[2] A. Kolmogoroff, "Normierbarkeit eines topologischen linearen Raumes," *Studia Math.*, **5**, 29–33 (1934); J. v. Neumann, "On Complete Topological Spaces," *Trans. Am. Math. Soc.*, **37**, 1–20 (1935); A. Tychonoff, "Ein Fixpunktsatz," *Math. Ann.*, **111**, 767–776 (1935); A. D. Michal and E. W. Paxson, "The Differential in Abstract Linear Spaces with a Topology," *Comptes Rendus, Warsaw Acad. Sci.* (in press). See résumé, *Comptes Rendus, Paris Acad. Sci.*, **202**, 1741–1743 (1936); see also forthcoming articles by E. W. Paxson in *Matematiceski Sbornik* and the Paris *Comptes Rendus.*

[3] For three equivalent definitions of boundedness in linear topological spaces see Kolmogoroff, loc. cit., v. Neumann, loc. cit., Michal and Paxson, loc. cit.

[4] For the definition see Banach, *Operations Lineaires*, pp. 26 and 53.

[5] That is, every Cauchy sequence of Y has a limit in Y. Cf. v. Neumann, loc. cit., p. 1.

[6] For a list of properties of convex sets see G. Birkhoff, "Integration of Functions with Values in a Banach Space," *Trans. Am. Math. Soc.*, **38**, p. 359 (1935).

[7] $\prod_{x\in U,\, U\in \mathfrak{U}} (U)$ denotes the intersection of all the sets U of $\mathfrak{U}$ containing x, while $\bar{U}$ denotes the closure of U.

[8] Cf. v. Neumann, loc. cit., p. 15, def. 12 (*b*).

[9] Cf. for example, Banach, loc. cit., p. 10. While (*s*) is metric, the weakly topologized Hilbert space is non-metrizable. Neither space is "normable."

INTERMEDIATES OF VITAMIN B_1 AND GROWTH OF PHYCOMYCES

BY WILLIAM J. ROBBINS AND FREDERICK KAVANAGH

DEPARTMENT OF BOTANY, UNIVERSITY OF MISSOURI

Communicated August 12, 1937

In studying the nutrient requirements of excised roots of tomato (*Lycopersicon esculentum*) Robbins and Bartley[1] found a solution of mineral salts, cane sugar and vitamin B_1 to be adequate for unlimited growth. No growth occurs if any one of the three constituents listed is omitted. However, the vitamin may be replaced by a mixture of 2-methyl-5-bromomethyl-6-aminopyrimidine (or 2-methyl-5-ethyoxymethyl-6-aminopyrimidine) and 4-methyl-5-hydroxyethylthiazole,[2] the two intermediates used by Williams and Cline[3] in synthesizing the vitamin. Furthermore, unlimited growth of excised tomato roots occurs in a solution of mineral salts, cane sugar and the thiazole[2] though the growth is less rapid than in a medium containing the vitamin B_1 or both intermediates. The pyrimidine will not replace the vitamin.

If the intermediates replace the vitamin for excised tomato roots can they be used as substitutes for vitamin B_1 for other organisms? A mixture of the pyrimidine and the thiazole referred to above has been found to cure polyneuritis in pigeons.[4] Neither the pyrimidine nor the thiazole alone is effective. The minimum curative dose of the intermediates is, however, several times the minimum quantity of B_1 which is effective.

Schopfer[5] has reported that *Phycomyces Blakesleeanus* requires vitamin B_1. We have found that a mixture of 2-methyl-5-bromo-methyl-6-amino-pyrimidine (or the ethyoxypyrimidine) and 4-methyl-5-hydroxyethyl-thiazole is as effective as molecularly equivalent amounts of B_1 in determining the dry matter produced by *Phycomyces* in a period of two weeks. Neither the thiazole nor the pyrimidine alone is effective.

The thiazole contains reduced sulphur. Can other sulphur compounds be substituted for the thiazole? Such a possibility is suggested by the report by Volkonsky[6] that *Saprolegnia sps.* require reduced sulphur and are unable to utilize sulphur in the oxidized form. Negative results, however, were secured with *Phycomyces* by the authors when the following sulphur compounds were substituted for the thiazole in a modified Coon's medium (0.5% $MgSO_4$, 7% H_2O, 1.5% KH_2PO_4, 10% asparagin, 10% dextrose) and the pyrimidine: *dl*-methionine, glutathione, thioglycolic acid, S-diphenyl-thiourea, thiopropionamide, allylthiourea, thiobarbituric acid, thiourea, 2-amino-4-methylthiazole hydrochloride, 2-amino-4-(p-diphenyl) thiazole, 1-chlorobenzothiazole, 1-mercaptobenzothiazole, 1-phenylbenzothiazole, 1-methylmercaptobenzothiazole (1-thiomethyoxybenzothiazole) and 5-amino-1-mercaptobenzothiazole hydrochloride.

Negative results were secured also when ethylene chlorhydrin or pimelic acid was substituted for vitamin B_1. Ethylene chlorhydrin is effective in breaking dormancy of plants[7] and pimelic acid has been reported to be an accessory growth factor for a strain of the diphtheria bacillus.[8]

Pyrimidine compounds are included in the hydrolytic products of nucleic acid. Will nucleic acid replace the pyrimidine? We have not succeeded in cultivating *Phycomyces Blakesleeanus* in the modified Coon's solution and thiazole[9] to which nucleic acid or the acid hydrolysate of nucleic acid was added.

It appears from these results and those reported earlier that the pigeon and *Phycomyces* differ from tomato roots in requiring either vitamin B_1 or both intermediates. The tomato roots will grow indefinitely in a solution of mineral salts, cane sugar and the thiazole though the growth is less vigorous than when B_1 or both intermediates are supplied. The pigeon and *Phycomyces* differ in that B_1, under the conditions of our experiments, is considerably more effective than equivalent amounts of the two intermediates for the pigeon but equally effective for *Phycomyces*.

It appears that there are heterotrophic organisms capable of continued growth in a medium lacking B_1. We have grown *Aspergillus niger* through six generations in a liquid medium containing mineral salts and pure cane sugar. The dry weight of the sixth generation was approximately equal to that of the first and spore production was normal. On this medium

Phycomyces will not grow unless B_1 or its intermediates are present. Since, in making the transfers of the *Aspergillus*, a few spores only were used in inoculating the new culture media the B_1 present in the original spores must have been fractionated to an inappreciable amount in the 6 successive transfers.

There are organisms (if we may refer to excised tomato roots as organisms) which require thiazole for continued growth. There are others, for example *Phycomyces Blakesleeanus* and the pigeon, which require vitamin B_1 or both intermediates. Are there organisms for which the pyrimidine alone is adequate? On this question we have no evidence.

Is the vitamin essential or do each of the intermediates function independently? When *Phycomyces* grows in the presence of the intermediates is vitamin B_1 synthesized or does the thiazole perform one essential function and the pyrimidine another? When the organism grows in the presence of the vitamin is the vitamin split into the intermediates which then function or does the entire B_1 molecule act in conditioning growth? The authors are of the opinion that the vitamin is required. This conclusion is suggested as the result of experiments on *Phycomyces* which show that the two intermediates are probably used in molecularly equivalent quantities. The organism was grown in the modified Coon's solution and the two intermediates. In each mixture of the intermediates one was supplied in small amount, the other in considerably larger amount. For each combination in which the thiazole was furnished in small amount and the pyrimidine in larger amount a similar mixture was prepared in which the quantities of the thiazole and pyrimidine were reversed. This will be made clear from the following table in which the dry weights of *Phycomyces* are given as determined at the end of two weeks' growth. The fungus was grown in 20 cc. of modified Coon's solution and a mixture of the thiazole and pyrimidine as given in the table.

AMOUNT IN 10^{-9} MOLE		DRY WEIGHT,
THIAZOLE	PYRIMIDINE	MGMS.
0.01	10.0	9
10.0	0.01	5
0.1	10.0	25
10.0	0.1	26
0.3	10.0	49
10.0	0.3	50
1.0	10.0	115
10.0	1.0	121
3.0	10.0	318
10.0	3.0	320
10.0	10.0	500
10×10^{-9} mole B_1		520

It appears that the dry weight formed in a medium containing thiazole and pyrimidine is determined by the one which is present in smaller concentration and is the same for equal limiting molecular concentrations of either the thiazole or the pyrimidine. For example, with 0.3×10^{-9} mole of thiazole and 10.0×10^{-9} mole of pyrimidine the yield was 49 mgms. and with 0.3×10^{-9} mole of pyrimidine and 10×10^{-9} mole of thiazole the yield was 50 mgms. Other pairs of combinations of the two intermediates show similar results. If the thiazole and pyrimidine functioned independently it would be expected that more growth would be secured in one of a pair of concentrations such as are given in the table. The fact that the two intermediates appear to be used by the organism in molecularly equivalent quantities is best explained by assuming that the vitamin is required and is synthesized from them by the organism.

Since the completion of the experiments on *Phycomyces* summarized here the writers' attention was called to a note by Schopfer and Jung.[10] They find that in a mixture of 4-amino-5-amino-methyl-2-methyl pyrimidine[11] and 4-methyl-5-hydroxyethylthiazole at amounts of 0.01 to 0.4 gamma per 25 cc. the development of *Phycomyces* is identical with that obtained with vitamin B_1. Our results confirm and extend those of Schopfer and Jung.

[1] William J. Robbins and Mary A. Bartley, *Sci.*, **85**, 246–247 (1937).

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

[3] R. R. Williams and J. K. Cline, *Jour. Am. Chem. Soc.*, **58**, 1504–1505 (1936).

[4] William J. Robbins, Mary A. Bartley, A. G. Hogan and L. R. Richardson, *Proc. Nat. Acad. Sci.*, **23**, 388–389 (1937).

[5] W. H. Schopfer, *Ber. Deutsch. Bot. Gesell.*, **52**, 308–313 (1934).

[6] Michel Volkonsky, *Ann. l'Inst. Past.*, **50**, 703–730 (1933).

[7] F. E. Denny, *Amer. Jour. Bot.*, **13**, 386–389 (1926).

[8] J. Howard Mueller, *Sci.*, **85**, 502–503 (1937).

[9] When the terms, thiazole or pyrimidine, are used in this paper without other qualification the 4-methyl-5-hydroxyethylthiazole or the 2-methyl-5-bromo-methyl-6-aminopyrimidine is meant.

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

[11] This pyrimidine is identical with the one we have used except that an amino group has been substituted for the bromine.