progress in several cognate fields would obviously result.

The analysis of the mechanisms involved in a variety of biological phenomena relating to the origin and control of cancer may therefore be only a matter of time.

CONCLUSIONS

1. A liver emulsion can be converted into a stimulator of spontaneous tumors in mice by incubation at 40 C for 10 days.

2. Following a period of stimulation of cancer, the same liver emulsion will inhibit the growth capacity of spontaneous tumors.

3. The conversion of stimulation to suppression of cancer can be brought about by storage in either the refrigerator or at room temperature.

4. Among the entities of the liver emulsion used (Bull II 456) are the following entities: adenosine, N^6-methyladenosine, and 5-methylcytidine.

5. The preliminary attempt to correlate the known biological characteristics of a spontaneous tumor inhibitor with the known biochemical constituents of the liver emulsion has been initiated.

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