Preface

In this book, the authors present current research in the study of the biology, classification and role in disease of protozoa. Topics discussed include the current and prospective tools for the control of cattle-infecting Babesia parasites; biological rhythms and cell behavior in paramecium; the use of protozoan tetrahymena as a cell model; anaerobic energy metabolism in protozoa; the biology of gregarines (protozoa: apicomplexa); the biology of parasitic protozoa cysts involved in human water-borne infections; and the effect of nickel toxicity to nutrient removal by selected indigenous protozoan species in wastewater systems.

Chapter 1 – Bovine babesiosis, a protozoal infection of cattle transmitted by Ixodid ticks, causes high direct and indirect economic losses around the world. The most important species are *Babesia bovis* and *B. bigemina* that infect bovines of tropical and subtropical regions. *B. divergens*, on the other hand, infects European cattle and bears growing public concern due to its zoonotic capacity. Depending on the *Babesia* species, acute infections can be accompanied by fever, hemoglobinuria, hemolytic anemia, abortions, respiratory distress syndrome, neurological symptoms and death. In addition to tick control, chemotherapy with anti-babesial drugs and live vaccine-based immunoprophylaxis are the main available tools for the control of these parasites. In spite of the usefulness of these methodologies, a number of drawbacks call for better and safer therapeutic interventions. This chapter gives an account of the currently used control methods as well as new perspectives that have arisen from the identification of parasite molecules that play critical roles at the host-parasite interphase and constitute attractive therapeutic targets.

Chapter 2 – The ciliated protozoan *Paramecium tetraurelia* displays well-characterized behavioral responses to external stimuli. These behavioral responses involve changes in the direction and frequency of the ciliary beat, which allows a cell to avoid unfavorable conditions. The change in the direction of the ciliary beat is instigated by a well-characterized calcium-based action potential, which is initiated by the external stimulus. However, under normal conditions the cells display spontaneous action potentials that cause the cells to continually alter the direction of swimming. A variety of biological processes in *Paramecium* are known to be modulated by biological rhythms; circadian rhythms display an endogenous, entrainable oscillation of about 24 hours, while ultradian rhythms are recurrent periods or cycles repeated throughout a 24-hour circadian day.

When the frequency of spontaneous behavioral responses is monitored over time in *Paramecium tetraurelia*, it demonstrates both a circadian and ultradian oscillation in the observed frequency. The molecular nature of these rhythms and how they control the frequency of spontaneous behavioral responses will be discussed, as well as the possible connection between the two different biological rhythms. The frequency of the behavioral response in *Paramecium* is an excellent system not only to study biological rhythms in unicellular organisms, but also to investigate the molecular relationships between different biological clocks.

Chapter 3 – Ciliated protozoa are quite large in size and are usually very motile. A single celled organism must perform all of the functions that multicellular organisms must perform but without specialized cells. Thus, their subcellular architecture can be quite complex, making them ideal organisms for the study of subcellular structure and how such structures are formed.

Tetrahymena is a non-pathogenic unicellular, free-living mobile ciliate protozoan. It is a typical eukaryotic cell, teardrop-shaped, and it is free-living in fresh-water.

Tetrahymena is an organism capable of independent life, but it is also a single cell with all features typical of "the cell" in general. Its relatively large size (about 50x25 μm) makes it suitable for detailed observations in the light microscope and conveniently small for electron microscopy.

The protozoan *Tetrahymena* is an established experimental model in biological studies and it has been extensively used for more than six decades as a toxicological model organism to test the toxicity of different substances using several endpoints. It has been used in evaluation of protein quality, in research concerning dynein, catalytic RNA, and telomerase, as well as in the determination of effects of carcinogens, insecticides, fungicides, mycotoxins, organic chemicals, antimetabolites, heavy metals and pharmaceutical drugs. It is usually used to demonstrate the levels of organization and cortical features that are characteristics of the ciliate taxa. It has proven extremely valuable as a model organism for many genetic and molecular biological studies. *Tetrahymena* species used as model organisms in biomedical research are *Tetrahymena thermophila* and *Tetrahymena pyriformis.*

Tetrahymena pyriformis is amicronucleate and contains only the polyploid macronucleus, which means that it is incapable of conjugation. Among the sexual species is *Tetrahymena thermophila* which is used much for generation of mutants and which has a higher optimal growth temperature (34^{0}C) than *Tetrahymena pyriformis* (28^{0}C). *Tetrahymena pyriformis* was the first protozoan to be cultivated axenically, in the absence of any other organism. Mainly this factor is responsible for its extensive use as a model cell system. Its biology and biochemistry have been studied extensively and so has its response to altered environmental conditions. Moreover, this protozoan has a short generation time and grows to high cell densities, even in large quantities for biochemical work. *Tetrahymena thermophila* has the potential to be used as part of a small battery of alternative tests that might reduce or replace the in vivo Draize ocular irritancy test of certain chemicals or chemical classes in the eyes of rabbits.

The purpose of this chapter is to outline the use of *Tetrahymena* as a model cell system in cytotoxicology and its rapid use as a genomic organism.

Chapter 4 – Energy metabolism plays a key role in all living organisms and is strictly related to their ecological niche and lifestyle. Anaerobic energy metabolism is broadly diffused among 'Protozoa': it is typical in commensal and parasite species, such as digestive tract symbionts and pathogens in general, but it is also present in free-living species adapted to anoxic or hypoxic habitats. Energy metabolism pathways in extant obligate or facultative anaerobes are varied and anaerobically functioning mitochondria or mitochondrion-related organelles seem to be ubiquitous in such organisms. In fact, it has been recently suggested that the endosymbiosis which gave origin to mitochondria in the eukaryote ancestor, occurred as early as the nucleus acquisition and that organelles such as hydrogenosomes and mitosomes are degenerate forms of mitochondria which evolved independently in different eukaryotic lineages. While mitochondria and hydrogenosomes are directly involved in ATP generation, however, mitosomes are not, but arguably still retain such essential functions that no eukaryotic organism could do without them.

This chapter critically reviews the present knowledge on the anaerobic energy metabolism in 'Protozoa', in view of the current (although still debated) picture of the evolution of eukaryotes and of their mitochondrion-related organelles.

Chapter 5 – Members of the Apicomplexan subclass Gregarinia (or Gregarinasina) parasitize mainly invertebrates. Because these protozoa are wide spread, common and sometimes large in size, they are often used as models in Parasitology laboratories. The number of gregarine species described up to date is scant compared to the number of potential arthropod hosts. In this chapter, the basic morphology and biology of gregarines is revised. In addition, the results of a new approach for the search of protozoa in invertebrates are discussed. The new strategy consisted in molecular detection instead of the standard microscopic method. Hence, a PCR-based survey for gregarines was conducted in 100 terrestrial arthropods collected in Alcalá de Henares (Madrid, Spain). The invertebrates included in the study were insects (75), crustaceans (20) and arachnids (5). Whole specimens were employed for DNA extraction. Real time PCR (targeting the 18S rRNA gene) and sequencing showed that one *Armadillidium vulgare* (Crustacea: Isopoda) was infected by a hitherto unknown protozoa, loosely related to *Gregarina blattarum* (86% identity by BLAST®). Based on its phylogenetic affinities, the new protozoa is likely to be a member of the suborder Septatorina. Further microscopic studies are underway to confirm the presence of gregarines in isopods and to ascertain which organ is infected by this new parasite. Although the prevalence of protozoa in invertebrates seems to be quite low, survey results are interesting. It must be underlined that gregarine infection has never before been reported in terrestrial Isopods, in spite of the long time that *Armadillidium* has been parasitologically studied and used as biological model. Since this crustacean is an organism easy to rear under laboratory conditions, it might be an excellent *in vivo* model system for studies on the biology of gregarines.

Chapter 6 – An important phase of the biology of biphasic parasitic protozoa as *Entamoeba histolytica* and *Giardia lamblia*, causative of human amebiasis and giardiasis, is the cyst that allows them to survive under adverse environment conditions outside the host. Consequently, the cyst is the form of the parasite that reproduces the infection in new hosts. Free living amebas capable to causing severe infections in humans as *Acanthamoeba castellanii* and *Naegleria fowleri* may also differentiate to cysts. Although infection with these potential pathogens is started by the trophozoite, cysts are capable to excyst in few minutes originating the invasive agent.

Two events are required for the morphological differentiation, the encystation that originates the cyst and the excystation that originates the trophozoite. Encystation occurs under unfavorable conditions for the trophic form which response producing a protective cyst wall with specie specific characteristics. When cysts are ingested by a new host or when environment conditions are optimal for its development, the trophyc form is restablished by means the excystation. The knowledge of the cysts may conduce to a best control of these serious infections. Here we present a review on the actual information of some parasitic protozoa cysts.