GENERAL INTRODUCTION
GENERAL INTRODUCTION

In recent years trend is increasing to understand the significance of morphological, biochemical and physiological features of organisms against the background of the environment they live in. Features peculiar to each group of organisms are considered as adaptations. The underlying principle of adaptations is to enhance the organism's ability to survive and reproduce in a given environment. Such studies whether explicitly stated or not form a major theme of biological researches nowadays. Adaptations of organisms fall into two categories. Morphological, behavioural etc. form the first category, while biochemical adaptation form the second (Hochachka and Somero, 1984). In the present work we have chosen two groups of parasitic protozoa to understand some of their biochemical and physiological adaptations, in the light of the peculiarities of the environments where they live in, by adopting various histochemical techniques.
II

Perusal of the literature reveals that biochemical organizations of various parasitic protozoa and physiological processes in operation in them have been excellently documented by Hyman (1940), Hutner and Lowff (1955), Baernstein (1963), Kidder (1967), Ryley (1967), von Brand (1973) and Gutteridge and Coombs (1977). These reviews have covered various aspects of biochemical organizations and physiology of a number of parasitic protozoa inhabiting different milieu of a large spectrum of hosts.... vertebrates as well as invertebrates. Further information is available in the still more recent works Prosser (1973) and Hochachka and Somero (1984).

Sophisticated techniques like fractionation, purification, isolation etc. of enzymes, labelling of the substrates and the metabolic end products provide reliable and detailed information on the biochemical organization and various metabolic pathways in cells and tissues. Histochemical techniques of various kinds also provide a fairly
detailed and reliable information on the subject. Of course, the findings obtained from these should be taken as indications rather than conclusive evidences. Nevertheless histochemical approaches to understand the biochemical organization and various metabolic pathways do serve the purpose to a large extent.

It is evident from the literature that histochemical studies have been carried out in recent years on a few species of ciliates, flagellates and sporozoea, while the rhizopods still remain untouched to a large extent (Seaman, 1955, 1959; Hunter, 1959; Bobyleva, 1963; Sharma and Bourne, 1963a, 1963b, 1964d; Amoji, 1975; Amoji and Rodgi, 1973; Frandsen, 1975; Ramachandran, 1975; Desai, 1985, 1987, 1989; Desai and Nadkarni, 1980, 1987; Wanda, 1984; Raikov, 1985; Mclaughlin, 1985; Fulop and Csaba, 1988; Hooli, 1988; Zwart, 1988; Mertens, 1989; Vinodini, 1991 and Hanagandi and Desai, 1995). Information available from these studies is mainly on certain


From the foregoing comprehensive studies it becomes clear that there are certain areas wherein the findings are not uniform and the views are not equivocal. Three of such areas are, (i) steroidogenic potentials, (ii) biosynthetic potential of ascorbic acid of these parasites, and (iii) the modes of nitrogen excretion. Following are some of the details.

Bloch, (1961), Hutner, (1964), Gutteridge and Coombs, (1977) and Kohidai, (1990) maintain that protozoa, whether free-living or parasitic, lack this ability and hence do depend upon exogenous supply of steroids. Recent histochemical studies have shown the presence of the key enzymes involved in steroid synthesis/inter-conversions in the trophozoites of *Stylocephalus conoides* (Desai, 1980; Desai and Nadkarni, 1987) and *Gregarina cuneata, Hirmocystis speculitermis, H. incola* and *Steinina termitis* (Hooli, 1988). These findings go in support of the views of the first group of workers.

Information on steroid excretion by protozoa is rather limited. Nothing for definite is known about steroid metabolism (break down) in lower animals and microorganisms (Grant, 1962). Occurrence of a functional glucuronic acid cycle associated with steroid excretion in the flagellates *Stigmomonas* and non-virulent *Leptomonas* spp. indicates the excretion of
steroids in conjugation with glucuronioiids
(Mayes, 1983).

As a side branch of glucuronic acid cycle is the process of ascorbic acid synthesis. This is the second area of divergent views. While the virulent forms of Leishmania and Trypanosoma lack the ability of synthesizing ascorbic acid, the non-virulent forms Strigomonas and Leptomonas can synthesize this vitamin (Prosser, 1973; and Mayes, 1983). Trypanosoma cruzi also can synthesize it (Clerk and Jorge, 1994).

Another area of divergent views is the mode of nitrogen excretion. Kidder, (1967), Dewey et al., (1957), Oser, (1965), Prosser, (1973) and Gutteridge and Coombs, (1977) consider that the protozoa in general are ammonotelic with the enzymes glutamate dehydrogenase and / or aminooxidase bringing about the production of ammonia. Contrary to this is the view of Seaman (1955; 1959), Hunter, (1959), Desai, (1987; 1989).
Vinodini, (1991) that urea cycle is present at least in some species, and in gregarines Stylocephalus spp. even urea so formed is further hydrolysed by the enzyme urease (Desai, 1987; 1989 and Vinodini, 1991).

As far as we are aware of the literature there are no comparative studies on parasitic protozoa thriving under totally different environmental conditions like the obligate anaerobic flagellate symbionts of termites and the aerobic ciliate parasites of the frog's rectum. The parasites chosen for the present study are the ciliates Nyctotherus cordiformis and Opalina ranarum from the rectum of the frog Rana tigerina and the flagellates Pseudotrichonympha indica, Spirotrichonympha flagellata and Holomastigotoides ovalis from the rectum of the termite Coptotermes sp. Such studies would reveal biochemical adaptations, of the parasites, of various types. Through deletion of certain enzymic reactions and alteration of the kinetic properties of persisting
enzymes, many parasites display an ability to exploit the unique intraorganism environment in which they live (Prosser, 1973).

As already mentioned, we have chosen these groups of protozoan parasites for the present study, to understand, some biochemical adaptations with respect to, i) respiratory pathways, ii) steroid biosynthesis and excretion and iii) the mode of nitrogen excretion. The techniques employed are histochemical in nature. The findings are presented in this work in the form of four chapters, each one beginning with a brief introduction, followed by the materials and methods, observations and finally the discussion. Our findings being based on histochemical techniques, do need confirmation by biochemical studies. Hence they are taken as suggestions. This point we have made amply clear throughout the thesis at appropriate places.

During the course of this study
chemicals of SIGMA Grade (U.S.A.) have been used.
The findings are supported by a number of important coloured photographs. To make the presentation more precise, the findings are also presented in the form of tables and figures. At the end of the thesis an up-to-date list of literature is given.

Following are the details of the contents of the four chapters.

Chapter-I: This chapter deals with the study of certain cytochemically demonstrable key enzymes involved in different pathways of carbohydrate metabolism and under study. On the basis of our findings the earlier concepts are either supported or certain modifications are suggested.

Chapter-II: This chapter deals with the steroid biosynthetic potentials of the chosen ciliates and flagellates, on the basis of the presence absence of the substrate cholesterol and certain key enzymes involved in steroid biosynthesis.
the light of our findings the relationship of these parasites and their hosts from the point of the mode of reproduction of the former are discussed.

Chapter-III: This chapter is concerned with the mode of excretion of steroids. This has been studied by detecting the presence / absence of the key enzymes of the glucuronic acid cycle in each of these parasites. In addition, the ability of the latter in synthesizing vitamin C or ascorbic acid is also assessed. Presence or absence of gulano-lactone oxidase in these organisms has been taken in deciding either way.

Chapter-IV: In this chapter the mode of nitrogen excretion in these protozoa has been described, on the basis of the presence / absence of the key enzymes of the Krebs-Henseleit or ornithine cycle. On the basis of our findings certain modifications are suggested to the general concept that all the parasitic protozoa are ammonotelic.