Comparative Effect of Three Nucleosides on Suppression of Cancer Growth in Mice

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It has been determined that three nucleosides, which were found in a liver emulsion, have effects on the growth of spontaneous tumors in mice. An 0.05 molar solution of adenosine inhibits the growth of spontaneous tumors in 5–8 month old mice but not in one aged 9–12 months. An 0.08 molar solution of 6-methyl adenosine also inhbits the growth of spontaneous tumors in 5–8 month old mice but not in one aged 9–12 months. An 0.02 molar solution of 5-methyl cytidine is a powerful inhibitor of spontaneous tumors in mice irrespective of age of host. The differential effect of the three nucleosides upon the growth of spontaneous tumors may be conditioned by a differential rapidity of growth of cancer depending upon the age of the cancer proband. That is, the age of the cancer probund may effect the growth capacity of a spontaneous tumor and its response to a nucleoside.

INTRODUCTION

Three nucleosides that are known to be present in a liver extract which has an inhibitory effect upon the growth of spontaneous tumors of mammary gland origin in mice have been tested upon this same type of cancer. They have been tested singly and with two of them in combination with each other, and in

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the same molar percentages as they occur in the original liver emulsion. It has been shown that 1. an 0.05 molar percentage solution of adenosine may serve as either an inhibitor or a stimulator of cancer depending upon the age of the solution; 2. an 0.08 molar percentage solution of 6-methyl adenosine is also an inhibitor of cancer; 3. an 0.02 molar percentage solution of 5-methyl cytidine is the best inhibitor of the three nucleosides tested; and 4. a combination of an 0.05 molar solution of adenosine and an 0.08 molar preparation of 6-methyl adenosine will stimulate the growth of cancer in mice. Thus the similarity of effect of these three nucleosides upon the growth of cancer as compared to that by the original liver emulsion is striking (Strong and Matsunaga, 1970).

MATERIALS AND METHODS

The source of mice with spontaneous adenocarcinomata is the same as that used in the previous communications on cancer control. This source is the well known C_3H/St inbreds together with the subline C_3HB/ST, characterized by a mutation at the microphalmic locus, mi. The mice were peridoically examined for tumors by palpation and were placed in an experiment as soon as it was determined that a progressively growing tumor was apparent. Measurements were made three times weekly by veniers for the two longest diameters of the tumors and these values were multiplied together and the data plotted consecutively in order to determine the fate of the tumor—either progressive or retrogressive growth based upon the average increments of growth at successive periods of time.

It has been determined that the age of the female at the time of onset of the spontaneous tumor is a factor in the determination of the growth pattern of that neoplastic disease. A spontaneous tumor in a mouse which is between 9 and 12 months of age will grow faster than one in a mouse which is between 5 and 8 months. This age factor of the cancer proband must now be taken into consideration in the evaluation of effect that the present three nucleosides have upon cancer in mice.

The nucleosides were put in distilled water in the molar percentage as they exist in the original liver emulsion as determined by Dr. Arnold Mittelman of Roswell Park Memorial Institute, Buffalo, N. Y. The solutions were placed in rubber stoppered serum bottles and kept in the refrigerator at 2.2°C when not in use. The serum bottles were wrapped in aluminum foil. The solutions were placed at room temperature before injections intraperitoneally into mice in order to avoid spasms which usually occur following the injection of cold materials in mice.

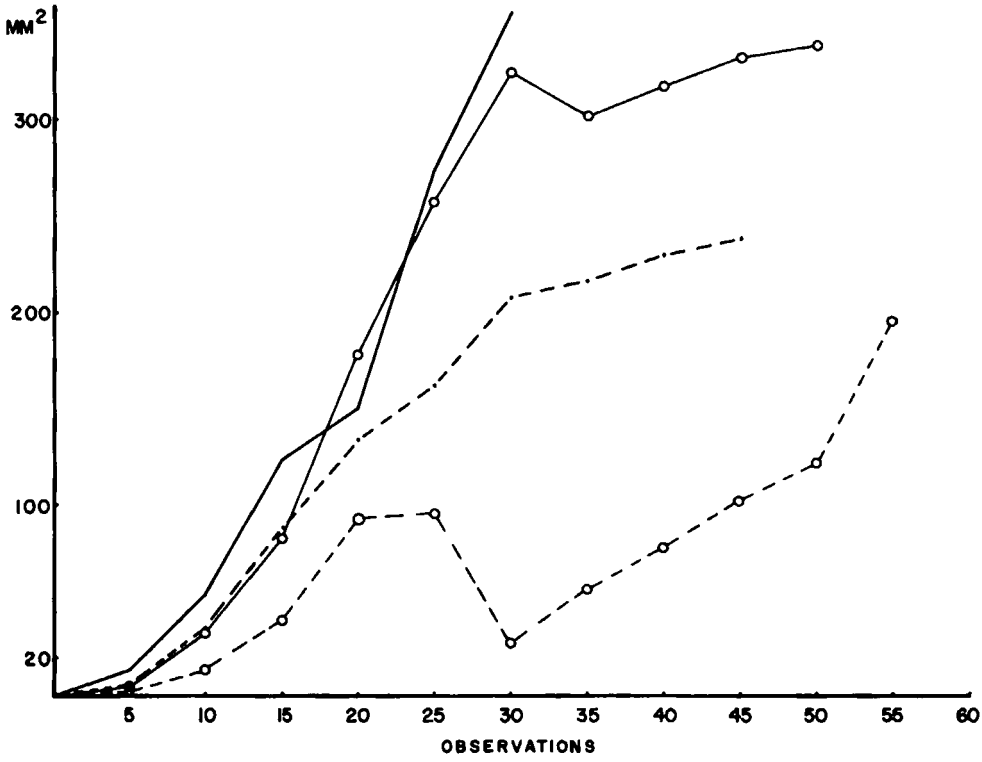

Fig. 1. Data obtained with 0.05 molar percentage solution of adenosine. Solid line: controls for mice between 9 and 12 months of age. Short dash line: controls for mice between 5 and 8 months of age. The two age groups of mice receiving the adenosine are on the two open circle curves as follows: Open circle and solid line, mice in 9–12 month old group; Open circle short dash line: mice of the 5–8 month age group. Ordinates: increments of tumor growth; abscissa: successive observations at the 5th, 10th, 15th, etc., periods of time.

RESULTS

The results are presented in a series of three figures. Figure 1 presents the data obtained by the use of a 0.05 molar percentage solution of adenosine. The controls for mice between 9 and 12 months of age are on the solid line and for mice between 5 and 8 months of age on the short dash line. The two groups of mice receiving the intraperitoneal injections of adenosine are on the two open

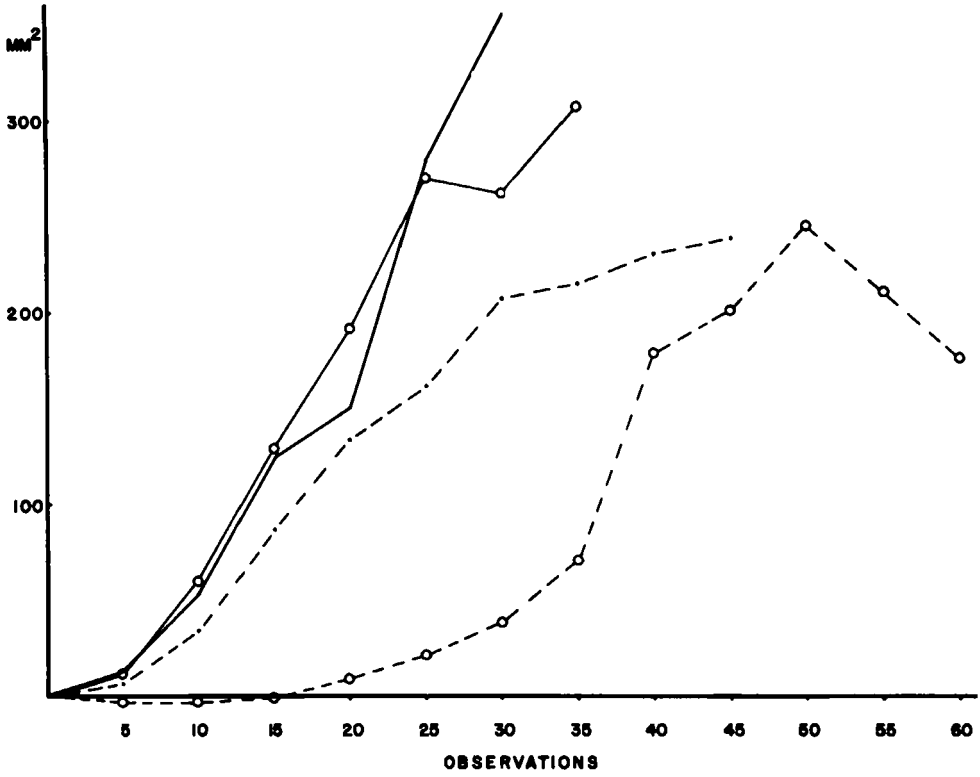

Fig. 2. Data obtained with 6-methyl adenosine on the growth of spontaneous tumors in mice. The controls at 9–12 months and 5–8 months of age are the same as those used for adenosine in Fig. 1, and are presented on the solid line for 9–12 months and short dash line for mice between 5 and 8 months of age. Mice receiving the 0.08 molar percentage solution of 6-methyl adenosine are at 9–12 months of age on the open circle and solid line and at 5–8 months of age on the open circle and short dash line. Ordinates: increments of tumor growth; abscissa: successive observation at the 5th, 10th, 15th, etc., periods of time.

circle curves, as follows: for mice of the 9–12 month age group are on the open circle solid line and for mice of the 5–8 month age are on the open circle short dash line. Thus a 0.05 molar percentage solution is an inhibitor of cancer growth in mice between 5 and 8 months of age but not in mice between 9 and 12 months of age.

Figure 2 is drawn from the data obtained with the effect of 6-methyl adenosine on the growth of spontaneous tumors in mice. The controls at 9–12 and 5–8 months of age are the same as those used for adenosine and are pre-

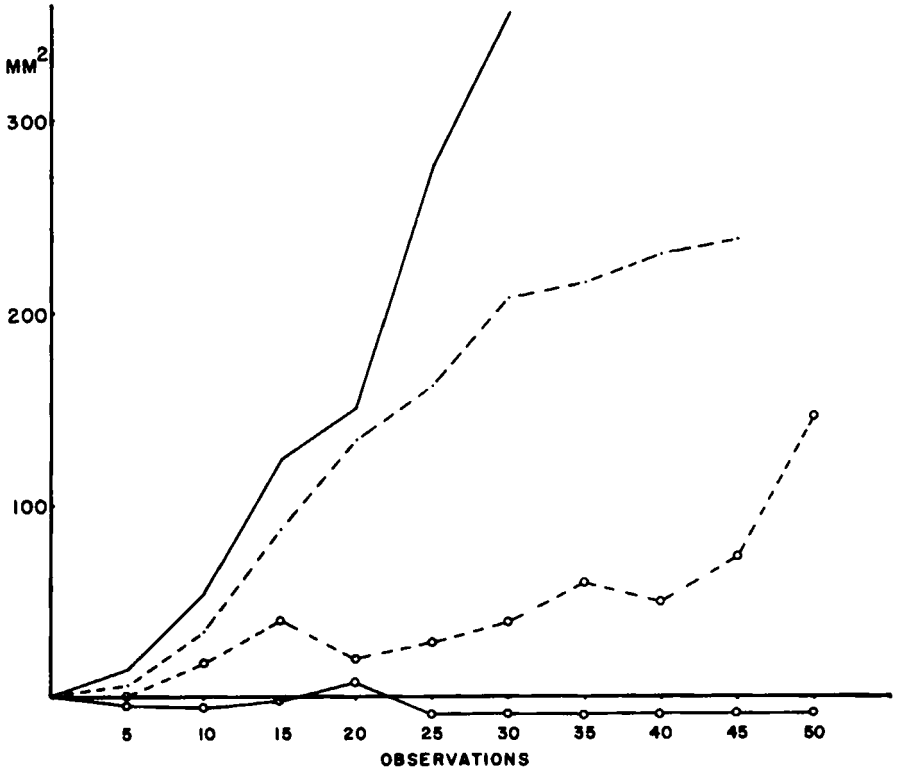

Fig. 3. Data obtained with 5-methyl cytidine. The growth rate of tumors for the controls are on the solid line for those mice between 9 and 12 months of age and on the short dash line for those at 5–8 months. The growth rate of tumors in mice receiving the 5-methyl cytidine are between 9 and 12 months of age on the open circle and solid line and at 5–8 months on the open circle and dash line. Ordinates: increments of tumor growth; abscissa: successive observations at the 5th, 10th, 15th, etc., periods of time.

sented on the solid line for 9–12 months and short dash line for mice between 5–8 months of age. Mice receiving the 0.08 molar percentage solution of 6-methyladenosine are at 9–12 months of age on the open circle and solid line and at 5–8 months of age on the open circle short dash line.

In this case also, as in adenosine, the 6-methyl adenosine is an inhibitor of tumor growth in mice between 5 and 8 months of age but not between 9 and 12 months.

Figure 3 presents the data obtained with the injections of 0.02 molar concentration of 5-methyl cytidine into spontaneous tumor bearing mice. The growth rate of tumors for the controls are on the solid line for those between

9 and 12 months of age and on the short dash line for those 5–8 months of age. The growth rate of tumors in mice receiving 0.02 molar solutions of 5-methyl cytidine are on the open circle and solid line for mice between 9 and 12 months of age and on the open circle and dash line for mice between 5 and 8 months of age.

In this case, the 5-methyl cytidine was found to be an inhibitor of cancer growth for mice of either age group. In fact, the mice between 9 and 12 months of age had the growth of their tumors completely suppressed in all mice beginning with the 25th period of observation (about 85 to 90 days).

DISCUSSION

It is becoming increasingly apparent from the analysis of the present data that the resolution of the problem that a specially prepared liver emulsion inhibited the growth capacity of spontaneous tumors even to a high percentage of complete regressions is imminent. This clarification has been made possible by the use of three nucleosides in pure form that are known to be present in the liver preparation.

By the use of these nucleosides, used singly, in pure form, and in the same molar concentration as they are found in the liver extract, a highly significant effect on the growth rate and fate of spontaneous tumors in mice has been obtained. In one case, by the use of an 0.02 molar preparation of 5-methyl cytidine in mice between 9 and 12 months of age, complete suppression of tumor growth was obtained and complete regression of all tumors in *all* mice that survived to the 25th period of observation also occurred.

A few comments on the differential effect that age of a mouse with cancer has on the end result may be profitable at this time. It has been known that a control mouse between 9 and 12 months of age will grow a spontaneous tumor faster than one between 5 and 8 months of age. There are many aspects of the "aging process" other than this differential growth rate of spontaneous tumors. Therefore, it is not wise perhaps to conclude that the differential effect of the nucleosides upon the growth capacity of tumors is exclusively an effect upon rapidity of growth. Perhaps it is, but final analysis must wait.

Another problem must receive further attention now. It has been convincingly established that there is a "transmissible entity" involved in the controlling mechanism for the growth capacity and fate of spontaneous tumors in mice. This influence on tumor control increases in potency between the 0 class and mice six generations removed from the injection of the original liver extract. It is not known what effect the age of the mouse has upon the potency of the "transmissible entity."

With the colony of mice now available, conclusions will have to be based on mice that have been established on a background of the injection of a liver emulsion in which a "transmissible entity" became at least detectable if not

actually induced. It is therefore imperative to analyze the effect of the nucleosides upon this background of the liver emulsion-"transmissible entity" relationship and the result, of course, is unpredictable and unresolved.

[It would be desireable to test out each of the nucleosides in mice with a background exposure of the same nucleoside over a period of generations. This procedure should also be followed with any agent that affects the "transmissible entity"-tumor growth relationship. The only agent so far discovered is neomycin in the drinking water, which is an inhibitor of tumor growth in mice of the 0 class (no treatment in ancestry of cancer proband) but will stimulate cancer in mice of the 0^7–0^{10} generations (seven or ten generations removed from the injection of the liver extract). It took twelve years to develop the present colony of mice with a genetic background of liver extract injections.]

But perhaps the present observations on the effect of the three nucleosides upon tumor growth may be indicating something about the nature of the "transmissible entity" and the controlling mechanism of cancer. It is clear that 5-methyl cytidine is a better inhibitor of spontaneous tumor growth than either adenosine or 6-methyl adenosine. Thus it may be that 5-methyl cytidine may have a greater effect upon the "transmissible entity" (or vice versa) than either of the other two nucleosides (adenosine and 6-methy adenosine) sine the only evidence, so far obtained, is that the "transmissible entity" is indicated by an effect upon the growth capacity of cancer. But this conclusion is not final. What is final, however, is that an 0.02 molar percentage solution of 5-methyl cytidine in distilled water and injected intraperitoneally is a powerful inhibitor of the growth capacity of spontaneous adenocarcinomata of mammary gland origin in mice.

CONCLUSIONS

1. An 0.05 molar percentage solution of adenosine in distilled water will inhibit the growth capacity of a spontaneous tumor in a 5–8 month old mouse but not in one between 9 and 12 months of age.

2. An 0.08 molar percentage solution of 6-methyl adenosine in distilled water will inhibit the growth capacity of a spontaneous tumor in a 5–8 month old mouse but not in one between 9 and 12 months of age.

3. An 0.02 molar percentage solution of 5-methyl cytidine in distilled water is a powerful inhibitor of spontaneous tumors in mice irrespective of age.

4. The differential effect of the three nucleosides upon the growth capacity of spontaneous tumors in mice may be conditioned by a differential rapidity of growth of cancer depending upon the age of the cancer proband.

5. The age of the cancer proband affects the growth capacity of a spontaneous tumor and its response to a nucleoside.

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