INTERMEDIATES OF VITAMIN B_1 AND THE GROWTH OF *TORULA*

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(WITH SIX FIGURES)

In previous papers (**2, 3, 4**) from this laboratory the effect of vitamin B_1 or its intermediates on the growth of excised tomato roots and of *Phycomyces blakesleeanus* Burgeff has been reported. Excised tomato roots (**2, 3**) required for unlimited growth either vitamin B_1 or thiazole[1] in addition to mineral salts and cane sugar. Pyrimidine alone did not permit growth to occur. *Phycomyces blakesleeanus* (**4**), on the other hand, did not grow (**3**) unless supplied with vitamin B_1 or with both intermediates. We have been interested in determining whether there are organisms which require pyrimidine but not thiazole. SCHOPFER (**5**) has reported that *Rhodotorula flava* and *Rhodotorula rubra* are such organisms. We have examined eight species of *Torula* and have found some which require an external supply of both intermediates for good growth under the conditions of our experiments, some which require pyrimidine but not thiazole and some which require neither.

Experimentation

A medium of the following composition was prepared:

KH_2PO_4	1.0	gm.
$MgSO_4 \cdot 7H_2O$	0.5	gm.
NH_4NO_3	0.05	gm.
Asparagine	0.5	gm.
Mineral supplements	0.1	cc.[2]
Dextrose (Cerelose)	50	gm.
Redistilled water	1000	cc.

The hydrion concentration of this solution was adjusted to pH 5.5 by the addition of Na_2HPO_4. Twenty-five cc. of the above solution were placed in 125-cc. Erlenmeyer flasks of pyrex glass. The solutions were divided into four groups. No addition was made to those of group I.

[1] Where thiazole is referred to in this paper the 4-methyl-5-β-hydroxyethylthiazole is meant and where pyrimidine is mentioned we mean the 2-methyl-5-ethyoxymethyl-6-aminopyrimidine. These compounds were used by WILLIAMS and CLINE (7) in synthesizing vitamin B_1 and are the intermediates in the formation of this vitamin.

[2] The mineral supplements were contained in a modification of HOAGLAND'S A to Z mixture prepared by adding to 18 liters of redistilled water: LiCl, 0.5 gm.; $CuSO_4 \cdot 5H_2O$, 1.0 gm.; $FeSO_4$, 1.0 gm.; H_3BO_3, 11.0 gm.; $Al_2(SO_4)_3 \cdot 18H_2O$, 1.0 gm.; $SnCl_2 \cdot 2H_2O$, 0.5 gm.; $MnSO_4 \cdot 4H_2O$, 7.0 gm.; $NiCl_2 \cdot 6H_2O$, 1.0 gm.; $Co(NO_3)_2$, 1.0 gm.; $TiOSO_4$, 1.8 gm.; KI, 0.5 gm.; NaBr, 0.5 gm.

To each flask of group II we added 30 units[3] (4.3 γ) of 4-methyl-5-β-hydroxyethylthiazole, to each flask of group III 30 units (5.0 γ) of 2-methyl-5-ethyoxymethyl-6-aminopyrimidine, and to each flask of group IV, 30 units of the thiazole and 30 units of the pyrimidine.

The flasks were sterilized at 12 lb. pressure for 20 minutes, and inoculated in triplicate with one drop of a suspension of *Torula.*

The *Torula* suspension was prepared by adding a loopful of *Torula* to 50 cc. of sterile distilled water. In removing the loopful of *Torula* care was taken to avoid including any of the wort agar on which the stock cultures were grown. The cultures were incubated at from 20° to 25° C.

The following *Torulae* supplied through the courtesy of F. M. CLARK, Department of Bacteriology, University of Illinois, were used: *Torula hansen,* #2500; *T. sphaerica* (Hammar), #2504; *T. cremoris* (Hammar), #2512; *T. rosea,* #2519; *T. fermentati,* #2539; *T. kefyr,* #2540; *T. sanguinea* (Schimon), #2546; *T. laurentii,* #2547. The relative development of these eight organisms after ten days is shown in table I.

TABLE I

GROWTH OF VARIOUS SPECIES OF *Torula* IN A MEDIUM OF MINERAL SALTS, ASPARAGINE AND DEXTROSE WITH THE ADDITION OF THIAZOLE OR PYRIMIDINE AS INDICATED

ORGANISMS	NO ADDITION	THIAZOLE ONLY	PYRIMIDINE ONLY	THIAZOLE AND PYRIMIDINE
Torula hansen	++	++	++	++
Torula sphaerica	+	+	+	+
Torula cremoris	–	–	–	–
Torula rosea	–	–	++++	++++
Torula fermentati	+	+	+	++
Torula kefyr	–	–	–	–
Torula sanguinea	–	–	++++	++++
Torula laurentii	+	+	+	++++

– = little or no growth. + = light growth.
++ = medium growth. +++ = fairly heavy growth.
++++ = very heavy growth.

The organisms did not respond alike. Two of them, *Torula cremoris* Hammar and *T. kefyr,* grew very little if at all in any of the solutions. Evidently none of the media used was suitable for the growth of these two species.[4] As a result no information on the significance of the thiazole or

[3] 1 unit is 10^{-9} mole of the substance in question.

[4] A mineral nutrient solution containing asparagine and dextrose which was supplemented with vitamin B_1 and nicotinic acid amide gave negative results with these two organisms. They grew satisfactorily on a medium of agar, mineral salts, asparagine, dextrose, vitamin B_1, and neopeptone.

of the pyrimidine in their development was secured. Two species, *T. hansen* and *T. sphaerica* Hammar, grew to the same extent in all four solutions as far as could be judged from the turbidity of the solutions. The growth of *T. hansen* was heavier than that of *T. sphaerica.* Either these two *Torulae* do not require thiazole or pyrimidine or they synthesize sufficient of these compounds from the constituents of the medium for maximum growth under the conditions used. On the basis of evidence presented later, we are inclined to believe the latter explanation to be the correct one.

The growth of *T. laurentii* (fig. 1) and of *T. fermentati* was markedly improved by the addition of the mixture of thiazole and pyrimidine but

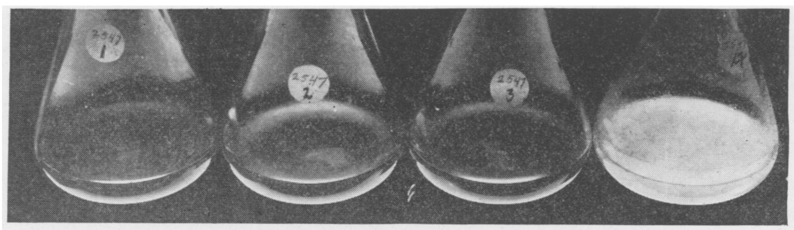

FIG. 1. *T. laurentii* grown in 1, basic medium (mineral salts, asparagine, and dextrose); 2, basic medium plus thiazole; 3, basic medium plus pyrimidine; 4, basic medium plus thiazole and pyrimidine.

little affected by either alone. The effect was greater for *T. laurentii* than for *T. fermentati.* These two species resemble *Phycomyces blakesleeanus* in requiring an external supply of both thiazole and pyrimidine, though the latter organism makes no growth in the absence of thiazole and pyrimidine while the two species of *Torula* under discussion grew slightly in the solution which lacked both thiazole and pyrimidine. It is possible that the small amount of growth of these two *Torulae* in the solutions lacking thiazole and pyrimidine or containing one of the compounds alone was made at the expense of small amounts of vitamin B_1 or of its intermediates carried in or with the cells of the original inoculum. Subcultures of these organisms from the growth in the absence of the intermediates to a solution of similar composition were not made. It would seem justifiable, however, to describe *T. laurentii* and *T. fermentati* as organisms requiring an external supply of pyrimidine and thiazole for good growth in the medium we have used. They may synthesize small amounts of the two intermediates, but if so the quantity formed under the conditions of our experiments was not sufficient for maximum growth.

T. rosea (fig. 2) and *T. sanguinea* grew very poorly in the solutions which lacked thiazole and pyrimidine and in those to which thiazole alone was added. They developed heavy growth in the solutions to which pyrim-

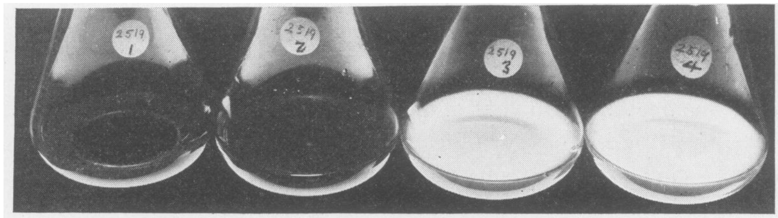

FIG. 2. *T. rosea* grown in 1, basic medium (mineral salts, asparagine, and dextrose); 2, plus thiazole; 3, plus pyrimidine; 4, plus thiazole and pyrimidine.

idine was added and in the solutions to which thiazole and pyrimidine had been added. Two possible explanations for these results may be offered. *T. rosea* and *T. sanguinea* may not synthesize either thiazole or pyrimidine and require only pyrimidine for normal growth, or they require both and synthesize the thiazole only. We are inclined to believe that the latter explanation is correct.

In order to substantiate in part the assumptions made on the ability of these various species of *Torula* to synthesize thiazole or pyrimidine, cultures of representatives of six species were sterilized at 12 lb. pressure for 10 minutes after 15 days' growth in the media described above. The sterile solutions containing the *Torula* cells and products of their metabolism

TABLE II

GROWTH OF *Phycomyces blakesleeanus* IN A MEDIUM OF MINERAL SALTS, ASPARAGINE AND DEXTROSE AND ADDITIONS GIVEN ABOVE IN WHICH VARIOUS SPECIES OF *Torula* HAD GROWN. COMPARE WITH TABLE I. FIGURES GIVEN ARE THE pH OF THE SOLUTIONS WHEN INOCULATED WITH THE *Phycomyces*

ORGANISMS	NO ADDITION	THIAZOLE ONLY	PYRIMIDINE ONLY	THIAZOLE AND PYRIMIDINE
Torula hansen	+++	+++	+++	+++
	3.6	3.5	3.5	3.5
Torula sphaerica	–	+	–	+++
Torula fermentati	+	+	+	+++
Torula laurentii	–	–	–	+
	3.6			3.8
Torula rosea	–	–	++	+++
	4.3	4.2	3.8	3.8
Torula sanguinea	–	–	+++	+++
	3.7	3.7	3.6	3.9

+++ = Aerial mycelium 2 cm. high, sporangiophores and sporangia.
++ = Submersed mycelium with few scattered sporangiophores.
+ = Submersed mycelium covering bottom of flask; no aerial hyphae.
– = Little or no growth.

were inoculated with spores of *Phycomyces blakesleeanus*. This fungus requires both thiazole and pyrimidine for growth. Its development would therefore demonstrate the presence of both intermediates. Its failure to grow in a culture solution in which a species of *Torula* had grown might be the result of a deficiency of one or both of the intermediates or of the development of some harmful constituent in the solution.

The relative growth of *Phycomyces* in these solutions in which a species of *Torula* had grown is given in table II. Some determinations of the hydrion concentrations of the solutions after the growth of the *Torula* and before inoculation with *Phycomyces* are also given in this table.

Phycomyces blakesleeanus developed fairly well in all four of the solutions in which *T. hansen* had grown. Aerial mycelium about 2 cm. high with sporangiophores and sporangia formed in all the culture solutions (fig. 3). The growth of the *Phycomyces* was probably limited by the con-

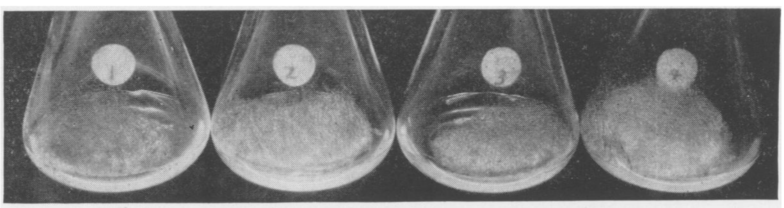

FIG. 3. *Phycomyces blakesleeanus* grown in solutions in which *T. hansen* had grown. From left to right: 1, basic medium; 2, plus thiazole; 3, plus pyrimidine; 4, plus thiazole and pyrimidine.

centration of asparagine (0.05 per cent.) in the original medium. We may conclude that *T. hansen* synthesized both thiazole and pyrimidine (or vitamin B_1) from the constituents of the medium. From the growth of this *Torula* (see table I) in the four solutions we may conclude also that the amount of the thiazole and pyrimidine synthesized was adequate for maximum growth in the medium used. Otherwise some increase in growth

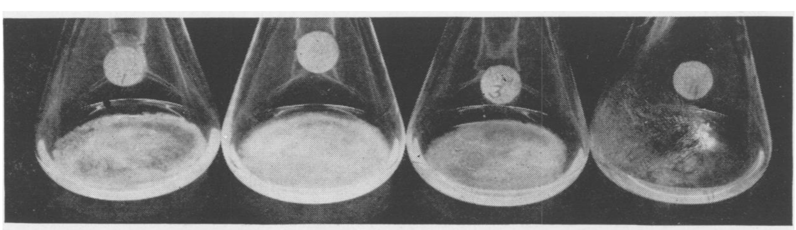

FIG. 4. *Phycomyces blakesleeanus* grown in solutions in which *T. fermentati* had grown. From left to right: 1, basic medium; 2, plus thiazole; 3, plus pyrimidine; 4, plus thiazole and pyrimidine.

would have occurred in the cultures to which thiazole and pyrimidine were added.

Phycomyces grew in all four of the solutions originally containing *T. fermentati.* The growth was greater in the solutions to which both thiazole and pyrimidine had been added (fig. 4). We conclude that *T. fermentati* synthesized both pyrimidine and thiazole but in smaller amounts per flask than *T. hansen* did. The amount of thiazole and pyrimidine synthesized was not sufficient for maximum growth of this *Torula* in the medium used (see table I) since the addition of the two intermediates improved its growth.

Phycomyces grew very little in the medium with no supplement or with pyrimidine in which *T. sphaerica* had grown but formed some submersed mycelium in the solution supplemented with thiazole (fig. 5). We conclude

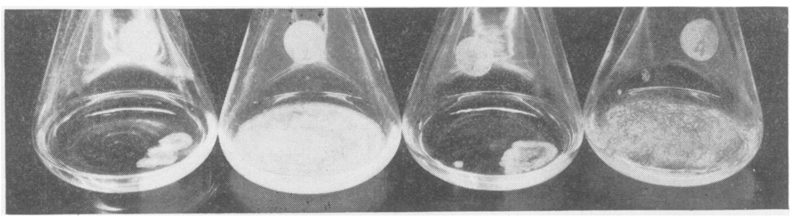

FIG. 5. *Phycomyces blakesleeanus* grown in solutions in which *T. sphaerica* had grown. Left to right: 1, basic medium; 2, plus thiazole; 3, plus pyrimidine; 4, plus thiazole and pyrimidine.

that this *Torula* may have synthesized a small amount of pyrimidine but little or no thiazole. The failure of *Phycomyces* to develop more luxuriantly in these solutions would not appear to be the result of any injurious substances formed by the *Torula* since the growth of the *Phycomyces* was as great in the mixture of pyrimidine and thiazole as it was in the solutions in which *T. hansen* had grown. These results are not entirely clear when compared with the relative growth of *T. sphaerica* (see table I). If pyrim-

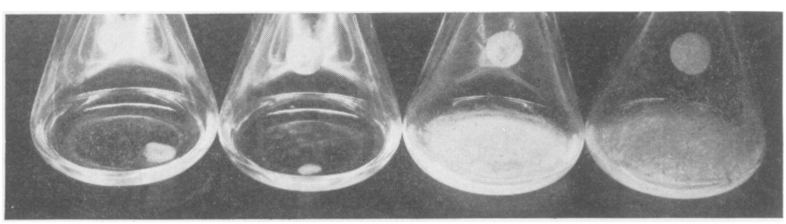

FIG. 6. *Phycomyces blakesleeanus* grown in solutions in which *T. rosea* had grown. Left to right: 1, basic medium; 2, plus thiazole; 3, plus pyrimidine; 4, plus thiazole and pyrimidine.

idine and thiazole are required for growth by this *Torula,* we should have expected greater development of *Phycomyces* in the medium containing no supplements and in the medium with pyrimidine.

Phycomyces made little or no growth in the solutions with no supplements and those with thiazole only in which *T. rosea* or *T. sanguinea* had been inoculated (fig. 6). The growth in the solutions containing pyrimidine was better. We conclude that these two *Torulae* synthesize little or no pyrimidine but form thiazole. The amount of thiazole per flask formed by the growth of *T. sanguinea* was somewhat greater than that formed by *T. rosea.* The relative growth of the two organisms in these solutions (table I) would suggest that the amount of thiazole formed by these two *Torulae* was adequate for their maximum growth in the medium used.

Phycomyces grew little or none in the solution with no addition, the thiazole solution, and the pyrimidine solution in which *T. laurentii* had grown. Its developument in the solution with both intermediates was poor. This suggests that this *Torula* had developed some material or materials which were injurious to *Phycomyces.* If this is correct our experiments would not permit us to draw conclusions on the formation of thiazole or pyrimidine by *T. laurentii.*

Discussion

These observations on the growth of eight *Torulae* in solutions lacking thiazole and pyrimidine and in solutions containing one or both of the intermediates, together with those on the subsequent growth of *Phycomyces blakesleeanus* in the same solutions, lend support to the hypothesis that vitamin B_1 is a fundamental growth factor. Some organisms make enough for adequate growth, others do not. Of those which do not, some synthesize neither the pyrimidine nor the thiazole in adequate quantities, others synthesize thiazole and some are capable of synthesizing pyrimidine. The result is that there are organisms which must be supplied with vitamin B_1 or both intermediates for satisfactory growth (*Phycomyces blakesleeanus, Torula laurentii, Torula fermentati*). There are organisms which must be supplied with pyrimidine or vitamin B_1 (*T. rosea, T. sanguinea*), and still others which must be supplied with thiazole or vitamin B_1 (tomato root).

It is possible to assume that the thiazole and the pyrimidine function in the organism as such, and that when vitamin B_1 is an effective growth substance it is split into its intermediates which then play their rôle in metabolism. As an alternate hypothesis we may assume that the vitamin molecule as such is essential and that the pyrimidine and thiazole synthesized by the organism or furnished from without are used to form the vitamin B_1 molecule. SCHOPFER favors the first of these possibilities. We have presented evidence elsewhere supporting the second hypothesis. The demonstration that *Torulae* which grew in a solution lacking B_1 or its intermediates synthesized both intermediates supports our assumption. It is supported also by the demonstration that a *Torula* which grew in a medium supplemented with pyrimidine only synthesized thiazole.

OKUNUKI (1) noted the favorable action of red yeasts on the development of certain fungi. WASSINK (6) observed good development of *Phycomyces blakesleeanus* in a maltose medium which had become contaminated with a red yeast. Without the contamination little or no growth of the fungus occurred in the medium. These results may probably be explained by the formation of vitamin B_1 or of thiazole and pyrimidine by the yeasts concerned.

Summary

Eight species of *Torula* were grown in a medium of mineral salts, asparagine, and dextrose, and the same medium to which the thiazole alone, the pyrimidine alone, or both intermediates of vitamin B_1 were added. Two species failed to grow in any of the four media; the growth of two was unaffected by the supplements; two grew distinctly better when both intermediates were used as supplements but were unaffected by either intermediate alone; the growth of two was much increased by the addition of pyrimidine or of pyrimidine and thiazole but unaffected by thiazole alone. By cultivating *Phycomyces blakesleeanus* in the solutions in which various species of *Torula* had grown it was demonstrated that a *Torula* which grew in the basic solution had synthesized thiazole and pyrimidine and one which grew in the solution supplemented with pyrimidine alone had synthesized thiazole.

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