SUMMARY

L-asparaginase (an antileukaemic agent) producing microorganisms from estuarine sediments and molluscs were screened and L-asparaginase positive strains were isolated, identified and were grouped into 5 groups depending on the specific activity. The effect of various ecological parameters on the growth of total as well as L-asparaginase producing microbial population were studied. Highest activity was shown by an *Aeromonas* Spp.

A cheap culture medium for *Aeromonas* has been formulated with starch and sucrose. The enzyme activity was slightly higher when sucrose was used as carbon source, rather than starch. The optimum cultural conditions for maximum enzyme production were worked out. The enzyme is constitutive and is not suppressed by feedback mechanism.

The L-asparaginase of estuarine *Aeromonas* was liberated from the bacterial cell by lysozyme treatment followed by osmotic shock and was purified by manganous chloride treatment, DEAE cellulose chromatography, gel filtration on sephadex G-200 and by hydroxyapatite chromatography. In the final step, the enzyme showed a
specific activity of 47.0 IU mg\(^{-1}\) protein. The purity of the enzyme preparation was found to be one hundred and forty two fold and the recovery was 8.33% and the \(K_m\) value of the enzyme was found to be \(1.75 \times 10^{-5}\) M.

The antitumor property of the L-asparaginase from estuarine *Aeromonas* was studied using Ehrlich ascites tumor and has been compared with that of Leunase.

*Aeromonas* L-asparaginase injected into the subcutaneous tumor mass caused a reduction in tumor growth. Complete reduction of the subcutaneous tumor mass without tissue necrosis was obtained with both Leunase and *Aeromonas* L-asparaginase injected in three intermittent doses of 10 units each at an interval of 24 hrs.

All the intraperitoneal tumor implanted animals survived when 75 units of *Aeromonas* L-asparaginase or Leunase were injected intraperitoneally in three intermittent doses of 25 units each.

Administration of *Aeromonas* L-asparaginase or Leunase resulted in marked increase in serum glucose levels. This increase was more marked during the early stages of therapy.
During L-asparaginase therapy, liver cholesterol level increased significantly but serum and kidney cholesterol levels remained unaltered. However, serum and kidney cholesterol levels increased at a later stage of therapy.

Serum and liver triglyceride levels increased with tumor growth, but no alteration was observed in kidney triglyceride levels. At a later stage of therapy, the serum triglyceride levels decreased with *Aeromonas* L-asparaginase and the value returned to near normal. A decrease in triglyceride levels of serum during therapy was an important observation and was observed only in *Aeromonas* L-asparaginase treated groups, when compared to Leunase. This reduces the secondary effects of tumor in inducing artherosclerosis, since this is an important risk factor in artherosclerosis.

The serum phospholipid levels increased during early stage of tumor growth while the phospholipid levels in kidney decreased. Serum phospholipid levels were elevated at a later stage of tumor growth, but those of the kidney and liver returned to near normal. Phospholipid levels in kidney decreased after ceassation of therapy. This decrease was more marked in groups treated with *E. coli* L-asparaginase.
In all the cases, administration of L-asparaginase caused a marked decrease in the protein content of serum, liver and kidney. Blood urea level increased with tumor growth and treatment with L-asparaginase brought this value to near normal.

There was a significant reduction in the level of protein bound hexose in kidney, right from the beginning of tumor growth, whereas the protein bound hexose level of liver decreased at a later stage of tumor growth. The protein bound hexose level of serum increased with the progression of tumor growth. Administration of L-asparaginase caused a reduction in the serum protein bound hexose. The protein bound hexose in kidney increased with L-asparaginase administration, and reverted to near normal after the cessation of therapy. The liver protein bound hexose level increased at a later stage.

Eventhough the protein bound fucose level in kidney and liver did not show any change with L-asparaginase therapy, serum fucose levels were found to be decreased at a later stage.

There was no significant alteration in sialic acid levels of serum and tissues immediately after
L-asparaginase therapy. Administration of *Aeromonas* L-asparaginase increased the sialic acid level in kidney. Sialic acid level in liver remained unaltered with both Leunase and *Aeromonas* L-asparaginase therapy.

Significant elevation in serum lactate dehydrogenase activity was observed with tumor growth and returned to near normal on treatment. Thus it would be possible that serum LDH can be used as a diagnostic index of tumor burden.

Administration of *Aeromonas* L-asparaginase and Leunase caused an increase in serum transaminase activity. But after the cessation of therapy, this elevated serum level returned to near normal. This reversal was pronounced in the case of animals treated with *Aeromonas* L-asparaginase, showing that *Aeromonas* L-asparaginase is less hepatotoxic than Leunase.

L-asparaginase therapy showed a significant reduction of tartarate labile acid phosphatase enzyme in serum, but at a later stage of therapy it was found to increase considerably and did not show any direct correlation with tumor burden and cannot be used as a tumor marker.
Serum alkaline phosphatase activity showed an increase with tumor growth and a significant increase was observed immediately after L-asparaginase treatment.

Of the enzymes studied, only LDH can be used as a tumor marker, but even this enzyme cannot be used to monitor cancer therapy.

From the above results it would be possible to suggest that L-asparaginase prepared from *Aeromonas* is superior to the commercially available drug, and hence can be recommended to replace this drug by *Aeromonas* L-asparaginase for the treatment of leukaemia.