PREFACE
A cursory review of the literature on "Gregarine" reveals that mostly these parasites infect a large number of non-chordate hosts, although a few species are reported from the protochordates, viz., the ascidians (Hyman, 1940). They do not seem to infect the craniate vertebrates. In the invertebrate group they have been reported from the following phyla: Coelenterata, Platyhelminthes, Annelida, Arthropoda, Mollusca and Echinodermata (Kamm, 1922). Among these, the animals that most commonly harbour the gregarines are the arthropod hosts.

In the present cytochemical work we have examined the different stages in the life-cycle of a cephaline gregarine Stylocephalus sp. to understand some of the aspects of their nucleic acids, viz., deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), the roles of certain key enzymes involved in such vital phenomena as protein digestion, metabolism of proteins and carbohydrates, the neurotransmitters and also some aspects of sex-chromatin etc. This gregarine species is the fore-gut parasite of the beetle, Tenebrio molitor (Coleoptera : S. Inserta).

The above mentioned hosts are abundantly available in and around Dharwad, 015° 17 North and 075° 3 East (Karnataka State, India), located at an altitude of about 2000’ to 2400’ MSL, in the transitory belt between the western Ghats and the Eastern plains of the Deccan plateau. The annual rainfall is about 800-900 mm. Temperature fluctuations during the summer and winter are from 40° and 20° C.
As we are dealing with different stages of the life-history of the above mentioned gregarine trophozoites, gamonts, gametes, zygotes and sporozoites, it is considered appropriate to describe in brief the general life-cycle of the gregarines in the preface.

The salient features of the life-cycle of the cephaline gregarines are:

1) Parasitisation of a single host.

2) Absence of schizogony or asexual multiplication.

3) Completion of trophic growth within the body of host while the reproductive stages are completed outside the host's body.

4) The initial stages during the trophic phase are cytozoic (inside the cell of the gut epithelium of the host) and the later ones are coelozoic (inside the lumen of gut).

When the food material contaminated with spores of gregarine is ingested by the natural host, the spores dehisce and liberate the sporozoites in the fore-gut of the host. These sporozoites soon invade the fore-gut epithelium and derive nourishment from this tissue and start growing. Gradually the tiny sporozoites develop into cephalonts in which three regions, the epimerite, protomerite and deutomerite are differentiated. Gradually, cephalonts make their way out of the gut epithelium but they remain suspended to the latter by means of their epimerite. At this stage they are known as trophozoites. The trophozoite is a conspicuous stage in the life-cycle of gregarines and con-
tains the same three parts as cephalonts with the nucleus in the deutomerite. In most of the species the epimerite is lost in the grown up trophozoites. These trophozoites in a majority of the species and genera remain in between the peritrophic membrane of gut lumen for an unknown period and complete their trophic growth in order to prepare for encystment and sexual reproduction. The trophozoites usually occur in biassociation (rarely multiple association), a condition known as syzygy. In a syzygy the anterior end of one trophozoite adheres to the posterior end of another trophozoite. In some genera such as Stylcephalus, the association is seen just prior to encystment and it is between the protomerites of the two gamonts. In a syzygy the anterior one is known as the primite and the posterior one is the satellite. In some genera such as Hirmocystis, three or more trophozoites may form a chain. Such chains appear to be temporary associations and they will break into pairs as the trophic growth ensues. Development of mature trophozoites into gamonts often involves noticeable changes. The epimerite is resorbed completely. The associated gamonts prepare for the encystment. After reaching maturity, they become sluggish and inactive and slowly start exhibiting the bending and rotatory movements. After sometime they bend in such a way that the tail end of the satellite embraces the head end of the primite and form a rounded mass. In the mean while a cuticular transparent cyst wall is secreted by them so as to form a perfectly spherical or oval cyst called as gamontocyst or gametocyst. Within the gametocyst each gamont undergoes gametogony. During this process the septum between the protomerite and deutomerite of each gamont disappears, representing some advanced stage. However the line of association between the gamonts is still present and the cysts at this stage are extruded out of the host's alimentary canal along with faecal matter, and further development oc-
curs outside the host’s body.

Gradually the line of association between the two gamonts in the cyst disappears indicating the beginning of formation of gametes. During this stage the nuclei of both gamonts/gametocytes undergo repeated divisions, giving rise to many daughter nuclei and soon form the gametes. Each gamont produces one type of gamete, i.e., either the macrogametes or the microgametes. Then the partition wall disappears allowing the fertilization of the former by the latter and formation of the zygotes. Soon each zygote envelopes itself by a transparent and resistant covering and becomes a spore. The spores are of different shapes such as barrel-shaped as in *Gregarina cuneata*, or biconical as in *Steinina termitis*, or hat-shaped as in *Stylocephalus* spp., etc. Subsequently within each spore develop eight sporozoites. The spores are liberated free either by the dehiscence of the cyst or through the sporoducts developed on the cyst. The spores get mixed up with soil, humus etc., or water in case of the cysts voided by the aquatic hosts. When a natural host ingests such a contaminated matter, the spores get entry into the latter’s alimentary canal where they dehisce and set free the tiny sporozoites. The sporozoites eventually pave their way into the gut epithelium. From this onwards the life-cycle of the parasite is repeated.

The Subphylum *Apicomplexa* is characterised by the zygotic meiosis. Only the zygote stage is diploid while all other stages of the organism are haploid (*Rai*ov, 1982).

**Specific Introduction:**

Scanning of the literature on gregarine parasites (*Apicomplexa : Sporozoea*) reveals that the exhaustive
studies made in the early decades were of taxonomic nature. Watson (1916), Kamm (1922), Bhatia (1938), Bhatia and Setna (1924), Kirby (1941 a and b), Karandiar and Rodgi (1955), Uttangi and Desai (1961), Desai and Uttangi (1962 a and b) and Ormier (1977) are some of the works of this category.

In recent years, Heller and Weise (1973), Hildebrand and Vincler (1975), Hildebrand (1978), De Cunha and Jurand (1978) and Waller et al. (1979) have made significant contributions to our knowledge on the ultrastructure of gregarines.

Joyet-Lavergne (1926), Ganapathy and Naiasimhamur (1955), Stein (1961), Nalochin and Seravin (1962), Bobyleva (1963), Schreval and Fouquet (1968), Loubes and Bouix (1970), Amoli (1975), Amoli and Rodgi (1973), Amoli (1975), Ramachandran (1976), Desai and Nadiamo (1978 and 1987), Desai (1980, 1985, 1986 and 1988). Raliov (review — 1982) and Hooli (1988) have significantly contributed to our knowledge on the cytochemical organization of different species of gregarines. For reasons unknown, these authors have not extended their studies to the nucleic acids, digestive enzymes, neurotransmitters of these protozoans.

Elucidation of sexual dimorphism on the basis of the differences in the intensities of the cytochemical colour reactions for the nutrient storage metabolites (i.e., carbohydrates and lipids in trophozoites enabled Stein (1961), Bobyleva (1963), Desai (1980) and Hooli (1988) to describe sexual dimorphism in septate gregarines of different genera and families.

Accumulation of more nutrient metabolites in one strain and relatively less in another strain of these ur-
organisms may appear to be a kind of phenotypic manifestation of sexual dimorphism. But the basis of differentiation of the two sexes, barring a few exceptions, is the function of the sex genes and sex chromosomes. In the field of chromosomal cytology of gregarines, there are quite a few reports by Jameson (1920), Weschenfelder (1938), Grell (1940), Sprague (1941), Narsimhamurty and Ganapati (1961), and Desai (1965a and b, 1966 and 1988), Canning and Anwer (1969) are there describing the mitotic/meiotic events in the organism's life-cycles, the chromosome numbers n and 2n etc. Except in the Stylocephalus gregarines (Desai, 1988), there is no information on the sex chromosomes in these organisms and protozoans in general, so far. If at all the sex chromosomes are there in the Stylocephalus gregarines as reported by Desai (1988), we expect a highly condensed X-chromatin body in the female stages. Hence it was felt worthwhile to study the nuclei of different stages of one species of Stylocephalus, particularly to check the presence/absence of any chromatin body comparable with the sex chromatin body noticed in the cells of the mammalian females.

Our knowledge on the carbohydrate metabolic pathways in gregarines rests mainly on the studies on Gregarina cuneata (Natochin and Seravin, 1962), Lecudina luzetiae (Shreval and Fouquet, 1968), Stylocephalus mesomorphi (Amo ji, 1975), Stenophora Conjuncta (Amoji and Rodgi, 1973) and Stylocephalus conoides (Desai, 1980 and Desai and Nadkarni, 1986). The points emerging from these studies are, i) these parasites have functional Krebs cycle; ii) Amoji (1975) alone has pointed out that gregarines can also adopt the hexose monophosphate (HMP) pathway besides the classical glycolytic route; iii) only Desai (1980) and Desai and Nadkarni (1986) have pointed out the "respiratory shift" from
the typical oxidative phosphorylation in the trophozoite, gametes zygotes to the anaerobic glycolysis type in the sporozoite stage only. In all these studies the activities of the key enzymes like isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH) and \( \alpha \)-glycerophosphate dehydrogenase (\( \alpha \)-GPDH) alone are considered as the cytochemical parameters. Detection of equally important enzymes namely cytochrome oxidase, catalase and peroxidase would make the above mentioned views more authentic. Extending studies of all these enzymes in all the stages of the gregarine's life-cycle would enable us to assess the validity of the proposed "respiratory shift" hypothesis by Desai (1980) and Desai and Nadkarni (1980). In the present work an attempt has been made in this direction.

Views on the nitrogen excretion in protozoa are not equivocal. While Seaman (1954 and 1959), Hunter (1959); Camargo et al., (1987) opine that at least some species have a functional urea cycle. Dewey et al., (1957), Kidder (1967), Prosser (1973) and Gutteridge and Coombs (1977) maintain that the protozoans, particularly the parasitic forms are all lacking this cycle. Contrary to this situation, recently, urease activity has been observed by cytochemical methods in a gregarine and a parasitic ciliate (Desai, 1987 and 1989). This necessitated to reexamine the nitrogen catabolism, whether it is by the action of glutamate dehydrogenase alone or through the Krebs-Henseleit urea cycle and also by the action of urease in not only the trophozoites but also in the other stages of a gregarine species already mentioned. Studies on this aspect also form a part of the present work.

The present study as outlined above is described in different chapters mentioned below.
Chapter I: Findings on the nucleic acids by cytochemical methods in different stages of a gregarine species, *Stylocephalus* sp. have been presented in this chapter.

Chapter II: This chapter deals with the presence/absence of the sex chromatin body in the nuclei of different stages of the gregarine under investigation. On the basis of the findings, the occurrence of sexual dimorphism in gregarines would be discussed.

Chapter III: This chapter deals with the study of certain cytochemically demonstrable key enzymes involved in different pathways of carbohydrate metabolism, and cellular respiration in different stages in the life-cycle of the gregarine species mentioned above. On the basis of the findings, certain modifications on the views proposed by the earlier workers are suggested.

Chapter IV: Two neurotransmitters *v,*, adrenaline and *acetylcholine* have been studied in different stages of the gregarine and the same are described in this chapter.

Chapter V: In this chapter are described three proteinases and also certain key enzymes of Krebs-Henseliet urea cycle in the different stages of the life-cycle of the gregarine *Stylocephalus* sp. Further, in these stages another enzyme *v,*, urease activity has also been examined and described. On the basis of the findings, the functional significance of them is suggested.

Limitations of the present work:

Since the present work is entirely of cytochemical
nature not much emphasis has been laid on the functional aspects of the findings. They do need support from biochemical and physiological studies. As already stated, cultures of gregarine parasite can not be raised owing to their inability to propagate asexually. So far our laboratory is not equipped with facilities to undertake biochemical work involving cell fractionation, sedimentation and isolation of cell organelles, nucleic acids etc. We do look forward for such facilities to reexamine these organisms on modern lines.