

in each high-dry field. In some areas the cells are arranged to form small cystic cavities containing a colloid-like substance, as in the "folliculoid" type of tumor. The vascular network is not prominent and there is very little discernible fibrous stroma. In many respects these resemble the ovarian tumors

found by Brambell¹¹ and by Furth¹² in the ovaries of mice following roentgen irradiation.

¹¹ Brambell, F. W. R., Parkes, A. S., and Fielding, U., *Proc. Roy. Soc., Lond., Ser. B*, 1937, **101**, 29.

¹² Furth, J., and Butterworth, J. S., *Am. J. Cancer*, 1936, **28**, 66.

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Development of Resistance to Pyrithiamine in Yeast and Some Observations on Its Nature.

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Since the development of resistance to various specific antimicrobial agents is a problem of much practical as well as theoretical interest, it has seemed advisable to attempt the production of variants of susceptible microorganisms which would be insusceptible or fast to pyrithiamine, and to study the means by which this fastness was achieved. It has recently been shown that pyrithiamine, the pyridine analog of thiamine, was a highly selective and effective inhibitor of the growth of microorganisms¹ and animals.² Only those organisms which were caused to grow more abundantly by the presence of thiamine were inhibited in growth by pyrithiamine. It was also shown¹ that the insusceptible species did not owe their resistance to the production of thiamine or other antagonist of pyrithiamine. If a fast strain of a susceptible species could be developed, it would be of interest to learn what alteration, if any, would occur in its thiamine requirements.

A pyrithiamine-fast strain of *Endomyces vernalis* was obtained in the following manner. Twelve successive transfers of the strain previously used were made in the synthetic medium,¹ to which had been added graded amounts of pyrithiamine ranging from 2 to 20

μg per cc. Consecutive transfers were made, beginning with the lowest concentration and terminating at the highest. After the yeast had been treated in this manner, its susceptibility to pyrithiamine was the same as that of the parent strain. It was then transferred 30 times in the synthetic medium plus 25 μg of pyrithiamine per cc. Care was taken to make each successive transplant as soon as growth was visible. Although in the first transfers growth appeared very slowly, after about the twentieth turbidity developed in 8 hours of incubation at 37°. The final culture obtained was labeled PF *Endomyces*.

PF *Endomyces* grew as well in a medium containing 25 μg of pyrithiamine per cc as it did in the absence of the compound. This was 25 times the amount of pyrithiamine which was sufficient to inhibit the growth of the parent strain half-maximally. However, the PF strain grew more slowly in the absence of pyrithiamine than did the parent strain. With the same size of inoculum, the PF strain required 40 hours to produce the same amount of growth made by the parent strain in 24 hours.

The PF strain had the same thiamine requirements as the parent strain. Thus it grew very poorly in the absence of thiamine, and was stimulated to grow either by thiamine or by its pyrimidine portion[†] alone. The quanti-

* With the technical assistance of J. Backstrom and J. Clifford.

¹ Woolley, D. W., and White, A. G. C., *J. Exp. Med.*, 1943, **78**, 489.

² Woolley, D. W., and White, A. G. C., *J. Biol. Chem.*, 1943, **149**, 285.

[†] Throughout this paper, the pyrimidine portion of thiamine refers to 2-methyl-4-amino-5-hydroxymethyl-pyrimidine.

TABLE I.
Effect of Thiamine and of Pyrithiamine on Growth
of PF *Endomyces*.
Colorimeter readings represent % of incident light
transmitted by the cultures.

Thiamine μg per cc	Pyrithiamine μg per cc	Colorimeter reading
0	0	72
0.01	20.0	54
0.01	0	61
0.003	0	69
0.001	0	70
0	0.5	54
0	0.1	54
0	0.05	56
0	0.025	60
0	0.01	67
0	0.005	69

tative requirements for these substances were the same as for the parent strain. The PF strain was thus the first organism observed which was insensitive to pyrithiamine and yet was stimulated by thiamine. Some data to illustrate these points are shown in Table I.

In the absence of thiamine, pyrithiamine was a growth factor for PF *Endomyces* (Table I). On a molecular basis, pyrithiamine was 50% as active as thiamine in promoting growth.

The fastness of the PF strain to pyrithiamine, as well as its ability to use the inhibitor in place of the vitamin, was partially explained by the observations that pyrithiamine was destroyed, and that the pyrimidine portion of thiamine was produced from pyrithiamine during growth. Apparently the inhibitor was cleaved between the rings by a system which had arisen in the PF strain. To demonstrate this, PF *Endomyces* was grown for 40 hours at 37° in the synthetic medium plus 1 μg of pyrithiamine per cc. The cells were removed and the culture filtrate was assayed for thiamine (or its pyrimidine half) according to the method described previously.¹ It was found to contain 0.5 μg of thiamine or its pyrimidine half per cc. This value agreed well with the finding that pyrithiamine had 50% of the growth-promoting potency of thiamine for the PF strain. When the PF strain was grown in the presence of 20 μg of pyrithiamine per cc, the percentage conversion to free pyrimidine was 39%.

Although the pyrimidine portion of thiamine had some effect in reversing the action of pyrithiamine on *Endomyces vernalis*,¹ it was less than 1% as active as thiamine in this respect. Since thiamine and its pyrimidine portion were equally effective as a growth factor, it was possible to determine thiamine in the presence of its pyrimidine portion by adding pyrithiamine to the basal medium. The growth effect observed under this condition was due almost entirely to the intact vitamin. By use of such a procedure, the active material in the culture filtrates was indicated to be the pyrimidine rather than thiamine.

Destruction of pyrithiamine was not the sole means by which the PF strain protected itself against the inhibitor, for when the filtrates obtained from cultures which contained originally 20 μg of pyrithiamine per cc were assayed for pyrithiamine with the parent strain, 20% of the inhibitor was found still to be present. This was sufficient to cause inhibition of growth of the parent strain and yet not to have an effect on the growth of the PF strain. The inhibition of growth of the parent strain was overcome by thiamine.

The production of the pyrimidine portion of thiamine from pyrithiamine was also accomplished by species naturally resistant to the inhibitor. When *Escherichia coli* was grown in a synthetic medium plus 1 μg of pyrithiamine per cc, pyrimidine activity equivalent to 47% yield from the pyrithiamine was found in the culture filtrate.

Summary. A strain of *Endomyces vernalis* was obtained by growing the yeast in media containing pyrithiamine. This strain was not affected in growth by concentrations of pyrithiamine 25 times greater than that required to inhibit the growth of the parent strain half-maximally. The new strain still required thiamine or its pyrimidine portion as a growth factor. In the absence of thiamine, pyrithiamine was 50% as active as the vitamin for growth of the variant. When grown in small concentrations of pyrithiamine and in the absence of thiamine, the new strain converted half of the pyrithiamine to the pyrimidine portion of thiamine.