Technical Papers

The Effects of Allithiamine on Some Thiamine-Requiring Organisms[1]

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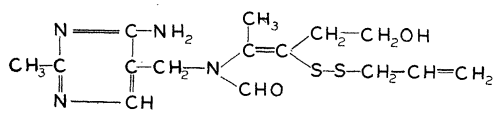
Matsukawa and Yurugi (*1*) prepared allithiamine by reacting thiamine with allicin under mild alkaline conditions. Fujiwara and Watanabe (*2*) found allithiamine to replace thiamine for rice birds and the rat. The structure of allithiamine is shown below:



Experiments with thiamine-requiring fungi. The value of allithiamine as a replacement for thiamine was determined for 5 thiamine-requiring fungi: *Endoconidiophora fimbriata, Mucor ramannianus, Phycomyces blakesleeanus, Schizothecium longicolle,* and *Thielaviopsis basicola.* Suboptimal amounts of allithiamine and thiamine chloride hydrochloride were added to a thiamine-free glucose asparagine medium. The cultures were incubated at 25°. The techniques used are described by Lilly and Barnett (*3*). The amount of growth was determined by harvesting and weighing the dry mycelium.

Allithiamine and thiamine were sterilized in 3 ways: (*a*) by autoclaving with the basal medium, (*b*) by autoclaving dilute distilled water solutions, and (*c*) by filtering dilute distilled water solutions using Pyrex sintered glass bacteriological filters. The activity of thiamine for the 5 test fungi was almost completely lost when thiamine was autoclaved in distilled water; the activity of allithiamine was not changed by this treatment.

On a weight basis, allithiamine was more active than thiamine for *E. fimbriata, M. ramannianus,* and *P. blakesleeanus*; and less active for *S. longicolle* and *T. basicola.* This was confirmed in repeated experiments. The results from a typical experiment for 2 fungi are shown in Fig. 1.

In view of the ready conversion of allithiamine into thiamine, when treated with cysteine, and the similarity of allithiamine and thiamine disulfide, it appears probable that fungi convert allithiamine into thiamine. This hypothesis was tested in the following way. *P. blakesleeanus* was grown on media containing 100 µg each of allithiamine and thiamine. The mycelium was harvested, washed, and dried at 50°. The 2 samples of mycelium were used as sources of thia-

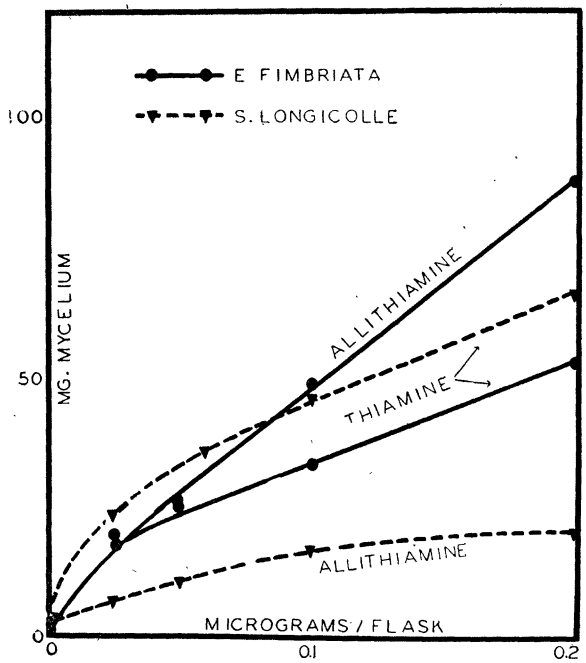


FIG. 1. Growth of *E. fimbriata* (11 days) and *S. longicolle* (14 days) on various concentrations of allithiamine and thiamine.

TABLE 1

MILLIGRAMS OF DRY MYCELIUM PRODUCED BY 5 TEST FUNGI WHEN THE BASAL MEDIUM CONTAINED 15 MG OF *P. blakesleeanus* MYCELIUM GROWN ON ALLITHIAMINE AND THIAMINE. CONTROLS WERE GROWN IN THE PRESENCE OF 0.1 µG OF ALLITHIAMINE AND THIAMINE

Species	Days of incubation	Allithiamine-mycelium	Thiamine-mycelium	Allithiamine	Thiamine
E. fimbriata	11	44	46	49	33
M. ramannianus	9			33	21
	11	30	29		
P. blakesleeanus	7	43	33		
	8			66	49
S. longicolle	12	40	44		
	14			17	46
T. basicola	11			17	32
	12	38	32		

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mine (or allithiamine) for 5 test organisms. Some of these results are reported in Table 1. The yields obtained with 0.1 μg of allithiamine and thiamine are included for the purpose of comparison.

Aqueous extracts, prepared from the allithiamine and thiamine-mycelium were treated with alkaline ferricyanide and cyanogen bromide. The thiamine activity of the treated extracts was destroyed equally for 2 test fungi: *E. fimbriata* and *P. blakesleeanus*. Since control experiments under the same conditions had shown that thiamine was inactivated while the activity of allithiamine was only slightly diminished, it was concluded that allithiamine is converted into thiamine by *P. blakesleeanus*.

Experiments with albino rats. Four weanling albino rats were fed a thiamine-free diet until two of them died. At that time, the 2 surviving rats were transferred to a diet containing allithiamine, but otherwise the same as the thiamine-free diet. After 2 wk on allithiamine, they were placed on a diet containing thiamine instead of allithiamine. The results for both rats were essentially the same. Figure 2 shows the results

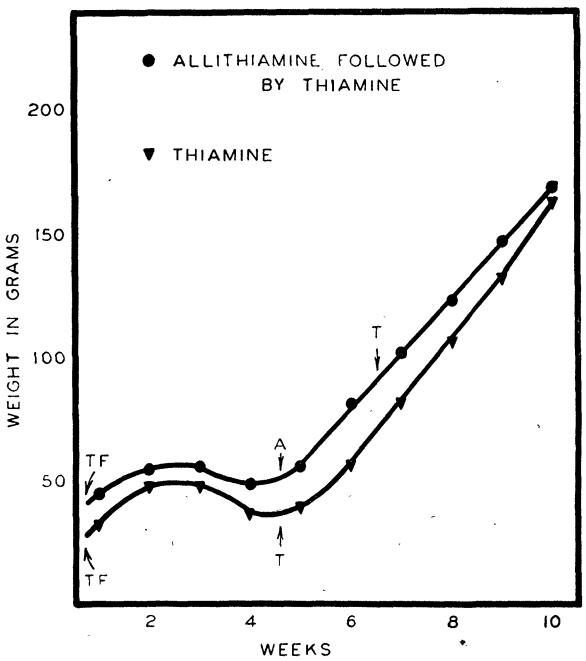

FIG. 2. Growth curves for 2 male rats kept on the thiamine-free diet for 4 wk, followed by allithiamine for 2 wk for 1 rat (circles) and by thiamine for the other (triangles). Time of change of diet is indicated by arrows: TF, thiamine-free diet; A, thiamine-free diet plus allithiamine; and T, thiamine-free diet plus thiamine.

for 1 of the 2 rats. Note that, after being placed on the thiamine-free diet, the rat gained weight during the 1st and 2nd wk, leveled off during the 3rd wk, and lost during the 4th wk. When transferred to the allithiamine diet, the rat gained rapidly and continued to gain after being transferred to the thiamine diet. For comparison, a typical growth curve for another rat from a series of experiments in which the rats were changed from the thiamine-free diet directly to the thiamine diet is also shown. On the basis of the above results, one may conclude that allithiamine may replace thiamine in the diet of the rat.

References

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2. FUJIWARA, M., and WATANABE, H. *Ibid.*, 156.
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The Effect of Cortisone upon the Therapeutic Efficacy of Antibiotics[1]

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It is well established that cortisone enhances various infections in animals and man and aggravates their severity (*1*). This property is attributable to a depression of the antimicrobial defenses of the host, rather than to a direct promotion of microbial growth or invasiveness (*2*). To protect a patient receiving cortisone against dissemination of latent or active infection, antibiotics are often administered. The question arises whether cortisone might also impair the therapeutic effectiveness of antimicrobial agents. If certain of these drugs acted in conjunction with normal antimicrobial defenses of the host then the depression of these defenses by cortisone might result in measurable impairment of the curative effect of these drugs. The experiments reported here were undertaken to explore this possibility.

The following laboratory model was used. White Swiss mice (15–19 g) were infected intramuscularly with a virulent strain of *Klebsiella pneumoniae*. The LD_{50} of this strain consisted of 50–100 organisms injected into the thigh muscle, in a volume of 0.1 ml. After infection with 10–500 LD_{50}, all animals died in 3–5 days with positive heart blood cultures. The antibiotics, aureomycin hydrochloride[2] and streptomycin sulfate were dissolved in suitable concentration in saline, and each dose was administered intraperitoneally in a volume of 0.2 ml. Antibiotic treatment was started 6 hr after infection, two doses were administered on the next day, and a single daily dose on the following 3 days.

Cortisone acetate was suspended in saline and a daily subcutaneous dose of 10 mg/kg was administered for 5 consecutive days, beginning 24 hr before infection. This dose of cortisone was not harmful to the animals (*3*) and permitted normal growth and weight gain. Alternate groups of mice received cortisone as shown in Table 1.

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[2] Supplies of aureomycin hydrochloride were made available by Dr. Stanton Hardy, Lederle Laboratories.