CHAPTER I
INTRODUCTION

The phylum mollusca constitutes one of the major divisions of the animal kingdom, and is of unusual interest both in regard to the diversity of organization and in the multitude of living species. The molluscs greatly vary in form, structure, habits, and habitats. They are highly adaptive and occupy all possible aquatic and terrestrial habitats. The phylum includes animals of wide diversity in form, such as the common slugs and snails, slow moving chitons, oysters and clams, swift darting squids, slithering octopuses, and the chambered nautilus. Molluscs have particular importance in that they form valuable fisheries in various parts of India as they are being used as food, as a source of lime, pearls and decorative shells, and as constituents of medical preparations. Thus, molluscs in general, have occupied a marked place in the affairs of man for time immemorial in his affairs of state and economy, of mind and aesthetic values, and of religion and rites of worship. In more recent times they have come to occupy prominent position in heraldy and royal insignia, and more conspicuously in the economy of vast section of the people. The gastropod molluscs constitute an important part of the ecosystem, and many aquatic animals thrive on them. Gastropods, including slugs and snails are the most successful of all molluscs, and are of special concern in that they serve as intermediate and as paratenic hosts of a variety of helminth parasites causing diseases in man and domestic animals. It is still not very clear why certain snail species are refractory to infection
while yet others are susceptible, and even among the susceptible ones, only certain age group snails are infected. This aspect is fascinating to investigate because, of late, susceptibility to trematode infection is being correlated with weak defence mechanism and it has been proved beyond doubt that haemocytes play an important role in internal defence against foreign materials. Obviously, haemolymph also plays a significant role in the defence mechanisms. Yet another aspect is to find out whether haemolymph can be treated as an organ system because in most of the studies in the past dealing with biochemical, physiological and metabolic changes, particular attention was given to determine the level of changes in specific organs such as the muscles, mantle, gills, digestive gland etc., but haemolymph was seldom considered as an organ system. Of late, it has been, however, shown that several parameters of blood can be taken as reliable indicators for diagnostic purposes, and also to monitor environmental pollution. The present investigation was, therefore, carried out on two species of freshwater gastropods for the following reasons, (i) to find out whether age, biotic and abiotic factors bring about any change in the haemolymph constituents particularly in haemocyte numbers since the haemocytes play a very significant role in cellular defence mechanisms of molluscs, (ii) to understand the various aspects of molluscan blood since very little work has been done in India, and (iii) to monitor pollution in freshwater environment as it has been suggested that quantitative determination of the levels of lysosomal enzymes and transaminases
can be employed as reliable indicator of the stress by environmental pollution.

The snail species selected for the present study are Indoplanorbis exustus (Deshayes), and Lymnaea acuminata (Lamarck) f. rufescens (Gray). Both the species serve as intermediate host for a large number of digenetic trematodes. A large number of cercariae are recorded from I. exustus, and a large number of trematodes of domestic and wild animals pass their larval stages through this molluscan species. L. acuminata f. rufescens is also recorded as the intermediate host of many flukes, and several cercarial forms have been recorded from this snail species (Rao, 1989).

It was reported that species of Lymnaea, L. stagnalis, is suitable as a test organism in toxicological studies (Canton and Sloof, 1977). This mollusc is easy to handle and culture, and is available in various quantities and stages of embryonic development. Canton and Sloof (1977) reported that L. stagnalis is a good biological indicator for establishing ecological limits for pollutants in surface waters. The adults are often used as test organism in acute toxicity experiments to measure mortality, immobility, and heart rate as criteria of toxicity (Batte et al., 1951; Patrick and Cairns, 1968; Sheanon and Trama, 1972; Knauf and Schulze, 1973; Polster, 1973).
Since gastropods have open circulatory system and the organ systems are bathed in haemolymph, any change in relation to abiotic or biotic stress is immediately reflected in blood and hence in the present study various haematological parameters of the two snail species were investigated in normal as well as in those under stress conditions. The haemolymph parameters studied were total haemocyte number, packed cell volume, haemoglobin (in I. exustus), and inorganic and organic constituents in three size groups of both the snail species. Moreover, the influence of various biotic and abiotic factors on total cell count was also investigated considering the importance of haemocytes in cellular defence mechanisms. To study the effect of pollution, copper was chosen as the pollutant because copper is the active ingredient in almost all molluscicide formulations, and the effect of copper toxicity was measured in terms of total haemocyte counts, and the activity pattern of selected phosphatases and transaminases.

The impact of pollutants on an organism is realized as perturbations at different levels of functional complexity (Moore, 1985). Xenobiotic induced sublethal cellular pathology reflects perturbations of function and structure at the molecular levels. In most cases the easiest detectable changes are associated with a particular type of subcellular organelle such as lysosomes, endoplasmic reticulum, and mitochondria. Cellular and subcellular responses to a variety of pollutants have been reported from a wide
range of animals. In molluscs, such responses reported include hepatopancreatic epithelial reduction in bivalves (Lowe et al., 1981; Couch, 1984), lysosomal disruption in mussels in response to copper and phenanthrene (Pickwell and Steinert, 1984, Moore et al., 1984), and inhibition of cellular immunity (Cheng and Sullivan, 1984). There are numerous studies in relation to accumulation and toxic effects of heavy metal ions including copper in molluscs (Menzel, 1979; Cunningham, 1979; Moore, 1985; Viarengo, 1985; Livingstone, 1985). Haemocytes are believed to play significant role in heavy metal metabolism, i.e., in the uptake, distribution to various tissues, and in the intralysosomal storage of metals (Galtstaff, 1964; Cunningham, 1979). However, very little information is available regarding the effects of toxic environmental chemicals on the immune functions in gastropods particularly when haemocytes are also involved in internal defence, and hence it was thought worthwhile (i) to study the effects of copper, ions on haemocyte number, and (ii) to examine the activity levels of selected lysosomal as well as non-lysosomal enzymes as xenobiotics can induce lysosomal destabilisation on molluscan cells, and quantitative measurements of the levels of both marker lysosomal and non-lysosomal enzymes have been indicated as reliable indicator of the presence of pollutants in the immediate environment.

Many trace metals are important in plant and animal nutrition, and as micronutrients they play an essential role in tissue metabolism and growth. Among the essential trace metals copper has
an important role and it is an essential component of many enzymes. However, not all of these enzyme activities are decreased in copper deficiency to the level that they are metabolically limiting. Copper is known to become toxic to aquatic organisms when the concentration exceeds tolerable limits. Copper sulphate is used in aquaculture for the treatment of ectoparasites and to eradicate certain diseases. Copper compounds are commonly used as molluscicides, and among them copper sulphate is the most important one. Copper sulphate is a less expensive molluscicide and is found effective in destroying the molluscan intermediate hosts of a variety of trematodes. However, it was reported that like all other major molluscicides copper sulphate has also certain disadvantages (see Ritchie, 1973). It was found to be totally or partially inactivated in natural waters due to adsorption by soil and organic materials, and is ineffective at alkaline pH's, and is toxic to other non-target organisms especially young fishes and certain aquatic vegetation (Cheng and Sullivan, 1975). In the present study, copper was chosen as the toxicant to study the effect of heavy metal pollution on the haemolymph for the following reasons: (i) copper is the main ingredient in almost all the molluscicides now in use, and (ii) very little is known about the pathophysiology and toxic mechanisms of cupric ions on gastropods and particularly so on total haemocyte counts, and hence in defence mechanisms.

The concept of haematological manifestation in response to abiotic and/or biotic stress is applied widely for identification
of stress factors and much of the information regarding the haemocytes and their variation due to stress has come from studies involving insects, crustaceans and molluscs. Molluscan haemocytes have been implicated in diverse functions such as wound repair, nutrient digestion and transport, excretion, and internal defence which include phagocytosis and encapsulation. Although the haemocytes in bivalve molluscs have been classified into granulocytes and agranulocytes (Cheng, 1981) differences continue to exist. Regarding gastropods, while Ottaviani (1983) reported two distinct haemocyte types, spreading and round, in Planorbis corneus which are not different maturational stages of a single cell type, Sminia et al. (1983) reported round and spreading haemocytes in L. stagnalis, and considered these cells as different maturational stages of a single cell. Renwrantz et al. (1979), Cheng (1980), Mohandas (1985), and Cheng and Downs (1988) have reported the occurrence of subpopulations of haemocytes in molluscs. Differences were also observed between blood cells of juvenile and adult specimens of L. stagnalis (and this difference was attributed to be one of the reasons for varying susceptibility to infection by larval trematodes) (Dikkeboom et al., 1984), and also in two different strains of Biomphalaria glabrata (Stumpf and Gilbertson, 1978). Several biotic as well as abiotic factors are reported to affect the number and distribution of circulating haemocytes. These factors include infection, snails size, age, host strain difference, temperature, wounding, heavy metal stress etc. (Sminia, 1981). In the present study the total
haemocyte counts in three size groups—juveniles, intermediate, and adults—of both the snail species were studied. Moreover, the effect of various abiotic and biotic factors on total haemocyte number was also investigated. The various factors selected for the study were temperature, pH, snail-conditioned water, and heavy metal—copper—stress.

It was reported that the organic in organic composition of molluscan haemolymph is variable (Bayne, 1973; Thompson, 1977). Burton (1983) reported that several factors shell size/age such as temperature, rainfall, photoperiodism, hibernation, starvation, oviposition etc. affect the biochemical composition of the haemolymph in molluscs. On gastropods, very little information is available concerning the influence of shell size/age on plasma metabolites, and hence to generalize the inorganic and organic composition of the haemolymph such a study on different age group snails is needed. In the present investigation the various inorganic and organic constituents in the haemolymph of the two snails species in relation to size/age were analysed and reported. The various inorganic constituents studied were haemolymph sodium, potassium, calcium, chloride, and ammonia while the organic constituents were urea, total carbohydrate, glycogen, total protein, and total lipids.

Molluscs generally have low enzyme level and information on their specific roles is sparse (Fried and Levin, 1973). Enzymes by themselves are not present in the haemolymph, unless they belong
either to the haemocytes or leak from intracellular confines of the
damaged tissues and hence serum enzymes levels were considered to
be of diagnostic value (Jyothirmayi and Rao, 1987). In the present
study, activity levels of lysosomal and non-lysosomal enzymes
- phosphatases and transaminases- in the haemolymph of normal and
copper exposed snails were estimated to understand the effect of
copper on the activity pattern of enzymes and also to examine its
diagnostic value. The enzymes selected for the present study were
acid phosphatase, alkaline phosphatase, glutamate-oxaloacetate
transaminase, and glutamate-pyruvate transaminase. Lysosomes and
cell membrane are the first target of pollutants because lysosomes
are concerned with the disintegration of foreign materials and the
cell membrane is the first barrier to a xenobiotic agent. Acid
phosphatase is a lysosomal marker enzyme and alkaline phosphatase
is considered by some as lysosomal enzyme and by others as plasma
membrane enzyme. During the period of stress, in the most likely
event of lysosomal membrane disruption, these enzymes are released
into the haemolymph thus increasing the enzyme level there. Hence,
these two enzymes can be treated as reliable indicators of stress.
It was reported that when copper accumulates in mammalian tissues,
a significant rise in serum transaminases and lactic dehydrogenase
occurs. Determination of the activity levels of serum glutamate
oxaloacetate transaminase and glutamate pyruvate transaminase has
therefore been proposed as an aid in detection of chronic copper
poisoning (Metz and Sagone, 1972).
The thesis is arranged in six chapters. The general introduction forms the first chapter. The second chapter is on total haemocyte counts and the factors affecting variability. In the third chapter the inorganic and organic constituents of haemolymph are reported. The effect of copper on the activity patterns of phosphatases, and transaminases forms the subject matter of chapters four and five. Summary of the work forms the sixth chapter, followed by the list of references.