enzyme. Further analysis of the detailed kinetics by the stopped-flow technique revealed that the observed species was a ternary complex of O_2 , the enzyme and tryptophan, an oxygenated form of the enzyme and an obligatory intermediate in the reaction (Ishimura *et al.*, 1971). The following reaction mechanism was therefore compatible with the above data: the ferrous form of the enzyme first combines with tryptophan and then reacts with O_2 to form an oxygenated intermediate, degradation of which is the rate-limiting step of the overall reaction. This reaction mechanism is consistent with the view that dioxygenase reactions in general involve a ternary complex of enzyme, O_2 and organic substrate (Nozaki *et al.*, 1968). A similar oxygenated intermediate has been demonstrated with protocatechuate 3,4-dioxygenase, a non-haem-iron-containing dioxygenase (Fujisawa *et al.*, 1971).

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Biosynthesis of Flavonoid Compounds: Enzyme Activities in Relation to Cell Growth and Differentiation

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Synthesis and degradation of secondary plant products can often be directly related to growth and development of certain organs or to processes of differentiation of special types of cells. One of the most widespread groups of secondary plant metabolites is that of flavonoid compounds. Most of the general aspects of their biosynthesis have been elucidated by feeding experiments with labelled precursors (Grisebach, 1968). Recently the relationship between organ development in young parsley plants and changes in the accumulation of flavone glycosides and in the activities of some of the enzymes related to their biosynthesis has been studied. As a result of these investigations, a high degree of correlation

between development of cotyledons and leaves and activities of enzymes involved in the formation of flavone glycosides was demonstrated (Hahlbrock *et al.*, 1971).

For experimental reasons more detailed studies on the relationship between cell differentiation, accumulation of flavonoid compounds and activities of the enzymes related to this biosynthetic pathway were carried out with cells grown in suspension culture. In contrast with parsley plants, cells grown in the dark in liquid medium (containing mineral salts, vitamins, sucrose and 2,4-dichlorophenoxyacetic acid) did not produce flavone glycosides. Induction of enzymes and subsequent formation of flavone glycosides were taken as an expression of cell differentiation and were initiated by treatment of dark-grown cultures with light (Hahlbrock & Wellmann, 1970).

All of the enzymes investigated exhibit a high degree of correlation with regard to changes in their activities during the initial stages of flavonoid biosynthesis. However, two groups of enzymes could be distinguished by the time-lag before reaching maximum activity. Group I consists of the three enzymes acting on substrates of the phenylpropanoid type (phenylalanine ammonia-lyase, cinnamate 4-hydroxylase and *p*-coumarate-CoA ligase), whereas group II comprises all those enzymes related exclusively to the formation of flavonoid compounds and their glycosides (chalcone-flavanone isomerase, UDP-glucose-flavone 7-*O*-glucosyltransferase, UDP-apirose, flavone-7-*O*-glucoside apiosyltransferase, UDP-apirose synthetase and *S*-adenosylmethionine-luteolin 3'-*O*-methyltransferase).

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Role of Indol-3-ylacetic Acid in the Induction of Enzymes during Seed Germination

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Biochemical morphogenesis has not attracted the same degree of attention in plants as in animals or in unicellular micro-organisms, notwithstanding the dramatic changes in the growth of plant tissues that occur under the influence of plant hormones. Differentiated single cells cultured in chemically defined media would be the ideal system for the study of the regulation of biosynthesis in plants. However, investigations with whole germinating seeds can also be considered as a practical approach for elucidating the

mechanism of homeostasis in developing plant tissues. Some of the results obtained on the effect of indol-3-ylacetic acid on enzyme induction in germinating seeds of Bengal gram (*Cicer arietinum*) are presented.

Previous findings on the degradation of phosphatidylcholine during germination of this legume with the concomitant appearance of a membrane-bound phospholipase D (Talwalkar *et al.*, 1964, 1969) have been supplemented by the demonstration of degradation of protein and nucleic acid (Hadi, 1966) as well as the activation of ribosomal function during germination (Hadi & Krishna Murti, 1968).

To explore whether amino acids arising from protein degradation, apart from enriching the amino acid pool, were being channelled into alternative pathways and utilized for the synthesis of physiologically active non-protein nitrogenous compounds, the activities of enzymes known to mediate the metabolism of amino acids were investigated in gram seedlings. Tryptophan oxygenase, glutamate dehydrogenase, tyrosine transaminase and indol-3-ylacetate oxidase were not detectable in the dormant seeds but made their appearance after 48 h germination. During this period there was 1000-fold stimulation of peroxidase activity compared with that of the dormant seed as well as significant increases in the pools of soluble carbohydrates and amino acids. Cycloheximide or azauridine, when present in the aqueous medium used for germination, not only inhibited elongation of the seedlings but also suppressed the induction of peroxidase and the accumulation of free carbohydrates and amino acids.

When induction was studied with seedlings *in vitro* incubated with substrate it was found that indol-3-ylacetic acid ($1\mu\text{M}$) or cyclic AMP (adenosine 3':5'-cyclic monophosphate) (0.1mM) added exogenously to the medium stimulated tryptophan oxygenase activity two- to three-fold in 48–96 h seedlings but did not affect the activity of tyrosine aminotransferase or glutamate dehydrogenase. The stimulation of tryptophan oxygenase activity by indol-3-ylacetic acid *in vitro* was inhibited by actinomycin D and cycloheximide. The stimulation of tryptophan oxygenase activity by indol-3-ylacetic acid or cyclic AMP was accompanied by increased incorporation of DL-[1- ^{14}C]valine into proteins. Indol-3-ylacetic acid also stimulated the incorporation of [8- ^{14}C]adenine into cyclic AMP in 48–72 h seedlings, from which it was inferred that indol-3-ylacetic acid presumably triggered a mechanism mediating the synthesis of cyclic AMP (Azhar & Krishna Murti, 1971a,b).

The relative rates of synthesis of cyclic AMP, indol-3-ylacetic acid, peroxidase and tryptophan oxygenase have been followed during germination to establish the correlation, if any, between enzyme synthesis and the appearance of indol-3-ylacetic acid

or its mediator cyclic AMP. Purification of tryptophan oxygenase from induced and non-induced seedlings has been carried out to enable investigations on the modulation of its activity by indol-3-ylacetic acid.

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Regulation of Phosphoglucomutase

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Phosphoglucomutase from at least two sources, *Escherichia coli* and rabbit muscle, is strongly and specifically inhibited by the high-energy metabolites GTP and acetyl-CoA. GTP acts as its magnesium complex. Detailed kinetic investigations reveal that the two inhibitors are non-competitive with glucose 1-phosphate, glucose 1,6-diphosphate and Mg^{2+} , and that they bind to the enzyme at separate sites. The inhibitors act in a hyperbolic manner, and do not induce sigmoid kinetics in the saturation curves for the substrates.

All previous workers with phosphoglucomutase have agreed that the enzyme consists of one polypeptide chain of molecular weight 62000. This enzyme therefore seems to be a unique example of an allosteric enzyme without subunits. We have reinvestigated the subunit structure to check this possibility. When rabbit muscle phosphoglucomutase, which gives a single band on polyacrylamide gels, is submitted to the sodium dodecyl sulphate-polyacrylamide electrophoresis technique, about 20–30% of the material migrates at a rate corresponding to a molecular weight of 68000–74000, but the remaining 70–80% forms a clear band at a position corresponding to a molecular weight of 31000. This behaviour is shown by both the phospho- and the dephospho-enzyme. Results obtained by a short-column sedimentation-equilibrium technique show that in buffer at pH 7.0, both in the presence and in the absence of guanidine hydrochloride, the molecular weight of phosphoglucomutase is 62000. Reduced and carboxymethylated phosphoglucomutase, however, shows considerable heterogeneity (molecular weights