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# *ORGANISMS REQUIRING VITAMIN $B_1$*

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In earlier articles[1] from this laboratory we have reported that excised tomato roots required an external supply of vitamin $B_1$ or of thiazole[2] for continued growth. The pyrimidine alone was ineffective. *Phycomyces Blakesleeanus* was found to require vitamin $B_1$ or both intermediates.[3] Neither of the intermediates alone was effective. *Phycomyces nitens* and two species of Torula[4] which we have investigated, *Torula fermentati* and *T. Laurentii*, resemble *Phycomyces Blakesleeanus* in requiring an external supply of both intermediates for good growth.

We have found some organisms also which grow well in a medium supplemented with pyrimidine alone, with a mixture of pyrimidine and thiazole, or with vitamin $B_1$. The thiazole alone is ineffective. Among these organisms are *Torula rosea*, *T. sanguinea*, *Phytophthora fagopyri*, *Schizophyllum commune*, *Sclerotium delphinii*, *S. Rolfsii*, *Sphaerulina trifolii*, *Pythium Butleri* and *P. polycladon*.[5] In a liquid medium composed of 0.5% $MgSO_4.7H_2O$, 1.5% $KH_2PO_4$, 1% or 0.5% Asparagine, 5% or 10% dextrose and certain mineral supplements, these seven organisms grow poorly or not at all. The addition to this medium of 30 units[6] of pyrimidine, a mixture of 30 units of pyrimidine and 30 units of thiazole or of 30 units of vitamin $B_1$ permits good growth.

None of the organisms mentioned above can be said to require an external supply of vitamin $B_1$ since they grow well in a medium lacking vitamin $B_1$ but supplemented with one or with both of the intermediates.

We have assumed on the basis of evidence presented earlier[3] that the vitamin $B_1$ molecule is the effective agent in determining the growth of *Phycomyces*, and that this organism when grown in a mixture of pyrimidine and thiazole synthesizes the vitamin molecule from the intermediates. Schopfer and Jung[7] are inclined to believe that the intermediates function as such and that *Phycomyces*, for example, when grown in a solution containing vitamin $B_1$, splits the vitamin molecule into the intermediates which then play their respective rôles in the metabolism of the organism.

We have found that *Torula rosea*, *T. sanguinea*,[4] *Phytophthora fagopyri* and *Pythium Butleri*[5] synthesize thiazole when grown in a medium supplemented with pyrimidine only. Furthermore, excised tomato roots form pyrimidine in a solution supplemented with thiazole only. This shows that some organisms which grow in a medium supplemented with pyrimidine alone or with thiazole alone form the missing intermediate from the elementary substances in the medium. This does not demonstrate that the molecule of vitamin $B_1$ rather than its thiazole and pyrimidine intermediates is the effective agent. It is, however, a result which would be anticipated on the basis of our assumption.

If we are correct in assuming that the vitamin molecule, as such, is the effective agent it might be anticipated that some of the more highly parasitic fungi may not be capable of synthesizing the vitamin molecule from the intermediates. For such organisms it would not be possible to replace the vitamin by its intermediates. They would require for growth an external source of vitamin $B_1$.

We have found certain species of *Phytophthora* which apparently require vitamin $B_1$ and cannot utilize the intermediates satisfactorily. *Phytophthora cinnamomi* and *P. capsici* are of this type. In our experiments these two organisms grew well in a medium of mineral salts and sugar supplemented with vitamin $B_1$. In the absence of the vitamin or with thiazole, pyrimidine or a mixture of the thiazole and pyrimidine we have secured little or no growth. It is possible that the synthesis of the vitamin from its intermediates is enzymatic and that these organisms lack the necessary enzyme.

Sinclair[8] and Schopfer and Jung[7] also have found that the two intermediates will replace the vitamin for *Phycomyces Blakesleeanus*. Neither intermediate alone is effective. Schopfer[9] has reported that *Rhodotorula flava* and *Rhodotorula rubra* are organisms which grow with pyrimidine alone, the thiazole alone is ineffective; and that *Mucor Ramannianus*[10] requires thiazole but not pyrimidine. We are inclined to believe on the basis of the evidence now available that the vitamin molecule is necessary for all of these organisms. Some kinds are capable of synthesizing from the elementary constituents of the medium sufficient for good growth. Others are not. Some synthesize enough of one of the intermediates for good growth but under the conditions of our experiments must be supplied with the other; some synthesize neither of the intermediates in amounts adequate for normal growth but are able to utilize the intermediates if supplied; and some not only do not synthesize either intermediate but are incapable of utilizing them when they are supplied.

The different groups of organisms in their relation to vitamin $B_1$ and its intermediates are summarized in the following table. The + sign indicates a positive growth effect of the substance given; the − sign, little or no effect.

| ORGANISMS | THIAZOLE | PYRIMIDINE | THIAZOLE AND PYRIMIDINE | VITAMIN $B_1$ |
|---|---|---|---|---|
| Group I | − | − | − | + |
| Group II | − | − | + | + |
| Group III | − | + | + | + |
| Group IV | + | − | + | + |

In Group I are included those organisms which require an external supply of vitamin $B_1$; for example, *Phytophthora cinnamomi* and *P. capsici*.

Group II includes organisms which require for good growth an external supply of vitamin $B_1$ or of both intermediates. The thiazole alone or the pyrimidine alone is ineffective. Examples are *Phycomyces Blakesleeanus*, *Phycomyces nitens*, *Torula Laurentii* and *T. fermentati*.

Group III includes those which require an external supply of vitamin $B_1$, of both intermediates or of pyrimidine. The thiazole alone is ineffective. Examples are *Phytophthora fagopyri*, *Pythium Butleri*, *P. polycladon*, *Schizophyllum commune*, *Sclerotium delphinii*, *Sclerotium Rolfsii* and *Sphaerulina trifolii*.

Group IV includes those which require an external supply of vitamin $B_1$, of pyrimidine and thiazole, or of thiazole. The pyrimidine alone is ineffective. Examples are *Mucor Ramannianus* and excised tomato roots.

A fifth group might be included comprising organisms which are unaffected by the vitamin or its intermediates in amounts which are effective for the organisms given above. This group includes many saprophytic organisms, for example, *Aspergillus niger*, and perhaps some parasites.

The members of a sixth group (*Rhizopus nigricans*)[5] are inhibited by amounts of the vitamin or of its intermediates which are favorable for other organisms.

These observations suggest that a biological method of detecting the presence of the vitamin or of its intermediates could be devised. Its success would depend upon the use of suitable organisms and further evidence of the specificity of vitamin $B_1$ and its intermediates for the organisms. It would also depend in part upon the presence of the vitamin (or intermediates) within certain limits, and the absence of injurious material which would inhibit the growth of the organisms. The scheme presented below has not been used on natural materials and is suggestive only.

I *Phytophthora cinnamomi*

Positive growth effect = vitamin $B_1$

No growth effect = both intermediates or pyrimidine alone or thiazole alone or no vitamin $B_1$ nor intermediates See II

II *Phycomyces Blakesleeanus*
Positive growth effect = both intermediates
No growth effect = pyrimidine alone or thiazole alone or no vitamin $B_1$ nor intermediate See III

III *Pythium polycladon*
Positive growth effect = pyrimidine alone
No growth effect = thiazole alone or no vitamin $B_1$ nor intermediate See IV

IV Tomato root or *Mucor Ramannianus*
Positive growth effect = thiazole alone
No growth effect = no vitamin $B_1$ nor intermediate

[1] William J. Robbins and Mary A. Bartley, *Sci.*, **85**, 246–247 (1937). William J. Robbins and Mary A. Bartley, *Proc. Nat. Acad. Sci.*, **23**, 385–388 (1937).

[2] When the terms, thiazole or pyrimidine, are used in this paper, the 4-methyl-5-hydroxyethylthiazole or the 2-methyl-5-bromo-methyl-6-aminopyrimidine is meant. These intermediates have been used in the synthesis of vitamin $B_1$.

[3] William J. Robbins and Frederick Kavanagh, *Proc. Nat. Acad. Sci.*, **23**, 499–502 (1937).

[4] William J. Robbins and Frederick Kavanagh, *Plant Physiol.* (in press).

[5] William J. Robbins and Frederick Kavanagh, *Am. Jour. Bot.* (in press).

[6] A unit is $10^{-9}$ Mole of the compound in question.

[7] William Henri Schopfer and Albert Jung, *Compt. Rend. Acad. Sci., Paris,* **204**, 1500–1502 (1937).

[8] H. M. Sinclair, *Nature*, **140**, 361 (1937).

[9] William H. Schopfer, *Compt. Rend. Acad. Sci., Paris*, **205**, 445–447 (1937).

[10] Werner Müller and William Henri Schopfer, *Ibid.*, **205**, 687–689 (1937).

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# *A CYTOLOGICAL STUDY OF COLCHICINE EFFECTS IN THE INDUCTION OF POLYPLOIDY IN PLANTS*

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A series of colchicine treatments of plant tissues was begun by the writer as an independent investigation in February, 1937, while employed at the Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N. Y. In a report at the annual winter meeting of the A. A. A. S. in 1936, Allen[1] mentioned that colchicine influenced mitotic activity in animal tissue. Soon thereafter Mr. E. L. Lahr,[2] a colleague in the