THOUGHTS ON PROBLEMS RELATED TO PLANT DEVELOPMENT

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For the past few years, the author has been interested in the developmental aspects of plant biology. The following is a short account of the research done or proposed.

1. What percent of respiratory energy is consumed in biosynthesis of macromolecules in growing cells. The cells isolated from the phloem of the Sycamore Maple (*Acer pseudoplatanus*) can be easily cultured in the modified White medium. These cells have a generation time of 2 days. They form a friable culture in a liquid suspension. This culture can be handled like a bacterial culture, thus is par ticularly suitable for biochemical and growth studies.

Associated with the growth of plant cells are the biosynthetic processes and respiration. It is interesting to estimate the extent to which the metabolic energy is utilized in biosynthesis of proteins and cell wall polysaccharides, the two classes of macromolecules constituting the bulk of the dry matter in plants. This can be accomplished by simultaneously measuring the respiration and the incorporation of isotopically-labeled precursors.

The R. Q. of the sycamore maple cultured cells in the logarithmic phase is close to unity, suggesting carbohydrates are the substrate for respiration. For each mole of hexose sugar respired, 6 moles of oxygen is consumed, resulting in the production of approximately 36 moles of ATP. By measuring 0_2 uptake, it is possible to estimate the amount of ATP phosphorylated.

In the same period, incorporation of isotopically-labeled sugar into the cell wall fraction in the presence of a swamping concentration of the carrier is measured, and the amount of sugars incorporated is calculated from the specific radioactivity of the sugar in the medium and the radioactivity incorporated into the cell wall. Since 3 molecules of ATP are needed to make one glycosidic linkage via sugar nucleotide (GDP-glucose) pathway, it is possible to calculate the amount of ATP consumed in the synthesis of cell wall polysaccharides. By comparison of this figure with the total amount of ATP produced in the same period of time, one can figure out the percent of energy used in wall synthesis.

Similarly, protein synthesis is measured by incorporation of a labeled amino acid, such as ^{14}C–leucine, in the presence of a known high concentration of the carrier. Assuming that the specific radioactivity of leucine in the protein percursor pool is the same as that in the medium, the amount of this amino acid incorporated

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can be readily calculated. Furthermore, an amino acid analysis of the gross protein in sycamore maple cells would reveal how many moles of other amino acids are incorporated into protein in conjunction with one mole of leucine. Therefore, the total amount of amino acids incorporated can be obtained.

Since these cells are grown in a medium with very little amino acids in the nutrient, they have to synthesize the building blocks of protein from sugar and nitrate. Theoretically, 15 moles of ATP are required for the synthesis of one mole of an amino acid: 4 moles of $NADPH_2$ (equivalent to 12 moles of ATP) for reduction of NO_3^- to NH_3, and one mole of $NADPH_2$ for the amination of α-keto acids. Finally, another 3 molecules of ATP are needed for the formation of each peptide link. Therefore, a total of 18 moles of ATP would be required for the addition of one mole of amino acid to the polypeptide chain. The total amount of ATP used in protein biosynthesis is thus obtained by multiplying the moles of amino acids incorporated by 18.

2. Sucrose as a transport sugar in germinating seeds. It is a well known fact that in green plants the photosynthetate is translocated in the form of sucrose (and oligosaccharides of the rhaffinose family in some tree species). In seeds the food in the storage tissues (endosperm and cotyledons) is digested and transported to the growing embryo. It has been shown that in castor been seeds, the oil in the endosperm is degraded to acetyl CoA via β-oxidation, then converted to sucrose via the glyoxylate cycle and absorbed by the embryo as such (2). Amino acids such as alanine, glutamic acid and aspartic acid are transformed into sucrose for translocation (5). Maltose resulting from starch hydrolysis is also converted to sucrose in the endosperm, cotyledons or scutellum (in cereals) and then translocated to the embryonic axis (3). It is thus clear that sucrose is the most important vehicle for transporting of the substrate needed for growth. The question arises as to why the carbon compounds have to travel in the form of sucrose in the stem or in the conducting elements of seeds. Since the exact physico-chemical nature of phloem transport is not understood, one can only speculate. It is probable that the sieve tubes are so constructed that their permease system (if present) can only recognize sucrose and the sucrose moiety of rhaffinose. Isolation and characterization of such a hypothetical permease system (protein) may help to elucidate the mechanism of transport in plants.

3. Is seed dormancy a state of gene repression? It has been stated that dormant seeds have low metabolic rates and dormancy may represent a state of gene repression (1). Recently, the author, in collaboration with Professor J. E. Varner, compared the ability of dormant and after-ripened seeds of wild oat (*Avena fatua*) to carry out biosynthesis of ribonucleic acid and proteins. Much to everyone's surprise, when allowed to imbibe water, dormant seeds synthesize proteins and RNA at a rate comparable to that of the non-dormant (after-ripened) seeds. Only

after non-dormant seeds had germinated, did the difference in the rate of biosynthesis become evident.[4] It thus appears that dormant seeds are metabolically active, and no general repression of genetic activity is conceivable. It is more likely that dormancy is due to some specific metabolic block, or the lack of one or a few crucial enzymes required for normal development.

4. Detection of metabolic activity in dry seeds. Freshly-harvested seeds of *Avena fatua* are dormant; they do not germinate when moistened. They acquire the ability (potential) to germinate after several months of dry storage at room temperature, i.e. they are after-ripened. The crucial changes which occur in the after-ripening process are unknown. Are the changes purely physical?. Or is a sophisticated type of metabolism possible in such a dry state? (The dry seeds have about 10% moisture content.) One of the most difficult tasks confronting the seed physiologists is to detect the metabolic activity in dry (unimbibed) seeds. Application of such powerful biochemical tool as radioisotope tracer technic calls for soaking of the seeds. When the seeds are soaked, they are no longer dry. The dilema is analogous to, as one President of the American Society of Plant Physiologists put it, taking the rectal temperature of a hibernating bear. One can't measure it comfortably without disturbing the system. Thus one is left with limited choice of tools. Exposure of the seeds (surface-sterilized in absolute alcohol) to the volatile radioactive metabolites appears to be a cute method. $^{14}CO_2$ could be fixed into organic acid by PEP carboxylase. Still more promising, highly specific radioactivity ^{14}C–ethanol might be taken up, converted to acetaldehyde, and acetate, which in turn enters the tricarboxylic acid cycle. If this happens, that is, if the alcohol dehydrogenase and TCA cycle are operative, the radioactivity in ethanol will inevitably become distributed in the organic acids, amino acids, proteins, and sugars.

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