[1] These experiments were aided by a grant to the senior author by the Committee on Effects of Radiation upon Living Organisms of the National Research Council.

[2] Hakansson (*Hereditas* **5**, 93–96 (1924)) discovered a 14-chromosome plant in the progeny of *Oenothera gigantea*, which was thought to have originated from the union of 7-chromosome gametes. But it is possible that the plant was a parthenogenetic diploid.

[3] Belling, John, and Blakeslee, A. F., *Amer. Nat.*, **58**, 60–61(1924).

[4] Gairdner, A. E., and Darlington, C. D., *Genetica*, **13**, 113–150 (1931).

[5] For an explanation of the gene symbols and a description of the characters referred to in this paper see Emerson, R. A., Beadle, G. W., and Fraser, A. C., *Cornell Univ. Agr. Expt. Sta. Memoir*, 180 (1935).

[6] Randolph, L. F., *Journ. Agr. Res.*, **50**, 591–605 (1935).

[7] The "haploid" derivatives of Aegilotricum are typical examples; cf. Katayama, Y., *Jap. Journ. Bot.*, **7**, 349–380 (1935).

Appendix.—Since this article was submitted for publication the current number of *Hereditas* was received, in which there was a paper by Heribert Nilsson (*Hereditas*, **25**, 1–8 (1939)) describing diploid derivatives of *Oenothera gigantea*, an autotetraploid form of *Oe. Lamarckiana* (cf. footnote 2). These derived diploids definitely exhibited certain characteristics of *Oe. gigantea* as well as certain characteristics of *Oe. Lamarckiana* and for this reason were appropriately designated *Oe. diplo-gigantea*.

THE SYNTHESIS AND DESTRUCTION OF VITAMIN B_1 BY PHYCOMYCES

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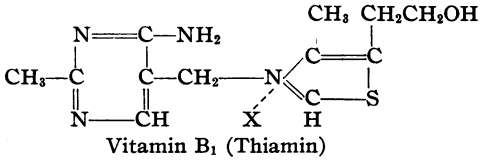
In an earlier communication[1] data relative to the *in vivo* synthesis of vitamin B_1 by the isolated pea root have been presented. For a fuller understanding of the physiological economy of the vitamin, some insight also into the modes of disappearance of the substance is indispensable. The accumulated evidence now permits us to present a picture both of *in vivo* synthesis of the vitamin by *Phycomyces Blakesleeanus* and of a mechanism by which the vitamin molecule is broken down by the latter organism.

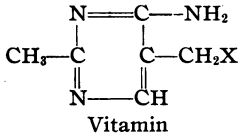
The experimental techniques were similar to those described in earlier communications.[1, 2] Stock cultures of *Phycomyces Blakesleeanus* and of *Phytophthora cinnamomi* were maintained on malt agar. The experimental cultures were, in all cases, made up with 10 cc. of medium ($MgSO_4.7H_2O$... 0.5 gm.; KH_2PO_4...1.5 gm.; asparagin...4.0 gm.; dextrose...100 gm.; distilled water...1 liter) to which was added the desired amount of vita-

min B_1 or related substances.[3] The experimental media were then autoclaved for 15 minutes at 15 pounds pressure, and inoculated, in the case of *Phycomyces*, with equal volumes of a sterile spore suspension, or, in the case of *Phytophthora*, with small pieces of mycelium. All cultures unless otherwise noted were allowed to remain 10 days at 25°C., the mycelium then filtered off, dried and weighed. All experiments were carried out in 3–5 fold replicate.

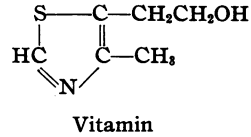
The structure of vitamin B_1 is shown below; the pyrimidine and thiazole portions of the molecule are also illustrated.



Vitamin B_1 (Thiamin)



Vitamin pyrimidine



Vitamin thiazole

It has been demonstrated repeatedly by various investigators[4] that *Phycomyces* is able to utilize not only the vitamin itself as a growth factor, but also equally well an equimolecular mixture of the pyrimidine (X = NH_2, Br, OC_2H_5) and thiazole fragments of the molecule. The *Phycomyces* assay determines then not only vitamin B_1 but also any uncombined pyrimidine plus thiazole which may be present in equimolecular amount in the sample under investigation.[5] *Phytophthora*[6] on the other hand utilizes vitamin B_1 as a growth factor but does not respond to a mixture of the two vitamin components. The *Phytophthora* assay, then, may be used for the determination of vitamin B_1 to the exclusion of any uncombined pyrimidine plus thiazole.

In the interpretation of experiments with *Phycomyces*, it has been assumed, but never demonstrated experimentally, that this organism synthesizes the vitamin from a mixture of vitamin pyrimidine and vitamin thiazole. That this is actually the case is shown by the following experiment. *Phycomyces* cultures supplied with an equimolecular mixture of pyrimidine (in all of the experiments reported in this paper, the 5-aminomethyl pyrimidine (R = NH_2) was used) and thiazole at different concentrations were harvested after 5 or after 10 days, and the vitamin B_1 in the medium and in the mycelium determined separately by the *Phytophthora* assay. From table 1 it may be seen that no significant amount of vitamin

B_1 was found at the end of 10 days if the initial amount of pyrimidine and thiazole was low. If, however, larger amounts of these substances were added initially, considerable amounts of vitamin B_1 were found at the end of the 10-day experimental period. It is also clear from table 1 that under certain conditions vitamin B_1 is formed and subsequently disappears.

TABLE 1

SYNTHESIS OF VITAMIN B_1 FROM PYRIMIDINE-THIAZOLE MIXTURE BY *Phycomyces*

GROWTH FACTOR SUPPLIED MOLS × 10^{11}	MG. DRY WEIGHT OF *Phycomyces* MYCELIUM AFTER 5 DAYS	10 DAYS	VITAMIN B_1 PRESENT (MOLS × 10^{11}) IN MEDIUM 5 DAYS	10 DAYS	IN MYCELIUM 5 DAYS	10 DAYS
Py + Th* 10	12	15	0.6	0.6	0.6	0.0
" 100	54	76	7.4	1.2	21	3.1
" 1000	58	96	21	34	131	370

*Py = vitamin pyrimidine; Th = vitamin thiazole.

In experiments of a similar type the course of this formation (from pyrimidine and thiazole) and disappearance of the vitamin was followed during a 10-day period. It was found that vitamin was synthesized rapidly after inoculation of the *Phycomyces* culture and that synthesis continued during active growth of the mycelium. A rapid disappearance of the vitamin immediately after cessation of growth was observed, and in numerous experiments the vitamin was found to be virtually or completely absent after 10 days. The points to be stressed in the present connection are (1) that *Phycomyces* does synthesize vitamin from pyrimidine plus thiazole, and (2) that vitamin is destroyed by the resting mycelium.

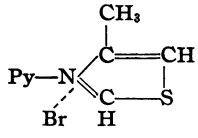
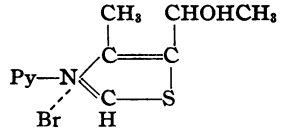
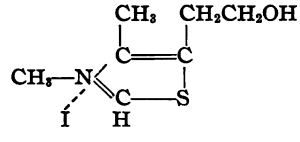
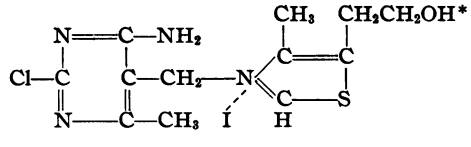
One might next inquire as to the fate of this vitamin which has disappeared. Experiments were made in which *Phycomyces* was allowed to grow for 10 days upon medium containing either vitamin B_1 (10^{-7} molar) or an equivalent mixture of vitamin pyrimidine and vitamin thiazole. At the end of 10 days both mycelium and medium from these cultures were assayed by *Phytophthora*, by *Phycomyces* and by *Phycomyces* in the presence of excess vitamin thiazole. The difference in the values from these two *Phycomyces* assays should be a measure[7] of excess vitamin pyrimidine over vitamin thiazole in the sample under investigation. In the experiment in which vitamin B_1 was added to the culture medium, the assay showed that, after 10 days, the vitamin had completely disappeared from both medium and mycelium. Practically no uncombined thiazole but a considerable quantity of free pyrimidine[8] was found both in the medium and in the *Phycomyces* mycelium. In fact, it was possible to account for, as free pyrimidine, more than half of the pyrimidine added initially as vitamin to the culture. A similar end result was obtained when *Phycomyces* was allowed to grow on the pyrimidine-thiazole mixture.

Since vitamin B_1 is broken down with resultant destruction of the thi-

azole portion, the free pyrimidine liberated should be available for combination with more thiazole. One would expect to find that the ratio of pyrimidine to thiazole optimal for supporting the growth of *Phycomyces* is not 1 to 1 (equimolecular)[4b] but rather that a given number of mols of pyrimidine should suffice for a larger number of mols of thiazole. In numerous experiments it has been found that when the concentration of

TABLE 2

VITAMIN ANALOGS AS SOURCES OF PYRIMIDINE

GROWTH FACTOR SUPPLIED (ALL SUBSTANCES 10^{-7} MOLAR IN THE CULTURE MEDIUM)	MG. DRY WEIGHT OF MYCELIUM PER CULTURE
Py + Th	80
	1
" + Th	70
	0
" + Th	75
	0
" + Py	1
	0
" + Py	2

* Kindly furnished by Professor A. R. Todd.

pyrimidine is small (10^{-8} molar) a ten times excess of thiazole will suffice for at least twice as much growth of mold as will a quantity of thiazole equivalent to the amount of pyrimidine used, whereas excess pyrimidine has no such pronounced effect. Similarly the addition of free thiazole to cultures containing vitamin B_1 itself results in a like marked increase of growth over that obtained with vitamin alone. A pyrimidine molecule

may then be available more than once for combination with vitamin thiazole. Thus the breakdown *in vivo* of the vitamin by *Phycomyces* appears to proceed by a mechanism which leaves the pyrimidine portion intact but which inactivates or destroys the thiazole half.

This mechanism apparently applies also to the case of analogs of the vitamin. Table 2 shows two vitamin analogs made up of the pyrimidine and a thiazole which is an inactive analog of the vitamin thiazole. These vitamin analogs, although themselves inactive, serve as sources of vitamin pyrimidine if the correct vitamin thiazole is present. In contrast to this, two vitamin analogs which are quaternary salts of the vitamin thiazole, but in which the group joined to the thiazole possesses a structure other than that

TABLE 3

DESTRUCTION OF THIAZOLE BY *Phycomyces* MYCELIUM IN THE PRESENCE OF PYRIMIDINE

MYCELIUM EMPLOYED		GROWTH FACTOR ADDED (10^{-7} MOLAR) AT BEGINNING OF PERIOD I	PERIOD II	MG. DRY WEIGHT* OF MYCELIUM PER CULTURE
Mycelium grown on Py + Th (each 10^{-8} molar) for 5 days.	A	0	0	9
		Py	0	9
		Th	0	17
Dry weight approx. 9 mg.		Py + Th	0	78
	B	Py (for 1 day)	Th	74
		Py (for 5 days)	Th	74
(pyrimidine containing)		Py (for 10 days)	Th	78
	C	Th (for 1 day)	Py	72
		Th (for 5 days)	Py	26
		Th (for 10 days)	Py	18
Mycelium from control cultures one month old.	D	0	0	4
		Th	0	4
		Py + Th	0	79
(pyrimidine free)	E	Py (for 5 days)	Th	75
		Th (for 5 days)	Py	81

*Weight taken 10 days after last addition of growth factor.

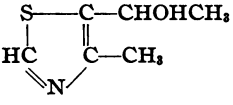
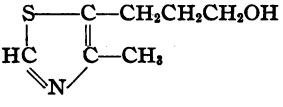
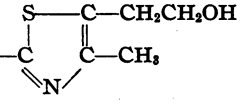
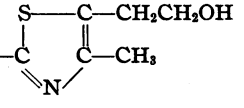
of the vitamin pyrimidine, apparently cannot be utilized at all as sources of thiazole in the presence of vitamin pyrimidine. Thus it seems that analogs of the vitamin are also broken down in a manner such as to make the pyrimidine but not the thiazole portion of the molecule available for recombination.

Next we may consider the question of whether the mechanism of thiazole destruction involves a preliminary dissociation of vitamin into free thiazole; that is, is it thiazole as such which is destroyed or thiazole in the form of a quaternary salt as in the vitamin? The data of table 2 suggest the latter alternative and this view is strongly supported by the evidence presented in table 3. *Phycomyces* mycelia were obtained (as indicated in

the table) either by a method which permitted the retention of a small amount of pyrimidine or by a method which left the mycelium pyrimidine free.[9] Uncombined pyrimidine or thiazole was allowed to remain in contact with these mycelia for varying periods of time as indicated and then the complementary portion of the vitamin was introduced sterilely. It may be seen first (3A) that addition of excess pyrimidine alone exerted no influence on the further growth of the mycelium. The addition of thiazole to pyrimidine containing mycelium brought about a two-fold increase in mycelium dry weight. This is in accord with the fact noted above that a small amount of pyrimidine may suffice for a large amount of thiazole. Uncombined pyrimidine is not affected in its ability to support the growth

TABLE 4

COMPETITION EXPERIMENTS

GROWTH FACTOR SUPPLIED			MG. DRY WEIGHT OF MYCELIUM PER CULTURE
Py + Th (each 10^{-7} molar)			60
" "	+ 	(10^{-5} molar)	58
" "	+ 	"	60
" "	+ NH_2— 	"	17
" "	+ CH_3— 	"	10

of *Phycomyces* even though the pyrimidine has been as many as 10 days in contact with mycelium (3B). We must then conclude that pyrimidine is not destroyed at an appreciable rate by resting *Phycomyces*. In marked contrast to this, thiazole is destroyed by contact with pyrimidine containing mycelium (3C) and has completely disappeared after 10 days, since addition of pyrimidine after this time resulted in no more growth than if the pyrimidine were not added at all. On the other hand, destruction of the thiazole apparently does not proceed in the absence of pyrimidine (3D and 3E). Thiazole remained quantitatively intact in these pyrimidine-free cultures even after 5 days of contact with mycelium, whereas in the pyrimidine containing cultures ca. 90% was destroyed after this time. Since

thiazole is destroyed by *Phycomyces* only when pyrimidine is present, we conclude that thiazole is destroyed primarily when it is combined in the form of vitamin.[10]

Further confirmation of this view and some additional insight into the mechanism by which the thiazole ring of the vitamin molecule is broken down is offered by competition[11] experiments. In these experiments *Phycomyces* was grown on a medium containing vitamin pyrimidine, vitamin thiazole and a large excess of inactive thiazole analog.[12] As may be seen in table 4, this large excess of added thiazole analog exerted no significant effect on the growth of the mold provided only that the analog in question was unsubstituted in the 2-position. If, however, the analog was substituted in the 2-position by CH_3 or NH_2, it exerted a marked depressant effect on the ability of the mixture to support the growth of *Phycomyces*. This depressant effect is not due to toxicity of the 2-substituted analogs. In accordance with the above views, we may suppose[13] that in the case of 2-unsubstituted thiazole analogs the corresponding inactive vitamin analog is synthesized *in vivo* but is again broken down, making the pyrimidine eventually available for combination with vitamin thiazole. In the case of 2-substituted thiazoles, on the other hand, the corresponding (inactive) vitamin analog, once synthesized, is not readily broken down, so that pyrimidine, once so bound, is not available for future combination with vitamin thiazole. It may then be postulated that the breakdown of the quaternary thiazole is initiated by a rupture of the ring adjacent to the 2-position, and that the ring is stabilized by the presence of a 2-substituent.

Summary.—Vitamin B_1 is synthesized from a mixture of vitamin pyrimidine and vitamin thiazole by *Phycomyces*. Subsequently the vitamin is broken down by the mycelium with destruction of thiazole and liberation of free pyrimidine. The thiazole portion is attacked presumably only when combined in the form of vitamin (as shown by the fact that its destruction takes place only in the presence of the pyrimidine half). Evidence is presented indicating that the first step in the *in vivo* degradation of the thiamin molecule involves an opening of the quarternary thiazole ring adjacent to the 2-position.

Acknowledgment.—The chemical portion of this work was made possible through a grant from the Research Corporation, for which the authors express their gratitude. The biological testing was carried out with the aid of the Works Progress Administration, Official Project Number 665-07-3-83, Work Project Number 9809.

[1] Bonner, J., and Buchman, E. R., *Proc. Nat. Acad. Sci.*, **24**, 431 (1938).

[2] Bonner, J., and Erickson, J., *Amer. Jour. Bot.*, **25**, 685 (1938).

[3] The additions of vitamin B_1 and/or related substances were made from concentrated stock solutions so that the dilution of the culture medium was negligible.

[4] *a.* Schopfer, W. H., and Jung, A., *Compt. Rend. Acad. Sci. Paris*, **204**, 1500 (1937);

b. Robbins, W. J., and Kavanagh, F., *Proc. Nat. Acad. Sci.*, **23**, 499 (1937); *c.* Sinclair, H. M., *Nature*, **140**, 361 (1937).

[5] It is probable that combined forms of the vitamin such as cocarboxylase are determined by *Phycomyces* and by *Phytophthora* as well. This would not alter the arguments presented here.

[6] Robbins, W. J., *Proc. Nat. Acad. Sci.*, **24**, 53 (1938).

[7] The values for excess pyrimidine obtained in this way are somewhat too high due to the fact that pyrimidine in the presence of excess thiazole may react more than once. In the present experiments, however, this error is sufficiently small to be disregarded.

[8] The work of G. M. Hills (*Biochem. Jour.*, **32**, 383 (1938)) indicates that, also with *Staphylococcus aureus*, the *in vivo* destruction of thiazole takes place more rapidly than that of pyrimidine.

[9] That these mycelia were actually pyrimidine free is indicated by the fact that addition of thiazole promoted no further growth (see table 3D and compare 3A).

[10] Preliminary experiments with *Phytophthora* indicate that this organism (which cannot combine thiazole with pyrimidine) also destroys vitamin with liberation of pyrimidine but does not attack uncombined thiazole.

[11] See reference 1, page 433.

[12] The very slight vitamin thiazole activity of the hydroxypropyl thiazole analog (see reference 2) may be neglected in evaluating the results of table 4.

[13] More direct evidence in support of this interpretation will be presented in a later communication.

TEMPERATURE AND THE CRITICAL INTENSITY FOR RESPONSE TO VISUAL-FLICKER. III. ON THE THEORY OF THE VISUAL RESPONSE CONTOUR, AND THE NATURE OF VISUAL DUPLEXITY

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Communicated March 7, 1939

I. In most vertebrates, including man, there occur two chief types of retinal photoreceptors, rods and cones. Evidence of various kinds has been very generally held to support the doctrine that the duplexity of the visual performance functions as obtained from the majority of vertebrates tested, including man, is a direct consequence of the specific differences in the excitabilities of retinal rods and cones.[1] Experimental data show unequivocally that, for certain vertebrates, in the production of the duplex performance contours there are indeed concerned the activities of two groups of sensory effects.[2] That the observable properties of the elements of sensorial effect in these 2 groups are determined by the quantitative differentiation of the primary receptors into two categories with respect to the photochemical basis of excitation[1, 3]—or indeed that the quantitative features