Studies on the Effects of Adenosine Upon Tumor Growth in Mice

LEONELL C. STRONG, Ph.D., and DEBRA ISHMAEL

Growth rates of spontaneous tumors of C_3H and C_3HB inbred mice were averaged together. Four series of tests using adenoisine as an inhibitor of tumor growth were used in a 0.05 molar concentration. The four series used were, (a) adenosine I, (b) adenosine II both for intraperitoneal injections, used singly and then combined for comparative analysis, (c) adenosine III, and (d) adenosine IV, both used for subcutaneous injections, of which series III was used in distilled water and series IV in physiological salt solution. Observations on tumor size were plotted at five time-period intervals in order to determine growth rates. It was found that adenosine IV, in physiological salt solution, gave the greatest deviation from from the controls both in inhibiting tumor growth and also in stimulating tumor growth in mice of the different age and generation classes as determined in a lineal descent from a mouse which had received a liver-emulsion injection. The subcutaneous injections of adenosine III gave only an early inhibition of tumors whereas the intraperitoneal injections of adenosine I and II brought about a constant inhibition of tumor growth during the life span of tumor-bearing mice. Variations in the effect of adenosine upon tumor growth were found to be dependent upon 1. the age of the mouse and 2. the generation of descent removed from the original liver treatment (Strong, 1969a). These tests for tumor growth, therefore, provided a close examination of some of the variables involved in the use of adenosine as an inhibitor and as a stimulator of spontaneous tumor growth rates in mice.

INTRODUCTION

Earlier papers by Strong and by Strong and Matusunga (1972a, b), have shown that adenosine is one of the three nucleosides, found in a liver extract, demonstrated to inhibit the growth of spontaneous tumors of mammary gland origin in mice.

From the Leonell C. Strong Research Foundation, San Diego, California

The present study has been concentrated on the use of 0.5 mM solutions of adenosine in distilled water and also in physiological salt solution, since adenosine is found in that concentration in the original liver extract (Strong and Matsunaga, 1972a). In their experimental observations on the effect of adenosine on tumor growth, Strong and Matsunaga also made use of the C_3H and C_3HB strains.

Previous work has suggested that adenosine has a similar effect on the liver extract on the suppression of spontaneous tumors, although less marked. The purpose of this paper is to present the evidence that adenosine as an inhibitor of cancer growth in mice is dependent upon several variables such as: 1. age, 2. the generation of the cancer proband removed from the injections of the liver emulsion into one of the cancer probands ancestry, 3. the injection technique, and 4. especially the use of a solution in physiological salt.

Preparation of the nucleoside was made up in 0.5 mM distilled water as well as in physiological salt solution. (See Strong, 1969a for a full discussion of the liver preparation.) All solutions were kept in the refrigerator when not in use and warmed to room temperature before injection into the mice to avoid spasms. The dosage administered was variable depending upon the physical appearance of the mouse and the surface examination of the tumor. Maximum dosage was limited to 0.40 cc. Following a complete regression of the tumor the mouse was never injected with more than 0.20 cc of the material, and this procedure never exceeded frequency once in three weeks.

The mice were examined three times weekly. Their tumors were measured with a caliper in the two longest diameters. The tumor size was calculated by multiplying the two longest diameters together. (It is recognized that this method is not an absolute measure of the tumor. However, Strong has used this method for many years and has obtained reproducible growth rates. It is, therefore, justified.) Variables such as cysts, hemorrhagic areas, and amount of connective tissue could not be determined.

The estimated effect of the treatment upon the spontaneous tumor is not dependent exclusively on growth rate but also upon percent regression of the tumors and the survival time of the mouse following the discovery of its tumor. Since many of the mice are still alive, their actual survival time is still not available.

Treatment consisted of injections of adenosine intraperitoneally in series I and II, subcutaneous in III, and a subcutaneous injection of adenosine dissolved in physiological salt in IV, three times a week.

RESULTS

The results are presented in one table and five figures.

Table I shows that 395 tumor-bearing mice were used in this analysis. Of

TABLE I.

Series		Mice used
Adenosine	I	60
	II	36
	III	32
	IV	20
Controls		247
TOTAL		395

these 247 mice were kept as controls. The remaining 148 were treated with adenosine according to the following series: series I, 60; series II, 36; series III, 32; and series IV, 20.

Figure 1 shows the data on growth rates of spontaneous tumors in series I (long dashed line), series II (solid line with dots), combined series I and II (short dashed line), and the controls (solid line). This is evidence that adenosine, when administered intraperitoneally, is an inhibitor of tumor growth. The data of series II, therefore, verify the data obtained in series I. Figure 2 presents the data on growth rates of tumors in combined series I and II (short dashed line), series III (long dashed line), and the controls (solid line). In this figure only mice between 5 and 8 months of age and belonging to one to two generations removed from the injection of the liver emulsion into the cancer probands' ancestors (generations 1 to 2 are included). Therefore, adenosine administered intraperitoneally to mice of series I and II is a mild inhibitor of tumor growth up to the twentieth period of observation. However, due obviously to mice with larger tumors dying first, this average size of remaining tumors is somewhat less than that of the controls. In series III, it appears that a subcutaneous injection of adenosine is a better inhibitor than that administered intraperitoneally.

Figure 3 presents data on growth rates of tumors in mice in the 5 to 8 month age group regardless of number of generations removed from the injection of the liver emulsion into an ancestor. The data obtained in series III (subcutaneous injection of adenosine in distilled water) show the greatest inhibition of tumors. The data in series IV (the same adenosine dissolved in physiological salt solution) show a pronounced stimulus to tumor growth.

Figure 4 shows the averaged growth rates of tumors for the controls (solid line), mice in series III (long dashed line), mice in combined I and II series (short dashed line), and mice of the IV series (long and short dashed line). All mice shown were between 9 and 12 months of age at the time they developed spontaneous tumors. At this age, all mice receiving adenosine in series I, II, III, and IV had the growth of their tumors inhibited. Note that all mice in this chart belonged to all generations removed from the original injection of the liver emulsion into one of the ancestors.

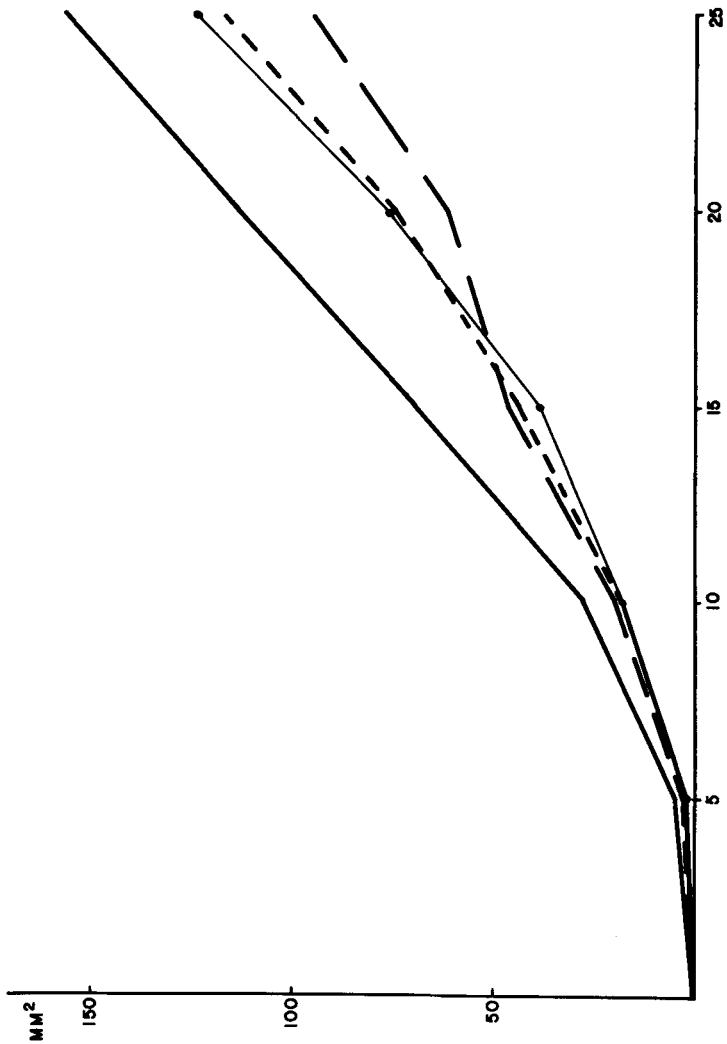

Fig. 1. Horizontal axis: time expressed in intervals of 5 successive periods of growth, (observations spaced 3 per week). Vertical axis: size of tumore in mm^2. Long dashed line: Series I; solid line with dots: seriesII; short dashed line: combined series I and II; solid line: controls.

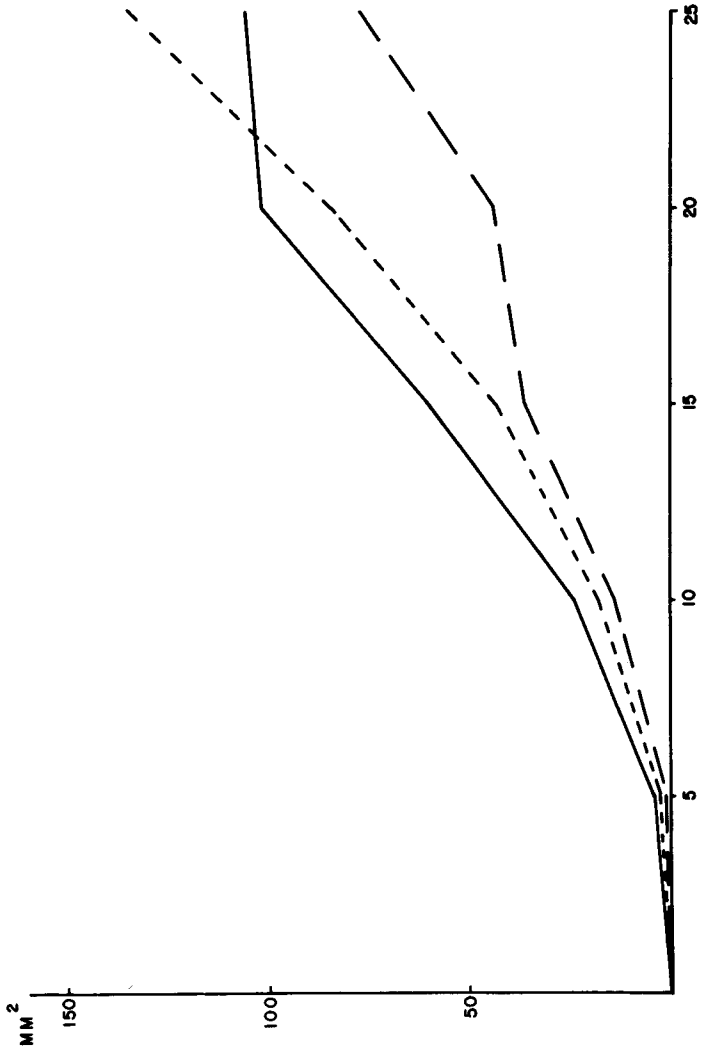

Fig. 2. Same axes as Fig. 1. Short dashed line: combined series I and II; long dashed line: series III; solid line: controls.

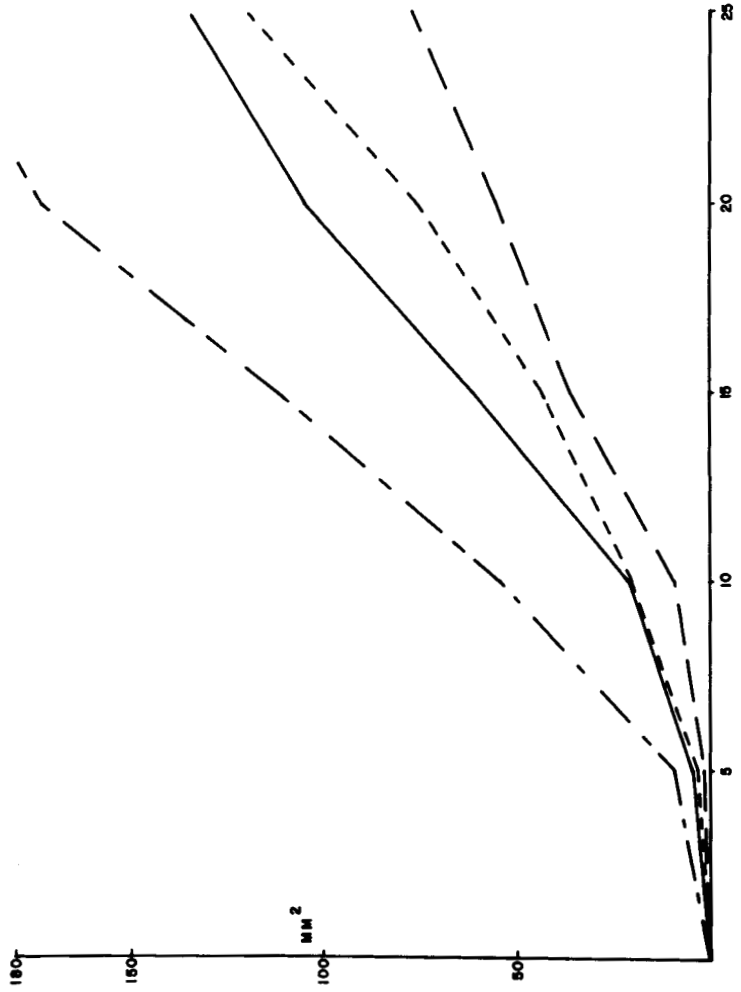

Fig. 3. Same axes as Fig. 1. Solid line: controls; short dashed line: combined I and II; long dashed line: series III; short and long dashed line: series IV.

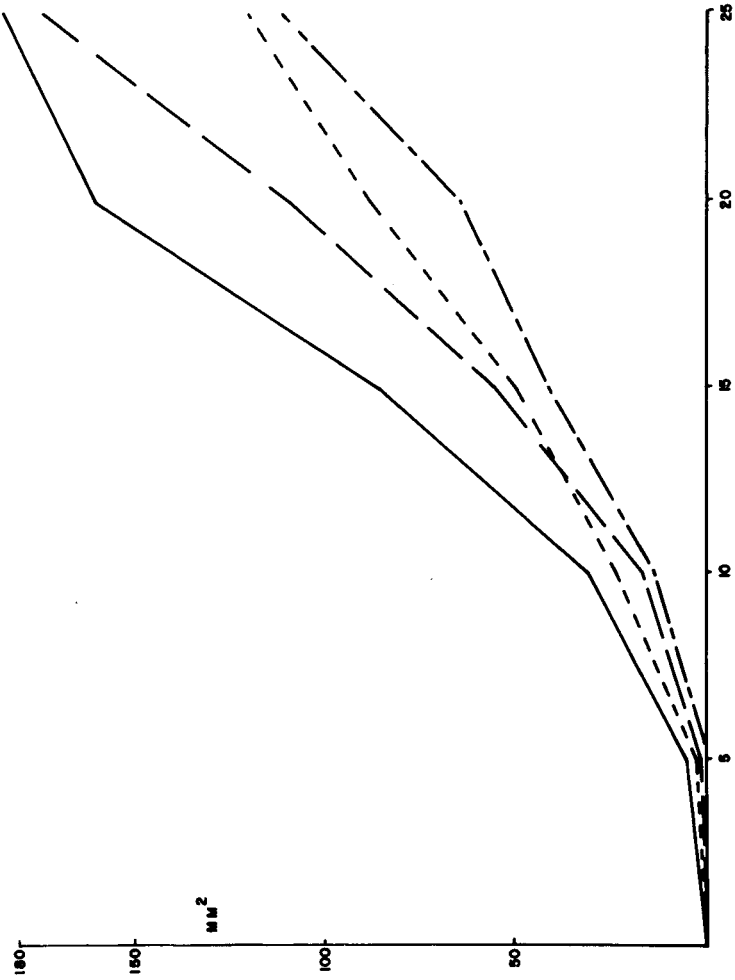

Fig. 4. Same axes as Fig. 1. Solid line: controls; long dashed line: series III; short dashed line: combined series I and II; long and short dashed line: series IV.

Figure 5 presents the data on growth rates of tumors in mice between 0_3 and 0_6 generations removed from the original liver-emulsion injection. Data are presented for the controls (solid line), combined series I and II (short dashed line), series III (long dashed line), and series IV (short and long dashed line).

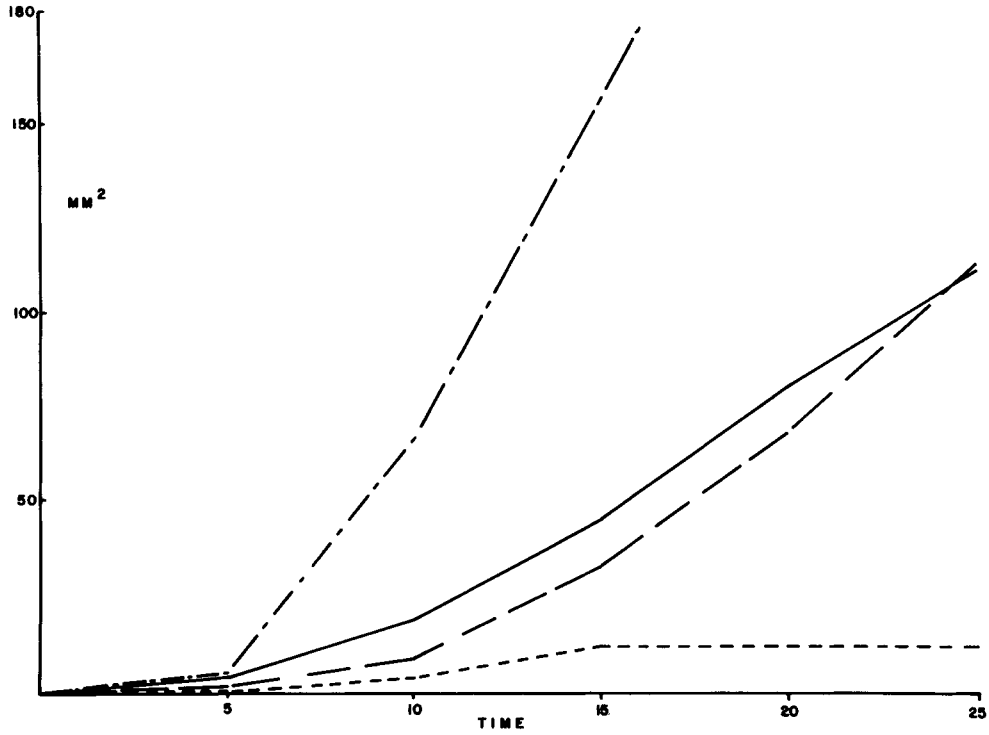

Fig. 5. Sames axes as Fig. 1. Solid line: controls; short dashed line: combined series, I and II; long dashed line: series III; short and long dashed line: series IV.

DISCUSSION

The age of the cancer proband and the number of generations from the original injection of a specially prepared liver emulsion into one of the ancestors of the cancer stocks have an influence on the growth rate and fate (eventual regression with or without recurrence) of spontaneous tumors of mammary gland origin in mice. This has been amply indicated in the data of Strong, 1969a, and of Strong and Matsunaga, 1971 a–c, 1972, a, b. The present data with adenosine verify these conclusions and indicate two more variables which have, at least, been partially resolved. These new variables on the suppression or stimulation

of growth of a spontaneous tumor in mice are 1. a subcutaneous injection of the nucleoside compared to an intraperitoneal administration, and 2. the use of a preparation of adenosine in physiological salt solution compared to one in distilled water. These studies have all been done with a single molar concentration only, i.e., 0.50 mM, which is the concentration of adenosine in the original liver emulsion.

There is a striking increase of inhibition of tumor growth when the adenosine was injected subcutaneously (series III, Figs. 2 and 3) as compared to the intraperitoneal injections of mice in series I and II, which were combined into a composite series since the data were shown to be very similar in the two series. However, the reverse of this comparison was obtained in the analysis of the data from Figs. 4 and 5 when the tumor-bearing mice of ages 9 to 12 months were used alone and generations 0_3 to 0_6 separation from an original injection into an ancestor of the tumor-bearing mouse were used. In this latter analysis only mice between 5 to 8 months were used.

Observations of the results obtained by using adenosine prepared with a physiological salt solution were even more striking. In Figs. 3 and 5, the data indicate a pronounced stimulation of tumor growth in series IV in tumor-bearing mice of 5 to 8 months in either total mice used (Fig. 3) or in those mice which were in the 0_3 to 0_6 generation of descent from the injection of the original liver emulsion into one of the ancestor of the tumor-bearing mice. Quite in contrast to this finding, the data on growth rate of tumors in series IV of Fig. 4 show that the greatest inhibition of tumor growth (as compared to combined series I and II and series III) was obtained in mice between 9 and 12 months of age.

The effect on tumor growth of separating a lineal descent, through continued in-breeding, from an original injection of a liver emulsion including adenosine as well as two other nucleosides, has led Strong and Strong and Matsunaga (1971c) to conclude that a transmissible entity, which is handed down to succeeding generations, is indicated to explain the data. Since the potency of the transmissible entity to suppress cancer varies from generation to generation it has been suggested that the entity is fluctuating. Again the numerous observations that have been made of studies of spontaneous tumors in mice also leads to the concept that cancer itself is a continuum of variability.

The present series of studies on the effect of several nucleosides found in the liver emulsion, including adenosine, on the growth and fate of cancer is entering into the analysis of the complex biologically variable state involved in this neoplastic condition, at least in mice.

Many of the variables, such as age of the mouse, number of generations removed from the injection of a liver emulsion, mode of application, and vehicle of preparation, are now known.

CONCLUSIONS

The inhibition of spontaneous tumor growth in mice by a 0.5 mM preparation of adenosine in distilled water has been verified (series II duplicates the growth rate of series I) (see Fig. 1).

A subcutaneous injection of a 0.5 mM preparation of adenosine gives more inhibition of tumor growth than the intraperitoneal route [see Fig. 2 where series III subcutaneous is compared to series I and II combined (intraperitoneal) and the controls – all mice 5 to 8 months of age and belonging to generations $E–0_2$; see also Fig. 3 where series IV has been added and all mice between 5 and 8 months have been included].

A subcutaneous injection of a 0.50 mM preparation of adenosine has less effect on inhibiting tumor growth when series I and II are compared to series IV where all mice are 9 to 12 months of age (Fig. 4).

In mice between 5 and 8 months and belonging to generations 0_3 to 0_6 removed from the injection of the liver emulsion into one of the ancestors, a subcutaneous injection of a distilled water preparation of 0.50 mM adenosine has little or no effect upon tumor growth (Fig. 5), whereas inhibition was obtained in series I and II and a pronounced stimulation in series IV.

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