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THIAMIN AND PLANT GROWTH[1]

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ALTHOUGH several investigators, including R. J. Williams (1920) and Lepeschkin (1924), had reported a favorable effect of vitamin B_1 or thiamin upon plant growth, their crude preparations contained other substances as well as thiamin, and their results could not be accepted as definitive. Indisputable evidence of the importance of thiamin for plants depended upon the availability of the pure compound. Isolated in crystalline form by Jansen and Donath as early as 1926, thiamin did not become generally available until 1934, largely as the result of the work of R. R. Williams and his associates.

Almost at once Schopfer (1934) demonstrated that *Phycomyces Blakesleeanus* will not grow in a medium of mineral salts, asparagine and sugar unless supplied with thiamin. The demonstration was so striking that further investigations with this organism and with others soon followed until it is now clear as the result of the work of Schopfer, Bonner, Knight and others that thiamin is as necessary in the physiology of plants as it is in the nutrition of animals.

My own interest in thiamin arose from a study of the nutrient requirements of excised roots, initiated in 1917 as the result of a paper by Jacques Loeb in which he suggested that a hormone is concerned in the formation of roots by the leaves of *Bryophyllum calycinum.* It was my opinion that Loeb had not eliminated sugar accumulation in the leaves as the "hormonal" factor, and the experiments on excised roots were initiated to attack that problem directly. In the twenty years since the original experiments a number of people have been associated with me and through their assistance one aspect or another of that problem and related ones

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have been attacked. W. E. Maneval worked with me in the earlier years; E. E. Naylor undertook a morphological and histological study of the origin of the roots of *Bryophyllum* leaves; Albert Saeger studied the necessity of accessory substances for the *Lemnaceae;* Virginia B. White, J. E. McClary and others have assisted in studies on excised roots of *Zea Mays;* Mary B. Schmidt and F. Kavanagh have cooperated in studies of excised tomato roots and of fungi.

Our studies have led us to the conclusion that growth substances are important for plants as well as for animals, that Loeb's original hypothesis is essentially correct, and that one of the important growth substances for plants is thiamin.

Thiamin, also called vitamin B_1 or aneurin, is a definite and specific organic compound of known structure, which is prepared synthetically in commercial quantity. It contains nitrogen and sulfur in addition to carbon, hydrogen and oxygen and is synthesized by combining a pyrimidine, 2-methyl-5-bromomethyl-6-amino-pyrimidine hydrobromide and a thiazole, 4-methyl-5-β-hydroxyethyl-thiazole.

On the basis of our present knowledge it appears that all or almost all plants require thiamin, some of them make it from more elementary materials; others do not and must be supplied with part or all of the thiamin molecule for normal growth. In other words, some plants are autotrophic in their relation to thiamin and others are heterotrophic. In fact, it is possible to divide plants into several groups on the basis of their relation to thiamin. Many kinds require no external supply of thiamin. This group comprises those plants which lack chlorophyll and grow in a medium of mineral salts and dextrose and most, if not all, green plants. It includes, for example, *Aspergillus niger, Agaricus campestris* and *Absidia glauca.* Of 36 saprophytic and parasitic fungi we investigated, the growth of 13 was unaffected by the addition of thiamin to a medium which lacked it. It has been demonstrated that some of these plants which require no external supply of thiamin synthesize it, and it is justifiable to assume that the others in this group also have the same power. It is important to note that the power of synthesis of thiamin is not limited to the green plant but is possessed by yeasts, bacteria and fungi also, because this observation eliminates chlorophyll as a factor essential for the synthesis of thiamin. The green plant is not the sole source of thiamin in nature.

Nearly 100 bacteria, yeasts and fungi are now known to require an external supply of thiamin or its intermediates for normal growth; and further investigation will doubtless materially increase this number. Some of these organisms are saprophytes, but most of them, as might be anticipated, are parasites; the fungi include representatives of the *Phycomycetes, Ascomycetes, Basidiomycetes* and *Fungi Imperfecti.* These plants which require an external supply of thiamin and are unable to make thiamin from the more elementary constituents of the medium differ considerably in their synthetic power. Some are able to construct the pyrimidine portion of the thiamin molecule but not the thiazole part; these must be furnished with the thiamin thiazole for growth. Very few organisms are known in this group. Others can make the thiazole but not the pyrimidine portion of the molecule; these are more common. Some are unable to construct either the pyrimidine or thiazole but can combine the two into the necessary thiamin molecule. *Phycomyces Blakesleeanus* is an example of this group. Finally, there are some plants, for example, most of the *Phytophthoras,* which are so specialized that they are unable to make the thiamin molecule from the thiazole and pyrimidine intermediates. These organisms must be supplied with thiamin as such and resemble in this respect the higher animal. *Rhizopus nigricans* requires no external supply of thiamin, and its growth is inhibited by the addition of thiamin to the medium.

It appears very probable that thiamin is necessary for the growth of all or nearly all organisms. Some of them, however, are capable of making the thiamin they need; some must be supplied with thiamin as such, and between these two extremes there are many gradations in synthetic power. Even closely related organisms may differ in their synthetic power, as has been found for the species of *Ustilago* by Schopfer.

Furthermore, a particular organism under one set of conditions may make sufficient thiamin for normal growth and under another set be unable to do so. We found this to be true for the fungus *Pythium Butleri,* which can not grow in a concentrated salt solution without thiamin but in a more dilute solution constructs sufficient for moderate growth.

The higher plant requires special consideration. Through the technique of cultivation of excised roots of higher plants under sterile conditions it has been possible to demonstrate that although an entire tomato plant synthesizes thiamin, the root does not; it depends upon the shoot for its supply of this essential material. Tomato roots synthesize the pyrimidine portion of thiamin but require an external supply of thiazole. We have maintained excised tomato roots in a solution of mineral salts, pure cane sugar and thiamin for 25 successive passages extending over a period of more than two years and in a solution of mineral salts, pure cane sugar and the thiamin thiazole through 19 passages extending over 20 months. In the same solution without thiamin or thiazole no growth occurs. This extended period of cultivation of excised tomato roots in a synthetic medium is of significance not only from the standpoint of their requirement for growth substances, but also because the nitrogen requirements of the roots during this period were met by nitrates, thus demonstrating that the root of a higher plant can synthesize its proteins from nitrates. Pea roots, as shown by Bonner, resemble *Phycomyces* and require both intermediates. It is clear that the parasitic relation of the root to the shoot involves more than sugar.

This observation raises the question as to how thiamin should be classified so far as plants are concerned. For the tomato plant, thiamin is a hormone, it is formed in the top of the plant and materially affects the development of the root; for *Phytophthora cinnamomi* it is not a hormone, since no part of the plant produces it. We are not justified in calling it a vitamin for the plant, if we hold to the original definition of this term, which limited it to animal nutrition. Depending upon the organism concerned, thiamin may be considered to be a hormone, a vitamin or something which is neither a hormone nor a vitamin. For the time being I have preferred to refer to it and similar substances by the general term "growth substance," meaning a specific organic substance needed in small amounts for the normal development of a living organism.

Thiamin is needed in small amounts. For example, *Phycomyces Blakesleeanus* shows a measurable response to less than three millionths of a milligram and excised tomato roots to one billionth of a milligram.

Functions of Thiamin. Although required in small amounts, thiamin is not to be regarded as merely a stimulant. It plays a definite and significant rôle in the metabolism of carbohydrates and probably in other metabolic processes also. Lohman and Schuster (1937) and others have demonstrated that thiamin combines with phosphoric acid to form thiamin pyrophosphate or cocarboxylase, which in association with a specific protein is concerned in the decarboxylation of pyruvic acid, an important intermediate in the metabolism of dextrose. There is some evidence to show that cocarboxylase is oxidized to thiochrome and that the thiochrome is ineffective or much less effective than thiamin or cocarboxylase, probably because the change of cocarboxylase to thiochrome is not readily reversible. We have found that a substance with the characteristic blue fluorescence of thiochrome appears in mineral-sugar solutions containing thiamin in which tomato roots have been grown and that thiochrome is much less effective with tomato roots than either thiamin or cocarboxylase. It appears, therefore, that one at least of the functions of thiamin is to serve as a precursor of a part of an enzyme system involved in respiration and that it is eventually rendered inactive.

It is probable that this is not the sole function of thiamin. As Williams and Spies point out in their excellent book on Vitamin B_1 it is reasonable to suppose that thiamin has a multiple function as a coenzyme in nature. The best-established ones are the two known reactions of its pyrophosphate, which in association with one protein can bring about a decarboxylation of pyruvic acid to acetaldehyde or in association with another protein can promote a simultaneous dehydrogenation and decarboxylation of pyruvic acid to form acetic acid. It may also have a purely oxidative function and be concerned in the transformation of carbohydrate to fat. At any rate, it seems clear from the work of Peters and others that in a deficiency of thiamin the catabolic metabolism of dextrose beyond the point of pyruvic acid is interfered with.

We have found that *Phycomyces* makes more growth in a medium lacking thiamin if the carbon source is acetaldehyde, ethyl alcohol or acetic acid than it does in a similar medium in which the carbon source is sugar. This might be anticipated if it is remembered that thiamin pyrophosphate is believed to function in the transformation of pyruvic acid to acetaldehyde and to acetic acid. By using carbon compounds simpler than pyruvic acid the need for the changes in dextrose to these simpler compounds is eliminated; in one sense the acetaldehyde, ethyl alcohol and acetate represent predigested food for *Phycomyces.* However, even with these simpler compounds the growth is distinctly limited and is materially improved by the addition of thiamin. In fact, we have been unable to maintain *Phycomyces* in a sodium acetate medium through more than the first transfer, which suggests that the growth made in the sodium acetate solutions is at the expense of thiamin supplied in the spores used as inoculum. If this is correct, the interesting conclusion follows that thiamin is more effective with sodium acetate as a carbon source than with sugar. Our inability to maintain *Phycomyces* in a sodium acetate solution, and the beneficial effects of thiamin on growth in solutions containing alcohol, acetaldehyde or acetate supports the belief mentioned above that thiamin functions also in ways other than the transformation of pyruvic acid.

These observations, though fragmentary and incomplete, suggest that *Phycomyces* may be used with advantage in determining more completely the changes which occur in the intermediary metabolism of carbohydrates. Furthermore, the presence of acetate or similar substances may affect the bio-assay for thiamin by *Phycomyces.*

The Specificity of Thiamin. The discovery of the enormous effect of thiamin on the growth of some plants naturally raised the question as to its specificity. This is particularly pertinent in view of the experience with the auxins. Investigations with *Phycomyces,* tomato roots, pea roots and *Staphylococcus aureus* have shown that thiamin is quite specific. No success has yet attended attempts to replace it with amino acids, various sulfur compounds or by other growth substances, including, among others, indole acetic acid, ascorbic acid, nicotinic acid, lacto-flavine, vitamin B_6, ethylene chlorhydrin, pimelic acid and pantothenic acid. In fact, most changes in its molecular structure render thiamin ineffective; in a few instances they reduce its potency materially. The majority of studies on the relation between structural modifications and effectiveness of thiamin for plants have been carried on by using modifications of the pyrimidine and thiazole intermediates, rather than analogs of the vitamin itself. On the basis of these results it appears, for example, that thiamin is rendered inactive for *Phycomyces* if hydrogen is substituted for the methyl group at position 2 on the pyrimidine ring, all other radicals remaining unchanged. Similar results are secured if oxygen is substituted for the amino group at position 6. Thiamin becomes ineffective for *Phycomyces* if a methyl group is substituted for the hydrogen in position 2 on the thiazole ring, or if acetate, acetamide, ethyl acetate, β-hydroxypropyl, γ-hydroxypropyl, hydrogen or methyl groups are substituted for the β-hydroxyethyl group in position 5; and the activity is reduced to 5 per cent. or 10 per cent. if an ethyoxy radical or chlorine replaces the hydroxyl in the hydroxyethyl group in position 5 on the thiazole ring. It is not possible to discuss here in detail the work which has been done by Knight, Schopfer, Bonner and others on the specificity of thiamin. In our own investigations with *Phycomyces* only one compound of nearly 50 pyrimidines and thiazoles tested was as effective as the vitamin intermediates themselves. This compound has an ethyl group in place of a methyl group at position 2 on the pyrimidine ring. It appears, however, that there is some difference between plants in their responses to different modifications of the thiamin molecule. We are not yet certain whether these modifications function as such or whether the plant is capable of transforming them into thiamin. The specificity of thiamin is doubtless associated in part with its function as a precursor of a part of an enzyme system.

The Biological Assay of Thiamin. Schopfer, Mieklejohn and others have used the growth of *Phycomyces Blakesleeanus* as a means of determining thiamin quantitatively. It is a useful method of considerable accuracy and sensitivity. Since it is being used by various investigators, the following comments based on our experience are pertinent.

Growth of *Phycomyces* in a mineral-glucose medium indicates thiamin or the pyrimidine and thiazole intermediates or both. Growth is not specific for the vitamin as such.

Growth is proportional to the amount of the vitamin and not to the concentration.

Growth is proportional to the amount of the vitamin or to the intermediate present in smaller molecular quantity, provided the mineral salts, sugar or nitrogen supply is not limiting.

The logarithm of the amount of thiamin per flask plotted against the logarithm of the dry weight of the mycelium is a straight line. In a suitable medium this linear relationship extends to 10^{-8} mole of thiamin or a growth of something over 500 mgms of mycelium.

The presence of excess thiazole may influence the results.

The Significance of Thiamin from the Standpoint of Symbiosis and Parasitism. The observations which have been made on the growth in mixed cultures of organisms which lack the ability to synthesize the thiamin molecule or a part of it and those which have the ability is most suggestive from the standpoint of parasitism and symbiosis. We have observed on occasion in our experiments the beneficial effects in a medium deficient in thiamin of a bacterial or pink yeast contaminant on the growth of organisms which require an external supply of thiamin. Our experiments showing that polyneuritis in pigeons may be cured by feeding sufficient amounts of the thiamin intermediates or by injecting them intraperitoneally are probably explained by the synthetic action of microorganisms in the intestinal tract. Schopfer has found that *Rhodotorula rubra,* which synthesizes thiazole but not pyrimidine, and *Mucor Ramannianus,* which synthesizes pyrimidine but not thiazole, grow successfully in mixed cultures in a medium lacking thiamin. In a medium lacking thiamin and biotin Kögl and Fries grew a mixed culture of *Ashbya gossypii* which can not make biotin but makes thiamin and *Polyporus adustus,* which synthesizes biotin but not thiamin.

In spite of the rapid progress which has been made in the last year or two in our knowledge of the relation of thiamin to plants and the light which has been thrown on the general problem of growth substances by this information, there is much more we should like to know about this material and its effect upon plants. What are the conditions influencing its production in the plant? Is the supply of thiamin associated with the shape and extent of the growth curve of a plant? What becomes of thiamin as it is used up by an organism? What functions does it play in the physiology of the organism? Where is it formed in the higher plant and where and how is it translocated? Is its production by microorganisms or its possible accumulation in an adsorbed condition in the soil of any significance in soil fertility, as suggested by Williams and Spies? However, in our enthusiasm, we must not forget that thiamin is not the only growth substance concerned in plant development. For one organism thiamin synthesis may be a limiting factor, while for another biotin, meso-inositol, vitamin B_6, nicotinic amide or some other growth substance may be important and still other plants may require an external supply of more than one. We have found that in a mineral-sugar solution thiamin is the factor limiting the growth of excised tomato roots. In a mineral-sugar-thiamin solution the ability of the tomato roots to synthesize vitamin B_6 becomes the limiting factor. Careful and critical work is necessary, lest we be set adrift in a maze which is none too simple at best.

In any event the investigations on the relation of thiamin to plants promise the possibility of elucidating important questions in general physiology through the study of more easily controlled material than the higher animal. Of all the growth substances probably involved in its development *Phycomyces Blakesleeanus* apparently lacks the ability to synthesize but one, thiamin; the higher animal is unable to make many. It would be difficult to imagine a simpler and more perfect arrangement than *Phycomyces* for studying the function of thiamin. The studies on the relation of thiamin to plants can not help but emphasize that the physiological mechanisms of fundamental processes are much the same in all living organisms, although details differ. It may not be a compliment, but it should be a salutary corrective of undue pride to realize that although *Phytophthora infestans* does not develop beriberi or polyneuritis it requires thiamin in the same form and probably for the same reasons as we do.

OBITUARY

JAMES PLAYFAIR McMURRICH

ON February 9, 1939, at Toronto, Canada, death from coronary thrombosis took suddenly from our midst a famous scientist, Professor James Playfair McMurrich, who was still actively engaged in writing and research, although in his eightieth year. His passing removes a notable and well-known figure from the ranks of biology and anatomy. But many happy memories of his inspiration and leadership remain, while a large number of accomplished researches and outstanding achievements are a lasting memorial of his exceptional ability combined with untiring application.

Professor McMurrich was born at Toronto on October 16, 1859, the youngest of eight children of the Honorable John McMurrich, M.L.C. and Janet Dickson McMurrich. He matriculated from Upper Canada College and early showed his brilliance of mind and interest in science by obtaining the degree of B.A. at the University of Toronto (1879) before he was twenty years old. Two years later (1881) he also obtained the M.A. and was beginning to write articles for scientific journals.

He now began his career as a teacher, and during the first three years he completed his work for the degree of Ph.D., which was awarded him by Johns Hopkins University in 1885. Later, in recognition of his attainments he received the honorary degree of LL.D. from the Universities of Michigan (1912), Cincinnati (1923) and Toronto (1930).

His academic career, while only one phase of his remarkable existence, was in itself a notable one. His fame early went abroad, and changes of position were rapid. The diversity of his earlier teaching posts gives evidence of his versatility and knowledge. He was successively professor of biology, Ontario Agricultural College, 1882–84, instructor in mammalian anatomy, Johns Hopkins University, 1884–86; professor of biology, Haverford College, 1886–89; docent and assistant professor of animal morphology, Clark University, 1889–92, and professor of biology, University of Cincinnati, 1892–94.

During this period an invitation came to him to become professor of anatomy at Yale. This he declined because he felt it outside of his province. When a similar invitation was proffered by the University of Michigan, however, he decided that its significance should not be unheeded, and he accepted, thus making a radical change in his career. This position he retained for thirteen years, 1894–1907, finally returning to his alma mater, the University of Toronto, in 1907, as professor of anatomy, which post he filled brilliantly until his retirement as professor emeritus in 1930 at the age of seventy.

Professor McMurrich made many firm and lasting friends amongst his colleagues, was loved and respected by his students and was stimulating to his staff. He worked consistently for the advancement of the universities to which he was attached, furthered the cause of science and promoted research. As a result of this policy he was instrumental in founding the School of Graduate Studies in the University of Toronto, which grew rapidly under his oversight, for he presided over its council in the honored position of the first dean for eight years, 1922–30, until his retirement.

His scholarship was profound, his memory phenomenal and his mind was forever active, keen and inquiring. His interests covered a great variety of subjects, many of them far beyond his professional field, and his accumulated knowledge was ever a source