INTRODUCTION

The interfaces between disciplines have been the horizons where the most exciting botanical breakthroughs have happened. Histochemistry, physiology, morphology, cytology and embryology help to enlighten extremely fruitful boundaries in the rapidly developing science of differentiation. The problem of how cells begin to differ is one of the most challenging and urgent enigma in modern biology. The colony of cells from a homogeneous population pass through a pragmatic complex of morphometric events to inherit their destiny and position before their physical performance can be detected. In some organisms, cells begin to differentiate with the first few cell divisions after fertilization. In others large number of divisions intervene before their fate is fixed. Thus the development of the fertilized egg into an embryo is related to an array of events resulting in structural and subsequent functional diversity.

Though the reproductive development of many angiospermic plants have been studied in considerable detail (Maheshwari, 1950; Wardlaw, 1955), little information concerns the physiological and metabolic drifts during plant reproductive cycle. Since angiosperm eggs are enclosed within an ovary and embryos in a seed, they have been less amenable to biochemical manipulation. For this reason very little
analytical embryology has been done with plant material. The application of histochemical techniques is one of the finer approaches contributing to the essence of these intricate processes.

A great deal of attention has been given by developmental botanists to the transition from vegetative growth to floral initiation in angiosperms (Chailakhyan, 1936). The subsequent development of floral organs involves a closely ordered series of stages of growth and redifferentiation of the reproductive organs. The question arises as to the steps by which the indeterminate apex of a vegetative plant becomes a determinate organ for reproductive development. Green leaves of a plant perceive the floral stimulus from the environment for the transformation via. chemical communication. This chemical(s) translate(s) the stimulus into the cellular reactions and a change is manifested. Ultimate expression of these diversified chemical reactions is flowering.

Recently the metabolism in the embryo sac and the endosperm around the time of fertilization have absorbed quite an interest embracing a number of problems. For example, Buell (1952) investigated the variation of the quantity of starch in the developing ovary of Dianthus chinensis. Bernstein (1943) studied the amylases and carbohydrates in developing maize endosperm. Takao (1960)
studied the embryogenesis of *Pinus thunbergii* histochemically.

Meiosis consists of a series of finely co-ordinated physiological, biochemical, cytological and morphological processes which accompany the principal event; the reduction of diploid parental chromosome set to the haploid one (Sauter, 1971). Meiosis during micro- and megasporogenesis provides an excellent system for studying the events associated with different meiotic stages because the meiocytes are easily accessible and are available in large numbers in a fairly well synchronised state.

Endosperm, the nutritive tissue for the developing embryos is a dynamic centre of the developmental influence on the embryos. Although normal development of the endosperm is deceptively simple, it presents a number of paradoxes that invite exploration. What makes the endosperm and their haustoria to be divergent from the other normal embryonal cells? How the endosperm haustoria maintain their turgocity - to remain fully expanded? Does the endosperm activity closely attuned to the physiological and developmental needs of the embryo at a particular stage, as for example when the basic nutritional support of the endosperm is exhausted. Do the endosperm haustoria remain active throughout the life of endosperm? Even though these cells are called haustoria, the evidence for their
haustorial function comes mainly from morphological data (Subramanyam, 1960). But do they play an active role in transporting food from the ovular tissue to the endosperm or embryo?

The notion of a developing organ implies accumulation of storage materials and utilization of products of their hydrolysis for synthetic processes. As a model system, the great diversity of embryos are no exceptions to this rule (Devine, 1950; Raacke, 1957) and some offer exceptional opportunity for the study of storage and metabolism of ergastic substances, for they often combine relatively large size with highly complex metabolic reactions.

Inclusion of nucleic acids in this study is due to their established importance in growth and differentiation. Histones play a role in DNA-dependent RNA and protein syntheses in microspores and two-celled pollen grains (Sauter and Maraquardt, 1967; Sauter, 1971). Histones are proteins rich in two basic aminoacids, arginine and lysine. They are located partially in the major grooves of DNA. When histones are ionically complexed with DNA, the melting or denaturation temperature of DNA rises. In this respect histones stabilize DNA against a rise in temperature. The suppression of DNA as a template for RNA synthesis is linked with this stabilizing effect, since histones involve in the
inhibition of strand separation of DNA and thereby exert the repressive action at the transcriptional level in the eukaryotic cell (Allfrey et al. 1963; Mirsky and Bert, 1973).

Proteins are the agents of biological specificity and not only permit the regulation of the multitude of cellular processes, but also the molecular differences that exist between individuals and species. Protein bound cellular sulfhydryls together with disulfides play a role in mitotic regulation (Rebhun et al. 1976). Mazia (1961) suggested that -SH proteins, connecting the protein moiety with the prosthetic group are essential for many reactions. Polysaccharides are the source of energy and body building materials and contribute to the chemical constitution of tissues and their differentiation. Study of these metabolites during different stages of reproductive differentiation should enhance our present knowledge of the dynamic relationship between tissues during morphogenesis.

Biochemical techniques for such work are valuable but suffer because data obtained pertains to whole organ homogenates where identity of specific tissues is lost. But with the advent of quantitative histochemical techniques (Ruthman, 1970; Troyer, 1980) with the use of a cytophotometer, analysis at the cell, tissue, and organ level has been possible. According to Briarty (1975) the area
calculated from the longitudinal section of an object bears a direct and quantitative relationship with the volume of that object. Therefore total amounts and concentration calculated per cell area are used as a measure of total amount and concentration per cell. Because measurements are relative, arbitrary units are used for expression.

Several angiospermic plants viz. *Stellaria media* (Pritchard, 1964 a,b), *Vanda* (Alvarez and Sagawa, 1965 a,b), *Chenopodium alba* (Gifford, 1963), *Zea mays* (Moss and Heslop-Harrison, 1967), *Paeonia* (Sauter, 1969), *Tradescantia* (Woodard, 1958), *Lolium* (Evans, 1975), *Paspalum* (Chao, 1977) and some members of Compositae and Orchidaeae (Poddubnaya-Arnoldi et al. 1964) are analysed for their histochemical paraphernalia during different developmental stages. We selected *Ottelia alismoides* Pers. and *Ceratophyllum demersum* L. to study the quantitative cytochemical turnovers in metabolites viz. DNA, RNA, histones, total proteins, proteins containing sulfhydryl-disulfide groups and insoluble polysaccharides at the following developmental patterns of the reproductive cycle: 1. Transition of vegetative shoot apex into a reproductive one, 2. microsporogenesis and male gametophyte, 3. megasporegenesis and female gametophyte, 4. endosperm and 5. embryogeny. The terms micro- and megasporogenesis depict the entire development of the pollen and the embryo sac (Stern and Hotta, 1968).
The plant materials *Ottelia alismoides* Pers. and *Ceratophyllum demersum* L. were selected for this study because of their hydrophytic nature, in possessing large cells with big nuclei which showed fair amenability to histochemical methods. In *Ottelia* each ovary produces with high synchrony a large number of ovules which facilitated the study of megasporogenesis; fertilization and embryogeny. The shoot apices of *C. demersum* have multiple sites of floral initiation. Sites differ in age and thus offer a progressive sequence of events in terms of cellular reactions viewed at any one moment. Its endosperm cells are fantastically large and are easy to dissect from the ovule.