INTRODUCTION

Species are the groups of interbreeding natural populations that are reproductively isolated from other such similar groups. The inability to interbreed is important because this establishes each species as a discrete and independent unit. Therefore, most organisms belonging to different species invariably exhibit strikingly different characteristics and hence pose little or no difficulty in identifying them. The concept of species — as sexually incompatible groups (Mayr, 1963) leaves no room for defining 'species' in the organisms which have a predominant asexual mode of life cycle. In such cases, the determination of a categorical rank becomes seemingly difficult. For example, in aphids, cladocerans and rotifers females of many species are parthenogenetic but return to sexual reproduction when environmental conditions change. In such organisms no nomenclatural recognition is given to such temporary clones (Mayr, 1969). In the case of permanently uniparental reproductive lines, the species category is applied on the basis of morphological differences which are used as indications of underlying genetic differences and probable species status.
Hydra, a fresh water coelenterate, offers a challenge to the biological concept of species as understood earlier. In hydra, asexual reproduction by budding is the normal and the primary means of propagation of its population. It resorts to sexual reproduction either under unfavourable environmental conditions or under the influence of one or more stimuli (Kanaev, 1952; Itô, 1952a,b,c; Burnett, 1961a; Burnett and Diehl, 1964; Park et al., 1965). Another important aspect adding to the taxonomic paradox of hydra is the tremendous regulatory property of structural organisation, endowed in this unique metazoan, as shown by the variation of number of tentacles, shape and size of the body column. This morphological variability within the clones has frequently been observed (Hyman, 1929; Ewer, 1948; Spangenberg and Eaken, 1961). Intraspecific variability even in the structure of hydra egg theca has been reported (McConnel, 1935; Spangenberg and Eaken, 1961). Due to a wide range of phenotypic variation found in this organism, problem of systematics becomes especially intriguing because no reliable morphological criteria have yet been found.
1. Taxonomic History of Hydra

Linnaeus (1758) placed all hydram under one specific name Hydra polyps. Attempts to classify hydra into species have been made since 1766, when Pallas gave species specific names to the hydram whose characteristics had already been defined by Trembley (1744). These species were viridissima, vulgaris and oligactis. He also added a fourth species, attenuata from Rosel's description, (Rosel, 1755). Although, Linnaeus (1767) recognized Pallas' description, yet without any justification changed the names to viridis, grisia, fusca and pallens respectively. Brauer (1908) pointed out that Linnaeus' names were wholly invalid and that Pallas' names had indisputable priority.

Annandale (1905, 1906) was the first to study hydram in India and was able to locate H. vulgaris in Calcutta, Bihar, Orissa, Bombay, Allahabad and Madras, and H. oligactis Pallas (1766) in Lahore. He also identified H. orientalis as a distinct species in 1905, but later (1911) concluded that it was only a variety of H. vulgaris.

Schulze (1914, 1917) selected some features of hydra as diagnostic characters and erected the sub-
genus *Chlorohydra* of those hydras which had symbiotic algae and embryotheca with polygonal plates. This subgenus was later (Schulze, 1917) converted to the genus *Chlorohydra* and *C. viridissima* (Pallas) was recognized as the correct name of the green hydra. He put all those hydras which were characterized by the differentiation of the column into a slender basal stalk region into the genus *Pelmatohydra*; and all those species with neither symbiotic algae nor stalk under the genus *Hydra*.

Hyman (1929, 1930, 1931a,b; 1938) carried out extensive taxonomic studies of the hydras of North America. She questioned the generic distinction suggested by Schulze (1917), since the presence of symbiotic algae in other groups had not generally been regarded as constituting a generic character. She felt that it was almost impossible to decide in many cases whether or not the stalk was sufficiently distinct from the body to assign a species status according to Schulze's distinction, since intermediate forms exist between fully stalked and stalkless forms (Hyman, 1929, 1930). The decision in such cases became purely arbitrary. Hyman also classified *H. cauliculata* under the genus *Hydra* although it was described as possessing a slender stalk (Hyman, 1938).
The characters considered to be reliable for the identification and description of a species by Hyman (1929) were: the general shape and form of the column both during contracted and expanded states, the lengths of the tentacles and column, the shape, size and internal structure of nematocysts; the shape of the testes; the forms of embryonic theca; the separation or non-separation of sexes; and the manner of origin of tentacles on buds. Hyman (1929) made attempts to resolve the synonymy which had crept in the identification and naming of hydra species, and also put forward a key to the known species of hydra (Hyman, 1931b).

Ewer (1948) criticized the use of symbiotic algae as a generic character, but felt that its retention was justified on the basis of the nature of embryotheca alone. She also questioned the validity of using the presence or absence of stalk as a generic character. Of the two Natal (Africa) species described by Ewer (1948), H. intaba was observed to possess a stalk only when fully grown, while in H. umfula the distinction between stalk and body was not clearly marked. This compelled Ewer (1948) to recombine the genera Pelmatohydra and Hydra under Hydra Linnaeus (1758) sensu Ewer (1948). Ewer also
questioned the idea of uniting different forms as sub-species of a single species, since very few breeding experiments had been done. For instance, Hadzi (1906) made unsuccessful attempts to fertilize eggs of *H. oligactis* Pallas with sperms of *H. viridis* Pallas. Schulze (1917) also failed to effect the fertilization of the eggs of *H. attenuata* Pallas with sperms of *H. oligactis* Pallas. Ewer (1948), therefore, suggested that the species be regarded as the ultimate taxonomic unit and raised all described sub-species and geographical races to the rank of species till their correct status could be thoroughly elucidated. For instance, Cordero (1941) had described an animal which he considered to be a sub-species of *H. attenuata* and named it *H. a. thomseni*, although it differed from *H. attenuata* in the mode of coiling of the thread of the holotrichous isorhizas, in the way in which the buds were borne and in the mode of origin of the tentacles on the buds. Moreover, no cross fertilization experiments between these two hydra types were done. Ewer (1948) therefore suggested that until full description of all species of hydra became available, it should be regarded as a distinct species *H. thomseni* Cordero.
In 1956, Caleb described a new species of hydra, *H. gangetica* from Allahabad (India). The characters utilized for identification were: shape and size of the column, number of tentacles, length of the column, number of buds produced by the polyp, position of the bud zone, hermaphroditic nature of the organism, number and shape of gonad and the nature of the embrotheca. On the basis of these criteria, however, this hydra type does not appear to be distinctly different from the hydra described as *H. vulgaris* by Annandale (1905).

In 1959, Forrest described a new species of hydra, *C. hadlevi* from North America, which she assigned to the genus *Chlorohydra* Schulze (1917). *Chlorohydra hadlevi* had a unique two chambered embrotheca, but resembled *C. viridissima* in all other characters. Forrest (1959) opted for the retention of the genus *Chlorohydra* to accommodate the two species *C. hadlevi* and *C. viridissima* pending further investigation.

Muscatine (1961, 1965) and Oshman (1967) employed various strain designations depending upon the geographic location of collection (e.g., Carolina, Florida, California, European) to distinguish between
apparently different clones of green hydra. Oschman (1967) found differences between algae from different strains of hydra and interpreted them as either indicative of differences in symbiotic species or simply adaptive changes resulting from a symbiotic habit with different strains of hydra.

Ball (1967) studied the hydras occurring in Britain. He supported the view of Forrest (1959) regarding the retention of the genus *Chlorohydra* pending further investigation.

Grayson (1971) abandoned the genera *Chlorohydra* Schulze (1917) and *Pelmatohydra* Schulze (1917) and transferred their species to the genus *Hydra* Linnaeus (1758). He argued that the usage of symbiotic algae as an important taxonomic character may be misleading. He suggested that *H. hadleyi* and *H. viridissima* may be conspecific. Since the taxonomic merit of most of the characters of hydra have not been adequately assessed, he considered it prudent to group all hydras under one genus *Hydra* Linnaeus (1758).

Cox and Young (1973) studied specimens from four separate localities in Kenya (East Africa) and suggested a new species *H. mariana* on the basis of
nematocyst characteristics and structures associated with sexual reproduction. It was also found that characters such as body length, tentacle number, tentacle length, nature of peduncle, and maximum number of buds produced per polyp varied in the four populations.

Muscatine (1974) defended the retention of *Chlorohydra* as a genus on the basis of two facts: (1) Green hydra (Carolina strain) are dependent on the algae for survival when food is limiting (Muscatine and Lenhoff, 1965). (2) Symbiotic algae are recognized by green hydra, and this association exhibits a very high degree of specificity in terms of both the algae and the host type (Pardy and Muscatine, 1973).

On the above basis, possession of symbiotic algae emerges as a genetically based character (Muscatine, 1974).

The most recent study on the identification of hydrazas (Campbell, 1983) utilizes three main characteristics: presence or absence of symbiotic algal cells, manner of origin of the tentacles on the bud, and shape of the holotrichous isorhiza.

Ewer (1948) and Grayson (1971) presented a list of the world species of hydra, which reveals that
twenty eight species of hydra have been well identified, seven from Europe, sixteen from America, five from Japan, three from Africa, five from Britain and one from India. Apart from these, thirty two other species have been incompletely described.

It is thus clear that the 28 hydras have been recognized as separate species mainly on the basis of morphological criteria. So far, no serious attempt has been made to determine the occurrence of different kind of hydras in different locations in India and to arrive at a logical conclusion regarding their taxonomic status based on characteristics at physiological, ecological or cellular levels. Very little is known about the geographical and phenotypic variations of Indian Hydra within the confines of a morphospecies. Also, almost no information is available about their molecular differences. A meticulous classification, therefore becomes extremely necessary, since hydra has emerged as an organism of choice for experiments in ecology, neurophysiology, development and differentiation. Moreover, since a number of new facts have emerged in the biology of hydra, a wider approach must be adopted to resolve hydra systematics.
2. Basic Form and Functions in Hydra

Hydra is a simple metazoan in which the adult displays many of the characteristics of the embryonic state. Thus, it remains in a state of perennial plastic phase, capable of showing continuous cell differentiation and retaining the infinite power of regeneration from any part of the body and even aggregates of its cells (Trembley, 1744; Sanyal and Mookerjee, 1959; Gierer et al., 1972; Smid and Tar­dent, 1982).

Topologically hydra appears as a cylinder made of two epithelial layers: epidermis and gastrodermis separated by a thin acellular membrane, the meso­lamella. It is beset orally by a hypostome and aborally the basal disc. Since morphology is a matter of cell groupings, the behavioural activities of cells which cause them to interact spatially are more direct determinants of form (Morgan, 1901; Child, 1941; Evlakhova, 1946; Campbell, 1967a,b,c).

In hydra body form and size is maintained by keeping a dynamic equilibrium between addition of new cells due to growth and loss of cells by slaughing at the oral and aboral ends and by tissue elimination through budding. In actively growing organisms, just
below the tentacle whorl, there is a band of tissue which appears stationary, and cells move up to the hypostome and tentacles and down to the bud region and basal disc. From the study of this continuing centrifugal movement of tissues, various workers (Brien and Reneir-Decoen, 1949; Burnett, 1961a,b; Mookerjee, 1966; Corff and Burnett, 1969; Mookerjee and Roy, 1971) have suggested that cell proliferation occurs in a localized growth zone just below the hypostome. However, studies involving incorporation of tritiated thymidine into DNA indicated a uniform distribution of cell division along the entire body column of hydra (Shostak et al., 1965; Campbell, 1967a,b,c; David and Campbell, 1972; Webster and Hamilton, 1972). In the steady state, cell proliferation balances tissue loss and hydra size remains constant (Otto and Campbell, 1977a).

Hydra grows asexually by budding off young polyps from the proximal part of the body column. Budding has been intensively studied from several points of view, for example, bud stages and fate maps (Sanyal, 1966; Otto and Campbell, 1977b), origin and migration pattern of cells (Tannreuther, 1909; Kanajew, 1930; Shostak and Kankel, 1967;
Shostak, 1968; Mookerjee, 1982), pattern formation and control of bud initiation (Li and Yao, 1945; Shostak et al., 1968; Webster and Hamilton, 1972; Moore and Campbell, 1973; Berking, 1979a). On the basis of the available evidence, it appears that new buds are formed from the double layered tissue of the parent animal by evagination without enhanced mitotic activity (Campbell, 1967a; Clarkson and Wolpert, 1967; Gustafson and Wolpert, 1967; Otto and Campbell, 1977a; Sanyal, 1967; Mookerjee, 1982).

The body of hydra is made up of seven basic classes of cells: epitheliomuscular, epitheliiodigestive, gland, mucous, interstitial, nematocytes and nerve cells. Out of these seven types, nematocyte and nerve cells are non-dividing cells which arise by differentiation from interstitial cells (Sanyal and Mookerjee, 1960; Lehn, 1951; Slutterback and Fawcett, 1959; Lentz, 1966; Burnett, 1968; Davis, 1974). Four types of nematocytes can be distinguished based on the distinct morphology of the nematocyst capsule. These are: stenotele, desmoneme, holotrichous isorhiza and atrichous isorhiza (Weill, 1934). Ultrastructural study by Davis et al. (1968) showed three main types of nerve cells: ganglionic, sensory and neurosecretory.
In sexual animals, interstitial cells differentiate to give rise to eggs and sperms (Lentz, 1966; Burnett, 1968).

Hydra has an extensive regeneration capacity. Removal of head and foot from the body column results in the regeneration of each at the appropriate end. This phenomenon is termed the polarity of regeneration. The dual capacity of regeneration of the middle piece in hydra has been demonstrated by Burt (1934) and Mookerjee (1962). Regeneration in hydra had been considered as an example of morphallaxis, that is reorganization of existing structures without new growth (Morgan, 1901). However, Lesh Lauri et al. (1976), and Kumar (1983) have shown that DNA synthesis does occur during regeneration in hydra. A large body of regeneration and transplantation experiments have led to a consistent model for pattern regulation in hydra (Webster, 1971; Wolpert et al., 1971; Bode, 1973; Campbell, 1974). A number of attempts have been made to isolate substances affecting pattern regulation in hydra (Lentz, 1965; Lesh and Burnett, 1964, 1966). Schaller (1973, 1979) has isolated, and Schaller and Bodenmüller (1981) have characterised a head activating substance from hydra. Berking (1977) has characterised an inhibitor which, depending on the
concentration, retards or inhibits the development of bud from bud analge. It also inhibits both head and foot regeneration. These activating or inhibitory molecules, termed 'morphogens' (Turing, 1952) are low molecular weight peptides and are active at a concentration of less than $10^{-10}$ M. The morphogens are distributed in a gradient along the body (Schaller and Gierer, 1973; Berking, 1977) with a maximum in head region, and are stored either in nerve or epithelial cells or both.

The control of developmental phenomenon in hydra has been attributed by various workers to the three cell types - nerve, interstitial and epithelial cells. Classically, developmental potency has been ascribed to interstitial cells (Kanajew, 1930; Tardent, 1954). The nerve cells have been viewed as controlling much of the patterning of hydra development, possibly through neurosecretory activity (Gierer et al., 1972; Schaller, 1976a,b). Some investigators have considered the epithelial cells important in carrying out morphological shaping function (Kanajew, 1930; Campbell, 1974). However, recent studies have revealed that the properties and functions of the different cell types undergo modulation to a certain extent under different conditions. Studies of 'epithelial hydra', in which
interstitial cells and its differentiation products have been lost by colchicine treatment (Campbell, 1976; Wanek et al., 1980), or hydra devoid of interstitial cells by nitrogen mustard treatment (Diehl and Burnett, 1964) or X-ray irradiation (Bhattacharya, 1970, 1972; Fradkin et al., 1975) show that the presence of interstitial cells is not an indispensable factor for regeneration. Thus epithelial cells are capable of autonomous region specific differentiation. Experiments involving chimeric combination of epithelial hydras with normal hydras also point to the epithelial cells as the major controller of morphogenesis (Campbell, 1979).

Recently, a great deal of interest has been shown in the quantitative investigation of the control of cell proliferation and differentiation. By combining appropriate pulse and continuous radioactive thymidine labelling technique, with tissue maceration technique of David (1973), the cell cycle and differentiation kinetics of stem cell, differentiating nematoblast and differentiating nerves have been determined (Bode et al., 1973; Campbell and David, 1974). They found that stem cells proliferate with a 24 h cell cycle. Stem cells committed to nematocyte pathway divide several times (18 h cell cycle) to yield nests
of 4, 8 or 16 nematoblasts and each cell in a nest differentiates a nematocyte capsule in 2-3 days. Stem cell committed to nerve pathway divides once and both daughter cells differentiate as nerve in about 6 h. The pattern of nerve differentiation along the body column of hydra is due to differences in nerve commitment in different regions (Venugopal and David, 1981). Sproull and David (1979) demonstrated that growth of the stem cell population was controlled by the cell cycle time of the stem cells and the self renewal probability (the fraction of stem cells in each generation which divide to yield more stem cells). The probability of self renewal is controlled by the density of stem cells in hydra. David and Campbell (1972) have characterised the epithelial cells with respect to their proliferation rate and cell cycle parameters in H. attenuata. The results indicate that more than 90 per cent of hydra epithelial cells are actively proliferating with a cell cycle equal to the tissue doubling time.

For a better understanding of hydra development, genetically altered strains of hydra have been isolated and studied (Lenhoff, 1965; Brien and Renier-Decoen, 1952; Haynes, 1967; Moore and Campbell, 1973a,b).
Spangenburg and Eaken (1961) examined variation in regenerative capacity among different hydra species and strains. More recently, Sugiyama and Fujisawa (1977a,b; 1978a) have reported the isolation of several strains of *H. magnipapillata* which showed greatly reduced regenerative capacity, or abnormal regeneration pattern.

3. Aims of the Present Study

Due to the commendable advances in hydra research, a better understanding of a number of aspects of hydra biology such as growth, bud development, control of cell proliferation and differentiation has been attained. There has, however, remained an enormous gap between the knowledge of the biology of hydra and its systematics. While the former has been studied in a great detail, hydra systematics has remained confined within the bounds of gross morphology. Due to the wide range of variations found at the phenotypic level, it is obvious that a purely phenotypic approach may have to be fortified by other approaches.

The unsatisfactory state of hydra systematics is due to the fact that most of the investigators relied heavily on morphological characters and a few
physiological characters for diagnostic purposes. (see Campbell, 1983). However, morphological characters could be an organism's response to environmental influences (acclimatization) or may indicate a deep seated genetic condition (Reisa, 1973). Thus, to make the concept of 'species' in hydra clear, it is necessary to study a wide range of cellular and biochemical parameters which may be used to develop a reliable key for identification.

The present study aims to analyse hydras collected from 15-distantly located places in India at: (a) morphological, (b) behavioural, (c) physiological, (d) cellular and (e) biochemical levels.

Species which are distributed over a wide geographical range often develop locally adapted populations, called ecotypes, having different limits of tolerance to temperature, light and other factors (Odum, 1971). Compensation along a gradient of conditions may involve genetic races (with or without morphological manifestation) or merely acclimatization. Although many species are able to compensate along an extensive gradient such as north south temperature gradient, complete adaptation is often accomplished from the ecological standpoint by a series of closely related species that replace one another along the
gradient. To find whether the different ecotypes of hyd r as are just 'locally adapted populations' or different species, analysis over a wide range will be carried out.

Form, in the biological sense exists from the molecular to the organismal level of organisation. That is, as a result of the activity of the molecular components or products of cells, proliferation and differentiation occurs. Differential activity of cell populations in turn is manifested as body regions. Finally, the appearance of body regions results in the gross structural differentiation constituting the characteristic form of an animal. The interaction and regulation of these processes at all levels are evident in view of the uniformity of the net process and the end result. Although the hydra types will be studied at the different levels of organisation, maximum emphasis will be given to cellular and molecular aspects.

Otto and Campbell (1977b) demonstrated that size of the polyp is dependent on the rate at which the polyps ingest food. Hecker and Slobodkin (1976) have further shown that size of hydra increases at low temperature and decreases at high temperature.
Thus, studies will be done to estimate the size of the various hydra types in terms of the length of the column and tentacles, under constant conditions of temperature and under a known feeding rate.

The shape of the column is a characteristic which has been adopted as a diagnostic character by some taxonomists (Schulze, 1917; Caleb, 1956) and criticized by others (Hyman, 1929; Ewer, 1948; Forrest, 1963; Cox and Young, 1973). Therefore, this character will be carefully analysed to find if this provides any basis of a reliable taxonomic character.

The number of tentacles have been shown to alter in response to certain feeding conditions (Otto and Campbell, 1977b) and also under the influence of certain chemicals such as lithium chloride, chloretone, 5-bromouracil (Ham and Eaken, 1958). Thus, the basic criterion for consideration of the number of tentacles as a species diagnostic character in a hydra requires that culture and feeding conditions must remain constant. This will be analysed further.

Wood and Novak (1982) have demonstrated that nematocytes free hydra possess epidermal cell mesogial junctions in their tentacles. These junctions resemble the normal basal junctions which anchor a nematocyte to
a surrounding battery cell and the underlying mesoglea. This suggests that sites for nematocytes positioning may be predetermined in hydras. Thus, investigation of the arrangement and density of nematocytes on the tentacles in hydras of different ecotypes may provide hitherto unknown clues for diagnostic purposes. Therefore, estimation of the nematocyst distribution and density of the nematocyst in the tentacles of hydras of various ecotypes will be taken in detail.

Bud position in hydra has been shown to be influenced by inhibitory processes involving cell communication over wide areas (Shostak, 1974a). The positioning of the bud has been shown to be influenced by the head (Burnett, 1961; Webster and Hamilton, 1972; Shostak, 1974b), by pre-existing buds and in some cases by other buds arising simultaneously (Berking and Gierer, 1977). It is possible that bud position may have diagnostic significance. Therefore, this feature will be analysed in detail to see if this can be utilized to identify different ecotypes.

A number of investigators have demonstrated that hydras show a number of well integrated fairly complex behavioural patterns, such as locomotory activity
(Trembley, 1744; Kepner and Miller, 1928), response to external stimulation (Passano and McCullough, 1964; Rushforth and Burk, 1971; Rushforth, 1971) and feeding behaviour (Marsical, 1971). Hence a comparative study of hydra behaviour between different ecotypes, will be made, which may provide a key to the organism's taxonomy.

Loomis (1954) reported that carbon dioxide tension (pCO$_2$) brought about gonad production in $H$. littoralis. Burnett (1961) confirmed the control of pCO$_2$ in the induction of sexual reproduction in $H$. littoralis, but found that this observation could not be extended to hydars in general. He found that at 8°C $H$. viridis never entered sexuality, while in $H$. pirardi low temperature induced sexuality in 100 per cent of the animals. On the other hand, at 20°C, $H$. pirardi remained asexual while in $H$. viridis sexual forms were observed. Although, it has been confirmed by a number of workers (Hyman, 1928; Burnett and Diehl, 1964; Park et al., 1964) that the stimulus for induction of sexual reproduction varies in different species, this has not been used as a criterion for the identification of species. Therefore, in this investigation, the stimuli inducing gonad production will be analysed and evaluated for use as a reliable index for classification purpose.
The shape of the gonads has been considered as a diagnostic character of importance by a number of taxonomists (Schulze, 1917; Hyman, 1929, 1930, 1931a,b; 1938; Ewer, 1948; Forrest, 1963; Cox and Young, 1973). Hence, this character will be critically studied to check its reliability in the identification of species.

Study of the effect of temperature on growth in H. littoralis (Park and Ortemeyer, 1972) revealed that the rate of bud production increased at high temperature and decreased significantly at low temperature. It was also shown that the increased budding rate at the high temperature was due to decrease in the time taken for bud development and detachment from the parent hydra. In H. viridis on the other hand, it was found that though growth rate was higher at 25°C as compared to 20°C, the rate of bud detachment was lower at higher temperature. Thus, it appears that temperature exercises significant effect on growth, which varies in different species. Therefore, an analysis of the temperature threshold as well as tolerance range of each ecotype will be done. In addition to this, the effect of high and low temperature on the various components contributing to the
rate of bud production will be studied. This is expected to elucidate whether there is any inherent differences in the intrinsic rate of increase of bud production between different ecotypes and whether this might be related in some way to their condition of existence in nature.

The cellular characteristics of hydra such as cell growth, migration and adhesion have been demonstrated to be causal factors in promoting the essentially dynamic morphology of hydra (Campbell, 1967a,b,c; 1974). Analysis of the dynamic tissue structure underlying the gross morphology of hydra in terms of cell composition, density of each cell type in an animal and determination of cell size and shape may provide a reliable index for classification of hydra. Therefore, studies will be undertaken to explore this possibility.

Molecular events during growth and regeneration of hydra have recently began to be understood. Significance of RNA synthesis (Brooks et al., 1977; Venugopal and Mookerjee, 1980a,b; Nangia and Mookerjee, 1982) and DNA synthesis (Lesh-Lauric et al., 1976; Kumar, 1983) during the regenerative process have been reported.
Rattan and Mookerjee (1979) demonstrated significant differences in the pattern of RNA synthesis during regeneration in *H. vulgaris* and dimethyl sulphate induced mutant hydra. Hence, the RNA synthesis pattern during growth and regeneration will be studied in some of the ecotypes, to examine the extent of similarities or differences at the molecular level.

Estimation of the total protein content per hydra of each ecotype will be made to analyse biochemically the size of hydra.

Due to the complexity of the problem of identification of species in hydra, a new approach based on multidimensional consideration has been adopted involving an in depth analysis at different levels of organisation. In the present study, attempts are made to develop a reliable key for the classification of hydra in terms of parameters at morphological, behavioural, physiological and molecular levels. This is also expected to lead to a clearer understanding of species problem in hydra.