Aspartate Carbamoyltransferase from Rat Liver

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1. Aspartate-carbamoyltransferase activity was concentrated from rat-liver preparations. Only L-aspartate, β -benzyl-L-aspartate and β -erythro-hydroxy-DL-aspartate were carbamoylated enzymically. The K_m for L-aspartate and carbamoyl phosphate have been determined by three methods: colorimetric procedure, radioactive assay with [^{14}C]aspartate and an assay with [^{14}C]carbamoyl phosphate. 2. The K_m for aspartate has been determined as a function of the pH; the pK of the functional group at the active site of the enzyme, pK_a , was at pH 9.0. Enzymic activity was diminished in the presence of *N*-ethylmaleimide, *p*-hydroxymercuribenzoate and the heavy metals Ag^+ , Hg^{2+} , or Zn^{2+} . The inhibitions could be prevented by mercaptoethanol. These findings suggested the association of a thiol group with the enzymic activity. 3. Enzymic activity was also decreased by sodium lauryl sulphate, urea and dioxan. Competitive inhibition (with L-aspartate) was manifested by maleate, succinate, oxaloacetate, β -erythro-hydroxy-DL-aspartate and β -benzyl-L-aspartate. The K_i for most of these inhibitions has been determined. 4. The properties of the liver enzyme are compared with those of *Escherichia coli* aspartate carbamoyltransferase and the implications of the findings are discussed.

Aspartate carbamoyltransferase catalyses one of the first reactions leading to the biosynthesis of pyrimidines, the transcarbamoylation of L-aspartic acid by carbamoyl phosphate to form carbamoyl-aspartate. In bacteria, the enzyme occupies a central position in the metabolic control of pyrimidine biosynthesis. The effectiveness of the feedback mechanism in regulating the synthesis of pyrimidines is due to the sensitivity of aspartate carbamoyltransferase to one of the end products of the pathway, CTP. The native enzyme may be separated into two types of sub-units: the larger, in which the catalytic activity resides, and a second, which possesses the regulatory sites (Gerhart & Pardee, 1962, 1963, 1964; Gerhart & Schachman, 1965). These studies have established the allosteric nature of *Escherichia coli* aspartate carbamoyltransferase.

Aspartate carbamoyltransferase from lettuce (Neumann & Jones, 1964), *Pseudomonas* (Neumann & Jones, 1964), erythrocytes (Curci & Donachie, 1964) and mammalian systems (Bresnick & Hitchings, 1961; Bresnick, 1963), however, does not behave in this manner. End-product inhibition is not manifested by CTP, but by other pyrimidine derivatives and only at high concentrations of both inhibitor and substrate. The enzyme has been partially purified from rat liver and some of its characteristics have been determined. The results

which are presented below establish clearly the fundamental dissimilarity between the *Escherichia coli* and liver enzymes.

EXPERIMENTAL

Animals. Male rats, approx. 175–200 g., were obtained from the Cheek-Jones Co., Houston, Texas, U.S.A., and were allowed free access to both food and water. The rats were killed by bleeding after aortic transection, the livers were removed, washed in cold 0.9% NaCl, and homogenized in cold 0.25 M-sucrose at 4° (1:4, w/v).

Materials. The amino acids and substituted amino acids were purchased from Calbiochem, Los Angeles, Calif., U.S.A.; β -benzyl-L-aspartate and cysteine acid from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. Sigma Chemical Co., St Louis, Mo., U.S.A., supplied the acetyl phosphate, carbamoyl phosphate, *N*-ethylmaleimide and *p*-hydroxymercuribenzoate. Sodium lauryl sulphate and dioxan were purchased from Fisher Chemical Co., U.S.A. Sephadex G-200 was obtained from Pharmacia Co., U.S.A. Hydroxyurea was a gift from Dr M. Lane of this Department.

DL-[3- ^{14}C]Aspartic acid (3.3 $\mu\text{C}/\mu\text{mole}$) and [^{14}C]cyanate (5 $\mu\text{C}/\mu\text{mole}$) were purchased from the New England Nuclear Corp., Boston, Mass., U.S.A. [^{14}C]Carbamoyl phosphate was prepared from the latter by the method of Spector, Jones & Lipmann (1957). The final product had a specific activity 0.0125 $\mu\text{C}/\mu\text{mole}$; the product was dissolved immediately before use.

Hydroxyapatite was prepared by the method of Tiselius,

Hjertenend & Levin (1956) as modified by Myazawa & Thomas (1965).

Enzyme assays. Three methods were employed in the assay of liver aspartate-carbamoyltransferase activity.

Procedure 1. The enzyme was incubated at 37° for 15 min. in a total volume of 1.5 ml. with 5 mM-L-aspartic acid, 0.7 mM-carbamoyl phosphate and 0.2 M-tris buffer, pH 9.2. At the end of the incubation, the enzymic reaction was stopped by the addition of 0.2 ml. of 4 N-HClO₄. The reaction tubes were placed in an ice bath for 5 min. to coagulate the denatured proteins, which were then removed by centrifugation. The carbamoylaspartate was colorimetrically determined in a portion of the deproteinized material by the method of Koritz & Cohen (1954) as modified by Gerhart & Pardee (1962). The colorimetric assay cannot be employed with high concentrations of thiol groups because of marked inhibition of colour development.

Procedure 2. Enzymic activity was ascertained by a radioactive assay in which DL-[3-¹⁴C]aspartate was utilized as substrate (Bresnick, 1965). The assay system consisted of: DL-[3-¹⁴C]aspartic acid, 2 μ moles (0.1 μ C); carbamoyl phosphate, 5 μ moles; enzyme; 0.2 M-tris buffer, pH 9.2, to 1.5 ml. The incubation was conducted at 37° for 15 min., after which time 0.2 ml. of 4 N-HClO₄ was added to stop the reaction. Phenol red was introduced as an internal indicator and 4 N-KOH was added until neutrality. The precipitate was removed by centrifugation and the supernatant fraction was added to a column (0.6 cm. $\times$ 5 cm.) of Dowex 50 (X8; H⁺ form; 200–400 mesh). [¹⁴C]Carbamoylaspartate passed through the ion-exchange resin whereas aspartic acid was retained. The eluate and washings were combined and the radioactivity was estimated in a Packard Tri-Carb liquid-scintillation spectrometer with the use of the scintillation solvent system of Bray (1960) at an efficiency of 75%.

Procedure 3. Enzymic activity was determined by a modification of the method of Lowenstein & Cohen (1956) in which advantage is taken of the acid-lability of carbamoyl phosphate while carbamoylaspartate is stable. The

assay system consisted of: [¹⁴C]carbamoyl phosphate, 2.4 μ moles (0.3 μ C); L-aspartate, 5 mM; 0.2 M-tris buffer, pH 9.2, to 1.5 ml. At the end of the incubation, 0.5 ml. of 4 N-HCl was added to stop the reaction, a portion was plated on planchets and was dried in an oven for 20 min. The radioactivity was then estimated in a Nuclear-Chicago gas-flow end-window counter at an efficiency of 25%.

The protein concentration of the enzymic extracts was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as the reference standard. The protein eluted from the Sephadex G-200 and hydroxyapatite columns was monitored by ultraviolet absorption at 240 and 280 m μ .

RESULTS

Purification of aspartate carbamoyltransferase

Aspartate carbamoyltransferase activity was concentrated from rat liver by two methods. A summary of the purification data is presented in Table 1. Method 1, i.e. hydroxyapatite, yielded the fraction with the greater specific activity and, accordingly, was employed more frequently.

The rat-liver homogenate was centrifuged at 100 000g for 60 min. in a no. 30 rotor in a Spinco L-2 ultracentrifuge at 5° to yield the soluble extract without any appreciable loss in enzymic activity. Solid ammonium sulphate was added to the soluble extract to 30% saturation and the suspension was stirred at 4° for 10 min. The suspension was centrifuged at 10 000g for 10 min. and the precipitate was dissolved in 0.01 M-imidazole buffer, pH 7.0, which contained 3 mM-mercaptoethanol. The ammonium sulphate fraction was dialysed for 4 hr. against the imidazole buffer-mercaptoethanol in a rocking dialyser. The

Table 1. *Purification of liver aspartate carbamoyltransferase*

	Volume (ml.)	Carbamoyl- aspartate (μ moles)	Sp. activity (μ moles of product/ 15 min./mg. of protein)	Yield (%)	Yield purification
Method 1					
Homogenate	2700	1320	0.04	100	—
Soluble extract	2200	1285	0.06	97	1.5
Dialysed 30% (NH ₄) ₂ SO ₄	285	1049	0.34	80	8.5
Hydroxyapatite	42*	400	1.82	31	45
30% (NH ₄) ₂ SO ₄	20*	228	6.85	17	43
Method 2					
Homogenate			0.06		
Extract + 30% (NH ₄) ₂ SO ₄			0.24		4
G200 combined			0.58		10
30% (NH ₄) ₂ SO ₄			2.84		47

* Only 100 ml. of extract was applied to the column. Yields have been extrapolated from these data.

dialysed fraction retained approx. 80% of the original activity and had specific activity 0.34, which was 8.5-fold greater than the original homogenate. A sample of the dialysed fraction (100 ml.) was applied to a column of hydroxyapatite, 4 cm. $\times$ 10 cm., which had been equilibrated with 0.05 M-phosphate buffer (pH 8.0)–3 mM-mercaptoethanol. The column was developed stepwise with increasing concentrations of phosphate buffer (pH 8.0)–3 mM-mercaptoethanol. The enzymic activity could be eluted with 0.3 M-phosphate. The specific activity of the enzyme increased to 1.82 μ moles of product/15 min./mg. of protein in a yield of 31%.

Solid ammonium sulphate was added to the hydroxyapatite fraction to 30% saturation and the suspension was stirred for 5 min. at 5°. The enzyme was sedimented after centrifugation at 10000 g for 10 min. and was dissolved in 0.05 M-tris buffer (pH 9.2)–3 mM-mercaptoethanol. The specific activity of the latter ranged from 4.5 to 7.8 with an overall yield of 17% and an overall purification of approx. 140-fold.

A second procedure for concentration of the enzyme was employed in the initial stages of the investigation. The 30% ammonium sulphate fraction (10 ml.) was applied to a column of Sephadex G-200, 4.0 cm. $\times$ 50 cm., which had been equilibrated with 0.05 M-tris buffer, pH 9.2, that contained 3 mM-mercaptoethanol. The enzyme, which was eluted with the void volume after development of the column with this buffer, was concentrated by a second treatment with ammonium sulphate (to 30%). The sediment was dissolved in the tris-mercaptoethanol buffer to yield a fraction with specific activity 2.84 μ moles of product/15 min./mg. of protein.

Properties of liver aspartate carbamoyltransferase

pH optimum. Aspartate-carbamoyltransferase activity at 5 mM-aspartate and 1 mM-carbamoyl phosphate exhibited a maximum at pH 9.2; 4% and 94% of this activity was manifested at pH 7.0 and 10.0 respectively.

Substrate specificity. The carbamoyl group of carbamoyl phosphate was transferred to L-aspartate, β -benzyl-L-aspartate and β -erythro-hydroxy-DL-aspartate. The most effective substrate was L-aspartate, the least effective β -erythro-hydroxy-aspartate. The list of compounds which were not carbamoylated under these conditions includes: D-aspartic acid, L-alanine, L-glutamic acid, glycine, O-phospho-L-serine, DL-cysteic acid, DL-homocysteic acid, L-cysteine, DL- α -aminobutyric acid, DL- β -aminobutyric acid, DL- β -methylaspartic acid, β -threo-hydroxy-DL-aspartic acid and L-ornithine.

Aspartate carbamoyltransferase did not catalyse

the reactions between L-aspartate and acetyl phosphate, ethyl urethane or hydroxyurea. The enzyme exhibited a particular specificity towards carbamoyl phosphate.

Michaelis constants. The K_m for aspartate and carbamoyl phosphate was determined with each of the methods for assay. The dependence of the velocity upon the concentration of the substrates as well as the Lineweaver-Burk representations (Lineweaver & Burk, 1934) are shown in Figs. 1 and 2. The K_m for aspartate, as determined by the colorimetric [14 C]aspartate and [14 C]carbamoyl phosphate procedures, was 3.0, 5.5 and 4.3 mM respectively. The K_m for carbamoyl phosphate, as determined by these methods, was 38, 22 and 54 μ M respectively.

The pK_m has been plotted against the pH and gives an inflexion point at pH 9.0 (Fig. 3). If K_m is equal to K_s , these data may indicate an ionization constant of the group in the enzyme to which the aspartate is bound, the pK_s (Dixon & Webb, 1964). The possibilities for a group with this pK include: SH^- ; α -ammonium; phenolic hydroxyl (tyrosine); ϵ -ammonium (lysine) (Dixon & Webb, 1964).

Effect of temperature. Aspartate carbamoyltransferase was gradually inactivated at temperatures at and above 50°. After 15, 30 and 60 min. at 50°, only 73, 53 and 16% of the original activity was observed. Over 90% of the original enzymic

Fig. 1. Velocity of liver aspartate carbamoyltransferase as a function of aspartate concentration. The enzyme, 22 μ g. of protein, was obtained after saturation of the hydroxyapatite fraction to 30% with $(NH_4)_2SO_4$. Carbamoylaspartate was determined by the colorimetric assay after a 15 min. incubation at 37° of L-aspartate (Asp) with carbamoyl phosphate, 0.7 mM. ●, $1/v$ against $1/[Asp]$. ○, $[Carbamoylaspartate]$ against $[Asp]$.

Fig. 2. Velocity of liver aspartate carbamoyltransferase as a function of carbamoyl phosphate concentration. The amount of enzyme employed in the assay was 22 μ g. [hydroxyapatite-(NH₄)₂SO₄ fraction] and the incubation was conducted for 15 min. at 37°. L-Aspartate was present at 7.5 mM; concentrations of carbamoyl phosphate were varied as indicated in the Figure. K_m was 0.44 mM. ●, [Carbamoylaspartate] against [carbamoyl phosphate]; ○, 1/ v against [carbamoyl phosphate].

Fig. 3. Michaelis constant for aspartate as a function of pH. See the legend to Fig. 1.

activity is lost after 5 min. at 60°. The enzyme could not be stabilized against the heat-inactivation by the addition of thiol groups, bovine serum albumin (addition of 6.0 mg.), or L-aspartate (up to 10 mM).

Thiol reagents. Aspartate-carbamoyltransferase activity was sensitive to thiol reagents. *N*-Ethylmaleimide and *p*-hydroxymercuribenzoate inhibited enzymic activity (Table 2). The latter was the more potent in this regard. At 0.7 mM, 17% and 42% inhibition respectively were recorded.

The inhibition by *p*-hydroxymercuribenzoate was determined as a function of the pH. At concentrations of inhibitor from 0.33 to 1.32 mM the shapes of the velocity-pH curves were generally similar to those of the uninhibited system.

Inhibition by heavy metals. Aspartate-carbamoyltransferase activity was inhibited by Ag⁺, Zn²⁺ and Hg²⁺. A 70–80% reduction in enzymic activity was observed in the presence (0.2 mM) of either Ag⁺

Table 2. Inhibition of liver aspartate carbamoyltransferase by thiol reagents

The reaction mixture, in final vol. 1.5 ml., included in Expt. A: L-aspartic acid, 5 mM; carbamoyl phosphate, 0.7 mM; enzyme [70 μ g. of protein, Sephadex G-200-treated 30% (NH₄)₂SO₄ fraction]; the inhibitors, *N*-ethylmaleimide or *p*-hydroxymercuribenzoate, as indicated; 0.2 M-tris buffer, pH 9.2. In Expt. B the amount of enzyme [hydroxyapatite-(NH₄)₂SO₄ fraction] was 32 μ g.

Additions (mM)	Carbamoyl-aspartate (μ mole)	Inhibition (%)
Expt. A:		
None	0.30	—
<i>N</i> -Ethylmaleimide, 0.7	0.25	17
1.6	0.20	33
3.2	0.17	43
4.8	0.14	53
Expt. B:		
None	0.29	—
<i>p</i> -Hydroxymercuribenzoate,		
0.1	0.25	14
0.3	0.22	24
0.7	0.17	42
1.0	0.09	69
1.3	0.05	83

Fig. 4. Inhibition of liver aspartate carbamoyltransferase by maleate (●) and succinate (○). See the legend to Fig. 1. The amount of carbamoylaspartate produced in the control assay was 0.23 μ mole.

or Hg²⁺; at 2 mM, Zn²⁺ inhibited enzymic activity by 87%. The inhibition was relieved by the concomitant incorporation into the reaction medium of mercaptoethanol at 0.6 mM. The concentrations of Ag⁺ and Hg²⁺ which was necessary for the production of 50% inhibition of enzymic activity were 0.75 and 0.5 mM respectively.

Agents which disrupt hydrogen and hydrophobic bonding. Enzymic activity was also sensitive to sodium lauryl sulphate, urea and dioxan. Over

Table 3. *Inhibition of liver aspartate carbamoyltransferase by structural analogues of L-aspartate*

The enzyme source was $(\text{NH}_4)_2\text{SO}_4$ -treated hydroxyapatite fraction, 22 μg . Carbamoylaspartate was determined by the colorimetric assay. The number of determinations is indicated within the parentheses after the structural analogue; the range of K_i is also indicated within parentheses.

	K_m (mM)	K_i (mM)	Type of inhibition
L-Aspartate (6)	3.0 (2.1-3.6)	—	—
Succinate (4)	—	3.3 (2.9-5.8)	Competitive
Maleate (4)	—	1.8 (1.6-2.4)	Competitive
Oxaloacetate (3)	—	6.8 (5.8-8.0)	Competitive
β -erythro-Hydroxy-DL-aspartate (2)	—	2.9 (2.7-3.1)	Competitive
β -Benzyl-L-aspartate (2)	—	3.1 (2.9-3.3)	Competitive

Table 4. *Compounds which do not inhibit liver aspartate carbamoyltransferase*

The compounds were tested at 64 mM at an inhibitor/L-aspartate or carbamoyl phosphate ratio 20:1, with 50 μg of the hydroxyapatite fraction.

C ₂ and C ₃ acids	C ₄ acids
Malonic acid	DL- α -Aminobutyric acid
L-Alanine	DL- β -Aminobutyric acid
Pyruvic acid	β -Methyl-DL-aspartic acid
Glycine	DL-Malic acid
DL-Cysteic acid	Fumaric acid
O-Phospho-L-serine	β -threo-Hydroxy-DL-aspartic acid
	DL-Homocysteic acid
	L-Asparagine
	L-Threonine
C ₅ acids	Analogues of carbamoyl phosphate
α -Oxoglutaric acid	Acetyl phosphate
L-Glutamate	Urethane
L-Glutamine	Hydroxyurea
	Pyrophosphate

80% inhibition of aspartate carbamoyltransferase activity was observed in the presence of 2 mM, 4.0 M and 4.0 M respectively of sodium lauryl sulphate, urea and dioxan.

Inhibition by structural analogues of aspartate. Enzymic activity was inhibited by the structural analogues of L-aspartate, succinate and maleate, which at 6 and 4 mM respectively diminished activity by 50% (Fig. 4). A number of other analogues proved to be effective inhibitors of aspartate carbamoyltransferase (Table 3). The compounds presented in Table 3 were competitive inhibitors (with aspartate) of the enzyme. The most effective of these inhibitors was maleate, which had K_i 1.8 mM as compared with K_m for aspartate 3.0 mM. The least potent was oxaloacetate with K_i 6.9 mM. The β -erythro-hydroxy-DL-aspartate, β -benzyl-L-aspartate and succinate possessed a similar K_i 3 mM.

The compounds which did not inhibit enzymic activity at inhibitor/substrate ratios 20:1 have

been tabulated in Table 4. In this Table are included several analogues of carbamoyl phosphate.

Effects of other substances. The [^{14}C]aspartate assay was employed to test the efficacy of several compounds as inhibitors of liver aspartate-carbamoyltransferase activity. The ineffective compounds included: EDTA (to 7 mM), UTP, CTP, deoxyadenosine or deoxyAMP (at inhibitor/aspartate = 1), CN^- or N_3^- (to 5 mM). Deoxyadenosine and deoxyAMP, in agreement with the results of Neumann & Jones (1964), did inhibit the development of the colour in the colorimetric assay. Pyrimidine ribo- and deoxyribo-nucleosides inhibited enzymic activity slightly only in the presence of high concentrations of L-aspartate, i.e. 14 mM.

DISCUSSION

Although aspartate carbamoyltransferase has been extensively purified and crystallized from extracts of de-repressed *Escherichia coli* (Shepherdson & Pardee, 1960), the enzyme from mammalian sources has only been partially purified from rat liver. The initial observations on the mammalian enzyme were reported by Lowenstein & Cohen (1956) with crude extracts. In view of the importance of this enzyme in the metabolic control of pyrimidine biosynthesis in bacteria, it seemed of importance to obtain comparative data with the mammalian enzyme. The procedure described herein offers a relatively simple means to accomplish this objective.

A comparison of a number of properties of the two enzymes is presented in Table 5. The *Escherichia coli* aspartate carbamoyltransferase may be precipitated from a soluble extract by saturation with ammonium sulphate.

The pH optimum for the *Escherichia coli* enzyme is markedly influenced by the substrate concentration, i.e. aspartate. At concentrations of aspartate < 5 mM, maximum activity is manifested at pH 6.8; at concentrations > 25 mM, maximum activity may be noted at pH 8.2 (Gerhart & Pardee, 1964). In the bacterial enzyme, the aspartate saturation

Table 5. Comparison of properties of the *Escherichia coli* enzyme with liver aspartate carbamoyltransferase

Source of enzyme.....	<i>E. coli</i>	Reference	Liver
(NH ₄) ₂ SO ₄ required for precipitation	40-50%	Shepherdson & Pardee (1960)	30%
pH optimum	6.8	Gerhart & Pardee (1964)	9.2
Nature of plot of [Asp] versus velocity	Sigmoidal	Gerhart & Pardee (1964)	Non-sigmoidal
Stability at 60°	Stable	Shepherdson & Pardee (1960)	Unstable
Effect of mercurials	Activation	Gerhart & Pardee (1962)	Inhibition
End-product inhibition by CTP	Yes	Gerhart & Pardee (1962)	No
Effect of ATP	Activation	Gerhart & Pardee (1962)	None
Effect of low [maleate]	Activation	Gerhart & Pardee (1964)	Inhibition
Effect of pyrophosphate	Inhibition	Kleppe (1965)	None

curve is uniquely affected by the pH (Gerhart & Pardee, 1963). Sigmoidal kinetics, which are reminiscent of the Bohr effect with the haemoglobins, are observed at alkaline pH values (Gerhart & Pardee, 1963). The mammalian enzyme does not exhibit the sigmoidal kinetics and, in this respect, behaves like the bacterial monomer.

The bacterial enzyme is subject to end-product inhibition by CTP (Gerhart & Pardee, 1962). The formation of the CTP-bound enzyme apparently favours the less active conformation of aspartate carbamoyltransferase, a concept which has been fully exploited by Changeux (1964) and Monod, Wyman & Changeux (1965). The pyrimidine end product, however, has little effect upon the liver enzyme although, at high aspartate concentrations, a small inhibition may be observed (Bresnick, 1963). No inhibition by CTP of enzymic activity has been reported in crude extracts from lettuce seedlings (Neumann & Jones, 1964) or erythrocytes (Curci & Donachie, 1964). It appears quite unlikely that the mammalian enzyme is subject to end-product inhibition, a conclusion which is in substantial agreement with that of Curci & Donachie (1964).

The liver enzyme, in contrast with the *Escherichia coli* enzyme, is markedly inhibited by mercurials and by thiol reagents, as first reported by Lowenstein & Cohen (1956) and confirmed in this paper. The thiol group of the mammalian enzyme is apparently associated with the catalytic activity.

The mammalian enzyme also differs from the bacterial aspartate carbamoyltransferase in the response to maleate (Gerhart & Pardee, 1964). At concentrations of maleate < 2 mM, significant stimulation of enzymic activity may be demonstrated in the latter (Gerhart & Pardee, 1964), whereas a marked inhibition is observed with the former.

The substrate specificity and inhibition data with the liver enzyme allow one to speculate on the nature of active site, i.e. one of the active sites. The substrate, L-aspartate, apparently interacts in

the 'chair' form with aspartate carbamoyltransferase. This inference is based on K_i for maleate, which is lower than K_m for aspartate, and on the finding that fumarate does not inhibit enzymic activity. Succinate, like aspartate, can exist in the 'chair' form as well as in other configurations, and hence possesses K_i similar to K_m for aspartate.

The site on the liver aspartate carbamoyltransferase to which carbamoyl phosphate is bound is very specific. Under our conditions, no substitute could be found which would either inhibit carbamoylaspartate formation in the presence of carbamoyl phosphate or would react with aspartate in the absence of carbamoyl phosphate. In addition pyrophosphate, a competitive inhibitor of the latter, with the bacterial aspartate carbamoyltransferase (Kleppe, 1965) exhibits little effect upon enzymic activity at inhibitor/carbamoyl phosphate ratios 40:1. Neither ethyl urethane, in which the phosphate of carbamoyl phosphate is replaced by an ethoxy group, nor acetyl phosphate, in which the amide of carbamoyl phosphate is replaced by a methyl group, can effect any enzymic alteration of aspartate.

The findings clearly demonstrate the differences between the properties of the liver aspartate carbamoyltransferase and the *Escherichia coli* enzyme. These differences are attended by the lack of a mechanism for control of enzymic activity in the liver (at least *in vitro*). If feedback control of pyrimidine biosynthesis does occur *in vivo*, it does so at a site distinct from aspartate carbamoyltransferase.

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