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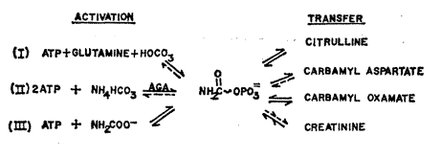
Carbamyl Phosphate

Many forms of life use this molecule to synthesize arginine, uracil, and adenosine triphosphate.

Mary Ellen Jones

Carbamyl phosphate is a low molecular weight, high energy intermediate in several important biosynthetic enzyme reactions. The formula for carbamyl phosphate, $NH_2CO{\sim}OPO_3^=$, indicates that it is a dianion at physiological *p*H and the squiggle sign (~) denotes the high-energy phosphate bond. This interesting compound, discovered only in 1955 (*1*) combines, as it were, ammonia, carbonate, and phosphate in a single molecule.

The enzyme reactions in which carbamyl phosphate participates are



The enzymes catalyzing the activation reactions are: (i) carbamyl phosphate synthetase from Basidiomyces, (ii) carbamyl phosphate synthetase from ureotelic vertebrate liver, and (iii) carbamyl phosphokinase. The enzymes catalyzing carbamyl transfer reactions are for citrulline, ornithine transcarbamylase; for carbamyl aspartate, aspartate transcarbamylase; for carbamyl oxamate, oxamate transcarbamylase; for creatinine, the enzymes have not been purified to define the exact substrate and are therefore not named. *N*-Acetyl glutamic acid (AGA) is a required cofactor for carbamyl phosphate synthetase.

Carbamyl phosphate may be formed biologically from ATP (*2*) by means of the activity of one of the three enzymes, in reactions which are essentially a fixation of ammonia and carbon dioxide or bicarbonate (left hand side of diagram). Therefore, carbamyl phosphate can be synthesized with the consumption of the energy resident in ATP, and then it can utilize this energy for the subsequent synthesis of carbamyl compounds; or it can be produced by the phosphorolytic degradation of certain carbamyl compounds and then be used for the production of ATP.

Chemically, at neutral *p*H in aqueous solution, carbamyl phosphate is in equilibrium with phosphate and cyanate (*3*, *4*) and biologically it serves as a cyanate to "carbamylate" amino compounds by introducing the NH_2CO- group as cyanate does chemically.

Biologically, carbamyl phosphate appears to be the universal donor of the carbamyl moiety in the biosynthesis of citrulline and carbamyl aspartate. These two amino acids are essential for the synthesis of arginine and uridylic acid, and therefore for the biosynthesis of protein and nucleic acid.

Carbamyl-Transfer Reactions for Energy Production

When carbamyl phosphate was discovered (*1*) it was already known to be the high-energy, intermediate compound occurring in the enzymatic synthesis of citrulline. The enzymatic reaction is catalyzed by ornithine transcarbamylase

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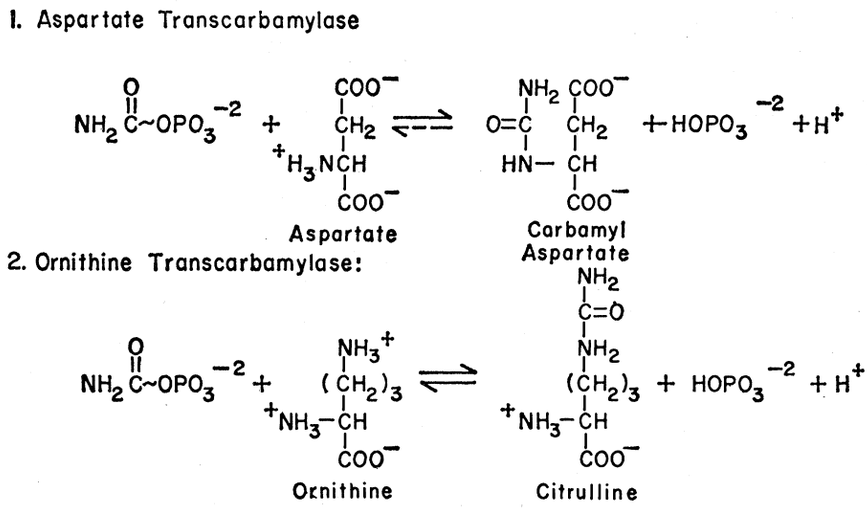


Fig. 1. Enzymes catalyzing reactions which utilize the carbamyl group of carbamyl phosphate, I.

(Fig. 1). This simple displacement reaction is reversible and specific for L-ornithine (*3*). In addition, carbamyl phosphate had already been implicated in the biosynthesis of carbamyl aspartate or ureidosuccinate (*4, 5*), the reaction at the top of Fig. 1. This latter reaction is the only essentially irreversible carbamyl transfer, as indicated by the dashed arrow, and also appears to be a simple displacement reaction (*6*).

In *Streptococcus faecalis,* carbamyl phosphate is not utilized for the synthesis of arginine since this organism cannot convert citrulline to arginine (*7*). Even though arginine is an essential amino acid for *S. faecalis*, arginine can be degraded by this organism (Fig. 2) to yield energy to the organism (*8*). Arginine is hydrolyzed to citrulline and ammonia (*9*); the citrulline is phosphorolyzed to carbamyl phosphate, which reacts exergonically with ADP to yield one mole of ATP (*1*). This utilization of carbamyl phosphate as an energy source is possible because the microbial enzyme carbamyl phosphokinase (reaction III, above), unlike the animal enzyme carbamyl phosphate synthetase (reaction II), is freely reversible; in fact, the reaction proceeds most readily, both kinetically and thermodynamically, toward synthesis of ATP (*5, 10*).

In a similar fashion it has recently (*11, 12*) been shown that two other microorganisms can degrade creatinine and allantoin to yield energy as ATP with carbamyl phosphate being the first high-energy phosphate intermediate formed, Figs. 3 and 4.

The degradation of creatinine in *Eubacterium sarcosinogenum* (*11*), has not been studied in detail as indicated by the several arrows. The organism, however, does degrade creatinine in the presence of phosphate to yield one mole of sarcosine, 2 moles of ammonia and one mole of CO_2 with the formation of 0.2 to 0.6 mole inorganic polyphosphate. The experiments of Szulmajster (*11*) indicate rather clearly that creatine and carbamyl phosphate are intermediates in this degradation and that the carbon source for the carbamyl phosphate is the guanido-carbon of creatinine, shown by the asterisk in Fig. 3. Therefore, this degradation of creatinine or creatine by *Eubacterium sarcosinogenum* appears to be analogous to the degradation of arginine by *S. faecalis* to yield an amino acid, ammonia, and carbamyl phosphate which in turn reacts in the living cell with ADP to form ATP. The phosphorolysis of carbamyloxamic acid (Fig. 3) which is the energy-yielding step in the degradation of allantoin (Fig. 4) by *Streptococcus allantoicus* has been studied by Valentine and Wolfe (*12*). The key intermediate is glyoxylurea which can be degraded either to a second mole of urea and glyoxylic acid, or it can be oxidized with NAD to carbamyl oxamate which undergoes phosphorolysis to carbamyl phosphate. This reaction from glyoxylurea to carbamyl phosphate, although involving free intermediates, is quite analogous to the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase. In the latter reaction, the reduction of nicotinamide-adenine dinucleotide (NAD) is accompanied by the formation of an energy-rich acylthioester whereas here an energy-rich carbamyl amide is formed on reduction of NAD.

Valentine and Wolfe have observed a phosphate-dependent degradation of urea (*13*) which requires glyoxylate that can react chemically with a mole of urea to form carbamyl glyoxylate; this in turn is oxidized to carbamyloxamic acid and phosphorylized to carbamyl phosphate. A direct phosphorylation of urea has not as yet been observed (*10*), although such a reaction is chemically feasible and might be the energy-yielding step in the degradation of creatinine by *Eubacterium sarcosinogenum* (*14*).

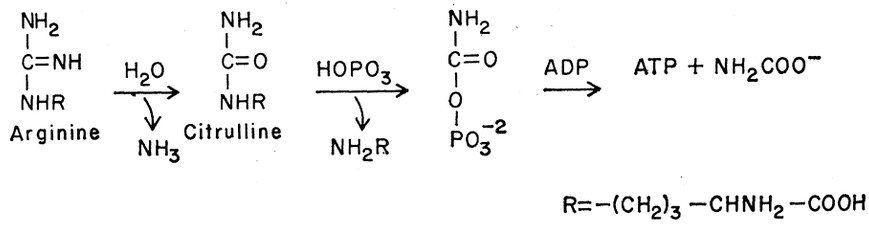


Fig. 2. Diagram of enzymatic reactions which degrade arginine to provide energy as ATP in arginine adapted *Streptococcus faecalis.* The enzymes catalyzing the reactions are from left to right arginine desimidase, ornithine transcarbamylase, carbamyl phosphokinase.

Carbamyl-Transfer Reactions for Biosynthesis: End-Product Control

In contrast to the microorganisms just discussed, which degrade a nutrient to yield carbamyl phosphate as the first high-energy product of metabolism, most forms of life synthesize carbamyl phosphate from ATP and utilize it for the biosynthesis of arginine and uridylic acid (Fig. 5). *Escherichia coli* and various mammals are the most extensively studied of these forms. *E. coli* exhibits clearly the interesting phenomenon of "feedback" control in which an essential nutrient inhibits its own biosynthesis (*15*). Feedback control operates when a sufficiently high concentration of nutrient (the "end product" of a given biosynthetic pathway) is attained within the cell. In the case of carbamyl

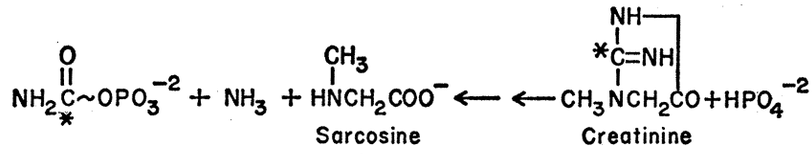
enzymes, the end products are arginine and uridylic acid (or a pyrimidine nucleotide derived from it). There are two types of feedback control: "end-product inhibition," in which the *activity* of an enzyme is inhibited by its end product, and "repression," in which the *synthesis* of the enzyme is inhibited.

These control mechanisms enable the cell to conserve its available energy sources to make products (or enzymes synthesizing these products) which are not available in the medium. Therefore, if uracil (*16*) or arginine (*17*) is present in the growth medium the biosynthesis of uridylic acid and arginine is halted. Physiologically, the two control mechanisms are geared to respond to varying amounts of nutrient. End-product inhibition is a more rapid response, sensitive to smaller concentrations of nutrient. Repression control requires time for the already existing enzyme concentration to be diluted by the synthesis of other proteins and higher concentrations of nutrient before enzyme synthesis is repressed (*18*). Repression control, unlike end-product inhibition, is frequently exerted on the biosynthesis of all the enzymes of a biosynthetic pathway.

The dramatic nature of repression is shown by the legend to Fig. 5. An approximate 200-fold increase in the specific activity of the ornithine transcarbamylase in *E. coli* occurs when one grows this organism in a low rather than a high concentration of arginine (*18*). It should be noted that *E. coli* grown on "normal" media containing relatively small amounts of arginine is still partially repressed and only when there is no arginine or very little arginine is a cell completely "derepressed"–that is, the cell is now synthesizing ornithine transcarbamylase at a maximal rate. This increase in specific activity of cell extracts from "derepressed" *E. coli* has been used as a first "purification" step for the isolation of nearly homogeneous ornithine transcarbamylase (*19*) and crystalline aspartate transcarbamylase (*20*). In fact, Shepherdson and Pardee (*20*) estimate that a single *E. coli* bacterium grown normally contains 50 molecules of aspartate transcarbamylase, whereas the organism grown on a low uracil medium contains 1000 times as much enzyme so that the enzyme now represents 7 percent of the total bacterial protein.

Although end-product inhibition of arginine biosynthesis occurs in the formation of ornithine from glutamic acid (*21*) and is not observed with ornithine transcarbamylase in *E. coli*, it is observed with aspartate transcarbamylase in the pathway of uracil synthesis. The actual inhibitory agent is cytidine triphosphate (*22*). Of particular interest is the fact that CTP, which acts like a competitive inhibitor of aspartate, appears to be bound to a site distant from the site responsible for enzyme activity. In lettuce-seedling extracts (*23*) and in *Neurospora* (*24*) a related but qualitatively different end-product inhibition of the pathway of uracil synthesis exists. Here the cytidine nucleotides do not inhibit the aspartate transcarbamylase but the uridine nucleotides do; uridylic acid is most effective; this inhibition is noncompetitive with respect to aspartate. In homogenates of rat liver and rat ascites cells (*25*) there is a competitive inhibition of this enzyme by pyrimidine nucleotides. This system is once again distinct, since the deoxyribosides of both purines and pyrimidines appear to be more potent than the ribonucleotides, while with *E. coli* the sugar moiety makes no difference in the amount of inhibition. Only in *E. coli* has this inhibition been shown to function in vivo. That such in vivo control might exist in a higher organism has been observed in a child patient (*26*) who excreted large amounts of orotic acid and had a megaloblastic anemia not responsive to vitamin B_{12}, folic acid, ascorbic acid, pyridoxine, or uracil. The metabolic defect therefore appeared to be in the conversion of orotic acid to uridylic acid. Oral administration of a mixture of uridylic acid and cytidylic acid rapidly reduced the excretion of orotic acid and returned the blood and marrow to normal; thus the enzymes participating in the synthesis of orotidylic acid, namely, aspar-

3. Creatinine degradation by Eubacterium sarcosinogenum



4. Oxamate Transcarbamylase

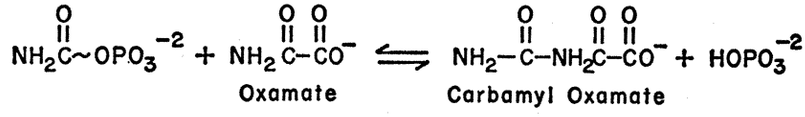


Fig. 3. Enzymes catalyzing reactions which utilized the carbamyl group of carbamyl phosphate, II. In the degradation of creatinine the several enzymes required have not been isolated nor is the exact identity of the intermediate substrates known; this is indicated by the double arrow. The starred carbon of creatinine (the guanido carbon) is the source of the carbon of carbamyl phosphate.

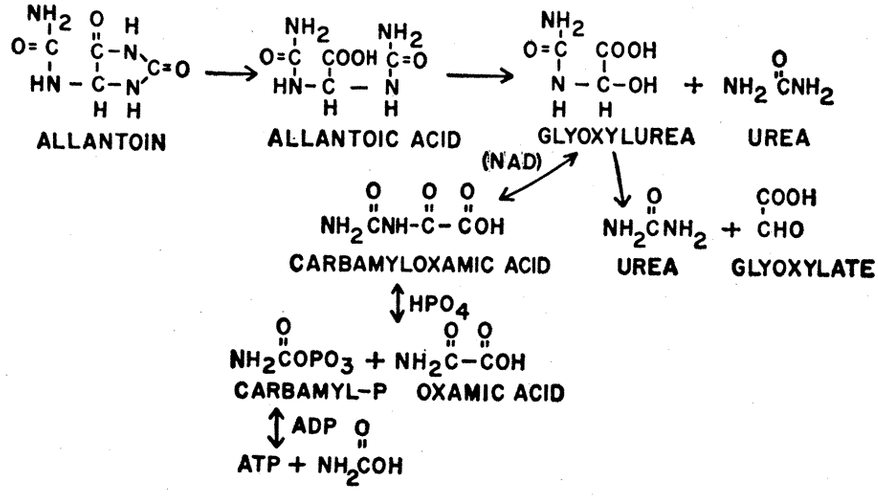


Fig. 4. Utilization of allantoin as energy source by *Streptococcus allantoicus*.

tate transcarbamylase and dihydroorotase, were repressed in this patient much as these enzymes can be repressed in *E. coli*.

Stadtman and co-workers (*27*), in studying aspartyl phosphokinase—the first enzyme of a series utilized in the biosynthesis of lysine and threonine—discovered two aspartyl phosphokinases, one inhibited by lysine and the other by threonine. It will be of interest to see whether there are two analogous carbamyl phosphokinases, one which would be repressed or inhibited by arginine and a second by uridylic acid. Gorini and Kalman (*28*) have isolated a mutant of *E. coli* whose carbamyl phosphokinase activity is repressed by uracil. Thorne and Jones (*29*) however, can find no end-product inhibition of carbamyl phosphokinase from *E. coli*. In *Neurospora* mutants, R. H. Davis hypothesizes two carbamyl phosphokinases to resolve the interplay of their nutritional requirement for arginine and uracil (*30*).

Although most bacteria and animals studied have utilized a pathway for uridylic and cytidylic acid synthesis where the sequence is carbamyl phosphate, carbamyl aspartate, dihydroorotate, orotodylate to uridylate, some of the higher vertebrates or certain organs of the higher vertebrates lack the enzyme capable of synthesizing carbamyl phosphate from ATP. This enzyme cannot be found in nonureotelic vertebrates such as the pigeon (*31*) and in ureotelic vertebrates its distribution is limited mainly, although not exclusively, to the liver (*32*). Aspartate transcarbamylase, however, is widely distributed (*33*), appears in the liver of the pigeon and rat, and is particularly rich in rapidly growing tissues such as intestinal mucosa, regenerating liver (*34*), and various tumor cells (*35*). The source of carbamyl phosphate in most of these tissues is unknown, and experiments of Dr. Sally Hager in my laboratory indicate that rat blood contains no more than $10^{-5}M$ carbamyl phosphate. Therefore, if the aspartate transcarbamylase of these non-hepatic cells participates in pyrimidine biosynthesis—as its high specific activity would indicate—some other enzyme source of carbamyl phosphate must be sought. It is possible that this might be a phosphorylase other than ornithine transcarbamylase, which is absent in pigeon liver and which in the rat is rather specifically localized in the liver. Therefore, the interesting bacterial studies on the degradation of creatine, allantoin, and urea may be of importance also in mammals.

At least two alternatives are possible. First, if the orotic acid pathway is the route for pyrimidine biosynthesis, an unknown or unsought enzyme must provide carbamyl phosphate either by utilizing ATP or by phosphorylizing a carbamyl compound other than citrulline. The second alternative is that there is no orotic acid pathway but that pyrimidine biosynthesis is achieved by some other route. The experiments with *Neurospora* (*36*) and with pigeon liver (*37*) suggest that a second pathway that excludes orotic acid as an intermediate may exist.

Finally, since carbamyl phosphate is closely related to and decomposes to cyanate, it would be expected that like cyanate it would react with hydroxyl groups to yield urethanes (oxygen- or *O*-carbamyl compounds), as well as with amino groups to yield ureido compounds (nitrogen- or *N*-carbamyl com-

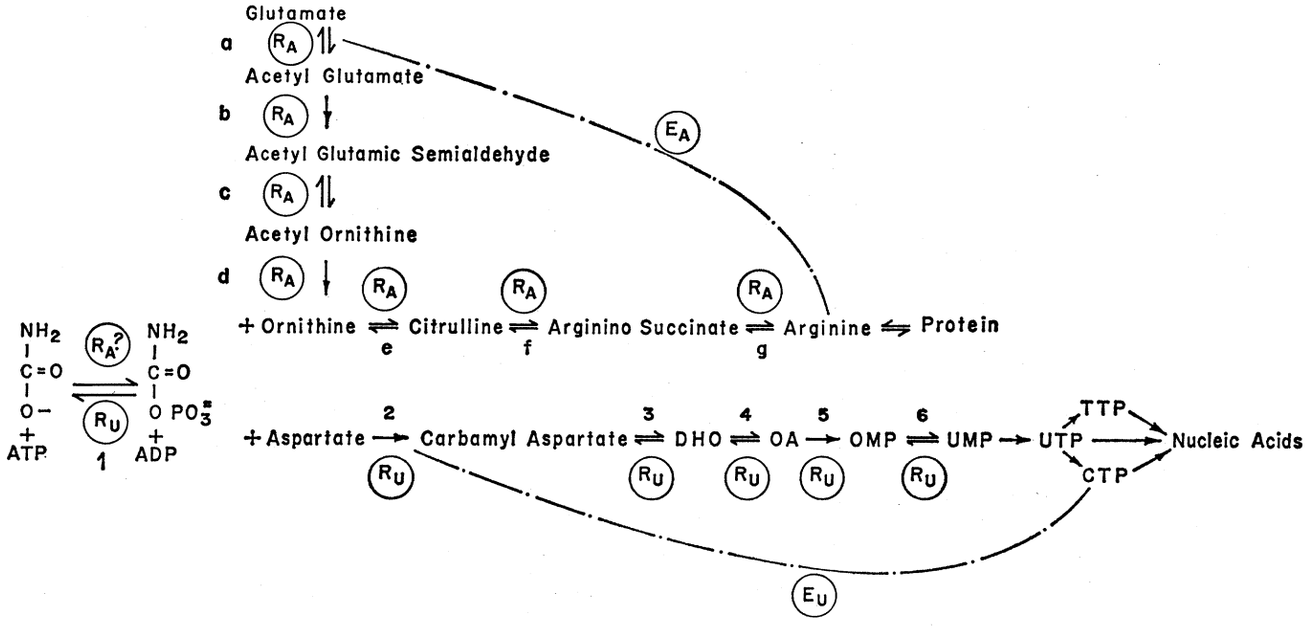


Fig. 5. Diagram of the biosynthetic pathway for arginine and uridylic acid in *Escherichia coli* indicating the position of end-product inhibition (*E*) and repression (*R*) control. Enzymes 1, 2, 3, 4, 5, and 6 are required for uridylic acid biosynthesis. All enzymes are subject to repression control by growing the cells on uracil as indicated by the symbol R_u Enzymes 1, a, b, c, d, e, f, and g are required for arginine biosynthesis and those enzymes which are subject to repression control due to high arginine in the media are labeled R_A. Enzyme 1 has not as yet been shown to be subject to such repression. End-product inhibition is observed at enzyme a in the arginine pathway and at enzyme 2, aspartate transcarbamylase, for the uridylic acid pathway. Ornithine transcarbamylase, enzyme e, levels were found (*17*) to vary as follows with the arginine content of the *E. coli* growth medium: (i) Low arginine: 104.0 units of enzyme activity per gram of dry bacteria; (ii) "normal" arginine: 8.0 units of enzyme activity per gram of dry bacteria; and (iii) high arginine: 0.5 units of enzyme activity per gram of dry bacteria. The abbreviations used here are: dihydroorotic acid, DHO; orotic acid, OA; orotidylic acid, OMP; uridylic acid, UMP; uridine triphosphate, UTP; thymidine triphosphate, TTP; and cytidine triphosphate, CTP.

pounds). Two *O*-carbamyl compounds are known to exist in nature (Fig. 6). They are carbamyl-D-serine (*38*) from a *Streptomyces*, and novobiocin, an antibiotic, which contains a 3-carbamyllyxoside residue (*39*). If novobiocin is treated with base the carbamyl group migrates from the 3 to the 2 position with concomitant loss of antibiotic activity (*40*). Therefore, the 2-carbamyl group is essential for antibiotic activity. Whether carbamyl phosphate participates in the biosynthesis of these compounds has not been tested.

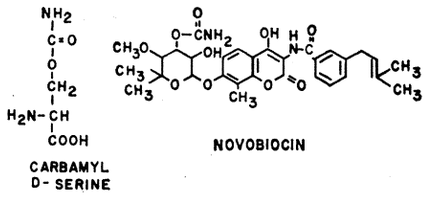


Fig. 6. Naturally occurring oxygen-carbamyl compounds.

Synthesis by Enzymes Utilizing ATP

There are three entirely different enzymes utilizing ATP to synthesize carbamyl phosphate (Fig. 7). The least studied of these enzymes was discovered recently by Levenberg (*41*) in mushrooms and appears to utilize the amide-nitrogen of glutamine as the source of the carbamyl phosphate nitrogen atom and some form of carbonate as the carbon source. The terminal phosphate of the ATP is incorporated into carbamyl phosphate and ADP is the other product. It seems likely that there should be several reactions in this synthesis and one step should be hydrolytic.

The other two reactions have been known for some time—the microbial reaction was studied in *S. faecalis*; it also occurs in many other microorganisms. The enzyme named carbamyl phosphokinase catalyzes the phosphorylation of carbamate by ATP to yield carbamyl phosphate and ADP (*10*). In the third reaction, studied mainly by Grisolia and Cohen (*42*) and by Metzenberg *et al.* (*43, 44*), the substrate is ammonium bicarbonate (*45*) and not carbamate. The reaction also differs in that it requires 2 moles of ATP and, as an essential catalytic cofactor, an *N*-acylglutamate. The natural cofactor is acetyl glutamate (*46*). This enzyme has been called carbamyl phosphate synthetase.

To return to the enzyme carbamyl phosphokinase, carbamate (*10*) was shown to be the substrate at 10°C and the equilibrium constant for the reaction was 0.037. The ΔF calculated from this value is 1.8 kcal (*p*H 9.5). Therefore, the reaction is endergonic and at *p*H 9.5, the phosphoryl potential of carbamyl phosphate is approximately 1.8 kcal greater than that of the terminal phosphate of ATP, and the reaction proceeds most easily both kinetically (*5*) and thermodynamically toward synthesis of ATP. The enzyme from vertebrates, carbamyl phosphate synthetase, is not only different in the substrates it utilizes (Fig. 8), but also it is partially irreversible. However, as shown by the second and third equations (Fig. 8), the enzyme will catalyze two partial reactions.

In the absence of added ammonia, and with the addition of ornithine and ornithine transcarbamylase to trap contaminating ammonia, one can observe the liberation of orthophosphate with larger amounts of enzyme than are required for carbamyl phosphate synthesis. Bicarbonate and acetyl glutamate are required. This cleavage of ATP to ADP and orthophosphate is thought to accompany the formation of an "activated CO_2" (*43*).

Since the phosphate bond of carbamyl phosphate is at a higher energy level than the pyrophosphate bonds of ATP, the observed formation of one mole of ATP from carbamyl phosphate and ADP would be expected (*43, 47*). This synthesis of ATP requires acetyl glutamate. The other products of this reaction are unknown. This partial backward reaction cannot be the same as that in microbial systems since not only is acetyl glutamate required but also about 50 times the amount of enzyme is needed for the rate of formation of product to equal that of the forward reaction. It is of interest that acetyl phosphate appears to replace carbamyl phosphate in this partial backward reaction (*48*).

The detailed mechanism for carbamyl phosphate synthesis with this synthetase remains unknown. Definitive experiments with O^{18} show that the first

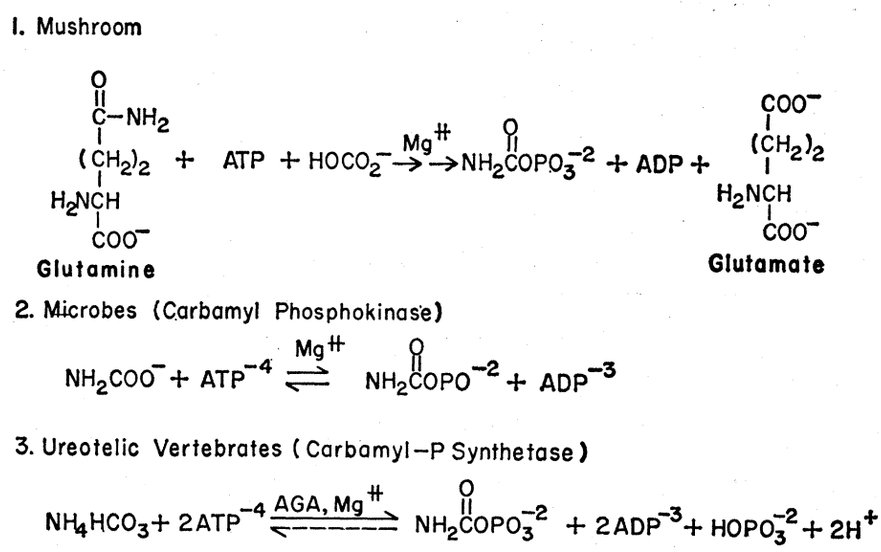


Fig. 7. Enzymatic syntheses of carbamyl phosphate utilizing ATP.

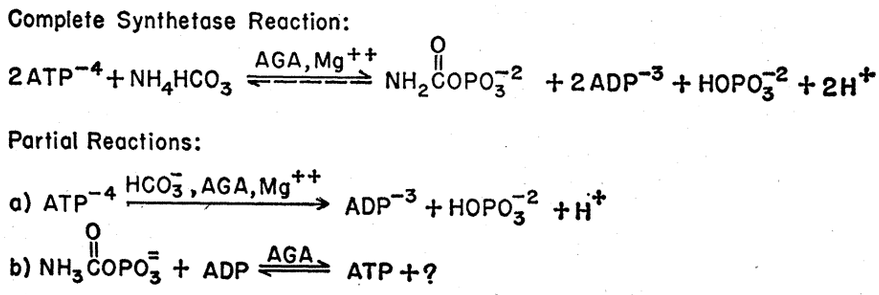


Fig. 8. Three reactions catalyzed by carbamyl phosphate synthetase.

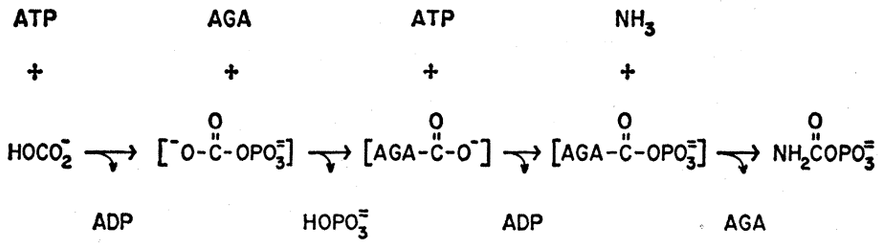


Fig. 9. Proposed mechanism for carbamyl phosphate synthesis with carbamyl phosphate synthetase.

step is an activation of carbonate (*45*). Metzenberg *et al.* (*44*) and Reichard (*49*) first studied the synthesis of carbamyl phosphate or citrulline in water containing O^{18}. These investigators found that both the phosphate residue of carbamyl phosphate and orthophosphate were labeled by the water in one of the four oxygens of the phosphate.

The direct participation of water in this reaction would allow neither the accumulation of high-energy intermediates, such as carbamyl phosphate nor the synthesis of citrulline. It therefore seemed likely that the observed incorporation occurred indirectly from the water labeled with O^{18} through its equilibration with the oxygen atoms of one of the substrates, the most likely being carbonate. To investigate this hypothesis (*45*), experiments were carried out with O^{18} water and O^{18} bicarbonate, under conditions where oxygen exchange between the two is minimal.

The equilibration of the oxygen atoms of carbon dioxide and the various species of carbonate with water below *p*H 8 occurs mainly through the reversible hydration of carbon dioxide (*50*). Above *p*H 10 there is a slow, base-catalyzed, bimolecular reaction with CO_2; between *p*H 8 and 10 both reactions occur simultaneously. Three changes were made in the incubation conditions to inhibit the exchange of O^{18} between water and bicarbonate: increasing the *p*H to reduce the CO_2, lowering the temperature to decrease the rate of exchange, and shortening the time of incubation of the experimental vessel. When these changes were made the incorporation of O^{18} from H_2O labeled with O^{18} into the two phosphate molecules was at a minimum (Table 1, experiment 2A). The original observations (Table 1, experiment 1) could be duplicated under the new conditions if carbonic anhydrase (Table 1, experiment 2B) was added to reestablish the rapid exchange of O^{18} between carbonate and water.

These facts imply that O^{18} water is not the immediate donor of O^{18} to orthophosphate or the phosphate residue of carbamyl phosphate, but rather that these atoms of O^{18} in the orthophosphate were derived from bicarbonate. The experiment to test this directly was performed once again under conditions where exchange between water and bicarbonate was at a minimum; however, the O^{18} was now in the bicarbonate. Each mole of phosphate formed contained nearly one atom of O^{18} (*45*). Thus all experiments are consistent with the view that irrespective of whether the O^{18} was initially in water or carbonate, the direct agent for transferring the O^{18} to phosphate was indeed carbonate.

These tracer experiments demonstrate that during this enzymatic synthesis of citrulline an oxygen atom is transferred from carbonate to each of two orthophosphate molecules. On the basis of these studies with O^{18}, we have proposed a mechanism (Fig. 9) for the carbamyl phosphate synthetase reaction. One oxygen transfer results from the cleavage of carbamyl phosphate between the carbon and oxygen atoms during the carbamylation of ornithine. The reaction in which the other transfer occurs is at an earlier stage in the synthesis of carbamyl phosphate and must be the first step requiring ATP. In the proposed mechanism shown here, carboxyacteylglutamate is the hypothetical product of this reaction in which there is first, a phosphorylation of bicarbonate by ATP to form carboxy phosphate and ADP. The formation of carboxy phosphate would account for the incorporation of an oxygen from carbonate into orthophosphate (*51*).

The second step requiring ATP in the carbamyl phosphate synthesis by this mechanism would be the phosphorylation of carboxyacetyl glutamate to yield the hypothetical phosphate derivative of carboxyacteyl glutamate which would finally undergo ammonolysis to yield carbamyl phosphate.

This proposed reaction sequence is consonant with all tracer experiments and provides for the catalytic function of acetyl glutamate. Dr. Marianne Grassl and I (*52*) have synthesized the trimethyl ester of carboxyacetyl glutamate and hope to utilize it to see if carboxyacetyl glutamate is a free intermediate in this synthesis of carbamyl phosphate. It is very possible that in the carbamyl phosphate synthetase reaction there are only enzyme-bound intermediates as is the case with glutamine synthetase (*53*) and the enzymes activating amino acids (*54*). Another suggestion (*55*) for the requirement of acetyl glutamate in this reaction is that unlike other coenzymes, it does not participate as a substrate in the partial reactions on the way to carbamyl phosphate synthesis, but is necessary so that the enzyme assumes a proper tertiary configuration compatible with catalysis.

There are three activation reactions which use ATP. Only one of these is freely reversible and represents the most direct synthesis, namely, the phosphorylation of carbamic acid. Although the vertebrate enzyme, carbamyl phosphate synthetase, utilizes the related substrate, ammonium bicarbonate, it requires 2 moles of ATP. This is in contrast to the high concentration of ammonium bicarbonate required to saturate the mi-

Table 1. Incorporation of O^{18} from O^{18}-water into phosphate produced during citrulline synthesis with carbamyl phosphate synthetase and ornithine transcarbamylase.

Experiment	Carbonic anhydrase	Fraction of one atom of O^{18} in phosphate
1	None	0.94
2A	None	0.20
2B	+	0.96

Experiment 1, a repetition of earlier experiments (*44*, *49*) which showed that one atom of O^{18} from water could be incorporated into each of the 2 moles of phosphate formed in citrulline synthesis was performed at *p*H 8, at 37°C for 60 minutes where oxygen exchange between water and bicarbonate is complete. In experiment 2A and B conditions were changed (*p*H 9, 30°C, 30 min) to minimize this oxygen exchange between water and bicarbonate. To show that the decrease in the labeling of the orthophosphate under these new conditions in experiment 2A was due solely to hindering the oxygen exchange between water and bicarbonate and not to other unknown effects, the exchange was reinstituted by the addition of carbonic anydrase in experiment 2B. Since Reichard (*49*) had demonstrated equal labeling of the orthophosphate and carbamyl phosphate formed in this reaction (see Fig. 7, reaction 3) we have simplified the experiment by adding ornithine and ornithine transcarbamylase so that both the orthophosphate and the phosphate group of carbamyl phosphate are now in the orthophosphate pool (note that release of phosphate from carbamyl phosphate in the enzyme reaction occurs with C-O cleavage and therefore does not introduce oxygen from water). A detailed report of this experiment (*45*) has been presented elsewhere.

crobial enzyme carbamyl phosphokinase and the reaction catalyzed by carbamyl phosphate synthetase is now only partially reversible. This change in mechanism is an obvious evolutionary advantage, since ammonia is present in low concentration in mammalian tissues and, in addition, is highly toxic to the organism (*56*). The synthetase found in mushrooms is a third, completely distinct enzyme requiring glutamine for its source of nitrogen. Here the reaction probably has a hydrolytic step and would therefore be expected to be irreversible. Carbamyl phosphate can also be formed phosphorolytically from citrulline, from carbamyl oxamate derived from allantoin, and from some metabolite of creatinine; it can be used for the synthesis of citrulline, carbamyl aspartate, and carbamyl oxamate. It seems probable that this will not be the complete list of reactions which utilize carbamyl phosphate as a carbamyl source (*57*).

The comparative biochemistry of carbamyl phosphate metabolism is a rich field open to future development. In addition to the microbial studies mentioned here, Cohen and Brown (*31*) have made a survey of the distribution of carbamyl phosphate synthetase and ornithine transcarbamylase in a large number of vertebrates and have discussed their evolutionary significance. Of particular elegance is the demonstration of the appearance of carbamyl phosphate synthetase and ornithine transcarbamylase in the metamorphosing frog (*58*).

References and Notes

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$$K_1 = \frac{\text{(carbamyl phosphate)}}{\text{(cyanate) (phosphate)}} = 3 \quad (5) \text{ and}$$

$$K_2 = \frac{\text{(urea)}}{\text{(cyanate) (ammonia)}} = 7 \times 10^4$$

[This latter figure is calculated from data in the paper of P. Dirnhuber and F. Schutz, *Biochem. J.* **42**, 628 (1948).)] $K_3 = K_1/K_2 = 4 \times 10^{-5}$. The latter figure is close to the value for the equilibrium constant for the reversible reaction of citrulline + phosphate $\rightleftharpoons$ carbamyl phosphate + ornithine of 1×10^{-5} determined by Reichard (*2*).
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