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Enzymatic Differences Between Carrot Taproot Secondary Phloem And Tissue Cultures Derived from Secondary Phloem.* (30819)

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Secondary phloem of the carrot taproot is highly differentiated and does not, under ordinary circumstances, give rise to any other tissue type. When however this tissue is aseptically excised from the root and maintained under tissue culture conditions, autogrowth occurs of dedifferentiated tissue which resembles carrot tumors(1,7). This dedifferentiated tissue can redifferentiate under proper cultural conditions and form a complete carrot(1,2).

The results reported here comprise the first part of a study designed to identify key enzymatic changes during dedifferentiation and redifferentiation.

Methods. All carrots (*Daucus carota*) used in these studies had healthy green shoots and unbroken taproots which ranged from 8-9 inches in length and 1-1½ inches in diameter.

Cultures initiated from secondary phloem were made by excising sections 2.0 × 3.5 mm cut as discs from the middle third of taproots which had been sterilized with a 5% (w/v) solution of sodium hypochlorite. These discs were planted on the surface of agar-solidified Heller's medium(3), modified by the addition of sucrose (3.0%), 2,4-dichlorophenoxyacetic acid (0.01 mμg%), and coconut milk (15.0 ml/100 ml). Cultures were incubated at 22-28°C under continuous illumination from warm-white fluorescent tubes.

TABLE I. Enzymatic Activity of Carrot Taproot Secondary Phloem and Cultured Tissue Derived from Secondary Phloem.

Tissue	Avg act units/mg protein	Std dev	p
Peroxidase			
taproot	.0476	.00367	<.001
culture	.445	.300	
Malic dehydrogenase			
taproot	.370	.0565	<.001
culture	.00135	.00147	
Acid phosphatase			
taproot	.0198	.00422	<.2 but >.1
culture	.0652	.104	

Enzymatic activity of filtrates prepared from normal secondary phloem and from new growth only from primary explants of secondary phloem discs grown in culture were compared. Standard methods(4) were used for assay of the following enzymes: acid phosphatase, alkaline phosphatase, glyceraldehyde-3-phosphate dehydrogenase, malic dehydrogenase, glucose-6-phosphate dehydrogenase, peroxidase, and aldolase.

Results and discussion. Only peroxidase, acid phosphatase, and malic dehydrogenase were present in either the cultured material or taproot phloem (Table I). There are significant quantitative differences between malic dehydrogenase and peroxidase activities of cultured material and taproot phloem (Table I): the cultured material contains more per-

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oxidase and less malic dehydrogenase. Differences in acid phosphatase activity in the two sample types (Table I) were not great enough to be judged significant.

If one considers that *change* in peroxidase activity may be a general indicator of differentiation in plant root material regardless of whether the direction is toward or away from further differentiation, then our results mesh with those of Avers and Grimm(5) who found that peroxidase activity of roots increased as differentiation into root hairs began. Reduced malic dehydrogenase activity in our cultured material is in keeping with the idea that the cultured material may be likened to tumors and that aerobic metabolism is reduced in both plant and animal tumor tissue(6,7). Our findings with malic dehydrogenase are the first in which reduction of activity of a Krebs cycle component has been measured directly. Whether activity of other enzymes in the cycle is similarly reduced remains to be shown.

Summary. Activity of 7 enzymes in carrot taproot secondary phloem and tissue in culture derived from secondary phloem was measured. Peroxidase, acid phosphatase, and malic dehydrogenase were present in both preparations but the cultured material contained more peroxidase and less malic dehydrogenase. Possible interpretations are discussed. Alkaline phosphatase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, and aldolase were not found in either the taproots or cultured material.

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Paper Electrophoresis of Serum in Rats Fed Various Carbohydrate Diets.*† (30820)

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The levels of free plasma amino acids, following protein meals, have been studied in swine(1) and rats(2) and found to correspond approximately to the amino acid composition of the ingested protein. The concentration of serum albumin is also related to the quality and quantity of dietary protein and has been found to decrease markedly in cases of protein malnutrition(3). Alterations in the electrophoretic pattern of specific serum protein fractions in the rat, due to type of protein in the diet, have been reported (4).

In addition to protein composition, Guggenheim *et al*(5) have shown that the nature of the carbohydrate fed exerts an influence upon the levels of free plasma amino acids. Previous studies(6) have demonstrated that the type of carbohydrate also influences protein utilization. Most methods which have been used to evaluate protein utilization have been based on standard procedures of nitrogen balance, growth, protein digestibility, and

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