INTRODUCTION

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A number of approaches have been made with a view to identify the biochemical differences between normal and neoplastic tissues of animal origin. The first empirical suggestion of a metabolic difference between normal and cancer tissue came from the observation of Warburg (1930) that tumour slices show a high rate of glucose utilization and lactic acid production under both aerobic and anaerobic conditions. However, the glycogen content was found to be lowered in hepatoma in spite of increased glucose utilization. Glycogenesis has been found to be reduced in hepatomas (Weber, Morris, Love and Ashmore, 1961; Sweeny, Ashmore, Morris and Weber, 1963; Nigam, MacDonald and Cantero, 1962). The enzyme α-glucan phosphorylase is markedly decreased in a number of mouse hepatomas (Nirenberg, 1958). Lesions have also been reported in the activities of phosphoglucomutase and UDP-glucose-glycogen-glucosyltransferase (Nigam, MacDonald and Cantero, 1962).

The enzymes concerned with the formation of glucose viz. phosphoglucomutase, α-glucan phosphorylase, glucose-6-phosphatase as well as hexose diphosphatase were found to be reduced (Nirenberg, 1958; Nigam,1965; Weber,1961; Shonk, Morris and Boxer,1965a; Weber and Cantero, 1955, 1956, 1959;Goldberg and Colowick,1965). On the other hand many enzymes concerned with glycolysis such as hexokinase, glucosephosphate isomerase, aldolase, triosephosphate isomerase, pyruvate kinase and lactate dehydrogenase were found to be increased in cancer (Sharma, Sharma, Donnelly, Morris and Weinhouse,1965; Bodansky,1954a,b;1955,1956,1961; Sibley and Lehninger,1949; Bierman, Hill, Reinhardt and Emory, 1957). These observations suggest an increase in glycolysis and a decrease in gluconeogenesis. Suggestions have also been made that differences in glycolysis are in part due to the differences in the availability of inorganic phosphate required for the regeneration of ATP. The effect of inorganic phosphate appears to be due to its facilitating effect on glyceraldehyde phosphate dehydrogenase (Racker, Wu, and Alpers, 1960). Suggestion has also been made that its primary effect is exerted over the control of phosphofructokinase activity (Wu, 1965).

Glucose-6-phosphate dehydrogenase activity has been found to be increased in Novikoff hepatoma (Sweeny,Ashmore, Morris and Weber, 1963). The increase in this enzyme associated with the decrease in glucose-6-phosphatase and hexose diphosphatase suggest the channelling of glucose-6-phosphate for energy production and ribose formation.

The suggestion of a disturbance in aerobic metabolism led to studies on the respiratory cycle. Though, Brown, Katz and Chaikoff (1956) found evidence for the operation of tricarboxylic acid cycle in tumour, Potter (1950) found a decrease in the activity of citrate synthase in tumour tissue which led him to hypothesize that in the face of this deficiency oxaloacetate may successively be converted to orotic acid and uracil and help in an increased synthesis of nucleic acids. Evidence was also obtained for a decrease in the activities of other respiratory enzymes such as succinate dehydrogenase and cytochrome oxidase (Schneider and Potter, 1943). Also the addition of substrates of the respiratory cycle to normal tissue was found to markedly stimulate respiration but to have no effect on tumour tissue (Greenstein, 1954). Further, while normal tissues had a wide range in the activities of these enzymes, tumour tissues showed only a narrow range pointing to a particular enzyme "profile" in all cancer tissues (Schneider and Potter, 1943; Carruthers and Suhtzeff, 1950). The inference seemed plausible that not only is the respiration rate in tumour tissue low but also that it represents its maximum respiratory potential.

Reference was made earlier to the suggestion that there may be a disturbance in oxidative phosphorylation and electron transfer system in neoplasm. Positive support for the same came from the observation of a decrease in cytochrome-C, NADH-cyt-C reductase and NAD-NADP transhydrogenase in tumour tissue (Reynafarje and Potter, 1957). In fact, NADH-cyt-C reductase was found to be altogether absent in some of the tissues. The above observations were consistent with the fewer mitochondria in cancer cells and an abnormal swelling of the same and decrease in oxidative phosphorylation (Emmelot and Bos, 1961).

A reduced level of NAD and NADP appears to be a common feature of tumour tissues (Kensler, Sugiura and Rhoads, 1940; Jedeikin and Weinhouse, 1955; Glock and McLean, 1955, 1957; Narurkar, Kumta and Sahasrabudhe, 1957). The low level in malignant tissues is explained in terms of a preferential utilization of the adenine moiety for the synthesis of nucleic acids (Sahasrabudhe, 1958) imposed by a rapid cellular division characteristic of neoplastic tissue(Morton, 1958; 1961). Deletion of enzymes involved in biosynthesis (Branster and Morton, 1956; Erbe, Preiss, Seifert and Hilz, 1966) or the elevation of enzyme, NAD glycohydrolase, (Erbe, Preiss, Seifert and Hilz, 1966; Williams-Ashman and Kennedy, 1952; Green and Bodansky, 1962, 1963; Waravdekar and Griffin, 1964) involved in the degradation has also been implicated.

The tumour tissues have been found to contain more free amino acids than in the normal tissues (Kotake and Ohsuka, 1953). This has been attributed to the greater capacity of tumour tissues to concentrate free amino acids and compete with other tissues for the same (Goodlad, 1964).

Tumour tissues have been shown to have higher levels of alanine, glycine and proline but less of aspartate and glutamine. Glutamine was found to be very low or almost absent in tumours (Roberts and Frankel, 1949; Kit and Awapara, 1953; Roberts and Borges, 1955; Roberts and Tanaka, 1956; Sassenrath, Welch and Greenberg, 1958). A variety of tumour tissues growing *in vivo* were also shown to possess significant peptidase activity (Wu and Bauer, 1963). Differences have also been found in the utilization of amino acids such as glutamine (Roberts and Borges,1955). Tumour tissues have generally been found to have a markedly reduced or no glutamine synthetase activity (Wu and Bauer, 1960; Abraham, Chaikoff and Cady, 1961; Brown, Katz and Chaikoff, 1956; El-Asmer and Greenberg, 1966). A number of tumour tissues have been found to have a greater arginase activity (Thomas and Britta, 1957; Edlbacher and Merz,1927; Hidekatsu, 1929). Slow growing tumour tissues were found to be richer in arginase than fast growing ones (Bach and Lasnitzki, 1946).

Auerbach and Weisman (1958) while working on Novikoff hepatoma found that tryptophan oxygenase, tyrosine aminotransferase, phenylalanine hydroxylase, threonine and serine dehydratase are either absent or present only in small amounts. However, Pitot, Potter and Morris (1961) found in hepatoma 5123 these enzymes at a significant levels of activity.

A similar increase in nucleic acid synthesis is suggested by several studies. The formation of purines and pyrimidines from labelled precursors is not affected by aminouridine in hepatoma as it is in normal liver, pointing to alternate pathways for their synthesis (Werkheiser and Visser, 1955). Uracil-2-C^{14} and C^{14} adenine and P^{32} are all incorporated in DNA or RNA or both at a higher rate in hepatoma than in normal liver (Rutman, Cantarow and Paschkis, 1954; Griffin, Davis and Tifft, 1952). At the same time ribonucleases were found to be lowered in many malignant cells (Ledoux, Brandli and Paepe, 1958). The capacity of tumour tissue to catabolize purine and purine nucleotide would appear to be reduced on the basis of the reported absence of one or other of the enzyme, deoxycytidylate deaminase, thymine reductase, xanthine oxidase and uricase in several tumours (Potter, Pitot and Ono, 1960; Ono Blair, Potter and Morris, 1963; De Lamirande, Allard and Cantero, 1958).

It will be evident from the foregoing that a considerable volume of work has been done on the normal and tumour tissues of animal origin, on the other hand very little work has been done on the metabolism of normal and tumour tissues of plant origin. Crown-gall tumour tissues have been reported to have reduced respiratory levels as compared to normal tissue (Neish and Hibbert, 1943a; White 1945; Link and Goddard, 1951; Eberts, Burris and Riker, 1951; Klein, 1952; Lioret, 1952, 1953;

Hildebrandt, Riker and Watertor, 1954). White (1945) found that aerobic rate of breakdown of carbohydrate is lower in tumours of sunflower than in the normal tissue. In contrast Link and Goddard (1951) and Klein (1952) reported that oxygen consumption was greater in tomato tumour than in normal tissues.

Investigations on the respiratory metabolism of mitochondria from callus and wound virus tumour tissues of Rumex acetosa L. revealed that callus mitochondria oxidized Krebs cycle acids at a rate two to three times greater than tumour mitochondria. Further the number of mitochondria per cell was significantly greater in the cells of callus tissue cultures (Gentile, 1963). No difference appeared, however, between the phosphorylating efficiencies of the mitochondria from callus and tumour tissues (Coles and Gentile, 1965).

In a series of papers Tamaoki and coworkers (1959a,b; 1960, 1961) reported the results of studies on the oxidative and phosphorylative activities of mitochondria from tomato. The differences in oxidative and phosphorylative activities found in the mitochondria isolated from normal and crown-gall tissue cultures were quantitative rather than qualitative in nature. Mitochondrial particles isolated from normal tissue always showed higher activities for DPNH oxidase, DPNH cytochrome-C reductase, cytochrome-C oxidase and diaphorase than did crown-gall particles. It was concluded from these studies that normal and crown-gall tissue cultures have virtually identical pathways for the transport of electrons from DPNH to oxygen. However, the rate of oxidation and the P/O ratio was less in mitochondria isolated from tumour tissue.

Lance (1961) reported that mitochondria isolated from tumour tissue of Scorsonera possess twice the succinic and malic dehydrogenase activity per unit of protein nitrogen as compared to normal tissue. Scott, Craigie and Smillie (1964) have compared the enzyme of hexose monophosphate shunt, glycolysis and TCA cycle in the homogenates of normal and tumour tissues of red beet roots. They found that the activities of phosphohexoisomerase, phosphofructokinase and 3-phosphoglycerate kinase were slightly lower in tumour tissue while glucose-6-phosphate dehydrogenase and phosphoribose isomerase activities were increased two to three fold in tumour. The activities of the remainder of the enzymes were similar in both types of tissues. They have also recorded a C_6/C_1 ratio of less than unity for both tissues and have suggested that tumours have a greater potential to oxidatively decarboxylate hexose to ribose-5-phosphate than normal tissues.

A number of investigators (Harvey, 1920; Riker and Keitt, 1926; Klein and Ziese, 1932a; 1933; Nagy, Riker and Peterson, 1938) have shown that catalase, peroxidase and oxidase (tyrosinase) activity to be higher in tumour tissues obtained from a number of plant specimens than in comparable normal tissues. The increased catalase activity found by Kelin and Ziese (1932a) was shown to be a function of the tumour tissues rather than the pathogen since the crown-gall bacteria itself possessed only a very slight catalase activity. Nagy, Riker and Peterson (1938) reported the presence of an active tyrosinase in tumour tissue but not in normal tissue of tomato. Levi, Michaelis and Hibbert, (1943) found tyrosinase and peroxidase to be far more active in tumour tissue of the beet than in normal tissue. Ascorbic acid oxidase on the other hand was less active in tumour tissue.

It is also observed that marked differences exist in nitrogenous constituents when normal resting cells are compared with actively dividing tumour cells (Klein and Keysener, 1932; Nagy, Riker and Peterson, 1938; Neish and Hibbert, 1940, 1943a,b; Klein, 1952). Neish and Hibbert(1943b) found that while about the same amount of non-protein nitrogen exists in beet tumour and normal tissue, tumours had three times more protein than that found in normal tissues. Water soluble protein was found by these authors to be 6 times in tumour than in normal tissue. The tumours, moreover, maintained 64% of the Kjeldahl nitrogen in the form of protein as compared to 39% of normal tissue. Lee (1952) compared the crown-gall, habituated and normal tissue of European grapes and found that crown-gall tissues had the highest concentration of total and soluble nitrogen while the normal tissue had the smallest amount of these constituents.

Morel and Duranton (1958) reported that when the tissues of Jerusalem artichoke tubers, which contain a large amount of arginine during the resting period, are cultivated in vitro the arginine disappears rapidly with an increase in proline, hydroxyproline, glutamine and glutamic acid. However, tumour tissues of the same plants showed that arginine undergoes very different transformation in these tissues, leading to various guanidylated compounds. Menage and Morel (1964) showed that in scorsonera crown-gall tissue also arginine metabolism is peculiar. No arginase activity was detected but the arginine was transformed into several amidines one of them was identified as γ-guanidino butyric acid.

Studies carried out in this laboratory on Rumex acetosa tissue showed that the level of oxidized pyridino nucleotide is low in tumour than in normal tissue (Maini, Srivastava and Ramakrishnan, 1966). The enzyme NAD glycohydrolase is present in tumour tissue but absent in normal tissue. In tumour tissue this enzyme is present in the form of two isoenzymes localized in different subcellular fractions (Srivastava, Maini and Ramakrishnan, 1969a). The enzyme nicotinamide amidohydrolase is also present in the tumour tissue and almost absent in normal tissue. The synthesis of this enzyme is induced by its substrate, nicotinamide and suppressed by 2,4-D if present in the cultivation medium (Srivastava, Maini and Ramakrishnan, 1969b). This enzyme has been shown to play an important role in the regulation of pyridino nucleotide level in these tissues (Srivastava, Maini and Ramakrishnan, 1970).

Arginine metabolism

The amidine group of L arginine represents but a small portion of the arginine molecule, it is however, endowed with high metabolic lability and with a large experimental literature. Most important exploration of arginine metabolism stem from Krebs and Henseleit's (1932) formulation of ornithine-urea cycle. This formulation was suggested by the observations that the rate of urea formation by liver slices was greatly accelerated by the addition of ornithine. Since addition of one mole of ornithine led to production of approximately thirty times as much urea, it was postulated that an intermediate was formed from ornithine, ammonia and carbon dioxide which would yield urea and also regenerate ornithine. It was subsequently found that citrulline also has a catalytic effect on urea synthesis. Krebs and Henseleit also suggested a primary function to arginine, as the immediate precursor of urea in the mammalian liver, mediated by arginase.

Studies with isotopically labeled compounds on intact animals (Foster, Schoenheimer and Rittenberg, 1939; Clutton, Schoenheimer and Rittenberg, 1940) confirmed the general mechanisms proposed by Krebs and Henseleit. There is now evidence for all or most of the reactions of this cycle in Neurospora, E.coli, Penicillium, several lactic acid bacteria (Srb and Horowitz, 1944; Bonner, 1946; Volcani and Snell,1948; Hogg and Elliott, 1951; Wu and Hogg, 1952; Abelson, Bolton and Aldous, 1952), invertebrates (Campbell and Bishop,1963) and plants (Coleman, 1958; Baker and Thompson, 1962) as well as vertebrates.

Apart from its role in urea cycle, arginine has also been implicated with growth of tumour tissues. It has been reported that arginine has a stimulatory effect on the growth of transplanted tumours, rat carcinoma, and guerin carcinoma (Gilroy, 1930; Suzuki and Miyao, 1933; Tokuyama and Nakahara, 1937; Biraben, Delmon and Ollivier, 1961). Bach and Lasnitzki (1947) observed that addition of arginine to the cultures of mouse adrenocarcinoma causes a significant increase in mitosis of the tumour cells. However, normal epithelial cells from mouse lung were unaffected.

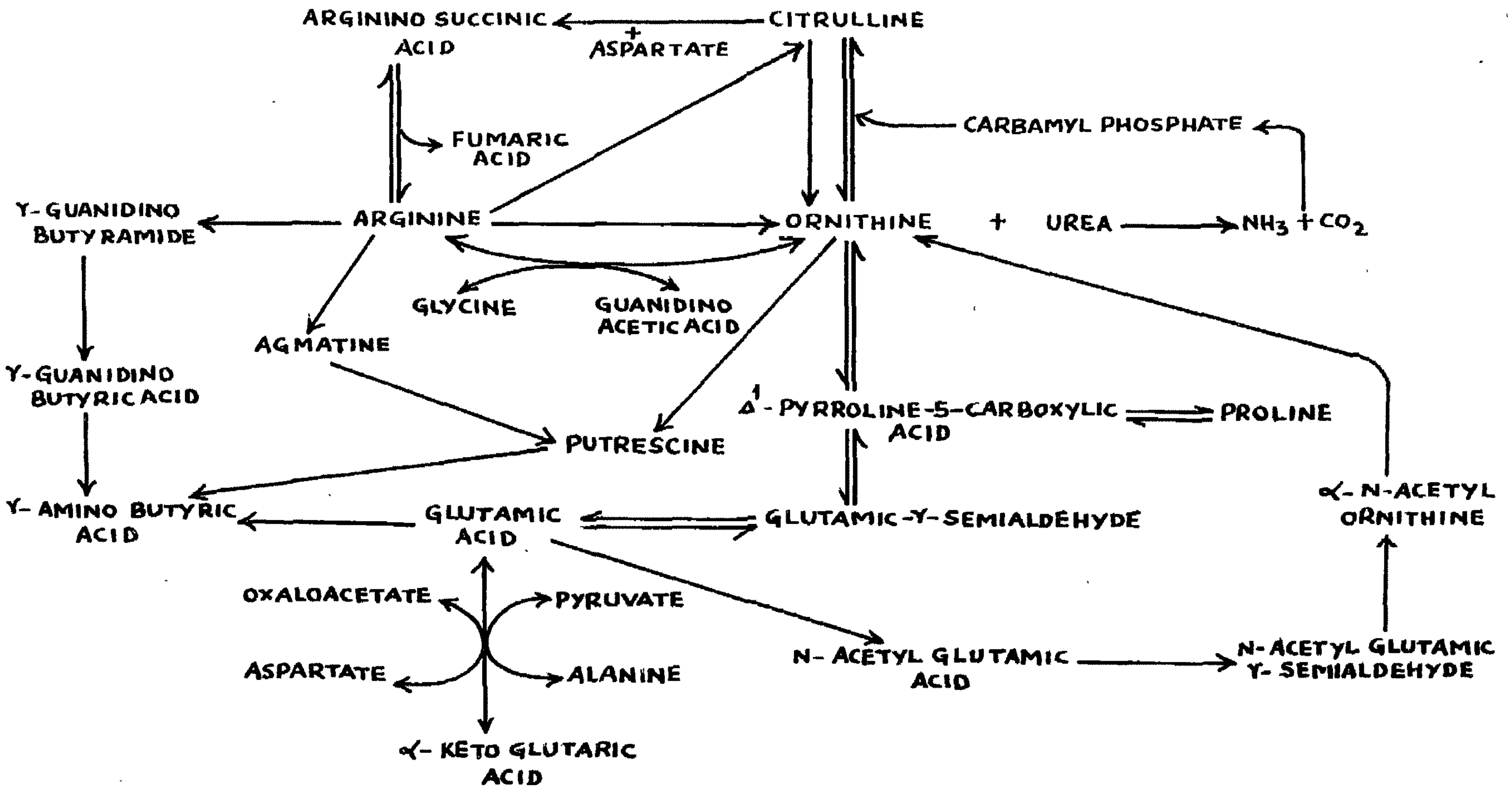
Arginine has also been shown to induce arginase activity in the freshly isolated embryonic chick and mouse cells but not in the established cell strains (Klein, 1960). However, Gustafson and Klein (1961) showed that arginine alone cannot induce the enzyme in the cells of mouse fibroblasts, sarcomas and carcinomas but addition of RNA along with arginine induces the formation of arginase.

Formation of arginine :

The formation of arginine from citrulline was demonstrated in livers and kidney preparations (Borsook and Dubnoff,1941; Krebs, 1942) and this conversion was found to be stimulated by either aspartate or glutamate. Studies by Ratner (1954, 1955, 1962) showed that arginine formation from citrulline specifically requires aspartate as well as ATP and the reaction occurs in two steps involving the intermediate formation of a compound identified as argininosuccinic acid (Ratner, 1962; Davison and Elliott,1952; Walker, 1953). Enzymatic clevage of the latter compound gives arginine and fumaric acid. The sequence of events is given in scheme - 1.

The citrulline required for the above reaction is obtained by the ornithine transcarbamylase reaction involving ornithine and carbamyl phosphate (Meister, 1965a). The ornithine in turn is obtained either from the breakdown of arginine or from glutamate or proline by a sequence of reactions shown in scheme - 1.

Several investigators have examined the mechanism of regulation of arginine biosynthesis in E.coli (Gorini,1960; Gorini Gundersen and Burger,1961; Vogel, 1961; Mass, 1961;Gorini and Mass, 1957; Mahler, Newmann and Marmur, 1963). There is a



Scheme 1. Interrelationship between arginine and glutamic acid metabolism

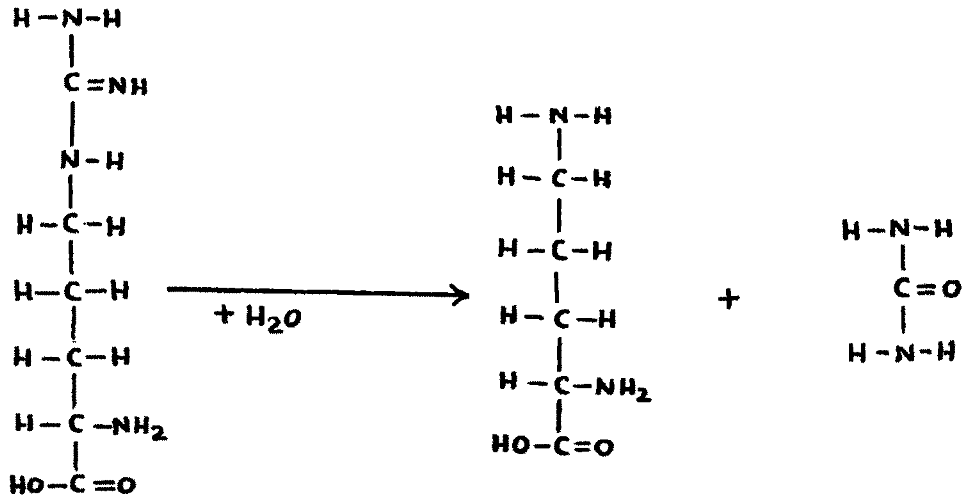
general agreement that arginine represses the synthesis of all the enzymes involved in the conversion of acetyl glutamate to arginine. The biosynthesis of arginine is also controlled by end product inhibition (Vyas and Mass, 1963).

Breakdown of arginine :

Arginine has been shown to be converted to citrulline and then to ornithine by arginine dihydrolase system involving arginine desimidase and citrullinase in certain micro-organisms (Oginsky and Gehrig, 1953; Slade, 1953). In streptomyces griseus the arginine is found to be converted to γ-guanidinobutyramide by oxidative decarboxylation, this compound is enzymatically hydrolysed to γ-guanidinobutyric acid (Meister, 1965a). Arginine can also be decarboxylated to agmatine (Gale, 1940) or oxidatively deaminated to the corresponding keto acid by L-amino acid oxidase (Boulanger, Bertrand and Osteux, 1957). It has also been reported that the guanidine group of arginine can be transferred to various acceptors like glycine, ornithine, canaline and hydroxylamine in presence of the enzyme transamidinase (Walker, 1956a,b, 1957, 1958; Ratner and Rochovansky, 1956). However, the hydrolytic clevage of arginine to ornithine and urea by the action of arginase is the most thoroughly investigated of the enzyme mechanisms concerned with arginine degradation.

Arginase

Arginase catalyses the reaction represented by the following equation. No intermediate steps have been observed in the reaction.



Arginine Ornithine Urea

The existence of a urea producing ferment in the mammalian liver was first mentioned by Richet (1894,1897). Kossel and Dakin (1904a,b) demonstrated the specific hydrolysis of arginine by extracts of liver. The metabolic role of this enzyme in the liver was made clear when Krebs and Henseleit (1932) established the cycle for conversion of amino nitrogen to urea by the stepwise conversion of ornithine, first to citrulline and then to arginine. The cycle was completed by the hydrolysis of the latter to ornithine and urea by arginase.

Distribution in nature :

The best source of arginase is the mammalian liver (Fuchs, 1931). According to Folley and Greenbaum (1946) the mammary gland is second to liver in its arginase content. Other sizable sources of arginase in the mammal are the testes and kidney. No arginase is found in the livers of birds and reptiles. This led Clementi (1914, 1919) to formulate the rule that arginase occurs in the livers of vertebrates that have a ureotelic metabolism but not in livers of animals that have a uricotelic metabolism. This rule has been amply verified.

The distribution of arginase is not limited to vertebrates. Baldwin (1935) showed that large amounts of arginase occur in the hepatopancreas of terestrial and fresh water gastropods.

Arginase also occurs in plants and microorganisms. Examples are jack bean (Stock, Perkins and Hellerman, 1938; Anderson, 1945), Lathyrus sativus (Cheema, Padmanaban and Sarma, 1969), Agaricus compestris, ergot, *A. niger* (Kiesel, 1922a,b), Neurospora crassa (Srb and Horowitz, 1944; Cabello, Urba, Prajoux and Basilio, 1959), Microsporum, Epidermophytum and Fusarium (Podgainaya and Sukhenko, 1959), *Bacillus licheniformis* (Ramaley and Bernlohr, 1966) and yeast (Edlbacher, Becker and Segessa, 1938).

Growth and neoplasm :

The possible significance of arginase for growth is reviewed by Baldwin (1935), who is of the opinion that the high arginase content of the testes which increases suddenly at puberty, points to a corelation between growth and high arginase content. The observation of high arginase content in the lactating mammary gland and more recent results on the arginase in neoplasms lessens the significance of the above view.

Edlbacher and Merz (1927) reported that all malignant mammalian tumours, sarcomas and carcinomas show a higher arginase activity than that of normal tissue. They also reported that mammalian and avian tumours differ in their arginase content corresponding to the differences in the metabolism of these two classes of animals. The relatively low arginase value of Rous chicken sarcoma represents, atleast one, if not the essential, difference in metabolism between this and mammalian tumour. Increased arginase content has also been reported in a number of other tumour tissues. Examples are gastric carcinoma (Kaiju,1939), spontaneous mammary tumours (Greenstein, Jenrette, Mider and Andervont, 1941), mammary adrenocarcinoma (Smith and Richterich,1957), transplanted mouse tumours (Sylven and Bois, 1960). However, a number of other tumours like rat and mouse hepatomas

(Greenstein, Edwards, Andervont and White, 1942; Greenstein and Thompson, 1944) and ascites hepatoma (Miura, Furiya, Aso, Imadome, Nishimura and Urushibara, 1961; Urushibara, 1961) have been reported to have low arginase activity.

Bach and Lasnitzki (1947) found that the arginase content of slowly growing tissues of the same tumour strain was twice as high as that of the fast growing tissue. This suggests that arginase activity resists the proliferation of the tumour. Therefore, the enzyme may be part of a defence mechanism that is called into action at the onset of malignancy and which, by its mode of action may influence the further course of the malignant growth.

Effect of nutritional state and endocrines :

The nutritional status of mammals particularly with respect to protein (Lightbody and Kleinman, 1939; Mandelstan and Yudkin, 1952; Ashida and Harper, 1961; Deosthale and Tulpule, 1969), Mn^{++} (Boyer, Shaw and Phillips, 1942; Mohamed and Greenberg, 1945), age (Lightbody, 1938) and sex (Greenberg, 1951) strongly influence liver arginase content. In general male animals have a higher arginase content than females.

An interesting endocrine control of arginase activity has also been observed (Fraenkel-Conrat, Simpson and Evans, 1942a,b). This apparently is exerted by the adrenocortical hormones. Both hypophysectomy and adrenalectomy caused a reduction in arginase activity (Kochakian and Robertson, 1950; Bach, Carter and Killip, 1958) which could be restored by administration of corticosteroids (Kochakian, 1944, 1945; Kochakian and Stettner, 1948; Folley and Watson, 1951). The results appear understandable on grounds of the protein catabolic influence of the adrenal cortex. Lowered adrenocortical hormone function results in a reduced requirement for arginase to metabolise nitrogen whereas increased hormone function leads to an increased requirement.

Localization :

Arginase occurs in the nuclei of mammalian liver cells as well as in the cytoplasm (Behrens, 1939; Dounce, 1942; Dounce and Beyer, 1948). Dounce and Beyer (1948) observed that the arginase content of the nuclei of rat liver cells was somewhat higher in concentration than in the cytoplasm. Whereas the activity of the whole liver homogenates could be increased by the addition of Mn^{++} the activity of the arginase in the isolated nuclei remained unchanged. These authors suggested that arginase is synthesized in the cell nucleus and passes out into the cytoplasm. However, Rosenthal, Gottlieb, Gorry and Vars (1956) obtained evidence that in rat liver homogenates 75% of the arginase activity was recovered in the nuclear and microsomal fractions. They concluded that the localization of arginase in the nuclei is a preparative artifact caused by the binding of arginase by nucleic acids and the enzyme is a constituent of the soluble fraction of cytoplasm. On the other hand Carruthers, Woernley, Baumler and Davis (1959) reported that in mouse liver the arginase activity was bound to microsomes. Montry, Litt, Kay and Dounce (1956) reported that arginase activity was highest in chromosomal fraction of rat and nucleoli of cat liver.

Purification :

Arginase of high purity has been prepared by a number of workers (Greenberg, 1955; Olomucki and Vervier, 1955; Robbins and Shields, 1956; Bach and Killip, 1958; Grassmann, Hormann and Janowsky, 1958; Bach, Hawkins and Swaine, 1963). Bach and Killip (1958) succeeded in crystallising the enzyme, although their product does not appear to be as pure as that of a number of amorphous preparations (Grassmann, Hormann and Janowsky, 1958). Greenberg (1955) and Greenberg, Bagot and Roholt (1956) achieved an activity of 4250 units/mg of protein nitrogen and a 350 fold enrichment over the starting horse liver extract.

Stability :

Arginase is a comparatively stable protein. Aqueous solutions of the enzyme at pH 7.0 can stand indefinitely at refrigerator temperature without loss of activity. The purified enzyme is unstable at high dilutions but is stabilized by ornithine or glycine. Mohamed and Greenberg (1945) reported that there was no detectable loss in arginase activity upon being left standing at 25° for 8 days. Dry, lyophilized preparations can be maintained unchanged indefinitely. Bach, Hawkins and Swaine (1963) reported that arginase purified from ox liver can be stored at -15° for an indefinite period.

In solutions below pH 6.0 and above 10.0 the enzyme becomes extreemly unstable. The unstability with increasing alkalinity commences at about pH 8.0 (Hunter and Morrell, 1933). Arginase is, however, quite stable to heat. Activating metal ions protect against heat inactivation in the decreasing order $Mn^{++} > Ni^{++} > Co^{++}$ (Greenberg, Bagot and Roholt, 1956). At a pH of 7.5 the enzyme has a half life of 177 minutes at 70° in the presence of Mn^{++} and 97 minutes at the same temperature in the presence of Ni^{++}. With Co^{++} the half life is 95 minutes at 65°.

Molecular properties :

Differences in substrate inhibition, molecular weight, Km and antigenicity of arginase from ureotelic and uricotelic species have been found (Mora, Tarrab and Bojalil, 1966). The rat liver arginase (Schimke, 1964; Kossmann, Hintz, Lange and Menne, 1966) is of interest because of its different electrochemical characteristics from that of purified horse (Greenberg, Bagot and Roholt, 1956; Greenberg, 1960) and calf liver arginase (Grassmann, Hormann and Janowsky, 1958). Whereas the latter are electro-negative in the physiological pH range, rat liver arginase is electropositive.

The sedimentation constant of horse liver arginase was found to be 5.95 Svedberg units (Greenberg, Bagot and Roholt, 1956). From this and the diffusion constant (4.2×10^{-7} cm^2 sec^{-1}) a molecular weight of 138000 was calculated. Grassmann, Hormann and Janowsky (1958) found a sedimentation constant of 3.84 Svedberg units for a Mn^{++} free solution of their enzyme preparation. In the presence of Mn^{++} anamalous results have been obtained which indicate that a number of molecular species are formed by a reversible association equilibrium of the enzyme (Bach and Killip, 1958; Grassmann, Hormann and Janowsky, 1958). This observation suggests that the molecular weight of 138000 is too high.

Data on the amino acid composition of arginase has been reported (Grassmann, Hormann and Janowsky, 1958; Greenberg, Bagot and Roholt, 1956). Noteworthy is the fact that the enzyme contains no methionine or proline. Arginase is probably a glycoprotein, it contains only 10% nitrogen. It was reported that in horse liver arginase, 75% of the dry weight represents amino acids and 107% of the nitrogen appears in the form of amino nitrogen. It was concluded that this low recovery of amino acid residues per 100 g of protein could be at best partially attributed to amino acid degradation during hydrolysis. Lenti (1953), however, reported that arginase does not seem to contain a sugar fragment as a part of its structure on the basis of the observation that neither amylase nor compounds capable of reacting with the carbonyl group modify the activity of arginase.

Bascur, Cabello, Veliz and González(1966) reported that human liver arginase could be separated into two protein fractions on CM-cellulose. On rechromatography, each of this fraction appears homogeneous and retains its adsorption-elution characteristics. Both arginase fractions have similar properties as far as their affinities for substrates, pH optima and thermal inactivation are concerned. However, they differ in their pH stabilities and their inhibition by ornithine and canavanine. Cabello, Prajoux and Plaza (1966) reported that purified preparations of arginase obtained from human liver and erythrocytes contain two proteins with arginase activity which separate on agar electrophoresis at pH 8.0 and 8.6. A slow and a fast moving components move towards cathode. The isoelectric pH was found to be 5.5 for erythrocytes and pH 7.1 for liver arginase. The decreased cathodic migration may be explained by additional charges furnished by the enzyme-bound Mn^{++} that dissociates as the pH decreases.

Hirsch - Kolb and Greenberg (1968) reported that rat liver arginase is comparatively stable enzyme and does not dissociate readily. Alteration in pH, removal of Mn and replacement of Mn by Co did not yield evidence of a change in molecular size. The results obtained with urea dissociated arginase lead to the reasonable conclusion that the rat liver enzyme is composed of four subunit peptide chains and acrylamide gel electrophoresis gave a single protein band, suggesting that the polypeptide subunits are probably identical.

Specificity :

Arginase is a highly specific enzyme. Normally it hydrolyses L(+) arginine to ornithine and urea. Not even D(+) arginine is attacked (Reisser, 1906; Edlbacher and Bonem, 1925).

The presence of the free α- NH_2 group is not essential for the activity of arginase. The existing evidence is that hydrolysis may be greatly slowed down but is not prevented either by the substitution or the replacement of the amino group of arginine. Felix, Muller and Dirr (1928), however, reported that the NH_2 group must be present, but one of the hydrogen may be replaced by an amino acid radical or by acyl group. Substitution by alkyl group inhibits the clevage. Felix and Schneider (1938) showed that the α- NH_2 group may be replaced by -N, $-CH_3$, CH_3Co-, or involved in a peptide link and still be acted upon. On the other hand, experimental observations have led to the conclusion that an unmodified guanidine group and a free-COOH group are prerequisite for the action of this enzyme.

Roche and Mourgue (1947) studied the effect of various guanidine derivatives on arginase activity and concluded that $-NH_2$ or -OH group α to a - COOH group is necessary for arginase activity and that a γ - guanidino group cannot replace the $-NH_2$ or -OH group.

Arginase has been found to act on α - N substituted products examples are octopine (arginine - α - N propionic acid) and its isomers (Akashi, 1937), α - N - benzyl arginine (Felix, Muller and Dirr, 1928) and α - Carbamido arginine (Hunter, 1938).

Evidence that substitution of the guanidino group completely blocks the action of arginase is given by the failure to split α - N - CH_3 arginine (Thomas, Kopfhammer and Flaschentrager, 1922) and arginine phosphate (Meyerhof and Lohmann, 1928).

It is generally agreed that the -COOH group of arginine must be present and free for arginase to act, for neither the methyl ester of arginine (Edlbacher and Rothler, 1925) nor the ethyl ester of arginic acid (Calvery and Block, 1934) could be hydrolysed, while only 50% of the available urea was split from arginyl arginine (Edlbacher and Burchard, 1931). The necessity for the - COOH group to be free is also emphasized by the fact that the protamines, clupin and clupeon are not attacked by arginase (Kossel and Dakin, 1904a b; Lieben and Lieben, 1934). Contrary to this, however, Richards and Hellerman (1940) found that agmatine, the amine of arginine is hydrolysed even more rapidly than arginic acid or α- N - p - toluene sulfonyl arginine. Kiesel (192~~2~~ a,4) and Baret and Mourgue (1957) found that arginase has no action on agmatine.

The length of the carbon chain is also an important factor in the specificity of arginase. Thus, the 4 carbon γ - guanidino butyric acid (Thomas, Kapfhammer and Flaschentrager, 1922; Baret and Mourgue, 1957) and the 6 carbon guanidino caproic acids are not hydrolysed (Thomas, 1914). Arginase has no action on - guanidino propionic acid or guanidino acetic acid (Edlbacher, 1917; Baret and Mourgue, 1957). On the other hand, the unusual oxygen containing analog of arginine, canavanine which is equivalent in length to arginine is reported to be split (Damodaran and Narayanan, 1940).

<u>pH activity</u> :

The pH activity curves of arginase are decidedly asymetrical. A careful study of the influence of pH on the arginase activity of a crude beef liver extract has been conducted by Hunter and Morrell (1933).

The influence of certain activating ions on the pH activity curves of purified beef arginase has been studied by Mohamed and Greenberg (1945). These curves were similar to that found by Hunter and Morrell (1933). Essentially the same form of curve was formed for untreated and Mn^{++} activated arginase. Co^{++} and Ni^{++} activated arginase showed a decided dip in the curve and flattened region of activity between pH 7 to 9. This has previously been observed by Hunter and Morrell (1933) and Hellerman and Perkins (1935).

Anderson (1945) reported that in jack bean arginase the optimum pH also varies with Co^{++} ion concentration increasing from 7.5 to 9.0 with rising concentration. The optimum pH with Mn^{++} is about 8.8 and is independent of the Mn curve.

In general the optimum pH is at nearly pH 10.0 although the combination of Co^{++} and Mn^{++} gives a curve which has its maximum over the range of pH 7 to 9.

Effect of temperature :

In temperature characteristics the arginase behaves like many hydrolytic enzymes of animal origin. The temperature of optimum activity is about 50° in phosphate buffer at pH 7.5 and for an incubation period of 15 minutes (Mohamed and Greenberg, 1945). As has been pointed out by Hunter (1934) the optimum temperature is greatly dependent on the time of incubation and the nature of the incubation medium. The thermal inactivation of arginase follows a first order reaction curve.

Shirakawa (1943) reported that apparent activation energy of decomposition of arginine by arginase is about 10500 cal/g mole at pH 7.0 and 8700 Cal/g mole at pH 9.0.

Activation :

This has been the most extensively studied phase of the properties of arginase. Earlier investigators (Waldschmidt-Leitz and Kocholaty, 1933; Reiss, Fleischmann and Schwarz, 1935; Salazkin and Solovev, 1931) considered the sulfhydryl compounds like glutathione and cysteine were specific activators of arginase but this view was discarded when it was found that Fe^{++} alone or used in conjunction with thiol or with certain other organic compounds was a more characteristic activator (Hellerman and Perkins,1935). They determined that arginase activity is not significantly altered by mercaptide forming organic mercurials (e.g. C_6H_5HgOH) nor it is enhanced by sulfhydryl compounds like cysteine or glutathione or organic reducing agents such as bisulfite, cyanide or hydrogen sulfide.

A number of investigators (Hellerman and Perkins, 1935; Badinaud, 1937; Stock, Perkins and Hellerman, 1938; Richards and Hellerman, 1940; Wiswell,1950; Roholt and Greenberg,1956;

Baret and Mourgue, 1960; Cabello, Basilio and Prajoux, 1961) observed that the activity of arginase may be enhanced by Co^{++}, Mn^{++}, Ni^{++} or Fe^{++}. The inactivation of arginase after treatment with such inhibitors as quinone, ferric ion or iodine could be restored to its maximum by the simple addition of Co^{++}. The general character of this observation has been verified by various investigators.

Activation by divalent metal ions was found to apply to other sources of the enzyme in addition to liver arginase. Edlbacher and Baur (1938) reported that yeast arginase was completely inactivated by dialysis at 0^{o} and reactivated to an equal extent by the addition of Mn^{++}, Co^{++} and Ni^{++} and Cd^{++} salts. Jack bean arginase also is activated by Co^{++}, Mn^{++} and Fe^{++} (Anderson, 1945).

Hellerman and Perkins (1935) found that when acting alone, Co^{++} was the most potent activating ion. Mn^{++} and Ni^{++} were somewhat less effective and Fe^{++} reactivated arginase after inactivation by iodine or quinone only when its use was preceded by cysteine or similar reagent.

In experiments by Mohamed and Greenberg (1945) it was readily demonstrated that the activation of arginase depends upon a measurable time reaction between enzyme and activating ion and not between substrate and ion. Incubating buffered solutions of arginine with Mn^{++} and then adding enzyme does not increase the velocity of splitting of arginine over the rate obtained when all the components are mixed immediately. On the other hand, incubation of arginase with Mn^{++} or Co^{++} leads to a progressive increase in activity, which eventually reaches a maximum value.

The experiments indicate that the activation of arginase by divalent cations concern the enzyme alone. A maximum level of activation is eventually attained for the reaction to go to completion. The time required to attain the maximum activation is dependent on the kind of ion, its concentration, the pH and the temperature. Increasing the pH toward pH optimum increases the rate of activation (Mohamed and Greenberg, 1945).

From these observations, the action of the metallic cations in the process of arginase activation might be assumed to be purely catalytic were it not for the fact that the presence of a metal ion is necessary for the maintenance of the enzymatic activity. Removal of the cation by dialysis causes a loss of enzymatic activity which can be restored by addition of the effective ion. Mohamed and Greenberg (1945) have suggested that the activation of arginase consists in the transformation of an inactive proarginase into active arginase as represented by the following reaction.

$$\underset{\text{(Inactive)}}{\text{Proarginase} + Mn^{++}} \rightleftharpoons \underset{\text{(Active)}}{\text{Arginase} - Mn}$$

Klein (1960) reported that in established animal cell strains cultivated <u>in</u> <u>vitro</u> addition of RNA enhances the arginase activity in the cells 5 fold.

Inhibitors :

Many reagents and physical conditions inhibit arginase reversibly or will inactivate the enzyme irreversibly.

An inhibition of arginase is produced by buffer solutions of citrate or borate (Mohamed and Greenberg, 1945; Lenti, 1946). A possible explanation of this effect is that these acids react to form complexes with the activating ions Co and Mn. This may greatly decrease the activities of these activating ions in solution. Phosphate ions, however, have been reported to activate arginase (Edlbacher, 1917).

A powerful inhibition of arginase activity by orinthine, a product of the reaction, was first observed by Grose (1920) and has been amply confirmed (Edlbacher and Zeller, 1936; Kitagawa, 1939; Bach, Crook and Williamson, 1944, Cheema, Padmanaban and Sarma, 1969). Urea the other product of reaction was found to inhibit in some cases (Vovchenko, 1936; Bach, Crook and Williamson, 1944) while it has no action in some others (Kitagawa, 1939). Edlbacher and Zeller (1936) found that glycine, alanine and lysine were also inhibitory. Of these lysine is the most powerful being nearly as active as ornithine. Kylstra (1960) and Cheema, Padmanaban and Sarma (1969) also reported that lysine is inhibitory.

A thorough kinetic study of the inhibition of arginase by amino acids was made by Hunter and Downs (1945). These investigators found that arginase is inhibited by all L-amino acids but not by D-amino acids, nor by amino acids having the amino group other than in the α position. The inhibition by mono amino acids was found to be non-competitive while the inhibition by ornithine and lysine was competitive. Mourgue and Baret (1956) also showed that liver arginase is inhibited competitively by lysine and ornithine and noncompetitively by other L-amino acids.

The role of various metal ions as activators has already been discussed in the earlier section, however, some investigators reported an inhibitory effect of metal ions e.g. Badinaud (1937) showed that Cu ions inhibit arginase activity. Greenberg, Bagot and Roholt (1956) found that horse liver arginase was strongly inhibited by Zn. Co has also been found to inhibit arginase (Angelis, 1954).

Mechanism :

The major variable in the kinetics of the arginase reaction is pH. This is logical in view of the amphoteric character of both the substrate and the enzyme. An additional factor is the influence of the metal ion activator on the dissociation of ionizable groups of the catalytically active centre of the enzyme.

The effect of pH on the enzyme reaction is represented in the variation of the Michaelis constant, Ks and Vm with pH. Plots of Ks against pH give a U shaped curve and Vm against pH a bell shaped curve (Greenberg, 1960). This has been studied quantitatively with Mn^{++} activated arginase only. Roholt and Greenberg (1956) on the basis of the effect of pH on Ks and Vm in Mn and Co activated arginase suggested that the active form of arginine attacked by the enzyme is zwitterion of the amino acid.

The minimum number of variables that could be involved in determining these curves include the dissociation of the α- NH_2 group of arginine, the dissociation of two acid groups associated with the catalytic activity of the enzyme and the dissociation of the two groups of the enzyme substrate complex (Alberty, 1956; Alberty and Massey, 1954). In the case of arginine an additional dissociation appears necessary in view of the fact that the acid branch of the pH activity curve is determined by not less than two dissociable acid groups.

Roche, Thoai and Verrier (1953) on the basis of adsorption studies proved the complex formation of the enzyme arginase with arginine in the presence of Co^{++}, Mn^{++} and Ni^{++}. Klotz (1954) has also proposed various ways for the metal-protein complex formation and it was believed that in case of arginase the metals stabilize the transition state complex.

Inspite of a large volume of literature being available on arginine metabolism in animal tissues, no detailed study seems to have been made in plant tissues. Studies were, therefore, undertaken to detect various enzymes involved in arginine and glutamate metabolism in normal and tumour tissues of plants cultivated *in vitro*. A detailed study of the enzyme arginase from *Rumex acetosa* tumour tissue was carried out in an attempt to prove its metabolic role and regulation in this tissue. Details of these studies are incorporated in this thesis.