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 -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, -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, 1965; 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 enzyme 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 amino-transferase, 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 maligant cells (Ledoux, Brandli and Paepe, 1958). The capacity of tumour tissue to catabolise 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 effieiencies 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 Klein 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 pyridine 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 pyridine nucleotide level in these tissues (Srivastava, Maini, Naik and Ramakrishnan, 1971).

Arginine Metabolism

The amidine group of arginine represents but a small portion of the arginine molecule, it is however, endowed with high molecular lability and with a large experimental literature.

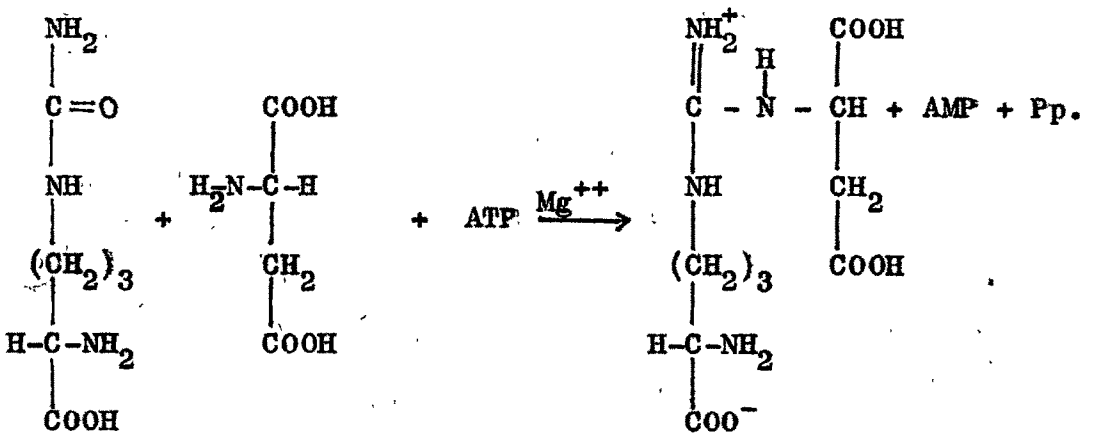
Most important exploration of arginine metabolism stems from Krebs and Henseleit's (1932) formulation of ornithine-urea cycle. This formulation was suggested by the observation that the rate of urea formation by liver slices was greatly accelerated by the addition of one mole of ornithine. Since addition 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 carbondioxide 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, 1952; 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.

The biosynthesis of urea presents a unique biological system in that it involves the primary fixation of both carbondioxide and ammonia. While the importance of this has been emphasized chiefly in relation to the highly specialized function of urea biosynthesis, it should be pointed out that the formation of carbamyl phosphate may be of more fundamental importance from the standpoint of biochemical evolution. Thus not only does carbamyl phosphate represent an energy rich compound formed as a result of the primary fixation of carbondioxide and ammonia but it also represents a component of two different systems, one leading to the synthesis of an amino acid, arginine, and the other to the synthesis of pyrimidines. Pyrimidine synthesis in relation to urea synthesis is discussed in a later section.

Formation of arginine :

The formation of arginine from citrulline was demonstrated in liver and kidney preparations (Borosook and Dubnoff, 1941; Krebs, 1942) and this conversion was found to be stimulated by either aspartate or glutamate. Studies by Ratner (1954, 1955a, 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 condensing enzyme or argininosuccinate synthetase catalyses the reversible, ATP dependent reaction (Ratner and Petrack, 1953a, 1956; Ratner, 1954) as shown below :

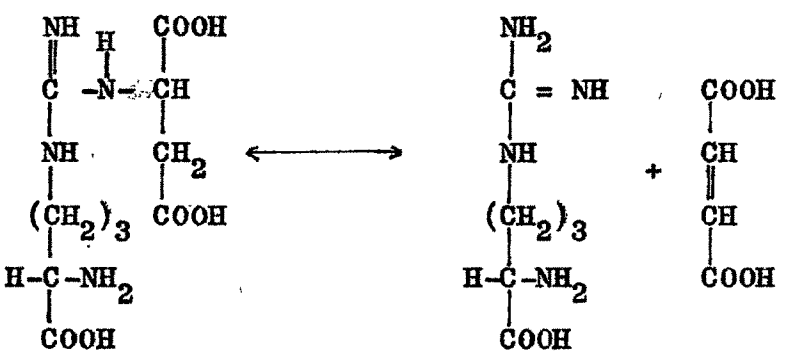


L-citrulline L-aspartate L-argininosuccinic acid

The enzyme has been partially purified from mammalian liver and kidney (Ratner and Petrack, 1953b; Ratner, 1955b) and is present in amphibian liver (Brown and Cohen, 1958). The enzyme is also found in yeast (Ratner and Petrack, 1953b; Ratner, 1954; 1955b) and presumably in other microorganisms.

The enzyme which catalyses the clevage of argininosuccinate to arginine and fumarate is widely distributed in nature. It occurs in mammalian kidney and liver (Ratner, 1954, 1955b) and in adult anuran liver (Brown and Cohen, 1958), yeast (Ratner, 1954), Jack beans (Walker and Myers, 1953), Pea seeds (Davison and Elliott, 1952), Chlorella (Walker and Myers, 1953; Walker, 1952) and E. coli (Ratner, 1954) and in Neurospora (Fincham and Boylen, 1955).

The enzyme has been studied extensively by Ratner and her coworkers (Ratner, 1954) who have shown the product of the reaction to be arginine and fumarate.



L-argininosuccinic acid

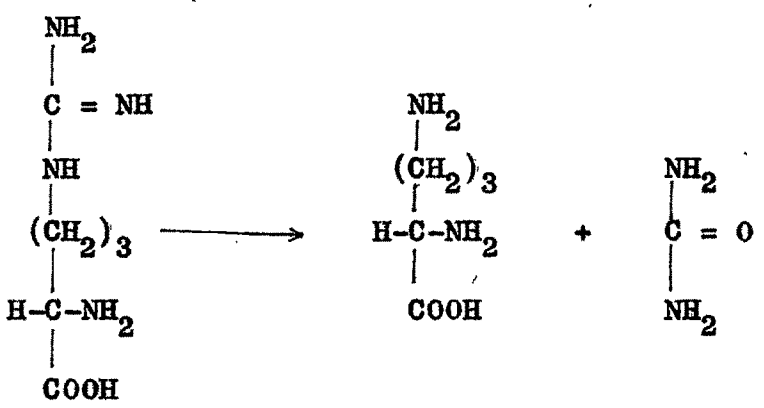
L-arginine + fumaric acid

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 microorganisms (Oginsky and Gehrig, 1953; Slade, 1953).

In Streptomyces griseus arginine is found to be converted to γ-guanidinobutyramide by oxidative decarboxylation. This compound is enzymatically converted to γ-guanidinobutyric acid (Meister, 1965). 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).

The hydrolytic clevage of arginine to ornithine, and urea by the action of arginase is, however, the most thoroughly investigated of the enzyme mechanisms concerned with arginine breakdown. The enzyme arginase catalyses the reaction as shown below.



L-arginine L-ornithine urea

Mammalian liver seems to be the best source of the enzyme arginase (Greenberg, 1951; Fuchs, 1921). It 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; Gabello, Urba, Prajoux and Basillio, 1959), Bacillus licheniformis (Ramaley and Bernlohr, 1966) and yeast (Edlbacher, Becker and Segessa, 1938).

Arginase is a highly specific enzyme, attacking only the L-form of the amino acid. Arginase of high purity has been prepared by a number of workers (Greenberg, 1955; Robbins and Shields, 1956; Bach and Killip, 1958; Bach, Hawkins and Swaine, 1963).

The arnithine produced by arginase reaction or produced from glutamate by the following pathway then condenses with carbamyl phosphate to form citrulline and thereby completing the urea cycle.

Glutamate ⟶ N-acetyl glutamate ⟶ N-acetyl glutamate-γ-semialdehyde ⟶ N-acetyl ornithine ⟶ ornithine.

The carbamyl phosphate required for the synthesis of citrulline is obtained by the reaction of carbamyl phosphate synthetase as shown below :

$$NH_4^+ + HCO_3^- + 2ATP \xrightarrow[\text{acyl glutamate}]{Mg^{++}} H_2N - CO \backsim P + Pi$$

The enzyme occurs in the particulate fraction of livers of vertebrates (Grisolia and Cohen, 1953). It is notably absent in avian liver. Studies with crude enzyme demonstrated the participation of ATP and Mg^{++} and N-acetyl glutamate as cofactors. As yet no definite role can be assigned for the action of glutamate derivatives for the synthesis of carbamyl phosphate. The available evidences, however, suggest that it is required in the CO_2 fixation step. Carbamyl phosphate synthetase has been purified from several sources (Issaly, Issaly and Reissig, 1970; Anderson, Weliner, Rosenthal and Meister, 1970). The purified enzyme shows the same requirement

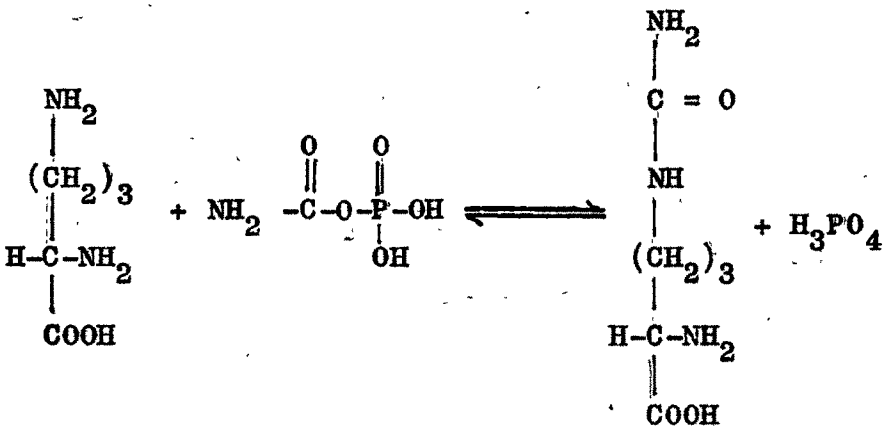
as the crude preparation for N-acetyl glutamate as cofactor. It has also been established that 2 moles of ATP are required for the synthesis of one mole of carbamyl phosphate by both mammalian (Metzenberg, Hall, Marshall and Cohen, 1957) and amphibian enzymes (Marshall, Metzenberg, and Cohen, 1958).

Streptococcus faecalis and other bacteria (Slade, 1953; Oginsky, and Gehrig, 1953; Knivett, 1954a,c; Korzenovsky and Werkman, 1953, 1954; Slade, Doughty and Slamp, 1954) possess an enzyme carbamate kinase which is related to carbamyl phosphate synthetase found in non-avian vertebrate liver. The presence of this kinase was deduced from observations on the substrate level generation of ATP which was linked to the anaerobic conversion of citrulline to ornithine (Knivett, 1952; Slade, 1953; Oginsky and Gehrig, 1953; Schmidt, Logan and Tytell, 1952; Knivett, 1954b). The bacterial enzyme catalyses the reversible synthesis of carbamyl phosphate.

$$H_2N\text{-}CO\sim P + ADP + H_2O \overset{Mg}{\rightleftharpoons} NH_4^+ + HCO_3^- + ATP$$

The bacterial enzyme differs from that in liver in that the former requires only one mole of ATP for the synthesis of carbamyl phosphate and is not dependent on an N-acetyl derivative of glutamic acid.

Mammalian liver contains an enzyme ornithine transcarbamylase which catalyses the synthesis of citrulline from ornithine and carbamyl phosphate (Burnett and Cohen, 1957; Cohen and Grisolia, 1948, 1950; Grisolia and Cohen, 1951, 1952, 1953; Grisolia, 1952; Smith and Reichard, 1956).



L-ornithine carbamyl phosphate L-citrulline

No intermédiate steps have been observed in this reaction.

The presence of this enzyme has been reported in various animal tissues (Cohen and Hayano, 1947, 1948; Grisolia and Cohen, 1951, 1953; Della, 1957; Burnett and Cohen, 1957; Pietra, Rogliani, Procaccini and Rogliani,1957; Cittadini, Andreucci and Festa, 1959; Reichard, 1960; Hager and Jones, 1967b; Brown and Brown, 1967; Grillo and Bedino, 1968) plants (Coleman and Hegarty, 1957; Kasting and Delwiche, 1958; Bone, 1959; Bernes and Naylor, 1959; Kleczkowski and Reifer, 1959; Kasting, 1963; Wielgat, Morawska-Muszynska and

Reifer, 1965) and microorganisms (Gorini and Maas, 1957; Smith, 1957; Bernlohr, 1966; Caravaca and Grisolia, 1960; Ravel, Grona, Humphreys, and Shive, 1959; Bechet, Wiame and Grenson, 1962; Dixon and Rose, 1964; Glebicki and Glebicka, 1958).

The enzyme has been shown to increase during post natal development in rats and it was concluded that this increase might be dependent on the developing thyroid gland (Illnerova, 1966). Dietary protein deficiency has been shown to produce a marked decrease in this enzyme which is reversed on protein repletion (Hutchinson and Labby, 1964). The enzyme is found to increase in various liver disorders except cirrhosis (Lamonnier, Levy, Charvel, Caroli and Raizman, 1963) and to be increased in several malignant tumours (Kondo, Kurino, Harabayashi and Kobayashi, 1961).

The enzyme is localized in mitochondria in several tissues studied such as livers of all vertebrates (Cohen and Hayano, 1948; Grisolia and Cohen, 1953; Charles, Tager and Slater, 1967) and mung bean (Bone, 1959).

The enzyme has been purified from several sources such as rat and bovine liver (Reichard, 1957; Burnett and Cohen, 1957; Grillo and Bedino, 1968) pea seedlings (Kleczkowski and Cohen, 1964). E. coli (Rogers and Novelli, 1962) Mycoplasma luminis 07 (Schimke, Berlin, Sweeney and Carrol,

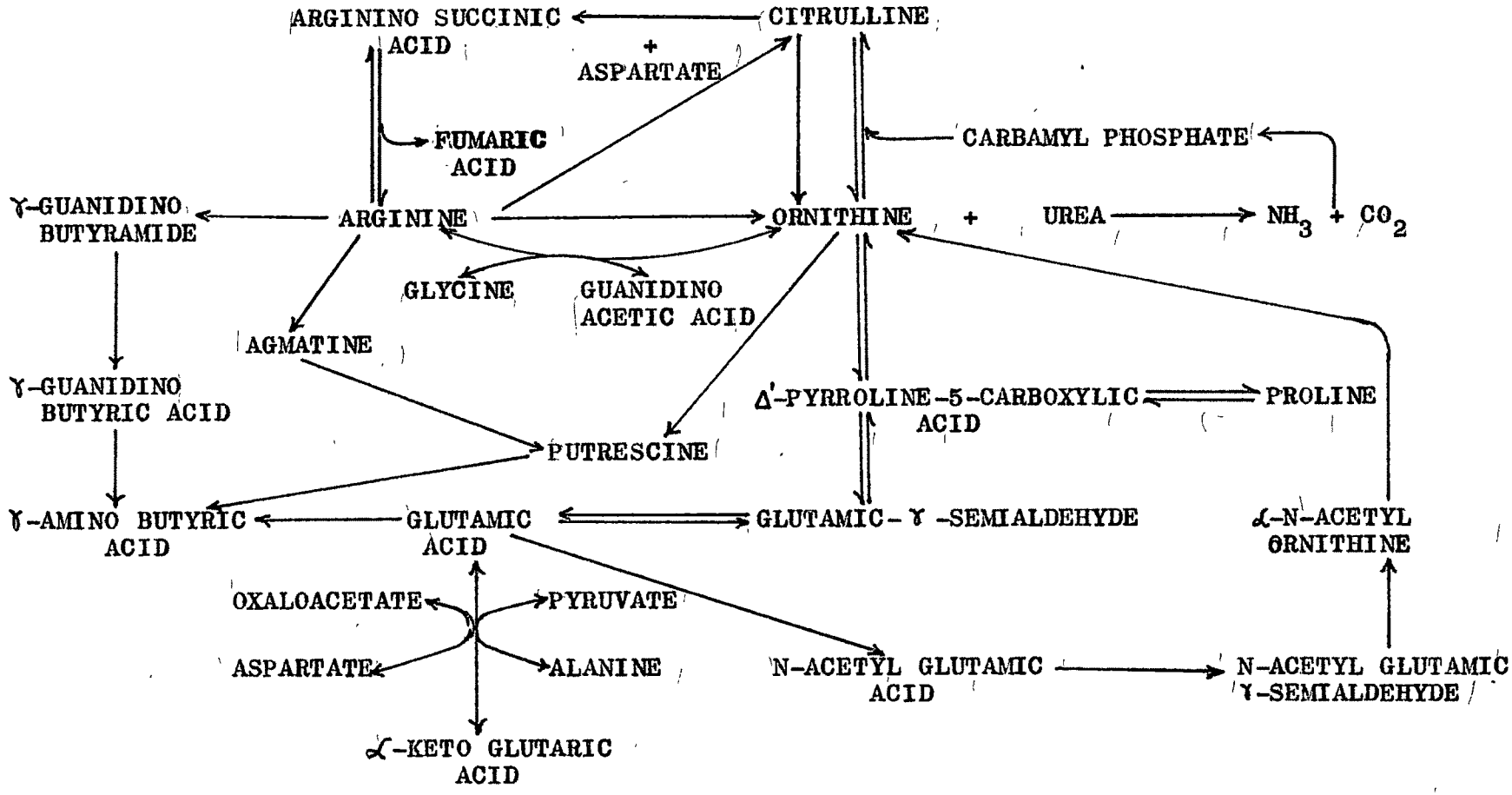
1966). The enzyme has been crystallized from Streptococcus D_{10} (Bishop and Grisolia, 1967). Recently Marshall and Cohen (1972a) have purified the enzyme from S. faecalis and bovine liver.

The unidirectional catalysis by the liver enzyme indicates that it probably evolved or became so modified as to serve the economy of those organisms which form urea via the urea cycle. While the reversibility of this reaction is thermodynamically possible operation of the urea cycle in a clockwise direction is maintained, in part, by failure of the liver enzyme to catalyse the reverse reaction under physiological conditions. The irreversibility of the total cycle is also maintained as a consequence of the positive free energy associated with the reversal of arginase reaction.

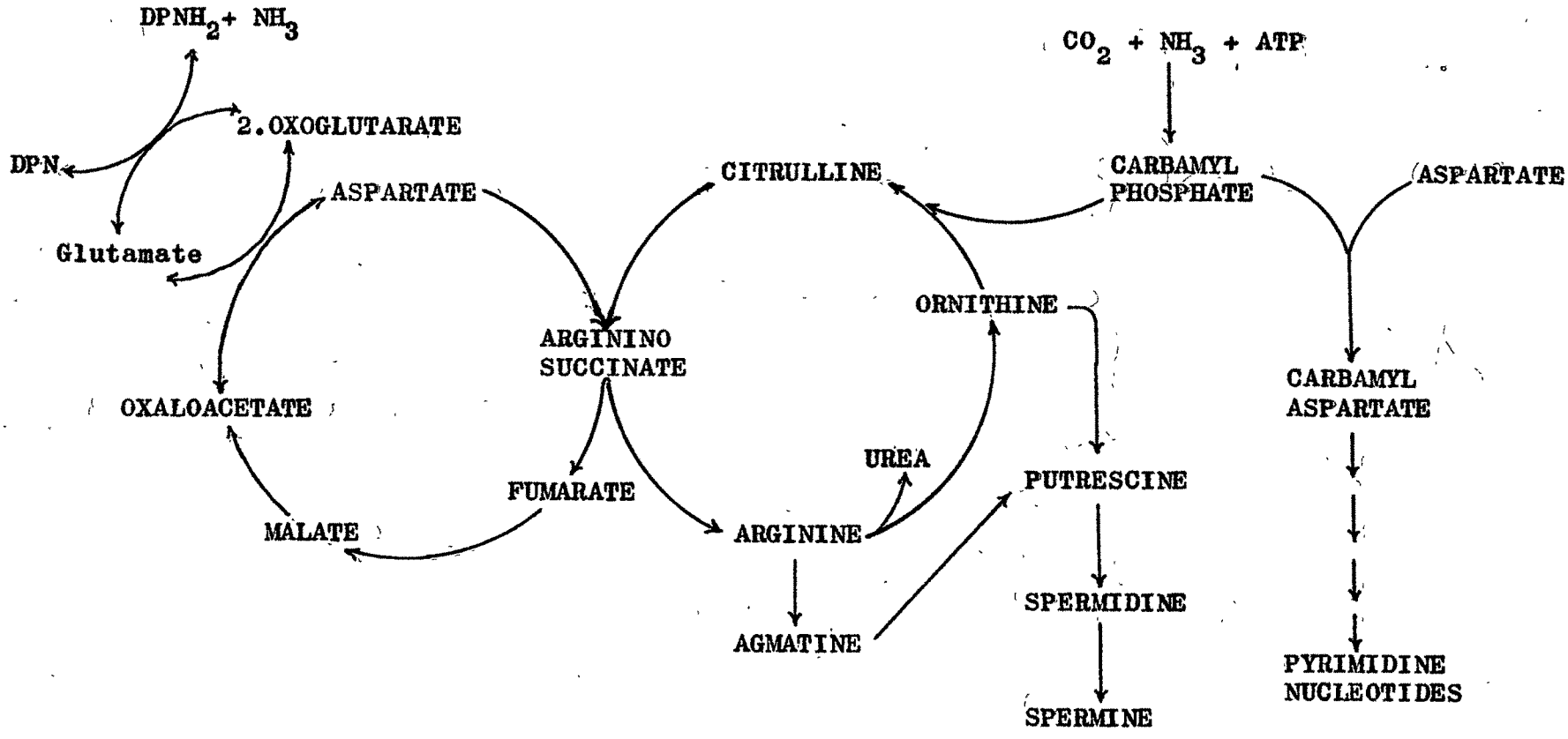
All the reactions of the urea cycle along with other related transformations are summarized in Schemes I and II.

Interrelationship of urea cycle with pyrimidine synthesis :

Carbamyl aspartate has been shown to be a precursor of pyrimidines in bacteria (Weed and Wilson, 1954; Lieberman and Kornberg, 1954) and animal tissues (Weed and Wilson, 1954; Reichard and Lagerkvist, 1953). The wide distribution of the enzyme, aspartate transcarbamylase, synthesizing carbamyl aspartate suggests that it is synthesized by most, if not all,



SCHEME - I : INTERRELATIONSHIP BETWEEN ARGININE AND GLUTAMIC ACID METABOLISM.



SCHEME - II : INTERRELATIONSHIP BETWEEN ARGININE, PYRIMIDINES, POLYAMINES AND ASPARTIC ACID METABOLISM.

tissues and represents the initial reaction in the synthesis of pyrimidines by these cells utilizing carbamyl phosphate as the carbamyl donor.

The relatively high aspartate transcarbamylase activity in regenerating liver and hepatomas is associated with a low activity of ornithine transcarbamylase and consequently of urea synthesis (Tung and Cohen, 1950; Greenstein, 1954). We thus have a case of biochemical dedifferentiation in what is highly specialized function viz. urea synthesis is lost in the case of hepatoma and decrease in the case of the regenerating liver but associated with an increase in the enzyme aspartate transcarbamylase which is concerned with a more fundamental function viz. growth and proliferation.

Carbamyl phosphate thus occupies a unique position in the liver in that on the one hand it can serve the highly differentiated and specialized liver cells for making arginine and urea and on the other hand it can serve as the precursor of pyrimidines. However, when the liver cell is dedifferentiated, as in the case of hepatoma, or in the early phases of regeneration, the need for pyrimidine synthesis is greater and the enzyme concerned with diverting carbamyl phosphate towards pyrimidine synthesis viz. aspartate transcarbamylase is increased.

Regulation of urea cycle :

Apart from its role in urea cycle, arginine, has also been implicated with the growth of tumour tissues. It has been shown to have 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 Olliver, 1961). In mouse adenocarcinoma arginine has been found to increase mitosis (Bach and Lasnitzki, 1947). The question arises, in the light of the above observations, whether arginine has any influence on nucleic acid metabolism.

Several investigators have examined the mechanisms of regulation of arginine biosynthesis in E. coli (Gorini, 1960; Gorini, Gundersen and Burger, 1961; Vogel, 1961; Maas, 1961; Gorini and Maas, 1957; Mahler, Newmann and Marmur, 1963). There is a general agreement that arginine represses the synthesis of all the enzymes involved in the conversion of acetyl glutamate to arginine. The biosynthesis of arginine has also been shown to be controlled by the end product inhibition (Vyas and Maas, 1963).

The biosynthetic sequences of arginine and uracil are connected through a common intermediate carbamyl phosphate and each sequence is controlled by its end product or a derivative of the end product through feed back inhibition

and enzyme repression. The question arises whether these control mechanisms constitute the only regulatory devices for arginine and uracil biosynthesis or whether any additional mechanisms regulating the formation of the common intermediate carbamyl phosphate exist.

Since carbamyl phosphate serves as a common precursor for both UMP and arginine, a special regulation of its formation must be obtained to assure a balanced supply of both the end products. This problem is handled in a variety of ways by different organisms and has been reviewed by Gots (1971).

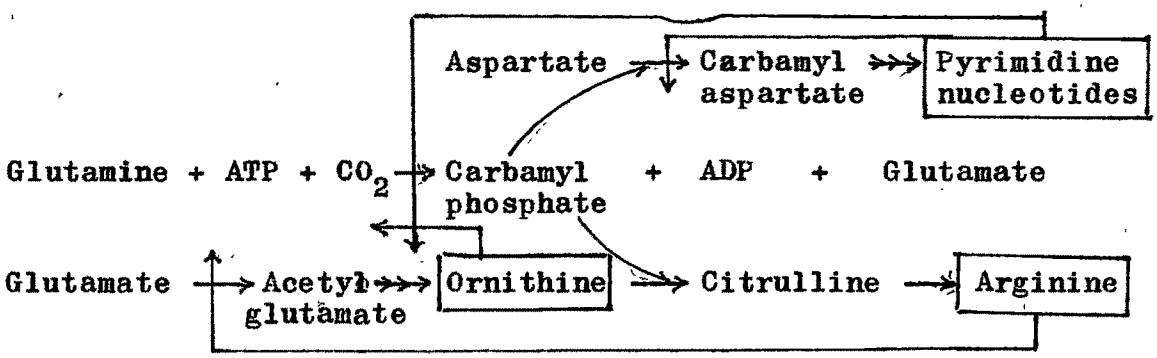
Carbamate kinase catalyses the synthesis of carbamyl phosphate from NH_3, CO_2 and ATP (Jones, 1965). A genetic deficiency of this enzyme was suspected to occur in bacterial mutants which had a requirement for uracil and arginine, but several attempts to show this were unsuccessful (Kanazir, Berner, Flaks and Cohen, 1959; Thorne and Jones, 1963).

Carbamyl phosphate synthesis is also achieved by another enzyme which uses glutamine as the NH_3 donor (Levenberg, 1962). It is the loss of this enzyme which creates the absolute requirement for uracil and arginine in the E. coli mutants (Pierard and Wiame, 1964). This enzyme is controlled by an accumulative type of repression requiring both uracil and arginine for a maximum effect.

In Saccharomyces cerevisiae there are two carbamyl phosphate synthetases, one under feedback control by arginine and the other by uracil and UTP (Lacroute, Pierard, Grenson and Wiame, 1965). Neurospora is similar to yeast in having two separate enzymes for the synthesis of carbamyl phosphate, but it differs in that the carbamyl phosphate formed by either one is not available for the other pathway. The complexities of this system have been thoroughly reviewed by Davis (1965, 1967). Mutational loss of one or the other of the two enzymes leads to an absolute requirement for either arginine or uracil. This has been interpreted as a channelling effect whereby the two modes for carbamyl phosphate synthesis gives rise to two spatially separate pools of carbamyl phosphate in the intact cell.

The enzyme carbamyl phosphate synthetase has also been found to be regulated by a compensatory control mechanism. In E. coli the enzyme is subject to strong feedback inhibition by pyrimidine nucleotides but not by arginine (Pierard, 1966; Anderson and Marvin, 1970). Consequently, when pyrimidine nucleotides become present in excess, the synthesis of carbamyl phosphate is curtailed and may become too low to support adequate synthesis of arginine. However, imposed deficiency of carbamyl phosphate will lead to an accumulation of ornithine which at high concentration is able to antagonise the inhibitory effects of pyrimidine nucleotides and thereby to

restore the activity of carbamyl phosphate synthetase, It is significant from the standpoint of cellular regulation that the carbamyl phosphate derived from this renewed synthesis will be available exclusively for arginine biosynthesis since feedback inhibition of aspartate transcarbamylase by CTP (Yates and Pardee, 1956) will prevent its use for continued synthesis of unwanted pyrimidine nucleotides.



In yeast Lacroute (1968) has reported that the pyrimidine controlled carbamyl phosphate synthetase occurs as a multienzyme complex with aspartate transcarbamylase. Both of the enzymes are coded by the same genetic region and both are strongly inhibited by UTP. The genetic control in the regulation of the synthesis of the carbamyl phosphate synthetase is reviewed by Fink (1971).

In mammalian systems the role of the NH_3 dependent carbamyl phosphate synthetase in the pyrimidine pathway was questioned by its limited distribution. The occurence of a

glutamine dependent synthetase was first suggested by Hager and Jones (1965) in vivo studies with Ehrlich ascites cells and such an enzyme was eventually isolated from extracts of these cells (Hager and Jones, 1967a), fetal rat liver (Hager and Jones, 1967b) and mouse spleen (Tatibana and Ito, 1967). It resembles the bacterial enzyme in its sigmoidal kinetics and its strong inhibition by UTP (Hager and Jones, 1967b; Tatibana and Ito, 1967). Further studies on the distribution of the two enzymes in rat liver suggest a compartmental isolation, whereby the NH_3 dependent form resides in the mitochondria for arginine and urea formation and the glutamine dependent form in the cytoplasm for pyrimidine synthesis (Kerson and Appel, 1968). No evidence for this functional compartmentalization could be found, since the NH_3 dependent synthetase was readily available for extra mitochondrial reactions and constituted a major source of carbamyl phosphate for pyrimidine synthesis (Nataley and Tremblay, 1969).

Studies reported in the preceeding paras indicate a control on the concentration of carbamyl phosphate associated with the regulation of the enzyme carbamyl phosphate synthetase. However, the question still remains whether this regulation is adequate enough to explain the entry of the available carbamyl phosphate towards arginine or pyrimidine biosynthetic pathway. It is possible that there

might be a second level of control at the point of entry of this common precursor for the two competing pathways. Of the two enzymes aspartate transcarbamylase and ornithine transcarbamylase involved in the balanced utilization of carbamyl phosphate the regulatory characteristics of aspartate transcarbamylase are well established (Gerhart, 1971) but no such role has yet been ascribed to the enzyme ornithine transcarbamylase though it is reasonable to assume that ornithine transcarbamylase which is a key enzyme in the biosynthesis of arginine may also be under some kind of control. In certain strains of E. coli (Ben-Ishai, Lahav and Zamir, 1964) uracil has been found to cause 2-3 fold increase in ornithine transcarbamylase activity.

Two types of controls have been demonstrated in the regulation of ornithine transcarbamylase in Wiame's laboratory. In case of Pseudomonads (Ramos, Stalon, Pierard and Wiame, 1967a,b) this reversible step is found to be controlled by the elaboration of two separate ornithine transcarbamylases. A biosynthetic function for one of these enzymes is apparent from the fact that its formation is selectively repressed by high concentration of arginine, whereas a catabolic function for the other enzyme is indicated by the fact that its formation is induced at high concentrations of arginine.

In Saccharomyces cerevisiae (Wiame, 1971) a peculiar type of control is obtained by the binding of the enzyme arginase with ornithine transcarbamylase. This binding provokes the inhibition of ornithine transcarbamylase without substantial modification of arginase activity. This binding is under independent and specific control by ornithine and arginine.

From the foregoing it is evident that there is a general regulatory interrelationship between arginine and nucleic acid metabolism. This assumes a great significance from the point of view of cancer biochemistry. However, most of the above mentioned work on regulation has been carried out in bacteria and no work seems to have been done on this aspect in cancer tissues.

Previous studies in this laboratory have shown that tumour tissue of Rumex acetosa has a high arginase and ornithine δ-keto acid transaminase activity (Srivastava and Naik, 1971). The most interesting observation made was the allosteric inhibition of the enzyme arginase by purine and pyrimidine bases on the one hand and ornithine on the other hand which may provide a mechanism for the regulation of arginine as well as pyrimidine biosynthesis (Naik, 1970).

Our preliminary studies also showed that the enzyme ornithine transcarbamylase is low in tumour than in corresponding normal tissue of Rumex acetosa. This observation along with that reported in the previous para would indicate the possibility of some sort of regulatory control on arginine metabolism in this tissue. Ornithine transcarbamylase being the first and the key enzyme of this pathway appears to be the probable choice for regulation. Studies were, therefore, undertaken to purify ornithine transcarbamylase from tumour tissue and study its kinetics with a view to demonstrate the regulatory characteristics of this enzyme, if any. The results of this study are reported in this thesis.