relatively great accumulation of radioactive phosphorus by the short branch roots, even by those which show none of the hypertrophy or dichotomous branching characteristic of typical mycorrhizae. Some of these branch roots resemble those pictured by Hatch (*1*) in his Plate III, A, B, and F, and in Plate XIII, B, and described as nonmycorrhizal or pseudomycorrhizal roots. These he believed to be of negligible importance in the absorption of nutrients. Although these roots appeared to be suberized almost to their tips and therefore had a very limited absorbing surface we found that they accumulated surprisingly large amounts of phosphorus. Measurements of radioactivity indicated that the tips of the main roots

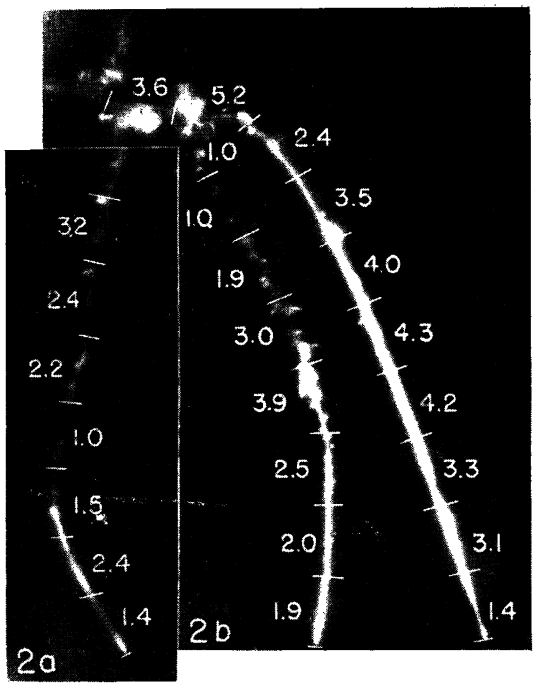

FIG. 2. Radioautographs of the roots pictured in Fig. 1. The numbers indicate relative radioactivity of 1-cm segments as measured by a Geiger counter.

did not contain as much radioactive phosphorus as the older portions bearing branches. A heavily suberized root bearing a few short branches or a small cluster of mycorrhizal roots apparently can absorb more phosphorus than an unbranched tip with a much larger unsuberized surface.

In a few instances autographs indicated that completely suberized roots absorbed considerable phosphorus, but microscopic examination of such roots showed that they were covered with a superficial layer of mycelium which apparently has a very large capacity to accumulate phosphorus. The few mycorrhizal branches which failed to show accumulation were dark and wrinkled, and apparently dead. A few unsuberized root tips showed very low accumulation of phosphorus, indicating that not all unsuberized root surfaces are equally active in absorption of solutes.

It appears possible that the increased accumulation of phosphorus by mycorrhizal roots may not result only from increased surface but perhaps may be related to a greater capacity for absorption because of high metabolic activity. Routien and Dawson found the oxygen consumption of mycorrhizal roots to be two to four times that of nonmycorrhizal roots. In preliminary experiments we have found that 10^{-3} M sodium azide, which may be expected to inhibit oxygen consumption, reduces the accumulation of P^{32} by both mycorrhizal and nonmycorrhizal roots. Study of the effect of respiration inhibitors was complicated by variations in proportion of mycorrhizal to nonmycorrhizal tissue in root samples, making quantitative comparisons difficult.

The results of these experiments show that mycorrhizal portions of roots of pine can accumulate much larger quantities of phosphorus than nonmycorrhizal portions. Root segments bearing short, unbranched, pseudomycorrhizal lateral roots appear to accumulate more phosphorus than typical unsuberized root tips. It seems possible that mycorrhizal roots have not only a greater surface, but also a greater capacity to accumulate phosphorus per unit of surface than nonmycorrhizal roots. Apparently in pine roots the region of maximum intake of minerals is not the root tips, as in roots of herbaceous plants, but the older regions where mycorrhizal or pseudomycorrhizal branch roots have developed.

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A Qualitative Analysis of the Amino Acids in Royal Jelly[1]

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Royal jelly, a secretion of the pharyngeal glands of the young worker of the honey bee, *Apis mellifera* L. (*6*), is the sole food of bee larvae which develop into sexually mature female adults, or queens. Larvae which develop into sexually immature female adults, or workers, are fed royal jelly for 2 days, then receive a mixture of pollen and honey during the remainder of their 5-to-6-day feeding period (*9*). Research toward an explanation of the role of royal jelly in the development of castes of *Apis mellifera* L. has been reviewed by Haydak (*6*).

Chemical analyses of royal jelly have shown that it is a complex mixture of substances having a protein content of 9 to 18% of the fresh material (*6*). The proteins were found by Abbott and French (*1*) to consist of an

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albumin and probably a globulin in the ratio of 2 to 1. Aeppler (*2*) by unstated methods and Townsend and Lucas (*10*) by chemical methods identified 7 amino acids in the hydrolyzed proteins of royal jelly (Table 1), but did not investigate the possibility of the occurrence of free amino acids in this substance. During the course of research on free amino acids in the blood of insects, an analysis of both free and combined amino acids in royal jelly was undertaken. Separation and identification of amino compounds was accomplished by the paper chromatographic method of Consden, Gordon, and Martin (*5*).

Approximately 0.5 ml of fresh royal jelly was suspended in 1 ml of distilled water and diluted with 10 ml of 95% ethyl alcohol in order to precipitate the proteins.

TABLE 1

FREE AND COMBINED AMINO ACIDS OF ROYAL JELLY

Amino acid or derivative	As free compound	As protein constituent
Alanine	+	+
Arginine	+	+*
Aspartic acid	+	+*
Cystine	–	+*
Glutamic acid	+	+
Glycine	+	+
Histidine	–	–*
Hydroxyproline	–	–
Isoleucine and/or Leucine	+	+
Lysine	+	+*
Methionine	+	+
Phenylalanine	–	+
Proline	+	+
Serine	+	+
Threonine	–	+
Tyrosine	+	+*
Tryptophane	–	–*
Valine	+	+
β-Alanine	+	
Glutamine	+	
Taurine	+	
Unknown	+	

* Previously reported (*2, 10*)

The precipitated proteins were separated by centrifugation, and the supernatant, which contained free amino acids, was drawn off. The proteins were washed with 1-ml aliquots of 95% alcohol until a negative ninhydrin reaction was obtained in the washings. The solution was evaporated to dryness by means of a jet of air and the residue dissolved in 300 μl of distilled water. Aliquots of 50, 100, and 150 μl were chromatographed.

The precipitated proteins were divided into two portions, one of which was hydrolyzed with 5 N NaOH and the other with 6 N H_2SO_4. Hydrolysis was accomplished by autoclaving for 12 hr at 15-lb pressure. The hydrolyzates were neutralized, then evaporated to 0.5 ml by means of an air jet and gentle heating. Aliquots of 50 and 100 μl were chromatographed.

It is evident from the data in Table 1 that significant quantities of amino acids and amino acid derivatives occur in the free state in royal jelly. The presence of cystine, histidine, hydroxyproline, phenylalanine, threonine, and tryptophane was not demonstrated. It is possible that extraction of amino acids from a larger sample of royal jelly would demonstrate the presence of histidine, for it is the least sensitive of the amino acids to identification by paper chromatography (*8*). Assuming complete extraction of free amino acids from the sample of royal jelly, an estimation based on the volume of aliquots chromatographed and on sensitivities of various amino acids to the method (*8*) indicates that, if amino acids not found in the free state do occur as such, they are present in less than the following quantities per ml of fresh royal jelly: cystine 0.032 mg, histidine 0.1 mg, hydroxyproline 0.004 mg, phenylalanine 0.02 mg, threonine 0.008 mg, tryptophane 0.008 mg.

Hydroxyproline, histidine, and tryptophane were not identified in hydrolyzed proteins of royal jelly. The latter two compounds were found in hydrolyzed proteins of royal jelly by Aeppler (*2*) and by Townsend and Lucas (*10*). Further research is needed to clarify this discrepancy.

The size and color intensities of proline spots occurring on chromatograms of free amino acids were greater than those occurring on protein chromatograms, indicating relatively large quantities of free proline in royal jelly. Auclair and Jamieson (*3*) have shown that pollen (dandelion, willow, and mixed taken from a beehive; and dandelion collected from blooms) is rich in free proline, which may explain its abundance in royal jelly since young worker bees consume pollen during their period of secretion of royal jelly.

The presence of free taurine in royal jelly is of interest. This compound, which is formed from cysteine in the animal body and is a constituent of the bile salts of animals, is known to occur in large quantities in muscle tissue of invertebrates, but its function there is unknown (*4*). Free taurine has also been found in blood of honey bee larvae and of two other adult insect species (*7*).

An amino compound of unknown composition was isolated as a free constituent of royal jelly. It has also been found free in the blood of honey bee larvae (*7*) and in pollen (*3*). Comparison of its chromatographic position with those of several known amino compounds has thus far failed to identify this interesting compound.

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