Studies on amino acid degrading enzymes, for both the essential and non-essential amino acids, represent an important approach in chemotherapy for cancer (Holcenberg, 1981; and Roberts, 1981). It has been established that the tumour cells develop an increased demand for certain amino acids, and in this respect some of the otherwise non-essential ones like asparagine, glutamine and arginine may become essential for these cells (Knox et al., 1969; Weber, 1974; Reitzer et al., 1976; and Holcenberg, 1981). Any system of treatment with drugs must, therefore, include one such enzyme in the schedule of drug administration. Acute lymphocytic leukemia in children and some adults has been treated with L-asparaginase in combination of vincristine and prednisone, as well as some other pharmaceuticals (Sutow, 1976; Trueworthy et al., 1978; and Chessels and Cornbleet, 1979).

Intensive research for L-asparaginase has been carried out among microorganisms and other higher forms.
during the last two decades (Wade et al., 1971; Imada et al., 1973; Mangatt et al., 1985; Mangatt, 1989 and Ali, 1989). From these studies emerged a fact that the capacity for elaborating L-asparaginase is of wide occurrence, in the living world, nevertheless, all the asparaginases do not have antitumour property. Besides the guinea pig serum L-asparaginase the others with antitumour property are those which have been obtained from Escherichia coli (Mashburn and Wriston, 1964), Serratia marcescens, Erwinia carotovora (Wade et al., 1968), Citrobacter freundii (Davidson et al., 1973), Vibrio succinogenes (Krautheim et al., 1982), Pseudomonas putida (Arima et al., 1972) and Aspergillus terreus (De Angeli, 1970). For large scale clinical trials only the E. coli enzyme has been used. Consequently, much data about biochemical characteristics and clinical fitness of L-asparaginase as a therapeutic drug for cancers is available on this enzyme.

Various asparaginases have been purified to homogeneity with conventional laboratory methods. The enzymes derived from E. coli II (Mashburn and Wriston, 1964), Erwinia carotovora (Distasio and Niederma, 1976), Citrobacter freundii (A) (Distasio et al., 1976) and Vibrio succinogenes (Distasio and Niederma, 1976) have been shown to be potentially therapeutically useful. Studies on the biochemical characteristics of the enzyme
include determinations of substrate specificity, $K_m$, molecular weight, thermal and pH optima and the inhibitors and activators of enzyme activity. Some leading researches have been briefly described here.

The purification and characterisation of L-asparaginase and L-glutaminase (deamidase - AG) from different species of *Pseudomonas* have been carried out by Nikolaev *et al.*, (1969), Katsumata *et al.*, (1972), Mardashev *et al.*, (1975 a and b), Roberts (1976), Davidson *et al.*, (1977 b), Pekhov *et al.*, (1985) and Pekhov and Zanin (1987). They were able to obtain *Pseudomonas* deamidase - AG in a crystalline homogenous state. This enzyme had a high affinity for L-asparagine ($K_m = 1.5 \times 10^{-5}$ M) and L-glutamine ($K_m = 2.2 \times 10^{-5}$ M) and a molecular weight of 156,000. Holcenberg *et al.*, (1976) studied the kinetics, inhibition and activation of L-asparaginase in different *Pseudomonas* species and found that L-aspartate was a competitive inhibitor of enzymatic hydrolysis of L-glutamine ($K_i = 1.6 \times 10^{-4}$ M) and L-asparagine ($K_i = 1.7 \times 10^{-4}$ M) while L-glutamate competitively inhibited the hydrolysis of L-glutaminate ($K_i = 1.7 \times 10^{-3}$ M). Badr-el-din and Foda (1976) demonstrated that the enzyme of *Pseudomonas* was highly sensitive to the action of 6-diazo-5-oxo-L-norleucine (DON) and azaserine. It was found that the process of enzyme formation was firmly linked to active cell growth,
as shown by the use of growth inhibitors. Kabanova et al., (1985) thoroughly investigated the thermostabilization of glutaminase (asparaginase) from *Pseudomonas aurantica* BKMB 548 and Lebedeva et al., (1986) studied its quaternary structures and some properties. Rowley and Wriston (1967) achieved a 240-fold purification of *Serratia marcescens* L-asparaginase, with an overall recovery of approximately 10%. Boyd and Phillips (1971 a) purified the L-asparaginase of *S. marcescens* and determined some of its properties such as kinetics of reaction, catalytic activity as a function of pH, boundary sedimentation velocity, electrophoresis on polyacrylamide gel, immuno-electrophoresis and immuno-depression. Acinetobacter glutaminasificans asparaginase was purified and a study of its properties like kinetic parameters and substrate specificity carried out by Holcenberg et al., (1972), Wlodawer et al., (1977) and Steckel et al., (1983) in order to understand the dual activity of this enzyme. Both L-asparaginase and glutaminase which showed similar *Km* and *Vmax* values were found to be competitive with each other for the substrate binding site and these two enzymes were inhibited by DON, L-methionine sulfoximine, azaserine and acivicine. Tanaka et al., (1988) determined the amino acid sequence of *A. glutaminasificans* L-asparaginase and Ammon et al., (1988) reported its preliminary crystal structure. Gaffar and Shetna (1977)
isolated an asparaginase from cells of *Azotobacter vinelandii* and purified it by standard methods of enzyme purification including affinity chromatography. This enzyme had an optimum activity at pH 8.6 and 48°C; approximate molecular weight of 84,000 and apparent Km was 1.1x10^{-4} M. Metal ions or SH-reagents were not required for enzyme activity. Guy and Daniel (1982) purified a specific D-asparaginase from *Thermus aquaticus* strain T 351 and found that this enzyme was present in the organism in larger amounts than L-asparaginase and had a molecular weight of 60,000, isoelectric point 4.8 and a Km 2 mM. Curran et al., (1985) purified and characterised a specific L-asparaginase from *Thermus aquaticus* T 351, which was highly substrate (L-asparagine) and stereospecific. It had a high Km = 8.6 mM, molecular weight of 80,000, isoelectric point 4.6 and pH optimum of 9.5. The activity of the enzyme was inhibited by some substrates, asparagine concentration above 20 mM, D- and L-lysine, L-aspartic acid, and D- and L-serine, and its half life at 85°C was 40 minutes. Arrhenius plot showed a change in slope at 55°C. Abdumalikov and Nikolaev (1967) were able to purify and separate glutaminase and asparaginase from yeast *Saccharomyces cerevisiae*. Characterisation of the *S. cerevisiae* asparaginase has been done by several workers (Jones and Mortimer, 1973; Dunlop et al., 1978; and Kim and Koon, 1983). *Asparaginase-II* of *S. cerevisiae* has been intensively
investigated by Pauling et al., (1981); Kim and Roon (1983); and Kamerud and Roon (1986). Foda et al. (1980 a) studied the properties of an L-asparaginase produced by Rhodotorula rubra and found that it was highly specific for L-asparagine and L-glutamine with virtually no activity towards their amides and amino acids tested. It was heat labile to temperatures higher than 40°C, had a Km value of $1.37 \times 10^{-2}$ M and $1.55 \times 10^{-2}$ M for L-asparagine and L-glutamine, respectively. Glutaminase was isolated, partially purified and the enzyme kinetics were studied in cell free extracts obtained from the commercial baker's yeast by Auerman et al. (1982 and 1984). Only little work has been done on the purification and characterisation of fungal asparaginases. An L-asparaginase, from Fusarium tricinctum was purified and its pH optimum, Km, molecular weight and amino acid composition was established by Scheetz et al. (1971). They obtained an activity of 1000 i.u/mg protein after final purification over initial activity of 5 i.u/mg protein. The enzyme was found to be specific for L-asparagine, it did not hydrolyze D-asparagine and L-glutamine and showed a broad pH activity profile with maximum activity at pH 8, Km of the enzyme was $5.2 \times 10^{-5}$ M, and isoelectric point 5.18. Amino acid composition of the enzyme was also determined. Mangatt (1989) purified another fungal L-asparaginase from F. solani and studied its biochemical characteristics. The enzyme had single substrate specificity for L-asparagine,
with no cation requirement, $K_m = 2.6 \times 10^{-3}$ M, thermal tolerance below $50^\circ C$ and pH optima of 7.

The antitumour property of L-asparaginase and L-glutaminase has been a subject of great interest and its different aspects have been studied. Special emphasis was laid on the testing of antitumour activity of the amidases isolated from different sources. Yellin and Wriston (1966 b) found that both guinea pig serum L-asparaginase and *E. coli* asparaginase given in sufficient doses caused prolonged regression of 6 C 3 HED lymphosarcoma in C3H mice. Broome (1968) found that L-asparaginase of agouti-serum and *E. coli* caused a profound lowering in the level of free asparagine in blood and tissues of treated mice. After studying the mechanism of tumour inhibition they found that the normal cells and cells of resistant lymphomas have a higher capacity for asparagine synthesis than sensitive cells, and they also utilize endogenous asparagine, preferentially for protein synthesis. Action of L-asparaginase from guinea pig serum on lymphocytes of normal and leukemic mice was studied by Kastrikin (1972), and he also suggested a morphological test for sensitivity of malignant cells to asparaginase in vitro.

The therapeutic potential of *E. coli* L-asparaginase was brought to light by the studies carried out with this enzyme. Roberts *et al.* (1966) found that one of the L-asparaginase components isolated from *E. coli* during
DEAE-cellulose column chromatography caused complete regression of the Gardner Lymphosarcoma. They also presented a number of factors which influenced the therapeutic usefulness of the enzyme. Schwartz et al. (1966) compared the two L-asparaginases from E. coli with relation to their action against tumors, and later in 1967 Campbell et al. found that of the two L-asparaginases of E. coli only the EC-2 enzyme served as a potent anti-lymphoma agent. The effectiveness of this EC-2 enzyme from E. coli a virtually limitless source presented the possibility of extending the study of inhibition of leukemias and other tumors by L-asparaginase to species other than small rodents as suggested by Boyse et al. (1967). A survey carried out by them on 109 derived leukemias of the mice revealed that sensitivity of suppression by asparaginase was a common property of transplanted leukemias of certain classes. Ho et al., (1969), Arens et al., (1970), Ujhazy et al., (1971) and Kondrat'eva et al., (1978 a) isolated L-asparaginase active in preventing lymphoma in mice from different E. coli strains, and showed their tumouricidal properties. Ryan and Sornson (1970) found, that there was a depression of glycine in the 6 C 3 HED tumour cells after administration of E. coli or guinea pig serum, while such a decrease in cellular glycine content was not observed in tumours resistant to L-asparaginase. They suggested
that the loss of cellular glycine may be playing a more important role in tumour inhibition than loss of cellular asparaginase because of the requirement of glycine in purine synthesis. Nakamura and Tanaka (1971) found that the L-asparaginase of *E. coli* A-1-3 KY 3598 caused complete regression of the Gardner lymphosarcoma in mice. Jaffe et al., (1971) presented the result of clinical studies of leukemia, in 29 children with *E. coli* L-asparaginase. Butianu et al., (1971) obtained encouraging results in five adult patients with acute lymphoblastic leukemia (ALL) treated with *E. coli* L-asparaginase and they discussed the place of asparaginase in the treatment of ALL. Studies concerning the tumouricidal effect of the L-asparaginase including mechanism, natural sources, its isolation from *E. coli* and therapeutic application have been reviewed by Stejskalova and Necinova (1971).

Therapeutically useful asparaginase was found in other microbial sources also, viz., *Serratia marcescens* (Rowley and Wriston, 1967; Pszczolska and Krzeminski, 1969 and Boyd and Phillips, 1971 b), *Mycobacterium tuberculosis* (Reddy et al., 1969), *Achromobacter* (Roberts et al., 1972), *Acinetobacter* (Holcenberg et al., 1975 and 1983; Vistica et al., 1978; and Sommerfeld et al., 1979); *Pseudomonas fluorescence* (Mardashev et al., 1975 b; Roberts, 1976; Davidson et al., 1977 b; and Kondrat'eva et al., 1978 b), and *Vibrio succinogenes* (Durden and Distasio, 1980 and 1981).
The enzyme L-asparaginase was considered to offer a new and promising approach for the problem of treating leukemia because it was found that the cells of certain tumors needed the amide L-asparagine for survival (Hill et al., 1967; Anonymous, 1968; Old et al., 1968; Adamson and Fabro, 1968 and Teulings and Bakkeren, 1970). A laboratory test to detect dependency of tumor cells on asparagine was developed by Hill et al. (1967). Renko and Laki (1969) presented some data concerning the mechanism of the inhibition of tumor growth by E. coli L-asparaginase and found experimental evidence for modification of sensitivity of fibrinogen to the fibrin stabilizing enzyme of L-asparaginase. It was suggested that the damaging effect of L-asparaginase on the fibrin clot probably played a crucial role in the inhibition of tumor growth by the enzyme in such a manner that the super stabilized, and hence morbid, fibrin fibres were unsuitable for playing the role of a matrix for formation of stroma and artery system feeding the tumor tissue. Giani and Nicolin (1969) presented the mechanism of action of L-asparaginase and presented clinical results in the treatment of various forms of leukemia by using the enzyme alone or associated with other chemotherapeutic drugs. Cooney and Handschumacher (1970) found that the L-asparaginase was therapeutically effective against over fifty neoplasmas of the mouse, three of the rat and canine lymphosarcoma. Lymphoid tumors were the most
susceptible but the Jensen sarcoma and the Walker carcinosarcoma of rats were both responsive to the enzyme. In general, virus-induced murine leukemias, except that induced by the Rauscher leukemia virus, were not susceptible to asparaginase. The importance of L-asparagine as an inhibitor of different kinds of tumours has been stressed by Burchenal and Karnofsky (1970); Hersh (1971), Crowther (1971) and Bauvarte et al. (1978) in separate reviews.

The effect of \textit{E. coli} and \textit{Erwinia carotovora} L-asparaginases on different transplanted tumours of the mice and rat have been intensively investigated. Many tumours which have been found sensitive to L-asparaginase include L 5178 Y (Jacobs \textit{et al.}, 1970; Hofer \textit{et al.}, 1970; and Keefer \textit{et al.}, 1985), Gardner lymphosarcoma (Weinberger, 1971; Carter \textit{et al.}, 1973 and Dolowy \textit{et al.}, 1974), L 522 (Zelleck \textit{et al.}, 1975), and L 5178 Y/L-ASE (Keefer \textit{et al.}, 1985) strains.

Treatment with L-asparaginases has been found quite effective in many cases of acute lymphoblastic or lymphocytic leukemia (Astaldii, 1970; Beard \textit{et al.}, 1970; Jamra \textit{et al.}, 1970; Mathe \textit{et al.}, 1970; Smirov, 1970; Biggs \textit{et al.}, 1971; Jacquillat \textit{et al.}, 1971; Ohnuma \textit{et al.}, 1971; Pratt \textit{et al.}, 1971; Saito \textit{et al.}, 1972; Mishikova and Cap, 1978; Nesbit \textit{et al.}, 1979; and Orzechowsko-Juzwenko, 1980). This success led to various types of clinical trials. Combination chemotherapy of the L-asparaginase by taking asparaginase with other agents
like vincristine, methotrexate, cytosine arabinoside, daunomycin, daunorubicin, prednisone, etc. has shown encouraging results against ALL (Hones, et al., 1977; Amadori, 1980; Greutziz and Schellong, 1980; Rak et al., 1981; Stein et al., 1982; Advani et al., 1983, Winkler et al., 1983; Gottlieb et al., 1984; Sanchetee et al., 1986; Blauw et al., 1986; Martell and Jacobs, 1987; Buchman et al., 1988; Ortega et al., 1988 and Van et al., 1989).

The L-asparaginase therapy has also been known to produce toxicity, immunogenicity and other side effects in acute leukemia and certain other lymphomas (Hansen et al., 1970; Mathe et al., 1970; Hersh, 1971; Jacquillat et al., 1971; Biggs et al., 1971; Mishikova and Cap, 1978). They have reported that the enzyme has hepatotoxicity and also causes depression of coagulation factors. Asparaginase suppresses both humoral and cellular primary immune responses including organ allograft rejection in vivo. As the enzyme is immunosuppressive and not myelosuppressive, its use in man should be considered in the preventive therapy of organ allograft rejection (Hersh, 1971). *E. coli* asparaginase causes marked decrease in spleen lymphocytes of B-cell lineage (Distasio et al, 1982).

The L-asparaginases have been modified with modulator and put to clinical use, and more pronounced results have been obtained in leukemias. These conjugated asparaginases are more active, stable, without significant
immune responses and have greatly increased plasma half lives (Bendich et al., 1983; Janka et al., 1984; Wileman et al., 1986; Ho et al., 1986; and 1987; Viau et al., 1986; Macewen et al., 1987; and Yulaev et al., 1987). Immobilised L-asparaginase has also been used in cancer treatment (Edman et al., 1983; Karskevich et al., 1986 and 1987; Mikhailin et al., 1986; Ampon et al., 1988; Karskevich et al., 1988).

The L-asparaginase therapy has been found effective in other types of cancers as well. These include tumours of the breast, colon, rectum, lung, melanoma, lymphoma and undifferentiated leukemia (Livingston and Carter, 1970; Hortobagi et al., 1980; and Prager and Braechtell, 1988), acute myeloblastic leukemia (Hansen and Canelllos, 1970 and Livingston and Carter, 1970 and Auber, 1988), non-Hodgkin lymphoma (Otten et al., 1981, Vecchi, 1986; and Yumura et al., 1989) and chronic myelogenous leukemia (Spiro et al., 1981).

The promising therapeutic results given by these L-asparaginases have led to several studies concerned with the optimization of their production from the potential microorganisms under cultural conditions. Some leading works include manipulation of the culture media (Roberts et al., 1968; Zanin et al., 1969; Beyart and Voets, 1970; Peterson and Giegler, 1972; Prusinier, 1975; Eremenko et al., 1975; Garaev and Golub, 1977; Barnes et al., 1977; Netrval, 1977; Smirnova et al., 1979; Cook et al., 1981
and Ling et al., 1982) and physical factors governing
growth of the organism (Boeck et al., 1970; Varrichio,
1972; Chaikovskaya et al., 1974; Peterson and Nelson,
1975; Maksimov et al., 1975; and Mangatt et al., 1986).
It appeared that the enzyme production was substantially
regulated by certain nutrients, especially the amino
acids in the medium, pH and aeration of the culture.
Since the enzyme production is constitutive, variation
in the amounts produced was positively inductive in
almost all of the microorganisms investigated in these
studies.

Further studies are being done for obtaining
new and potent sources of the amidases from the microbial
world. Since these enzymes are still under active investi-
gation, their full therapeutic spectra remain to be
deefined. At the present time, L-asparaginase appears to
be most effective for the induction of remission in
acute lymphoblastic leukemia.