6.1. SUMMARY

1. The present investigation was carried out on the haemocytes and haemolymph of the mussel *Lamellidens marginalis*. 
2. Studies were confined to the following aspects: In vitro spreading activity pattern of haemocytes at different temperature periods, chemotaxis towards different bacterial strains, both Gram negative and Gram positive, the role of serum in chemotaxis, in vivo phagocytic capability, activity pattern of selected lysosomal enzymes, and the effect heavy metals on the release pattern of selected haemolymph enzymes. 
3. Spreading activity of haemocytes at different temperature was studied. It was found that at 32°C higher number of haemocytes was spread than at 24°C, 26°C, 28°C, and 30°C, both at 1 and 2 hour periods. 
4. Chemotactic attraction towards the Gram negative *E.coli*, and *Vibrio alginolyticus*, and the Gram positive *Micrococcus sp.*, and *Bacillus sp.* was studied. 
5. Of the four bacterial strains, the association with *E.coli* was higher than with both the Gram positive strains. Hence, *E.coli* was chosen for further experiments. 
6. The results showed that there was greater chemotactic
attraction with live bacteria than with killed-bacteria, and higher association at 2 hour period than at 1 hour period.

7. These results confirm the ability of haemocytes to recognise nonself substances, and to be selective in attachment.

8. The time taken for selectivity reaction suggests that the rate of cellular defense mechanism increases with increase in exposure time.

9. The attraction of higher number of haemocytes towards live bacterial cells shows the capacity of haemocytes to distinguish the nature of, foreign substances, and confirms the role of as primary function.

10. Higher number of haemocyte-bacteria association in the presence of serum (with-serum incubated haemocytes) than in the absence of serum (with-saline incubated haemocytes) indicates the role of serum in the activities of haemocytes.

11. In in vivo studies the number of phagocytosed haemocytes was higher in animals maintained at 27°C than in those maintained at 32°C and 22°C, and the number was found to decrease with increase in time.

12. The maximum phagocytic rate at 27°C shows the optimum temperature suitable for maximum activity in vivo.

13. Haemolymph glycogen level in both live, and heat-killed bacteria challenged animals was higher at 4 hour post-injection.
14. The increase in haemolymph glycogen level at 4 hour post-injection suggests the possible digestion of bacteria both live and killed by haemocytes and subsequent release of glycogen into haemolymph.

15. Activities of two enzymes, acid phosphatase and alkaline phosphatases origin were assayed in haemolymph.

16. The result indicated lower ACP activity at 2 and 4 hour post-challenge in live-bacteria injected animals, and higher at 24 hour, but little change was noticed between untampered, saline, and heat-killed bacteria injected groups. ALP activity pattern showed no significant change among test groups.

17. The reduction in ACP activity at early time-periods in live E. coli injected animals suggests neutralization of enzyme with E. coli cell wall resulting in the formation of spheroplast. The increase at 24 hour suggests the increased synthesis of ACP by the animal to counter the bacterial challenge.

18. The no change in ALP suggests that the release of lysosomal enzymes not be through membrane destabilisation.

19. Effect of exposure to sublethal concentrations of heavy metals on the activity pattern of haemolymph ACP and ALP in animals challenged with E. coli was investigated.

20. Heavy metals selected for the experiments were mercury (HgCl2) and copper (CuSO4 2H2O).

21. Results of ACP activity in Hg exposed group showed the following. At 2 hour post-injection live bacteria injected
animals showed lower activity but no significant change was noticed in other groups at any time-period.

22. Regarding haemolymph ALP activity in Hg exposed animals there was no change in activity in the untampered, and in those injected with heat-killed bacteria. In live bacteria injected animals at 2, and 8 hour post-challenge, reduction in enzyme activity was noticed, but increase at 4 hour.

23. In Cu exposed animals, there was, in general higher haemolymph ALP activity at all time-periods, and no change was noticed between the test groups.

24. Haemolymph ALP activity in Cu exposed animals showed no significant change.

6.2. CONCLUSION

Haemocytes of Lamellidens marginalis show reaction to non-self substances. They are also selective, showing more reactivity to live bacterial strains than to dead ones and greater attraction towards E.coli. This could be due to presence of specific receptor sites on live E.coli. Thus, variation exists in the degree of chemotaxis by haemocytes to different bacteria due to variation in the chemical nature of receptors on them. It was also found that serum plays a major role in recognition process by haemocytes to bacteria.
In in vivo studies maximum phagocytosis was noticed at ambient temperature indicating efficient defense mechanism. However, the results indicate that activity of haemocytes on Gram negative bacteria is often influenced by the formation of a complex between the lysosomal hydrolases and bacterial cell wall which protects the bacteria from lysis at early time-periods.

The effects of Hg ions and Cu ion on enzyme activity exposure suggest the influence of different heavy metals on the internal defense physiology, in different ways. However, further investigation is required on this aspects to draw clear conclusions on the resistance potential of haemocytes, and the molecular mechanism of interaction between haemocytes and heavy metals.