MYCORRHIZAL SYMBIOSIS IN APLECTRUM, CORALLORHIZA AND PINUS

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(WITH NINE FIGURES)

Introduction

When plants of different types are closely crowded together or anatomically engaged, in addition to the ensuing competitions, several special relations including immunities, antagonisms, toxicities, parasitism, or symbiosis may arise.

A secretion by one plant which inhibits the growth of a neighboring organism, such as the substance found in the tissues of orchids by Noel Bernard which limits the growth of the hyphae of endophytic fungi, results in an immunity. Similar antagonisms exist between some fungi growing together in the soil in which a secretion from one limits the growth of the other (**11**).

This constructively defense mechanism varies widely. In one instance described by Stoller (**21**) *Psalliota campestris* produces a diffusible oxidizing substance, acting as a quinone which protects the mushroom against microorganisms producing sulphides.

The action of the "humoral" secretion in such cases is to be distinguished from toxicity by the fact that in the first case the secretion simply blocks some of the processes of cell-metabolism but does not destroy the mechanism; in toxic action a theoretical secretion causes irreversible and fatal changes in the cytochemical set-up of living cells of the organism affected. In parasitism, in addition to possible anatomical and cytochemical damage, a fatal extraction of nutritive material may ensue. Further distinction between parasitism and symbiosis will be made in the following pages.

A much heightened interest in the bacteriostatic secretions of molds, of which penicillin is the most notable because of its sterilizing action in surgery, now prevails. This secretion specifically inhibits the growth of gram-positive bacteria but does not destroy them; secretions such as patulin exert similar action on both gram-positive and gram-negative bacteria [see also Burgess (**2**)].

Scope of the present paper

The senior author had previously made some studies of mycorrhizae of some heaths and orchids [MacDougal (**14, 15**); and MacDougal and Lloyd (**16**)] and when isolated segments of mycorrhizal pine roots which were capable of extended existence with a nutritive program identical with that of a chlorophylless plant were found, an arrangement for collaboration in the study of the implied symbiosis was made with Dr. Dufrenoy early in 1940. Grants in support of the research were awarded by the American Philosophical Society and some of the results have been published and other material is in press [MacDougal and Dufrenoy (**17, 18, 19**)].

The present paper presents additional results obtained by a study of the action of mycorrhizal roots of the pine, when included in an intact system with the tree, and when grown in isolated soil cultures. A re-examination of the endophytic mycorrhizae of two orchids, *Corallorhiza maculata,* and its near relative, *Aplectrum hyemale,* in a perspective of known facts as to auxins and vitamins has made available some cytochemical features of value in the estimation of the cytochemical relationships in the two types of mycorrhizae involved.

Morphogenesis of mycorrhizae

ENDOPHYTIC MYCORRHIZA OF CORALLORHIZA

No record of the seedling stages of *Corallorhiza maculata* are available,[1]

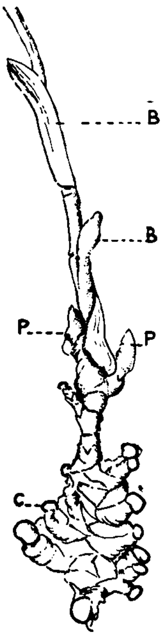


FIG. 1. Underground rootless stem of Corallorhiza with lower part of scape. C, coralloid branches. P,P, buds in autumnal condition; B,B, reddish bracts carrying traces of chlorophyll.

but the adult plant consists of a glistening white underground stem with irregularly placed thickened branches. No roots are to be seen. The older basal part of the axis dies away after the manner of a rhizome, which action may involve the branches as illustrated in figure 1. One or more scapes arise from the upper internodes. Foliar organs are represented by ap-

[1] Subsequent to the completion of the MS. of the present paper we have noted the record of observations by Dr. D. G. DOWNIE (Trans. and Proc. Bot. Soc. Edinburgh 33: 380–382. 1943) on the symbiotic germination of seeds of *Corallorhiza innata* by association with a Basidiomycete. Later hyphae entered and formed characteristic coils in the basal cells of the slowly growing protocorms. Trichomes apparently similar to those of the coralloid branches of adult plants were developed.

pressed bracts in which a trace of chlorophyll and a few stomata present a capacity for transpiration and photosynthesis but little above zero.

The outer walls of some epidermal cells are extended into thick short trichomes suggestive of root-hairs. Hyphal branches pass outwardly through both types of epidermal cells, forming an absorptive system. The fungus develops dense swirls of hyphae surrounding the nuclei in the cortical cells. Generally the occupied cells show a random arrangement, but in a species collected by the senior author in Arizona two or three of the external layers not including the epidermis were filled; although the walls were intact a con-

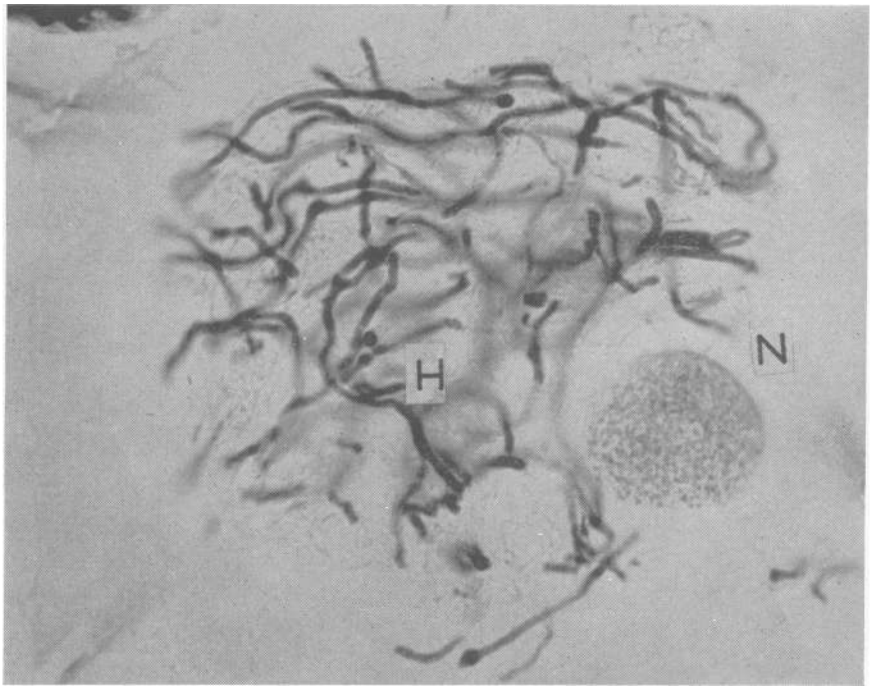


FIG. 2. N, nucleus of cortical cell of Corallorhiza with portion of surrounding hyphae, H; stained blue with 2–6-dichloro-quinone-chloro-imide. × 715.

tinuous mycelium was formed [MACDOUGAL (15)]. In this case the hyphae invaded only the outermost half of the cortex, which is densely filled with starch and phenolic material.

Anatomically the whole arrangement is one by which soil-solutions may be absorbed by the external hyphae, elaborated, and translocated by the fungus (probably Rhizoctonia) to the cells of the higher plant. Functionally the metabolic possibilities are parallel to those offered by mycorrhizae of the pines.

Nuclei within the hyphal clumps were hypertrophied and granular, a modification which has been taken by many authors to imply a mechanism of exchange between the fungus and the orchid (fig. 2). This arrangement presents some contrasts with that of pine mycorrhizae in which palmated hyphal branches entering the cortical cells engage finely vacuolated masses of cytoplasm near the nuclei. The disintegration of hyphal clumps in the cortical cells of the orchid takes place ahead of the death of the older tissues so that the material in the hyphae is liberated in living cells and thus becomes available to the higher plant conformable to the non-parasitic action of the fungus (fig. 5).

Reactions to tests for auxins demonstrated that some compounds of this kind were included in the substances yielded.

Cytochemical tests indicate that the hyphal branches in the outer part of the clumps are the site of a high indophenol (cytochrome) oxidase activity which may be a source of energy necessary for the transfer of material between the fungus and the higher plant (fig. 5).

Evolutionary development of mycorrhizae

Although some seed-plants upon which ectotrophic mycorrhizae are formed undergo great modifications of the shoot, yet roots are retained to be felted by the fungus partner. Plants in which the fungus partner inhabits the tissues of the higher form display much more advanced morphological alterations, inclusive of loss of roots and photosynthetic apparatus. Chlorophylless species to the number of over two hundred in the Burmanniaceae, Gentianaceae, Triuraceae, and Orchidaceae are included in this category. Many are reduced to small irregular underground stems and the scanty flower stalks which do not emerge from the heavy humus which they inhabit. [For example, see Degener (**4**)].

While some of the types exemplifying extreme reductions may have originated early in the history of the family to which they belong, it is not to be assumed that any correlation exists between the degree of modification and the antiquity of the adaptation; some of the alterations may have been of mutative occurrence, as exemplified by the occasional coralloid mycorrhiza of *Aplectrum hyemale* native to the northern United States and Canada.

Mutative origination of stem-mycorrhizae in Aplectrum

The greater number of plants of this species consist of an ovoid corm of three internodes attaining full size in late summer, at which time a single elongated, plaited, and ribbed leaf arises from an upper internode and persists through the winter. Early in the spring a scape arises from the axil of the leaf, bearing a loose raceme of flowers, and concurrently one or more offsets arise from a lower internode which reach a length of 2 to 4 cm. The terminals become apogeotropic and, turning upward while undergoing thickening, develop corms, thus effecting vegetative reproduction (fig. 3).

Twelve to twenty thick roots emerge from the base of each corm and attain a length of 5 to 10 cm. which bear a dense crop of large root-hairs extending from base to within 2 mm. of the root-cap, both root-hairs and cap being persistent. The outer walls of the epidermis and the walls of the root-hairs soon become slightly suberized. The 4 to 8 layers of cortical cells contain dense clumps of hyphae of a fungus which has been identified by J. T. CURTIS (**3**) as *Rhizoctonia neottiae* Wolff. It is probable that the plants examined by MACDOUGAL contained a different species, as indicated by the lack of color of the hyphae.

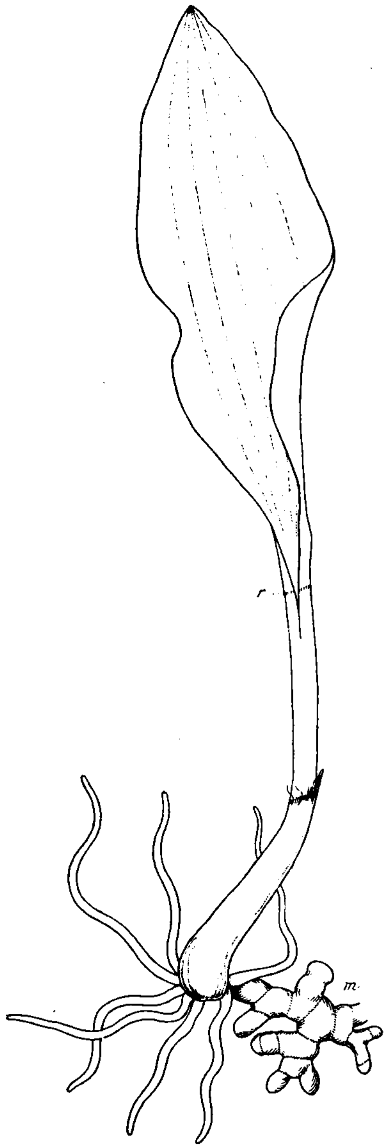


FIG. 3. *Aplectrum hyemale* with normal form of mycorrhizal roots, and with mutative coralloid offset, *m.* *r*, abscission zone of leaf.

The hyphae traverse the cortex, and branches pass *out* into the soil through the root-hairs and epidermal cells in functional duplication of the arrangement in the underground *stems* of Corallorhiza.

The entrance of the fungus into the tissues of the individual plant is through a set of trichomes on the lower flank of the offset near the parent corm from which it originates. Here hyphal branches originating in the nearby roots, or from the soil, enter the trichomes and pass into the cortex of the offset, in which the growth of the hyphae follows the extension of the offset to its termination in a young corm with roots; progressive occupancy of the roots follows as described above.

Swirls of hyphae are formed in the lower quadrant of the cortex of the offset as the fungus extends to enter the roots of the young plant. No external branches are sent out through the epidermis of offsets in normal, or typical plants. The suggestion lies near that a secretion may be formed in the outer cortex and epidermis which inhibits growth of hyphal branches through these tissues, as outlined in the introduction to this paper, and as is known to occur in other parts of the plant.

It is in this stem-fungus association that the possibilities of a mutation lie; a mutation which has been seen to alter plants of Aplectrum so that they were adjusted to form coralloid or mycorrhizal stems anatomically similar to those of Corallorhiza, with which it is closely related [Gray (**6**)]. That a parallel evolutionary path might be followed is supported by the reduction of the corms and foliar surfaces of plants which are formed at the terminals of coralloid offsets. Subsequent to the first record [MacDougal (**14**)] plants with coralloid stems were obtained from Vermont and Minnesota (fig. 3).

Ectendotrophic mycorrhiza of the Monterey pine

The seasonal activities of the root-system of the Monterey pine reflect the conditions of the climate, especially in the fact that elongation may begin during the winter season in which the soil temperature is not too low for growth. Apical growth of the terminals may be initiated upon repletion of the soil moisture at any time, beginning in November and December, and continue until the following summer with but haphazard connection with longitudinal growth of stems. Lateral short rootlets arise irregularly from initials in the pericycle and under favorable conditions become thickly mantled with hyphae of the symbiotic fungus before their final length of about 2 to 4 mm. is attained. Characteristic coralloid branching ensues and occasionally renewed growth exposes whitish tips.

The entire root system is in contact with the mycelia ramifying in the soil, hyphae of which mantle and penetrate the short roots only, the rapidly extending leaders and primary branches remaining free from investment. In seeking a theoretical cause of this differential action Hatch (**9**) records that while the main roots of *P. strobus* are diarch, the short roots which become mycorrhizal are monarch, lacking a root-cap, have only four layers of cortical cells, and reach their limited extension by slow growth. These features are seen in pure cultures and hence are not due to the action of the fungus. Short roots have been seen to originate in the pericycle and endodermis of roots of Monterey pine, but nothing can be said as to the inciting agent. As will be discussed in a subsequent section, the perennity of the main roots is possible only by a type of respiration in which phosphorus linkages in the form of dehydrogenases is maintained in the apical meristem. Theoretically, the failure of this mechanism would account for the dwarfing and reduction of the short rootlets, but no supporting evidence is available.

In the external adjustment of the mycelial mantle to the rootlets the arrangement is similar to that of the ectotrophic mycorrhizae of the heaths. An additional feature of pine mycorrhizae is one by which branches of the hyphae which traverse the middle lamellae of cortical cells penetrate the inner walls of the cells in which the nuclei appear as large globular bodies with radiating strands of cytoplasm enclosing a network of anastomosed vacuoles, similar to the arrangements which have been described under the name of "the Golgi apparatus."

The arrangement in question is one which yields the maximum area of surface contact of vacuolar solution and cytoplasm, making possible a high rate of respiration and a high rate of translocation of material introduced by the fungus. It would appear that a similar arrangement was recognized by CHARLES DARWIN in the "aggregations" in the cells of the leaf-hairs of insectivorous plants, facilitating the translocation of introduced material in solution.

Each rootlet enters into a separate association with the investing fungus. Hyphae from the mycelial felt in the soil encounter the rootlets, not all of which may be so engaged. The fungus does not traverse the axis of the root from one branch to another; such longitudinal extension of the fungus in the tissues of the root is purely incidental, and generally takes place only at the end of the season when exfoliation of the cortex has begun. By reason of this manner of association, a root-system of a tree might be richly provided with mycorrhizae in one season, and devoid of them in the next by conditions in the humus unfavorable to the fungus partner.

This last-named condition has been brought about experimentally by the removal of seedlings, in the second season after some mycorrhizae had been formed, to water cultures in which the association does not occur.

The anatomical arrangement described justifies the designation of *ectendotrophic,* and it is one which is seen to furnish a highly efficient interchange between the two symbionts.

The coralloid branching supposedly resulting from the action of auxins produced by the hyphae on the endodermis and pericycle of the monarchic short roots are the only morphological effects of the association. The seasonal death of the mycorrhizae may leave the root system entirely free and a new crop of lateral rootlets are infected *de novo* by the hyphae ramifying through the soil.

Cyto-physiology of mycorrhizae

CYTOCHEMISTRY OF HYPHAE

No comprehensive analysis of the chain of metabolic processes in the hyphae of the fungal symbiont of the pines can be made, but a few of the more important features can be detected. Free-hand sections of fresh material vitally stained with neutral red prepared in Ringer's solution or in reagents for oxidases were employed. Other results of importance were secured by killing mycorrhizal roots in Helly's fixative, imbedding in paraffin, sectioning with a microtome, then staining with haematoxylin, acid fuchsin, or molybdenum blue as decribed below.

In vital staining with neutral red in Ringer's solution the vacuolar solutions became pink-purplish, and red precipitates flocculated, a reaction taken to indicate an acid solution rich in polyphenols and presumably the site of a high cytochrome and polyphenolase activity.

In microtome sections of imbedded mycorrhizal roots stained with haematoxylin, acid fuchsin, or molybdenum blue the cytoplasmic meshwork enclosing the vacuoles are clearly defined; the mitochondria appear clearly as well as the several nuclei in each unit of the hyphae.

Cytoplasmic strands connecting the separate units through the central pores of the cross walls were seen. Pads of material at the pores, staining black by haematoxylin after fixation in Helly fluid (fig. 4), and red in acid

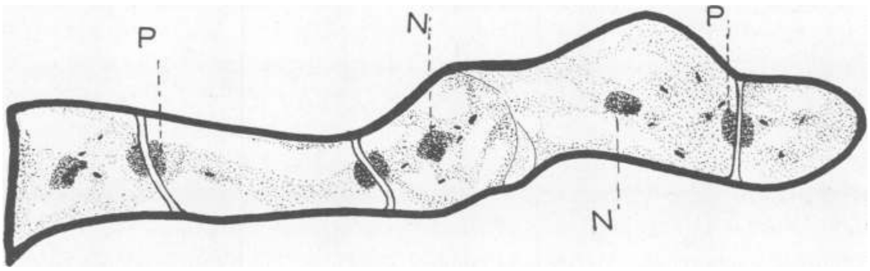


FIG. 4. Sketch of chain of cells of hyphae of pine mycorrhiza. N,N, nuclei with connecting mesh of cytoplasm. P,P, pads or cushions of material at pores in cross walls.

fuchsin and methyl green, negative their characterization as "optical illusions" by BULLER (**1**). These bodies from their histochemical character may be considered homologous to the callose drops at the sieve plates of the red algae, and to the sieve plates in the conducting elements in the phloem of seed-plants, *i.e.*, in elements through which translocation occurs.

The occurrence of auxins in the hyphae surrounding the nuclei in Corallorhiza and the release of this substance in mature cells is noted in a subsequent paragraph. The localization of oxidases in the outer part of the swirls of the nuclei has also been noted (fig. 5).

When sections of the heavy stems of Corallorhiza are fixed in Helly fluid and placed in a boiling solution of triketohydrindene hydrate, hyphae developed a purple color, while the nucleolus of the cortical cell was of a deeper tinge, with the body of the nucleus uncolored. The presence of proteids and amino acids was indicated.

IDENTIFICATION OF SITES OF OXIDASE ACTIVITY

The best fixative was found to be Helly's fluid, made up as follows: a stock solution of 25 gm. potassium bichromate and 50 gm. mercuric bichloride in one liter of distilled water was kept on hand. A mixture for treatment of pieces of tissue was made by adding 1 ml. neutralized 40 per cent. formaldehyde to 9 ml. of the stock solution. After twelve hours' immersion a brown sediment might develop, in which case the material was transferred to fresh solution of the same concentration for twelve hours. The sections were then placed in a 3 per cent. solution of potassium bichromate for a few days, then washed in running water for a few hours. Some of the results described were made by staining hand sections, and others by the paraffine method.

The mercuric chloride of the reagent was reduced in regions of high activity, with the result that the cytoplasm around vacuoles was marked by the presence of heavy black dots of mercuric precipitates. Another test was one in which hyphae in sections treated with paraphenylediamine and thymol was followed by an indophenol blue reaction, demonstrating oxidase activity.

This test gave marked positive reactions in the hyphal felt clothing coralloid roots, the color extending into the outermost layers of the cortex. The cytochrome oxidase indicated is present in the apical meristem of normal roots. The pericycle and endodermis are the site of phenoloxidases and lipidoxidases with the resultant production of tannins and of suberization, which constitute an effective barrier to the penetration of the stele by the fungus (figs. 5, 6).

Definite zonation of cytochemical features of the heavy stems of Corallorhiza does not exist. This, as will be noted later, is true of auxins as well as of the oxidases. Fresh sections treated with the reagent giving a nascent indophenol blue, stains some of the hyphae in the outer part of the swirl surrounding the nuclei a general blue, interpreted as a reaction of cytochrome oxidase, with some granules of deeper shade taken to be phospholipids. The reaction is seen only in certain cells supposedly in active growth.

Auxins in mycorrhizae

Much evidence is available to the effect that substances promoting growth in secondary meristem originate chiefly in leaves and buds, from which region they are translocated downward in the main axis as far as the root-system. This scheme is perhaps never strictly correct except under experimental conditions, as external agencies may induce intensive respiration and localized formation of these substances.

The continued growth of isolated segments of mycorrhizal roots would imply that these substances were obtained independently of the action of the stems or shoots.

Hitchcock and Zimmerman (10) found that normal roots might absorb from the soil and transmit to distant parts of the shoots added substances such as alpha-naphthaleneacetic acid, indole-acetic acid, indole-butyric acid, indole-propionic acid, phenylacetic acid, and the methyl and ethyl esters of these acids. In confirmation of the above results, seedlings grown aseptically in a nutrient solution containing 1 part per million indole-acetic acid developed the red color when crushed in the HCl and iron perchloride reagent.

The auxin in question occurs but sparingly in soils, however, and is known to be formed by various fungi. The development of a pronounced red color in the hyphae when fresh sections of mycorrhizae were treated with the reagent was taken as evidence of its origin in the fungus. It was found that the addition of a few drops of amylic alcohol to the reagent would aid in the detection of traces of this growth promoting substance.

The color effect was most marked in the closely woven hyphal mantle, but it appeared in the vacuoles of hyphae intermeshed with the cortical cells as well as in the endodermis, pericycle, and some companion cells. That the auxin was produced in the fungus and translocated to the root was confirmed by the fact that crushed fresh materials from fungus-free leaders did not give the reaction, although it was noted that prolonged treatment was fol-

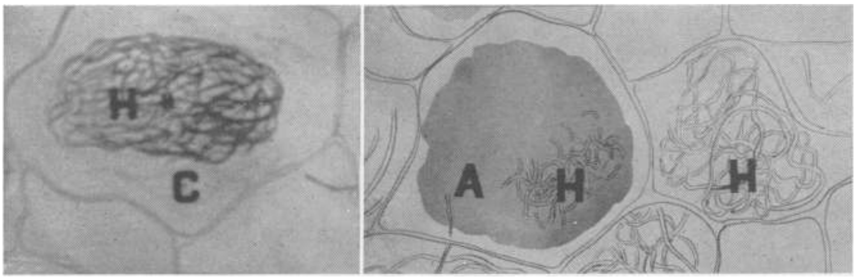


FIG. 5. Sketch of cells of cortex of Corallorhiza, with hyphae, H,H,H. Cell at left is from a fresh section treated with nascent indophenol blue. The central mass has become yellowish, while the peripheral portion has taken a pale blue, C, indicative of cytochrome oxidase, a reaction in actively growing cells. The contents of the fresh cell in the center with disintegrating hyphae, H, have become reddish after treatment with HCl and iron perchloride, A, by the presence of an auxin released by the hyphae.

lowed by the development of a reddish color, of a tinge different from the above, in the walls of wood cells.

As a supplemental test the reagent was applied to the tissues from the outer layers of "knees" of *Taxodium distichum.* Many cells of the cortex yielded a bright cherry red as well as the wood parenchyma, and the vacuoles of young cambium derivatives contained a reddish solution.

Sections from the thick mycorrhizae of Corallorhiza treated with the hydrochloric acid, perchloride of iron reagent, gave no perceptible color in the thin hyphae; but in cells of the cortex where the clumps of hyphae near the nuclei were disintegrating, the large vacuole contained a dense solution of reddish color tinged with purple, as if the auxin had been released with other soluble material by the fungus to the cell.

The actual amount of growth in the thick stem-mycorrhizae of the orchid is very small in comparison with the mycorrhizae of conifers and the supply of auxin found would be adequate. In what region this substance is metabolized has not been determined. Examination of the bud at the stage when the scape begins rapid elongation would probably bring out more positive evidence.

TEST FOR VITAMIN B_1

The blue color in vacuolar solutions following treatment of sections with the molybdic reagent is of a shade indicative of ionic or loosely held phosphorus as is the case in vitamin B_1, which includes pyrimidine and thiazol (fig. 5).

TEST FOR VITAMIN B_6 (PYRIDOXIN)

Fresh sections of coralloid roots immersed in a veronal (diethysodium-barbiturate) suspension of dichloro-quinone-chloro-imide results in the formation of pyridoxin-indophenol, in the vacuolar solutions, which comes down as a blue precipitate. The treatment should be carried out at temperatures not above 5° C. with the light excluded. (Phosphate or carbonate buffers at pH 7.6.) Pyridoxin will be found most abundant in the hyphae of the outer mantle in pine mycorrhizae (fig. 6).

KARYOLOGICAL FEATURES OF ROOTS

Cytological examinations of the tissues of roots of Pinus fixed in Helly's fluid confirmed the conclusion that the structure of the chromosomes, their behavior in leading roots under normal conditions, or after several years of isolated cultures, and in mycorrhizal roots was uniform. In the more actively growing tips, nuclei never reached the truly quiescent stage; after a telophase, and previous to the next prophase, nuclei retained a network of banded materials, with many chromocenters in the length of each chromosome. As the prophase occurs, the chromosomes lose their banded appearance, and the molybdenum reactions for nucleo-proteins at first obtained in the chromocenters, extend to include the whole chromosomes.

The resting nuclei of the differentiated tissues contain 4 nucleoli and 24 prochromosomes, corresponding to the 24 (the diploid 2n number) chromosomes seen in karyokinesis, as in the pines in general, 12 being the haploid number in the megosporangium (fig. 7).

It is to be added that neither the structure nor chemical reactions of the nuclei are demonstrably modified in pine mycorrhizae, in which the hyphal branches entering the cortical cells are palmated and are in close contact with masses of cytoplasm minutely vacuolated. Clumps of hyphae enclose the nuclei of cortical cells or the endophytic mycorrhizae of orchids; and the nuclei here are hypertrophied and densely granular (fig. 2).

PLASTIDS

Two types of nucleo-protein-phospholipid inclusions occur in pine mycorrhizae—amyloplasts, and mitochondria—the last-named only occurring in the fungus. Both are capable of division and both give cytochemical reactions of phospholipids, are affected by acetic acid, alcohol, or lipid solvents, unless the lipids are previously oxidized to fatty acids by fixing reagents. As plastids become differentiated to take on the function of synthesizing starch they become more resistant to solvents. These bodies are on the average bulkier in pine cells than the mitochondria.

In sections treated with the molybdic reagent both amyloplasts and mitochondria become blue (by the phosphorus reaction). Further staining with acid fuchsin results in the mitochondria becoming a bright red, while amyloplasts take on shades of purple of varying depth. Included starch grains become pure blue (figs. 4 and 7).

Phosphorus metabolism in mycorrhizae

The assignment of an all-important rôle to phosphorus is justified by the fact that neither carbon nor nitrogen can be metabolized into cell-constituents without its action. In particular no proteins could be built up; the

Fig. 6. Sketch of partial cross section of coralloid root, vitally stained with 2–6-dichloro-quinone-chloro-imide. Hyphae, H, become a light blue as a reaction to pyridoxin (vitamin B_6) present. Hyphae traverse the cell walls but do not enter cavities of outer cells (I) rich in oxidized catechol compounds. Palmate branches are seen to enter cells of median cortex. Vacuoles of inner cortex take on various shades of red, indicative of catecholase activity, T, constituting a regional barrier to the fungus.

desoxy-pentose-nucleotides are essential constituents of nuclei, while the ribose nucleotides are equally important in the respiratory systems of living cells. Special attention was given to tests for compounds into which this element might be bound. The molybdic reagent which is especially useful for such tests was made up as follows: A. 20.9 ml. sulphuric acid was added to distilled water of the highest possible purity to make up 250 ml. 3 N solution in which 6.41 gm. of ammonium molybdate [$(NH_4)_6MO_7$] was dissolved. B. 15.75 gm. sodium bisulphite was dissolved in 85 ml. of water, to which was added 0.5 gm. 1-amino-2 naphthol sulphonic acid ($C_{10}H_9O_4NS$) and then 5 ml. 20 per cent. sodium sulphite solution which was stirred until complete solution was obtained.

Small amounts for treatment of fresh or paraffine imbedded sections were made by adding 1 ml. of B to 5 ml. of A. The best results were obtained by placing the preparations in a refrigerator at 5° C. for 24 to 48 hours. A progressively deepening blue color develops, appearing first at the site of ionic phosphorus in the vacuoles of the hyphae; later mitochondria become blue by the presence of this element in the phospholipidic boundary phase; nuclei throughout the section become a slaty blue at chromocenters made of nucleo-proteins. In general the density of the color was greatest at the site of ionic and loosely bound phosphorus. The deeper blue shade in the vacuoles was one which may be taken to indicate the presence of vitamin B_1.

Only through phosphorus linkages, achieved through phosphorus esterification, do most of the molecular structures coming into play in the cell metabolism acquire some degree of physiological activity or efficiency, as expressed in components of a respiratory system.

The hyphae in mycorrhizae are rich in proteids, polypeptides, or alpha amino acids, or more specifically in substances involving the radical COOH–CH–NH_2 as made evident from the purple color the mycelium develops when sections of mycorrhiza are heated in a solution of ninhydrin (tricetohydrindene hydrate). The ectendotrophic mycorrhizal fungus is beautifully demonstrated as a purple network in between the cortical cells, which remain unstained; in the root only some phloem elements stain as a whole suggesting a translocation of soluble nitrogenous materials. From this histochemical picture it may be inferred that soluble forms of nitrogen from the mycorrhizal hyphae are mostly metabolized, presumably through carboxylation and phosphorylation, into the nucleoproteins which build up the tissues of the mycorrhizal roots.

As metabolism is so strictly dependent on phosphorylated compounds of the nucleotides or pentose nucleic acid-like components, it may be surmised that actively growing meristematic and postmeristematic tissues should show a high content in such compounds. In the course of tissue differentiation, as the affinity for basic dyes becomes less marked, so also the nucleic acid contents diminish.

Features of metabolism indicative of symbiosis

Hyphae coming from the investing felt around the short roots traverse the walls of the cortex with but little effect on the nuclei and cytoplasmic masses. Palmate branches penetrate some of the cells to make an arrangement facilitating nutritive exchanges, but the fungus does not penetrate the stele (fig. 6).

The stele in its earlier stage of differentiation from the meristem consists of elongated prophloem elements, the vacuolar solutions of which have a notably higher pH (staining orange with neutral red) than the surrounding tissues; the xylem with a conversely low pH, and the endodermis and pericycle, have vacuolar solutions which become purple; they are characterized by a high polyphenolase activity, as is made conspicuous by the chromaffin reaction when sections are immersed in solutions containing potassium bichromate. The large vacuoles in both pericycle and endodermis are seen to contain catechol derivatives and catechol oxidases (figs. 6 and 8).

Absorption of salts by mycorrhizae

Mycorrhizal associations are formed by short lateral rootlets of conifers only, the leaders and secondary branches displaying the general features of

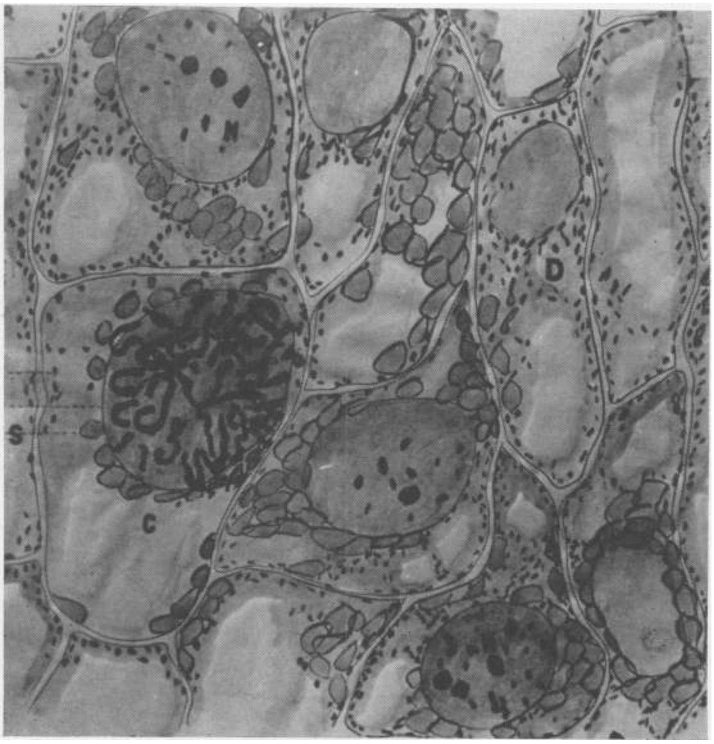


Fig. 7. Sketch of part of a longitudinal section of a main root of Pinus fixed in Helly fluid, imbedded in paraffin, stained with haematoxylin, and treated with I in KI. In one cell the nucleus, C, is at prophase surrounded with starch grains, S. Four nucleoli and 24 prochromosomes are present in others; amyloplasts enclosing starch grains and mitochondria appear in all cells.

a rapidly elongating apical region followed by a zone of root-hairs. As has been noted, young trees may survive for months or even years with such simple or normal absorption apparatus, without the formation of mycorrhizae. It is therefore possible to make valid comparative measurements of the rate or amount of absorption by mycorrhizal and non-mycorrhizal plants. In the most recent contribution to this subject Routien and Dawson (**21**) describe results in which mycorrhizal plants (*Pinus echinata*) were found to show an increased absorption of K, Mg, Ca, Fe, and ionic phosphorus, which is to be connected with a heightened rate of oxygen intake—two to four times greater than in non-mycorrhizal plants, with correlated energy release and carbonic acid production.

Absorption of organic compounds by roots

The long cherished notion that only ionized material could be absorbed by roots has been gradually modified until it is now established that such complex substances as hormones, auxins, and vitamins may be taken from the soil by normal roots.

The present paper is concerned chiefly with absorption by mycorrhizal systems, in which hyphae act as the absorbing organs, capable of taking up nitrogen not only as NO_3 but as NH_3 or NH groups, linked into complex cyclic molecular structures. These humus derivatives are metabolized into nucleo-proteids, phospho-lipids and the various components of the cytoplasm and walls; surplus proteins may be accumulated in the hyphae. Uronic carbon in the soil may be in part residual or it may be synthesized by microorganisms. Nothing is known as to its movement into hyphae, but it has been assumed that the polyuronides of the cell-walls of the cortex broken down by the action of the fungus are re-metabolized. Reliable evidence of the presence of any carbohydrate except glycogen in hyphal cells is lacking.

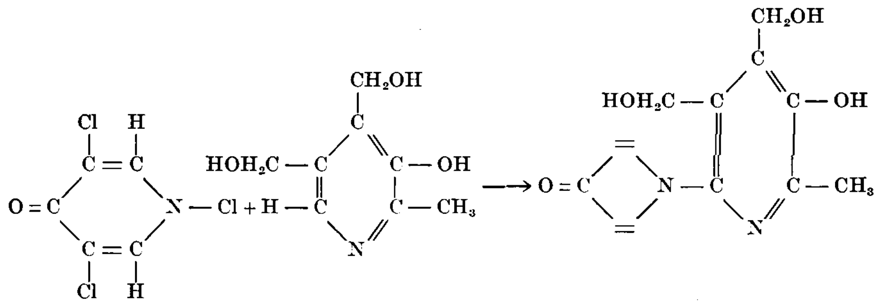
Growth-factors and carbon metabolism

All the process of survival and growth in the excised root tips must depend ultimately on the catalysts, which govern the process of biological oxido-reductions, whereby energy is obtained, enabling the plant to carry on the process of living.

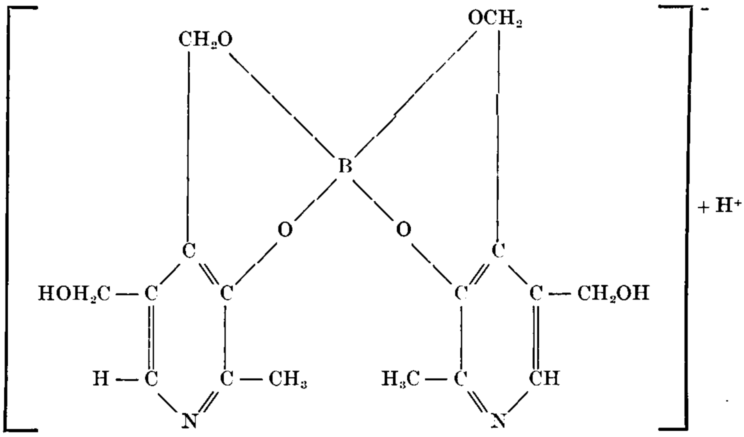
Two types of such systems may be distinguished: (1) The protein-alloxazin system, where the alloxazin derivative is so firmly bound to the protein that it can be set loose only by dilute acid hydrolysis. To this type belongs one of the components of the vitamin B_2 complex: "riboflavin." (2) The protein-pyridine nucleotide systems, where the pyridine nucleotide represents a "prosthetic group" easily removed by washing, and which has long been described as the "co-enzyme" or "coferment."

The best known coferments which assist in the transference of hydrogen, are the so-called "hydrogen carrying coferments," which are a tri- or bi-phospho-pyridine-nucleotide. The former contains 3, and the second 2 mol. phosphoric acid, besides which, each of these two nucleotides consists of a molecule of the amide of nicotinic acid, 1 molecule of adenine, and 2 molecules of pentose. To survive and grow, isolated roots must synthesize, or obtain from their mycorrhizal partners, or from the soil, these pyridine nucleotides. Pyridine has been assumed to be a significant source of nitrogen for soil organisms, thriving in humus; it may yield nicotinic acid by the introduction of a carboxylic group at carbon 3 in the cyclic chain. It is to be noted that two of the vitamins, nicotinic acid and pyridoxin (vitamin B_6) are pyridine derivatives.

2–6-dichloro-quinone-chloro-imide condenses with pyridoxin in the presence of appropriate buffers to yield indophenol blue.



This reaction does not occur in the presence of a borate ion, while building a complex involving the 3-hydroxy and 4-hydroxy methyl groups.



Pyrimidine, one of the two parts of the molecule of vitamin B_1, has recently been found to protect carboxylase against the enzymatic action of the phosphatase which tends to destroy it.

Pyrimidine, as associated with thiazol in the molecule of vitamin B_1 or thiamin, plays a dual rôle—that of a growth substance and that of a control of cell fermentation.

Elaboration of carbohydrates

As to vitamin B_1 or thiamin, made up of the two constituents pyrimidine and thiazol, it plays a most essential rôle in the metabolism of carbohydrates, since, when phosphorylated into the diphosphate of thiamin, it becomes the co-carboxylase.

Vitamin B_1 has been chiefly considered in connection with the decarboxylation process; Evans called attention to the fact that vitamin B_1 also controls the reversible reaction of carboxylation, being apparently directly concerned with the C–C linkage whereby pyruvic acid can unite with carbon dioxide to yield oxalacetic acid: enzymatic fixation of carbon dioxide in oxalacetate has been recently confirmed by KRAMPITZ *et al.* (**13**).

Pyruvic acid can unite with carbonic acid to yield oxalacetic acid $COOH \cdot CO \cdot CH_3 + CO_2 \rightarrow COOH \cdot CO \cdot CH_2 \cdot COOH$. This carboxylation of pyruvic acid may then be followed by the following:

4 oxalacetic acid → alpha ketoglutaric acid + 2 malic acid + 3 CO_2.

The widespread occurrence of the carbon dioxide effect may be taken to indicate that carbon dioxide takes part in a fundamental metabolic process, common to many organisms. The synthesis of oxalacetate is a reaction sufficiently fundamental to explain the use of carbon dioxide, although, experimentally, it should be possible to replace carbon dioxide by malate, by fumarate, or asparagine or other substance which can serve as a source of oxalacetate.

Carbon dioxide is an active metabolite in heterotrophic cells; it can condense with pyruvic acid to form oxalacetic acid. By this reaction carbon dioxide is "assimilated." Oxalacetic acid has at least two important functions in cell life: it acts as a hydrogen carrier in energy-giving processes, and is a parent substance to a number of cell constituents.

Through carboxylation, pyruvic acid acquires two additional carboxyl groups. These lend to the two middle C atoms the high reactivity which is necessary for the catalytic function. Both carboxylic groups were demonstrated by SZENT GIORGYI to account for the absorption of the C_4 dicarboxylic acid on protoplasmic surfaces. Their high absorbing capacity of the traces of C_4 dicarboxylic acids present, saturates the enzyme and maintains respiration, a function of especial importance in the isolated root cultures.

STARCH SYNTHESIS

Synthesis of starch from phosphorylated soluble carbohydrates with the release of phosphorus in simple form as set forth in the reversible equation of HANES, takes place in the amyloplasts with a consequent reduction of the osmotic pressure of the cell liquids.

In the reversible equation, defined by C. S. HANES: "Starch + inorganic phosphate ⇄ glucose-1-phosphate," the equilibrium is determined by the divalent ions,

$$(HPO_4)^{=}/(C_6H_{11}O_5 \cdot OPO_3)^{=}$$

The metabolic turn-over of phosphate may therefore involve a series of recurrent phases, which might be as follows: (1) absorption of inorganic phosphate from the soil; (2) introduction of inorganic phosphate into ester linkages within the mycorrhizal fungus; (3) generation of energy-rich phosphate bonds by oxidation-reduction; (4) taking over and distribution of such bonds by the catalysts in the fungus tissue; (5) utilization of these energy-rich phosphate bonds, and concomitant liberation of inorganic phosphorus; (6) translocation of such inorganic phosphorus to the pine tissue, where the above phases, 2 to 5, would again occur.

In other words, the mycorrhizal association would be one wherein the fungus partner would obtain inorganic phosphate (as well as nitrogen and carbon) from the soil, and be able to build high energy phosphorus linkages, thereby maintaining a high potential level; it would then release inorganic phosphate which would eventually be carried into the irreversible group acceptors of the pine tissue.

In accordance with widely accepted views phosphate current can be used to do osmotic work, to synthesize cytoplasmic materials, nucleic acid, and to carry on organic chemical synthesis in cells; all such reactions involve group donators and group acceptors. The force which drives the group into the final acceptor may be the difference in potential levels.

Histochemical map of mycorrhizae

Determination of the sites of many important metabolic transformations as described in previous sections has yielded some identifications of regions of the principal activities of the symbionts. In some cases the appearance of untreated sections furnish reliable indications of the nature and action of substances in a tissue. Thus the brown discoloration of the vacuolar contents of outer cortical cells engaged with mycorrhizal mantle is to be attributed to the irreversible oxidation of polyphenols, mainly pyrocatechol, to the corresponding quinone.

Under normal active cells, pyrocatechol is protected by reducing substances; but if these substances become oxidized, the catechol is also oxidized and undergoes molecular aggregation of the gummy, brownish catechol tannins which stain red in neutral red in living cells and retain the color after death.

Vacuoles in one and the same cell may differ in the composition of the solutions or colloidal dispersions which they contain. Some positive reactions indicative of their character were obtained by vital staining. It is important that the reagent be isotonic and isoionic to the vacuolar solutions in this treatment. To this end neutral red was added to a Ringer's solution made up by adding 6 gm. sodium chloride, 0.075 gm. potassium chloride, 0.1 gm. calcium chloride, and 0.1 gm. sodium bicarbonate to 1 liter of distilled water. Cells of mycorrhizae remained alive in this reagent at ordinary room temperatures during the period of observation with accumulation of the dye in the vacuoles, the nucleus and cytoplasm being unstained.

The development of the capacity of the vacuolar fluid to absorb neutral red is taken to be concomitant with the formation of polyphenols; a similar intensification of reaction occurring with either 2–6-dichloro-quinone-chloroimide, potassium bichromate, or the molybdenum reagent.

The progressive effect of increasing polyphenols was observed in sections of the apical parts of roots. No vital stain was absorbed by the meristematic cells, but as differentiation proceeded, vacuoles enlarged and coalesced and their contents became reddish, the elongated cells about the xylem becoming deep purple and the phloem a light orange red, indicative of polyphenols and also of the higher pH in the phloem, this difference also being applicable to the color in the hyphae (fig. 8).

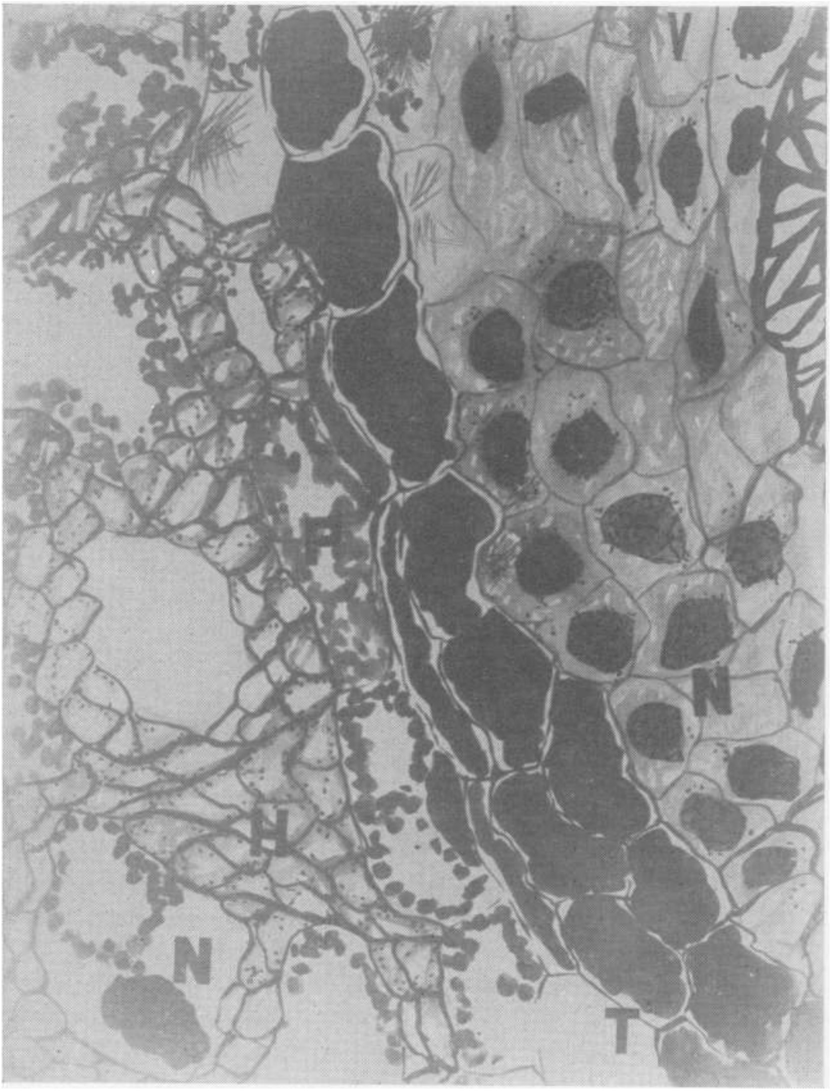


FIG. 8. Sketch of section in apical region of coralloid root of pine in isolated soil culture, collected June 5th, fixed in Helly fluid and stained with acid fuchsin and methyl green. Hyphae, H, at left have finely vacuolated cytoplasm staining pink, with numerous mitochondria becoming red. The endodermis contains yellow phenolic precipitates, P, and the vacuolar contents of the pericycle are rich in catechol tannin, T, which stains deeply. Crystals of calcium oxalate are seen in some of the older (upper) cells. Parenchymatous cells of the stele to the right are in a meristematic condition with large nuclei, N, and many small vacuoles, V. Terminal portions of wood cells are at the upper, right margin.

COACERVATES

In addition to the series of colloidal interphases of the first order as implied in the preceding paragraphs, interphases of a second order come into existence when two phases separate from within a vacuolar solution. When such two phases become permanently separated by a membrane so that each phase and the membrane or interphase differ in physico-chemical properties, especially in oxidation-reduction potentials, bodies or masses are formed which are now termed *coacervates.*

Further examination revealed that the membrane was phospho-lipidic and alveolated by the retention of some of the water in which its material was dispersed; the brown central core was composed of oxidized phenols. The nature of these bodies, known as coacervates, was established by DE JONG (**12**) and has been discussed by DUFRENOY and REED (**5**).

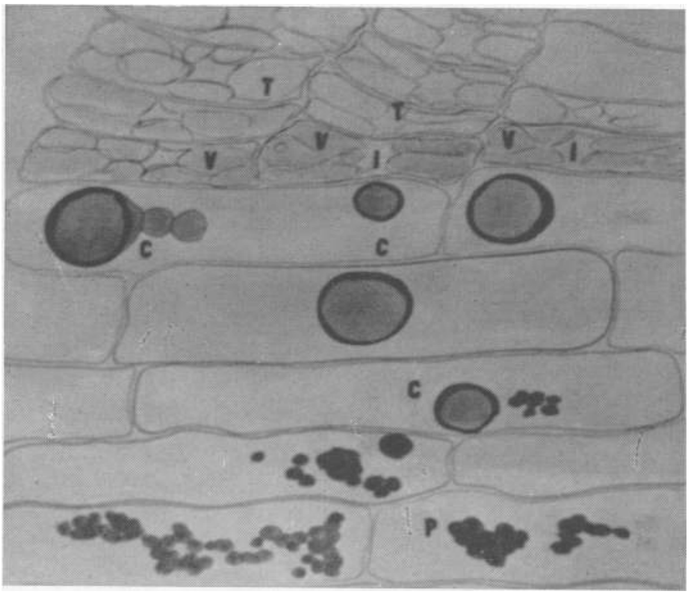


FIG. 9. Sketch of part of a longitudinal section of the apical region of the main root of pine stained vitally with neutral red. Initial cells as well as young derivatives, T,T, did not take the stain. The vacuolar contents of older cells, V,V, became reddish, with the uncolored nuclei, I,I, compressed into star-shaped bodies. In cells of more advanced stage large spherical inclusions, C,C,C, showed a differential staining of the boundary phase and the core characteristic of coacervates and indicative of colloidal structure. Phenolic material in solution in other cells came down as precipitates.

When cells of the root-cap of pine were vitally stained with neutral red, the presence of small globular bodies was revealed. The limiting membranes of these bodies became a deep red, while the enclosed central mass was of a lighter red. Post-vital staining with 2–6-dichloro-quinone-chloro-imide brought into view large spherical refringent bodies, the boundary layers of which were dark brown while the central part was a lighter hue, which may be interpreted as indicative of a strong polyphenolase activity.

The vacuolar colloids have been generally considered as electronegative, but some of them (phenolic compounds or their derivatives) may be assumed to be amphoteric. Electro-positive charges may be supposed to develop within the vacuolar colloids in connection with oxidation-reduction phenomena. Where an oxidase system exists in high concentration, there might be a locus for the attraction of cations; and conversely, where the dispersal of cations is concomitant with oxidation or dehydrogenation, phenolic compounds form molecular linkages, resulting in the formation of larger and larger molecular aggregates. If these aggregates remain firmly bound to the suspending saline liquid or serum, there may result a process of formation of definite physical bodies such as that described by BUNGENBERG DE JONG as due to coacervation. These condensation products show the microchemical characteristics of polyphenols; more specifically, inasmuch as they form pyridoxin-indophenol complex with 2–6-dichloro-quinone-chloro-imide in phosphate or veronal buffers, they may be considered to be composed largely of pyridoxin. They exhibit the following characteristic physicochemical activities (fig. 9):

1. A high level of oxidase, as evidenced by the rapid oxidation of polyphenols or amides to their colored derivatives, and concomitant high reducing power, as evidenced by the reduction of the silver ion from silver nitrate to metallic silver, or of mercuric chloride, to mercury, or of osmic acid to osmium.
2. Attraction of cations, explaining staining with basic dyes.
3. Dispersion of anions, explaining why phosphate anions move out into the suspending liquid.
4. The formation of the precipitation membrane at the periphery of the condensed colloids, explaining why phosphatids or phospho-lipoids, being amphoteric, remain at the periphery.

Under what may be considered as the "normal condition" the vacuolar solution appears to be a random distribution of ions and of molecules, where an rH lower than 12 is maintained, so that phenolic compounds such as catechol are protected from permanent oxidation to quinone derivatives and polymers. The vacuolar solution contains both the oxidizable polyphenols and the system which can catalyze their oxidation, in other words, the polyphenol oxidase. As previously mentioned, these polyphenols are normally protected from oxidation by the presence of the ascorbic-dehydroascorbic, or the dihydroxy-maleic acid systems, and chiefly by the presence of such thiol containing groups as cystein and glutathione, the latter of which is able to act as a "reservoir of hydrogen." Under conditions as yet undefined, the random distribution of substances no longer prevails, and cations become concentrated about some focus from which anions are repelled. The site of cation concentration is also the site for a high level of oxidase activity and can be demonstrated in the case of catechol oxidase or polyphenol oxidase, which is a copper protein whose activity is a function of the concentration of Cu^{++}. Therefore we must expect to find a strong positive response for the ortho-diphenols such as catechol and their derivatives and for phenols such as pyridoxin; a strong reaction for polyphenol oxidase and a correlative strong reaction for Cu may be expected in the vacuolar inclusions. Also a negative reaction for anions such as PO_4, while a positive reaction for bound phosphorus could be expected in amphoteric phospholipids at the interphase between the vacuolar inclusion and surrounding vacuolar solution. As a result of the high activity of polyphenol oxidase and dispersion of the dehydrogenase system (involving probably nucleo-proteins with $-SH$ active groups) oxidation and concomitant polymerization of the orthodiphenols to gummy derivatives composed of glucosides of the anthocyanol type would be possible as may be observed in epidermal cells of some flowers. These molecular linkages resulting from carbon bonds, through dehydrogenation should be reversibly broken by hydrogen donors which would therefore appear to be solvents of the inclusions, *e.g.*, alcohols, and, in general, liquids with methyl or ethyl groups. The previous "fixation" of plant material with oxidizing solutions which contain potassium bichromate protect these inclusions against solvents.

Discussion

A study of ectendotrophic mycorrhiza under natural condition and in experimental cultures yields ample evidence that the fungus is not parasitic. Isolated segments of the root system, with mycorrhizal branches, a few cm. in extent, have been seen to carry on an existence for many seasons similar to that of a chlorophylless plant. The older parts of the main axes die away, the leaders extend, new mycorrhizae are formed and surplus starch is continuously accumulated. A small fraction of the salts and organic material derived from the soil may be absorbed by the zone of fragile root-hairs following the extension of the leaders, but the greater part of the material is taken up by the hyphae and, after some metabolization, is translocated into the cortical cells of coralloid roots.

Auxin (indole-acetic acid) is formed in abundance in the hyphae; vitamin B_1 and vitamin B_6 may be traced from the origin to their presence or effects in the root.

The implied processes may be keyed to the metabolism of phosphorus. This element absorbed from the soil in inorganic form enters into water-insoluble combinations, such as the nucleo-proteins from which it may be freed by phosphatases; acting concomitantly with dehydrogenases from the hyphae it passes into the root-cells, making similar combinations from which it is freed by respiration to migrate to the stele, where it is again bound in stable unions in a region of low pH and low oxidase activity.

In the over-all nutritive cycle, mycorrhizae add to the material entering the roots and passing upward to furnish energy and structural material to the tree; leaves, fruits, bark, twigs, etc., are cast off to contribute to the general layer of humus which is the habitat of the fungus. A minute fraction of the mycelia becomes engaged with the roots to which simple and metabolized soil substances are transmitted.

Hyphal branches extending in the middle layers of cortical cell walls, have been assumed to metabolize for their own nutrition plastic materials, or more precisely, the polyuronides from the cell walls. In addition, such mycorrhizae as that of the pine send other modified branches into the cell which become enmeshed in a reticulum of cytoplasm amidst a cluster of minute vacuoles. This arrangement with the chemical reactions described indicate a region of active translocation of amino acids.

An analysis of the metabolic processes of mycorrhizae makes it evident that practically all of the material which enters into the construction of cells of isolated roots or of chlorophylless plants is translocated from the fungus. Metabolization of this material in the higher plant, in addition to nitrogenous material, produces cellulose, sugars, and starch, not found in the fungus, in the significant presence of plasts. Glycogen, known to occur in hyphae, but not identified in the observations described here, is non-diffusible but, if present, its products by the action of amylolytic ferments might be translocated to be synthesized as starch. The protoplasmic organs of hyphae include the main components of cells of higher plants except carbohydrates and plasts. Auxins, and vitamins of the B group are seen to accumulate in, or to be originated by, the fungus and to be transmitted to other parts of the mycorrhiza.

Lastly, it is to be said that the presence or action of the fungus is not necessary for the germination of pine seeds. The seedling may not form a mycorrhizal alliance with the fungus until the second season, but the implied symbiosis is indispensable for the normal maturity of the tree.

The non-necessity of the fungus for germination of terrestrial orchids has been wrongly taken as a proof of parasitism of the fungus by J. F. CURTIS (3).

A review of previous work by the senior author and the results of recent examinations of the mycorrhiza of an orchid, *Corallorhiza maculata,* supports the view that the fungus (Rhizoctonia) which enters most numerously into nutritive associations with species of this family sustains a symbiotic relation similar to that of the elements in the pine mycorrhizae, although the morphological engagements of the fungus and seedplant are widely different. The mycorrhiza of orchids is of the endophytic type which in some cases is accompanied by striking modifications of the roots and shoots.

Parasitic fungi may infect the roots and stems of some orchids, but when the association follows a regularized pattern accompanied by a loss of absorbing organs and photosynthetic apparatus, the assumption that the fungus yields nutritive material which is usually furnished by these organs is acquired by symbiosis with the fungus, is not only allowable but inevitable.

Adult individuals of Corallorhiza have no roots; (whether these organs are formed in germination is not known) and its leaves, represented by bracts, contain so little chlorophyll as to be functionally negligible.

The amyloplasts, however, are seen to function normally as evidenced by the dense accumulation of starch in the coralloid stems.

The fungus partner gains entrance to the cortex of the stem at some early stage, and the hyphae follow the development of this tissue in the early post-meristematic stage. Once infected the partnership is for life; unlike the pine mycorrhiza, as the fleshy stems die at the base after the fashion of a rhizome, and extend at the apices of the branches, the extending hyphae send branches outwardly which emerge from the epidermal cells, constituting the absorbing mechanism of the system. The principal processes of metabolism of pine mycorrhiza, including a dense accumulation of starch, are duplicated. In this case, however, the hyphae form dense swirls against the nucleus, which shows a modified granular structure; interchange between this organ and the hyphae appears to take place instead of by a vacuolar mechanism as in the pine. Positive reactions for auxin are shown only in the older cells, in which the fungus appears to disintegrate and yield this and other contents to the cortical cells.

Summary

1. Results of a comparative study of the endophytic mycorrhizae of terrestrial orchids and of the ectendotrophic mycorrhizae of pines are presented.

2. The possible mutative origin and vegetative propagation of "stem-mycorrhizae" as exemplified by Aplectrum is described.

3. Association of pines with symbiotic fungi can not be connected with any heritable morphological alterations. Short lateral rootlets are invested directly by hyphal branches of mycelia in the soil; such association may not be made until the second season.

4. No plants of Corallorhiza with roots or other than stem-mycorrhizae have been seen. Results of observations of germination, development of seedlings, and beginning of symbiotic association with fungi are not available.

5. The mycelium of the endophytic mycorrhiza of Corallorhiza is in the cortex, to which it gains access in an unknown early stage; in its growth it keeps pace with the differentiating meristem, sending branches outwardly through the epidermal cells of underground coralloid branches in simulation of the arrangement of root-hairs. Symbiotic fungi of other terrestrial orchids with roots traverse these organs in a similar manner, sending hyphae out into the soil through the durable root-hairs and other epidermal cells.

6. The absorption of inorganic phosphorus from the soil by the fungus, and the stages in its metabolism terminating in the stele of the root, identifiable origination in hyphae and translocation of auxin, vitamins, and amino compounds to the root-tissues, together with the capacity of isolated segments of mycorrhizal roots to survive and grow like a chlorophylless plant, establishes the non-parasitic character of the fungus.

7. The distribution of the hyphae in the cortex of pines does not extend beyond the endodermis in which catechol tannins are formed; the middle lamellae of the outer cortex is traversed and its disintegration products are available for absorption.

8. Palmated branching hyphae enter cortical cells and become closely engaged with a cytoplasmic mass characterized by minute vacuoles sugges-

tive of a mechanism of exchange. Hydrolyzation products of polyuronides, of starch, and of other diffusible compounds may be absorbed by the fungus.

9. The mycelium of the endophytic mycorrhiza advancing toward the growing apex of coralloid branches forms dense swirls of hyphae enveloping the nucleus which is hypertrophied and densely granular, constituting an arrangement for possible exchanges. The masses of hyphae disintegrate in the older parts of the stem, their contents becoming available to still living cortical cells as exemplified by reactions for auxin. Layers and islands of cells containing oxidized catechol form a barrier to the extension of the hyphae.

10. The hyphae of the fungus partner and the cells of the symbiotic seed plant are richly endowed with mitochondria appearing as short rods or cylindrical bodies, giving reactions for phospholipids. No cytochemical results yield any clue as to their rôle in the activities of the cell. The cells of the higher plant also include plasts which may be differentiated to synthesize proteins or starch; these capacities are exhibited by the plasts of isolated pine roots in soil cultures.

11. Carbohydrate metabolism takes place mainly in cortical cells and in the presence of plasts. Auxins and vitamins are accumulated by, or originate in, hyphae and are translocated to the roots.

12. Pads of material at the pores of cross walls of hyphae demonstrated by diverse reagents and stains were observed. Possible similarity of composition and function with sieve plates of higher plants is noted.

13. The limited initial growth of the short laterals of pine roots which become invested by the mycelium of the symbiotic fungus, may be connected with the lack of cytochrome oxidase which is active in the meristem of rapidly elongating leaders. The subsequent profuse coralloid branching of the short roots is in the presence of such oxidases produced by the hyphae.

14. The phenolic compounds in the pericycle and endodermis which are firmly held in suspension or solution in the vacuolar solutions or coacervated into spherical masses with a phospholipidic membrane in the endodermis and pericycle, are neutral to the protoplasm in these elements. When allowed to escape from the vacuoles the phenolic substances oxidize to tannin which "fixes" the protoplasm as seen in the brown peripheral layers of old mycorrhizae.

CARMEL-BY-THE-SEA
CALIFORNIA

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