DISCUSSION

DISCUSSION

Arginase, a wide spread enzyme in nature, catalyses the hydrolysis of L-arginine to ornithine and urea serving as a terminal link in the chain of reactions that ureotelic animals, some plants and microorganisms use for the disposal of ammonia (Cabello, Basilio and Prajoux, 1961).

Most malignant mammalian tumours, sarcomas, carcinomas and embryonic tissues have been shown to have high arginase activity compared to normal tissue (Edlbacher and Merz, 1927; Klein and Ziese, 1932 b; Kaiju, 1939; Smith and Richterich, 1957). Fuchs (1921), however, reported a decreased arginase content in carcinoma tissue of liver. Rous chicken sarcoma (Edlbacher and Merz, 1927) and mouse hepatomas (Greenstein, Edwards, Andervont and White, 1942) also have been shown to have low arginase activity. In scorsonera crown-gall tumour tissue no arginase activity has been detected and the arginine is found to be metabolised by pathways involving various guanidine compounds (Menage and Morrel, 1964).

In the present case the arginase activity is found to be high in tumour tissues than in the corresponding normal tissues. The activity in Parthenocissus and tobacco tissue is found to be low compared to Rumex tissue and no evidence could

be obtained that in the former two tissues arginine is metabolised by any other pathway. The very low activity in these two tissues can partly be ascribed to the inhibitory metal ions present in the tissues and partly to the presence of some inhibitory material in the residue fraction of their homogenates. It has, however, not been possible to solubilize the inhibitor from the residue fraction of Parthenocissus normal tissue homogenate.

The fact that arginase is known to be sensitive to metal ions and the ashed supernatant of the parthenocissus normal tissue homogenate showed inhibition, suggested that the low activity in these tissues may be due to the presence of various metal ions in the cultivation medium. Cultivation of these tissues in the absence of trace elements raised the specific activity of arginase in all the cases. Of the various trace elements Cu, Zn and Ni are found to be responsible for the decrease in enzyme activity.

The enzyme in the present case purified 81 fold from Rumex tumour tissue is found to be very unstable in contrast to that isolated from other sources (Kocholaty and Kocholaty, 1941; Mohamed and Greenberg, 1945; Bach, Hawkins and Swaine, 1963) which is active even after a storage period of several months. The enzyme activity in the present case is completely lost in 6-8 hours at 0°. $MnCl_2$ is able to stabilize the enzyme only partially when added during purification. However, any

further addition of $MnCl_2$ or albumin could not afford any protection towards inactivation.

Regarding the characteristics of the Rumex enzyme the pH optimum is found to be 10.5 using carbonate-bicarbonate buffer. Arginase from other sources have been shown to have pH optimum between 9.0 - 10.5 (Edlbacher and Simons, 1927; Felix, Muller and Dirr, 1928; Vovchenko, 1936; Mohamed and Greenberg, 1945, Roholt and Greenberg, 1956; Bach and Killip, 1961; Splittstosser, 1969). Mohamed and Greenberg (1945) and Lenti (1946) reported that citrate and borate buffer inhibit arginase activity. A possible explanation for this effect was provided that these acids form complex with the activating metal ions. In the present case also the activity is found to be affected by the nature of the buffer used. With tris-HCl buffer the activity is high compared to other two buffers whereas with glycine-NaOH buffer the activity is almost half that in case of carbonate-bicarbonate buffer. This is in contrast to the data of Robbins and Shields (1956) for bovine liver arginase where the activity in glycine-NaOH buffer has been shown to be more than in carbonate-bicarbonate buffer.

A major variable in the kinetics of the arginase reaction is the pH. This is logical in view of the amphoteric nature of both substrate and enzyme. Mohamed and Greenberg (1945) from an examination of the titration curve of arginine suggested that it is possible that the monovalent cation is

the only form of the arginine susceptible to attack by the enzyme. The only possible alternative is that it is the dipolar ion of arginine that reacts to form the active enzyme substrate intermediate. Similar reasoning leads to the conclusion that the active form of the enzyme is either in the isoelectric or anionic state. However, these authors favour the view that the active form of arginine is the monovalent cation and the active form of arginase is anionic. In the present case also it is found that a specific ionic form of the enzyme and substrate is involved in the formation of active enzyme-substrate complex and the extent of ionization is affected by the nature and concentration of buffer ion used. The role of buffer ions is further evident from the studies on the sequence of addition of components of the assay system. When the reaction was started by adding buffer in the last, an ineffective complex was formed giving only about 30-40 percent activity. Further studies along these lines suggested the possibility that there are three sites on the enzyme molecule and atleast two of them bind with the substrate and one of these sites combines with the substrate through Mn only when it is ionised by the buffer. The other site seems to regulate the binding of Mn to the active site. This is, however, only a tentative explanation and other possibilities cannot be ruled out.

The enzyme arginase has been shown to act in most of the cases on L-arginine (Greenberg, 1951; Bach, Hawkins and Swaine, 1963; Ramaley and Bernlohr, 1966) and in some cases on D-arginine (Felix and Morinaka, 1924; Stock , Perkins and Hellerman, 1938) and Canavanine (Kitagawa and Eguchi, 1938; Damodaran and Narayanan, 1940). In the present case the enzyme acts on L-arginine but has no action on canavanine, agmatine, γ-guanidino butyric acid, guanidino acetic acid and p-tosyl arginine methyl ester. Hunter (1938) and Hunter and Woodward (1941) reported that free guanidino and carboxyl groups of arginine are essential for the action of arginase. The α-NH_2 group can be substituted or even replaced by a -OH group without loss of activity. The length of carbon chain is also important for the enzyme activity (Thomas, 1914; Thomas, Kapfhammer and Flaschentrager, 1922). In the present case the presence of guanidine, carboxyl and α-NH_2 group seems to be essential for enzyme activity.

The Rumex enzyme shows an absolute requirement for Mn ions. In contrast to the characteristics reported from other sources (Hellerman and Stock, 1936; Anderson, 1945), the Rumex enzyme is not activated by Co and Ni ions but is inhibited by Zn, Cu, Ni and Co ions. The effect of a particular activating ion on an enzyme may vary not only with the substrate and with pH but also with the purity and age of the enzyme. Partially purified arginase is activated

by Co, Mn and Ni (in descending order of effectiveness) but the highly purified enzyme is activated by Mn only (Bach and Whitehouse, 1954). It has been suggested that the metal may act as a binding link between enzyme and substrate combining with both and so holding the substrate at the active centre of the enzyme. Hellerman and Perkins (1935) and Hellerman and Stock (1936) suggested that in case of arginase Mn forms a chelate compound with two or three groups in the substrate and with a similar number of groups in the enzyme. The data obtained in the present case by altering the sequence of addition of assay components also suggest a similar mechanism.

The substrate concentration curve in the present case is found to be sigmoidal or 'S' shaped. The enzyme is inhibited by ornithine, one of the products of reaction but not by urea. In this respect it resembles the enzyme reported from other sources (Mourgue and Baret, 1956; Bach, Crook and Williamson 1944; Edlbacher and Zeller, 1936). It is also inhibited by γ-guanidino butyric acid, guanidino acetic acid, lysine and agmatine which are very similar in structure to arginine. The inhibition by these compounds is found to be competitive. The enzyme has also been found to be inhibited by various drugs. In this respect it resembles the enzyme from goat liver (Iyer, Arora, Krishnamurti and Malaviya, 1953).

It has been reported that arginase is inhibited by oxygen due to the oxidation of the cysteine group in the enzyme molecule

and ascorbic acid and cysteine are able to reverse this inhibition (Salazkin and Solovev, 1931). The Rumex enzyme is also inhibited by Hg^{++} ions, p-CMB and iodoacetate. The inhibition of p-CMB can be reversed by cysteine showing the involvement of -SH groups in the enzyme molecule.

The most important observation made in the present case is the competitive inhibition of arginase by purine and pyrimidine bases which bear no structural similarity to the substrate arginine. In order to inhibit the enzyme activity the molecule must have a ring and a polar group in the 2nd position of the ring. The 9th position of purine ring must also be free.

Further studies on the kinetics of the inhibition by agmatine, ornithine, lysine, guanidino acetic acid on the one hand and xanthine, guanine and uracil on the other showed that in both cases the double reciprocal plots do not follow a normal Michaelis Menten kinetics but there is a significant deviation from straight lines in the plot. Also the Hill plots resulted in straight lines and the slope of the lines in presence of inhibitors deviated significantly from that of without inhibitor. The slope n of the lines obtained in Hill plots has been interpreted by a number of workers as a function of the interaction of substrate or effector binding sites and of the strength of this interaction (Taketa and Pogell,1965; Atkinson, 1966; Corwin and Fanning, 1968; Changeux,1963). On

this basis one might assume an interaction of at least two binding sites for arginine. However, when reciprocal of the reaction velocity was plotted against varying inhibitor concentrations a homotropic cooperative effect was also observed when the enzyme was saturated with xanthine, guanine or uracil (in the presence of substrate) indicated by a significant concave upward curvature. This suggests the cooperative interaction of the inhibitor binding sites. In case of agmatine, ornithine, lysine and guanidino acetic acid no such concave curvature was obtained eventhough the Hill plots showed a significant increase in n value. Thus no conclusive evidence could be obtained from these studies about the binding sites of the two groups of inhibitors. The plots of asymptote slopes against inhibitor concentration, except in the case of agmatine did not give a straight line which may again indicate cooperativity. Attempts made to desensitize the enzyme by urea or differential heat-treatment in presence of inhibitors, however, did not give any indication of the difference between the inhibition by these two groups of compounds.

Monod, Wyman and Changeux (1965) in their plausible model, distinguish between K system and V system to explain the effect of an allosteric inhibitor by its trapping an enzyme form with either reduced affinity toward the substrate or reduced maximum reaction rate. It seems that in the present case the enzyme belongs to K system.

The fact that the kinetic studies suggested the allosteric interaction of atleast purines and pyrimidines with the enzyme may be interpreted in terms of a regulation of urea production in this tissue by these compounds. Purines and pyrimidines are also known to be metabolised to urea (Smellie, 1955). No urease activity has been detected in this tissue. Thus the level of urea in the tissue seems to be controlled by purines and pyrimidines. It has also been reported that arginine level controls the synthesis of uracil by affecting Aspartate transcarbamylase activity in E.coli (Ben-Ishai, Lahav and Zamir, 1964). These two mechanisms thus may control nucleic acid and arginine production. In the early growth period when the synthesis of nucleic acids proceeds at a high rate the arginine level is kept low by arginase whereas when the level of purines and pyrimidines increase in the tissue they control urea level by inhibiting arginase. Work along these lines is being continued to see whether arginine has any control on uracil biosynthesis in this tissue.