one percent, and was inactive as a paramorphogen. There is no evidence that inositol is directly involved in the paramorphogenic activities of the other chemicals, since high concentrations of inositol have no effect on paramorphogenesis by sodium desoxycholate, Tergitol, or sorbose with *Neurospora,* or on that by desoxycholate with *Syncephalastrum.* Furthermore, bio-assays of *Neurospora* mycelium grown in the presence of Tergitol 7, or of sorbose, showed no significant variations from the normal inositol content. In addition, sorbose has no significant effect on the requirement of the *inositolless* mutant for inositol. These findings suggest that the paramorphogenic agents tested do not influence either inositol utilization or inositol synthesis by *Neurospora* or *Syncephalastrum.*

In addition to theoretical considerations, the use of paramorphogens provides a new and valuable tool for handling many colonies on microbiological plating media. Sorbose has been selected as the standard colonial paramorphogen, because colonies on sorbose show no tendency to form extensive aerial mycelia, and because, in comparison with the other compounds, the effective concentration range of sorbose is wide, and survival is significantly better. Media containing 0.8 percent sorbose and 0.1 percent sucrose or 0.2 percent sorbose and 1.0 percent glycerol have been used effectively. The value of the sorbose technique in mutation studies has been demonstrated, using a strain of *Neurospora* which has a wild-type growth rate and which produces exclusively uninucleate microconidia (*1*). Microconidia were treated with X-rays (20,000 r) and plated directly into complete medium containing sorbose. Nineteen hundred colonies arising from the treated microconidia were isolated and examined for mutants; 24.2 percent morphological variants and 3.1 percent biochemical mutants were recovered, with considerably less labor than is required by other techniques. In 500 control isolates 0.2 percent morphological and no biochemical mutants were recovered.

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Purine Metabolism in *Tetrahymena* and Its Relation to Malignant Cells in Mice[1]

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ONE OF THE PRIME GOALS of studies involving metabolic inhibitors has been the discovery of useful chemotherapeutic agents which could be directed against invading organisms of man. Accordingly, if tests on bacteria *in vitro* seemed promising the compound was subjected to tests on the same microorganisms and others *in vivo.* When metabolic inhibitors which blocked reactions peculiar to the invader but not to the host were discovered (e.g. sulfonamides for *p*-aminobenzoic acid), then useful treatment resulted. Following these initial findings (*6, 18*), a long list of compounds have been tailored to inhibit many natural metabolites, and great strides have been made toward an understanding of competitive inhibition of specific enzymes by antimetabolites (*19*).

While certain encouraging reports have appeared in the literature concerning the retardation of the growth of neoplastic tissue following the injection of folic acid antagonists (*5, 15, 17*), nitrogen mustards

[1] Aided by a grant from the Williams-Waterman Fund for the Combat of Dietary Diseases, a grant recommended by the Committee on Growth acting for the American Cancer Society, and a grant from the American Cancer Society (Massachusetts Division). The authors wish to acknowledge the valuable aid given them by Philip S. Thayer, Charles S. Keevil, Jr. and Howard E. Sullivan, all of Amherst College.

(*1, 3, 17*), and recently 2,6-diaminopurine (*4*), the differential between neoplastic inhibition and host toxicity is exceedingly narrow or nonexistent. This narrowness of differential effect is what is to be expected when inhibitors of metabolites essential to the host are employed. Our knowledge of the biochemical pattern of cancer cells, and indeed of animal cells in general, has been too incomplete to formulate a rational attack on cancer with any assurance that the inhibition would be specific for the cancer. In other words, no single, clear-cut qualitative biochemical difference between normal and malignant cells was known.

Our work on the animal microorganism *Tetrahymena geleii* has demonstrated its biochemical similarity to the vertebrates in regard to amino acid (*10*) and vitamin requirements (*13*). There are, however, a few striking differences between the protozoan and the vertebrate, such as the total lack of sucrose-splitting enzymes, and presence of guanine and uracil requirements in *Tetrahymena*. The purine and pyrimidine metabolism has been subjected to intensive investigation, since it was felt that these very differences from the mammalian metabolic pattern might prove to be useful tools. It has been pointed out that, while the mammal can synthesize guanine from adenine (*2*), it cannot make use of dietary guanine for the reverse reaction. *Tetrahymena,* on the other hand, cannot synthesize guanine but requires this purine in its diet (*11*). A similar situation exists in the case of uracil, which, while required by the protozoan (*11*), fails to be metabolized by the mammal when it is added to the diet (*16*).

During a comprehensive study by Hitchings and co-workers (*8, 9*) of substances designed to interfere with nucleic acid synthesis, compounds were produced which provided tools for attacking these problems. These authors have pointed out that such substances might be expected to have chemotherapeutic effects even on the basis of the difference in rate of nucleic acid synthesis between host and parasite (*8*).

Studies on substituted pyrimidine (*12*) have shown that competitive inhibition results when certain substitutions are made and inhibition indices from < 20 to > 1000 were found.

Inhibition indices (based on complete inhibition) from 0.075 to 300 were found when a number of substituted purines were tested (*14*). Evidence was presented which seems to indicate that the most powerful purine inhibitor encountered (5-amino-7-hydroxy-1H-v-triazolo [d]pyrimidine) produces its effect by being incorporated in the nucleoprotein, and this incorporations depends upon its structure, which is that of a guanine analogue. Thus the guanine analogue, which we will call guanazolo, has an inhibition index (calculated for half-maximum growth) of 0.02, the 2,6-diaminopurine analogue (5,7-diamino-1H-v-triazolo[d]-pyrimidine) which we will call diaminazolo, has an index of 27, while the hypoxanthine analogue (7-hydroxy-1H-v-triazolo-[d]pyrimidine) is inert.

The suggestion was made (*16*) that if invasive organisms could be found with a purine metabolism similar to that of *Tetrahymena,* then guanazolo should inhibit their development without being metabolized by the host. Tests on mice showed that the compound was nontoxic when repeated doses were given over a three-day period.

With this background we were ready to determine whether or not cancer cells were similar to normal cells in this phase of their purine metabolism. Accordingly we chose a rapidly growing transplanted mouse adenocarcinoma[2] (Eo771) for our initial test and both guanazolo[3] and diaminazolo for the possible inhibitors, on the theory that if the cancer cell had lost its ability to synthesize guanine (or gained the ability to metabolize guanine), then guanazolo should cause growth retardation while diaminazolo should be relatively ineffective in moderate concentrations. This theory appears to be correct, and the number of mice in this series of experiments has now reached over 300.

Fig. 1 illustrates the striking difference in tumor size between the control group and the guanazolo-treated group. The number of mice in this initial experiment was small (eighteen divided into three equal groups), and the standard deviations of the control group and those which received the diaminazolo overlapped to an extent which indicates little or no significant difference between the two. Accordingly no

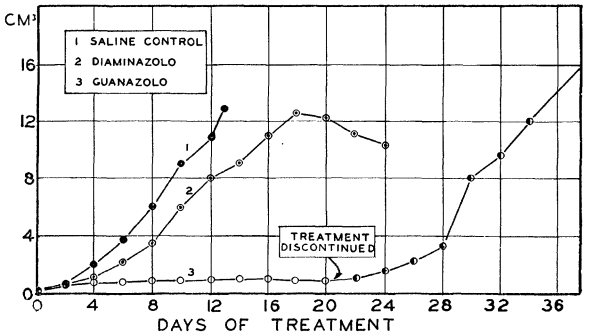

FIG. 1. Adenocarcinoma Eo771. Eighteen inoculated mice (C57 black) were used in this experiment (6 in each series). Dosage 500 γ twice daily, subcutaneous. Size of tumors (cm^3) averaged in each series. Treatment started 6 days after inoculation.

[2] Purchased from the Roscoe B. Jackson Memorial Laboratory.

[3] All but one sample of the guanazolo used was obtained through the cooperation of Dr. Hitchings. The remaining sample was sent to us by Dr. R. O. Roblin, Jr. of American Cyanamid Company.

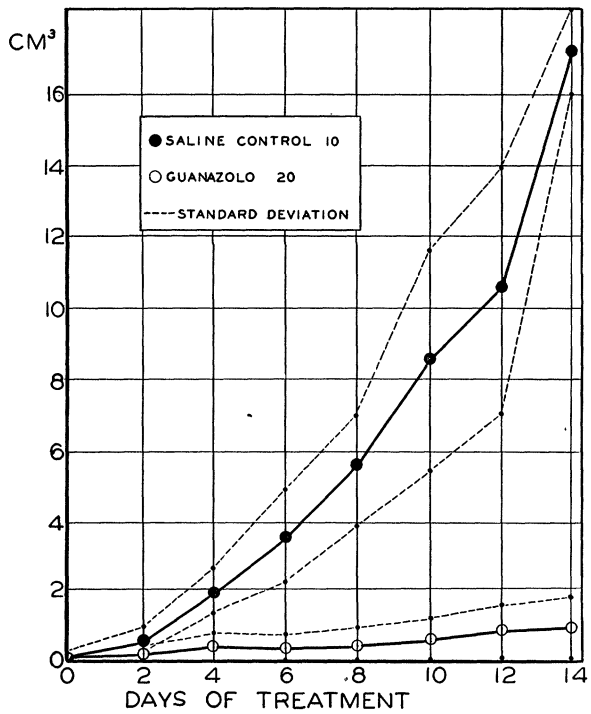

FIG. 2. Adenocarcinoma Eo771. Thirty inoculated mice (C57 black) were used in this experiment (10 control; 10 given 500 γ of guanazolo twice daily subcutaneously; 10 given 500 γ of guanazolo twice daily intraperitoneally). Since there was no detectable difference in the route of administration the tumor sizes of all the guanazolo-treated mice were averaged.

further tests were run using diaminazolo. Almost complete cessation of tumor growth resulted during guanazolo treatment (the tumors were six days old when treatment was instituted), but growth resumed shortly after the treatment was discontinued.

Subsequent experiments employing substantial numbers of tumor-bearing mice have completely duplicated the results of the first experiment on this type of malignancy. Graphic representation of one such series of 30 mice is shown in Fig. 2. The treatment in this case was divided equally (10 each) between saline control, 0.5 mg of guanazolo twice daily administered subcutaneously, and the same amount of guanazolo given intraperitoneally. No difference in the effect on the tumors was found between the two routes of guanazolo treatments.

TABLE 1

LYMPHOID LEUKEMIA

Effects	Length of treatment (days)	Ten mice treated*	Ten control mice (untreated)
White blood cells (ave.)	5	9,600	11,080
" " " "	12	5,911	20,175
Percent lymphoblasts (ave.)	10	0.8	32
" " "	13	1.0	21
No. palpable tumor masses	13	1	10

* With 500 γ guanazolo twice daily, starting 3 days after inoculation. The particular preparation of guanazolo (17–3) used in this experiment produced a leukopenia after prolonged treatment. That a contaminating substance in this preparation produced the effect was shown by observations on normal mice treated with 17–3 and normal mice treated with another preparation (17–4). No leukopenia developed after four weeks of treatment with 17–4 (1 mg twice daily), whereas leukopenia did develop with 17–3. There was, however, complete retardation of adenocarcinoma Eo771 and spontaneous mammary cancer in C3H mice following the administration of 17–4.

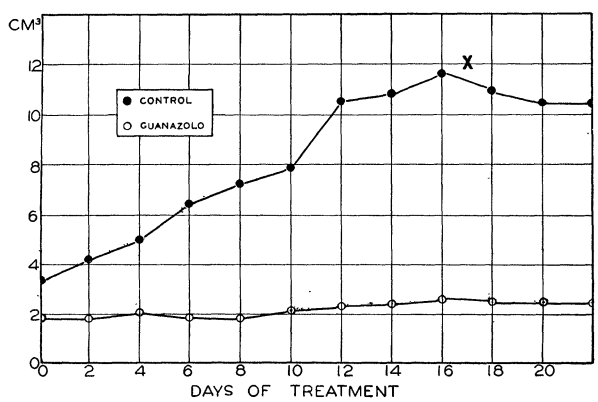

FIG. 3. Spontaneous mammary cancer in C3H mice. Twelve mice were used in this experiment (6 control; 6 given 1 mg of guanazolo twice daily intraperitoneally). The initial segregation into the two series was random, and the inclusion of the largest tumor in the control group (dead at 15 days, marked X on curve) raised the average of the tumor size of the control series over the treated series at the start of the experiment.

Pathological findings[4] show that this level of treatment does not cause the death of the malignant cells, but only checks their growth.

Due to the extreme activity of guanazolo as an antiguanine, it has been difficult to reverse its activity. Massive doses of guanylic acid, calculated on the basis of *Tetrahymena* activity ratios, administered together with effective levels of guanazolo, have given indications of inhibitor release, but the guanylic acid is so insoluble that the effective blood levels are much below those required for complete release. The data at hand, however, are consistent with the theory that the effect of guanazolo on the cells of Eo771 is the same as in *Tetrahymena*.

With the evidence of a real biochemical difference between the cancer cells of Eo771 and the normal mammalian cells, and the mechanism of the reaction

[4] Control and treated mice were sent to Dr. Joseph C. Aub, director of the Cancer Commission of Harvard University and the pathological studies were made by Dr. William Emerson.

between the inhibitor and enzyme system indicated, it was of the utmost importance to test the general occurrence of this difference in other types of malignancy. We have had the time and opportunity to investigate only two other cancers. One was the spontaneous mammary cancer of strain C3H[5] mice, the results of which are shown in Fig. 3. The other was a lymphoid leukemia (lymphoma 2) in strain A mice,[6] the results of which are shown in Table 1.

These results strikingly illustrate the soundness of the view expressed by Greenstein (*7*) when he said (p. 367): "It seems probable that a cancerous tissue can be described by a chemical pattern which is largely similar to that of nearly all other cancerous tissues regardless of their etiology, histogenesis, or even species wherein found." It would be hazardous to assume that *all* malignant cells, unlike the normal, metabolize guanine and therefore will become inhibited by guanazolo. But the fact that the first three types selected show the same biochemical difference from the normal cells would, on the basis of chance alone, offer fantastic odds in favor of uniformity, at least in this respect, among the various cancerous tissues.

Experiments are in progress with guanazolo on other types of malignancy, on the most effective dosages, on the effect of diet (purine intake) and on the effect of age of the tumor before the administration of the inhibitor. The details will be reported later.

[5] Purchased from the Roscoe B. Jackson Memorial Laboratory.

[6] Sent to us by Dr. Lloyd W. Law of the National Cancer Institute.

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