The Effect of Allicin from Garlic on Tumor Growth*

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SUMMARY

Allicin, alliin, and a garlic extract were tested on solid and ascites mouse tumors carried in proper hosts. Preincubation of certain concentrations of enzymatically prepared allicin with tumor cells resulted in complete inhibition of tumor growth. Single or multiple treatment, as high as 30 mg. of either allicin or alliin per kg. of mouse, did not produce any consistent and significant tumor inhibition. The garlic extract also was an ineffective tumor-inhibitor. Only allicin inhibited the yeast cells and their respiratory mutants. The stability of allinase was increased to 20–34 days by storage at −25° C.

The suspected medicinal value of garlic (*Allium sativum* L.) is now believed due to the properties of its active principle, allylthiosulfinicallyl ester, allicin (6). Allicin is produced by enzymatic degradation of alliin, S-allyl-L-cysteine-S-oxide. Inhibition of sulfhydryl enzymes is associated with the presence of the −SO−S− grouping (11). The role of sulfhydryl groups in cell division and mitoses has been reviewed by Barron (1): an increase in −SH is associated with cell division as well as growth, while agents which poison or oxidize −SH compounds inhibit cell division.

Sulfhydryl-deficient diets may inhibit tumor growth in some animals, whereas the addition of cysteine or glutathione may have the opposite effect (9, 10). Therefore, interference with sulfhydryl-carrying components might block necessary steps for division and growth of cells.

Von Euler *et al.* (7) reported that pure alliin had growth-inhibiting action on various tumors, with inconsistent complete inhibition in rats of Jensen sarcoma and of a benzpyrene-induced sarcoma. No regression of Ehrlich ascites tumors in mice was noted following a single intratumoral injection of 20 mg. of alliin. In 1957, Novinov, Levy and Chochlov (4) reported that their synthetic alliin was ineffective, at concentrations higher than those used by von Euler, on transplanted and induced tumors. Recently Weisberger and Pensky (8) reviewed some of the evidence implicating abnormalities of metabolism of sulfhydryl compounds in human leukemia and presented their own results on Sarcoma 180 (S-180) tumors in Swiss mice and the Murphy-Sturm lymphosarcoma in Wistar rats with a series of analogs of allicin. They concluded that their compounds have tumor-inhibiting effects.

The present study was undertaken to evaluate the effect of alliin and allicin itself upon mouse tumors and upon a microbiological screening system for potential tumor-inhibiting agents.

MATERIALS AND METHODS

a) Preparation of allicin.—On treatment of alliin[1]—$CH_2=CH-CH_2-SO-CH_2-CH(NH_2)-COOH$—with allinase, allicin ($CH_2=CH-CH_2-SO-S-CH_2-CH=CH_2$), pyruvic acid and ammonia are produced.

The enzyme allinase was prepared by the procedure of Stoll and Seebeck (5), except that the garlic bulbs were homogenized (Omnimixer) in water and the final crude enzyme preparation was redissolved not only in M/15 phosphate buffer of pH 6.4 but also in 0.9 per cent saline after the pH of the enzyme preparation was raised to 6.2–6.4 with 2 N NaOH. The activity of the alliinase was determined by measuring its capacity to split alliin (DL-alliin) to allicin, ammonia, and pyruvic acid (5). The pyruvic acid liberated was determined as the 2,4-dinitrophenyl hydrazide (5). Since alliinase is quite unstable at 0–4° C. (5), attempts were made to increase its stability by storage at lower temperatures. The results of these experiments are shown in Chart 1. Alliinase stored in M/15 phosphate buffer of pH 6.4 at 0–4° C. lost its activity rapidly (curve *A*), whereas the enzyme stored in the same buffer or in saline at −25° C. was stable for 20–34 days (curves *B* and *C*).

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The following procedure was employed for the enzymatic preparation of allicin. The required amounts of DL-alliin (assuming 50 per cent conversion to allicin) were dissolved in 0.9 per cent NaCl to which was added alliinase solution previously adjusted to pH 6.2–6.4 with NaOH. (Alliin in 0.9 per cent NaCl and alliinase in solution in 0.9 per cent NaCl were added in the ratio of 1.0–1.5 ml., respectively, the same ratio employed for assay of enzyme activity). The enzyme solution and alliin were mixed and immediately incubated in a water bath at 37° C. for 8–10 min. The resulting solution, which contained the allicin, was injected immediately into mice bearing tumors. In some cases the alliin was dissolved in 0.9 per cent NaCl and frozen. When allicin was needed, an alliinase preparation was thawed, added to the unfrozen alliin, and the mixture incubated at 37°C. for 8–10 min. for immediate use.

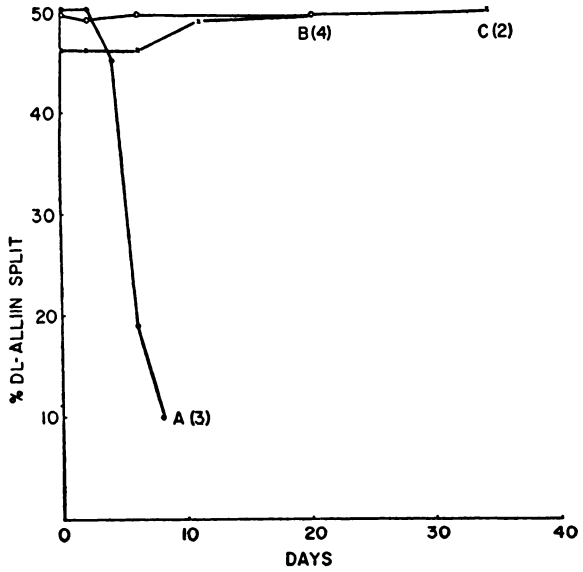

CHART 1.—Stability of alliinase. *A*. Alliinase stored in phosphate buffer, pH 6.4, at 4° C. *B*. Alliinase stored in phosphate buffer, pH 6.4, at −25° C. *C*. Alliinase stored in 0.9 per cent NaCl (adjusted to pH 6.4) at −25° C. Number of samples indicated in parentheses.

b) *Preparation of water extract of garlic.*—In a few experiments an aqueous extract of garlic was employed for chemotherapeutic purposes. This was made by homogenizing 10 gm. of cleaned garlic bulbs in 50 cc. H_2O (Omnimixer) for 3 min. The homogenate was then spun at 16,000 r.p.m. (International multispeed attachment and rotor No. 269) for 20 min. at 0° C. The supernatant fraction was filtered and stored at −25° C. until ready for use. As required, aliquots were thawed at room temperature.

c) *Anti-tumor study.*—The tumor-inhibiting effects of allicin were studied by *in vitro* preincubation with saline-suspended tumor cells for 15 min. prior to intraperitoneal inoculation into Swiss mice. Allicin, alliin, and garlic extract were tested at the maximum tolerated dose on solid and ascites mouse tumors carried in proper hosts. Concentrations greater than the maximum reported resulted in excessive deaths. The material was given by intraperitoneal or intravenous injections or oral intubation usually once daily, beginning the day after tumor transplantation and continuing for 7 or 14 days. The effect of a single injection was also studied. Control animals, usually fifteen per group, were given injections of 0.1 M phosphate buffer or saline, depending upon the solvent used in the reaction mixture. Inhibition of S-180 was determined on the 9th day following tumor transplantation by determining the average diameter of the tumors. Adenocarcinoma C-755 inhibition was determined on a wet-weight basis on the 16th day following transplantation. Inhibition of ascites tumors was determined by calculating the number of days that 90 per cent of the animals survived.

The materials were also tested for inhibition of respiratory-deficient yeast mutants and their parents, with an agar-disc assay method (3). Since a characteristic of neoplasms is an increase in the role of glycolysis, chemicals which preferentially inhibit the respiratory mutant and not the wild type yeasts may have carcinolytic or carcinostatic effects.

RESULTS

Preincubation of 5×10^6 cells of Sarcoma 180 ascites tumor or Ehrlich-2 carcinoma ascites cells with allicin (2–810 μg/0.2 ml suspension) prior to intraperitoneal injection into Swiss mice did result in complete carcinolytic action, with one exception at the 16 μg. or higher concentration per inoculum (Table 1). The corresponding controls survived less than 3 weeks.

Only slight inhibition was found in animals treated subsequent to tumor inoculation; therefore, many of the results obtained following treatment with intermediate doses of allicin are omitted from the data reported in Table 2. Up to 40 mg/kg of animal weight of synthetic alliin, the specific principle of garlic, or garlic extract (.02–.05 ml/injection) did not have any significant tumor-inhibiting properties. Necrosis and the collapse of veins followed repeated intravenous injection of allicin.

Six inhibition tests with *S. cerevisiae var.*, *S. ellipsoidue*, *S. cerevisiae 1741*, and *S. carlsbergensis*, each with the synthetic alliin, resulted in no inhibi-

tion of any yeast parent or respiratory mutant derived from them with a concentration of 86 μg. of alliin per disc. A concentration of 86 μg. of the enzymatically prepared allicin per disc resulted in inhibition of all the yeast parents; whereas, in the six mutants derived from them, two were not inhibited, one was inhibited to the same extent as the parent yeast, and three were inhibited to a greater extent than the parent.

DISCUSSION

Although allicin is a potent bactericidal agent (2) and has been shown to preferentially inhibit sulfhydryl enzymes without affecting many others (11), it has not been possible to demonstrate a consistent and significant inhibition of transplantable mouse tumors either by the injection of alliin, which in the presence of a specific enzyme might split in the animal body to allicin, or by the enzymatically produced allicin. The *in vitro* incubation of ascites with allicin prior to intraperitoneal injection into mice has tumor-inhibiting effects which might be attributable to either a combination of allicin with cysteine or the oxidation of sulfhydryl to disulfide in —SH-containing enzymes by the labile oxygen (2). Weisberger and Pensky (8), using analogs of allicin, concluded that these analogs might have tumor-inhibiting effects.

TABLE 1

THE INHIBITION OF ASCITES TUMOR FORMATION BY PREINCUBATION WITH ALLICIN

5×10^6 cells/mouse

Tumor	No. mice	Conc. of allicin (μg/0.2 ml suspension/ mouse)	Survival time (days)
Ehrlich-2	5	810	90*
"	5	81	90*
"	5	16	60*†
"	5	8	25
"	5	4	22
"	5	2	20
"	26		20
Sarcoma 180	10	810	90*
"	10	81	90*
"	10		15

* Killed. † One with solid tumor.

TABLE 2

EFFECT OF ALLICIN ADMINISTRATION* UPON TUMORS

Tumor system	Results†	No. animals	Deaths	Dosage (μg/mouse/day)	Total no. injections	Animal wt. changes‡	90 per cent survival mean (days)		Per cent inhibition
							Exp.	Control	
Ehrlich-2 ascites	—	5	0	600	7		27	24	
L1210 ascites	±	10	0	645	7		15	12	
"	±	6	0	86	7		14	11	
S-180 ascites	±	7	0	860	1§		25	18	
"	±	9	4	800	6		23	19	
"	—	9	0	700	7		22	19	
"	—	7	0	345	7		20	19	
"	—	6	0	172	7#		18	19	
"	—	7	0	258	7#		20	19	
"	—	9	0	700	7§		19	19	
S-180	—	7	0	860	1	−0.1/+0.7			−24
"	—	7	0	700	7	−2 /+0.3			14
"	±	7	0	43	7	−1.2/−0.6			34
"	—	7	0	86	7	−1.1/−0.6			22
"	—	8	0	700	7§	−1.1/−0.2			13
"	—	8	0	700	7#	−1.2/−1.2			7
"	—	7	0	516	7‖	−1.3/−0.7			−10
C-755	±	8	2	700	14	−1.5/+1.6			55

* I.P. unless otherwise noted.

† In the ascites tumors, ± indicates 20–50 per cent increase in survival time of experimental group; — indicates less than 20 per cent inhibition. Per cent inhibition of S-180 was determined by mean diameter, and for C-755 it was determined by mean tumor wt. A negative number indicates the extent to which tumors of the treated group exceeded the control group.

‡ Av change of treated/av change of control.

§ I.V. # Orally. ‖ Subcutaneously.

Although our results with allicin are similar to their results with analogs, the absence of significant and consistent inhibition in tumor-bearing mice leads to the conclusion that alliin and allicin do not appear promising for cancer chemotherapy.

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