Studies on Carbamoyl Phosphate-L-Ornithine Carbamoyltransferase from Ox Liver

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Carbamoyl phosphate-L-ornithine carbamoyltransferase, EC 2.1.3.3 (ornithine carbamoyltransferase), which catalyses the reaction:

has been partially purified from rat liver (Grisolia & Cohen, 1952; Reichard, 1957), from ox liver (Burnett & Cohen, 1957) and from *Streptococcus lactis* (Ravel, Grona, Humphreys & Shive, 1959). Some properties of the enzyme have been studied and certain differences between the preparations have been observed. However, since different buffers and assay systems have been used, the extent to which these differences are significant is difficult to assess. Brown & Cohen (1960) have suggested an evolutionary significance in the distribution of urea-cycle enzymes. The present study was undertaken to find out whether or not ornithine carbamoyltransferase showed variation in its properties when prepared by identical procedures from different sources. The properties of the ox enzyme are reported as a first step in this programme.

Reichard (1957), on the basis of isotope-exchange data, has suggested that the catalytic reaction proceeds by a single displacement reaction rather than via the formation of an enzyme-carbamoyl intermediate. A reaction of this sort is most simply explained by having the substrates bound simultaneously at separate sites on the enzyme. Kinetic data are presented which support this hypothesis and suggest that carbamoyl phosphate is bound almost entirely by the phosphate group.

MATERIALS AND METHODS

Chemicals

L-Ornithine. L-Ornithine (L. Light and Co. Ltd.) was twice recrystallized from mM-EDTA. It was found to be pure by paper electrophoresis in 0.45M-phthalate buffer, pH 3.2, and by descending paper chromatography with butanol-acetic acid-water (50:11:25, by vol.).

Carbamoyl phosphate. The dilithium salt was prepared by the method of Jones, Spector & Lipmann (1955). The product, which was free from inorganic phosphate as determined by the method of Lowry & Lopez (1946), was 75% pure by

analysis of inorganic phosphate liberated on hydrolysis. This was confirmed by the amount of citrulline formed after incubation with excess of ornithine and ornithine carbamoyltransferase at pH 8.3 (see the Results section).

Ammonium sulphate. This was twice recrystallized from mM-EDTA. For the fractionation procedures it was stored as a saturated solution at 4°, adjusted to pH 7.0 or 8.5 with saturated sodium hydroxide solution.

Sodium chloride. This was purified by precipitation from a saturated solution in de-ionized distilled water with redistilled acetone.

Cetyltrimethylammonium bromide. Cetyltrimethylammonium bromide (The General Chemical and Pharmaceutical Co. Ltd., Sudbury, Middlesex) was used without further purification.

Iodoacetic acid. Iodoacetic acid (recrystallized four times) was a gift from Dr B. R. Rabin.

All other reagents were of AnalaR grade.

Buffers

All pH measurements were made at room temperature. Glycylglycine was twice recrystallized and shown to be pure by paper chromatography with butanol-acetic-water (50:11:25, by vol.). Glycylglycine-sodium hydroxide buffer, pH 8.3 and 10.4, was prepared in 25 ml. batches by dissolving 3.3 g. of the peptide in de-ionized distilled water, adjusting the pH with saturated sodium hydroxide and making up to the final volume.

For the pH-activity curve, 1M-glycylglycine was titrated with saturated sodium hydroxide and samples were transferred at convenient pH values into the reaction tubes. The ionic strength was adjusted with 1M-sodium chloride before making up to the final volume.

Tris-hydrochloric acid buffers for the pH-activity curve were prepared from 1M-tris (recrystallized) and conc. hydrochloric acid in a manner similar to that used for glycylglycine.

Tris-hydrochloric acid buffer, pH 7.5 and 10.02, for column chromatography was prepared in 500 ml. quantities by adding 10 ml. of 1N-hydrochloric acid to 12 ml. of 1M-tris and making up to volume.

Enzyme assay

The standard unit of ornithine carbamoyltransferase activity is defined as the quantity (in mg.) that will catalyse the formation of 1 μ mole of citrulline/min. at pH 8.3 in glycylglycine buffer, 10.2, at 37°, under conditions of saturating concentrations of substrates (25 mM for both ornithine and carbamoyl phosphate). The reaction was started by the addition of the carbamoyl phosphate solution, which was prepared 1 min. before starting the reaction. The final reaction volume was either 1 ml. or 10 ml. A

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progress curve was obtained by transferring 0.1 ml. or 1 ml. samples respectively at suitable time intervals to 0.5N-perchloric acid. If necessary, precipitated protein was removed by centrifuging. Samples of the supernatant were assayed for citrulline by the method of Archibald (1944). A similar assay procedure was used where components of the reaction mixture were varied. The pH of the ornithine solution and other buffering additives was always adjusted to the desired value beforehand. All initial velocities were measured from the slopes of the tangents to the progress curves obtained during the first 4% of the reaction and are expressed as μ moles of citrulline produced/min.

Purification of ornithine carbamoyltransferase

Ox liver was obtained from the slaughter-house packed in ice and used within 1 hr. of death.

Stage 1. Liver (300 g.) was homogenized (for 3 min.) to a cream with 600 ml. of 0.1% cetyltrimethylammonium bromide at room temperature (18–20°), strained through two layers of cheese-cloth and centrifuged for 35 min. at 800g at 2°. The precipitate was resuspended in a further 300 ml. of cetyltrimethylammonium bromide and recentrifuged. The cooled supernatants were combined and the residues rejected.

Stage 2. Cold saturated ammonium sulphate, pH 7.0, was added to the combined supernatants to give 50% saturation; the mixture was stirred and kept for 30 min. at 0°. The precipitate was removed by centrifuging (for 35 min. at 800g) and the concentration of ammonium sulphate in the supernatant was increased to 75% saturation. After 30 min. at 0° the precipitate was collected by centrifuging as above and dissolved in 25 ml. of tris-hydrochloric acid buffer, pH 7.5 and *I* 0.02. This enzyme solution was dialysed for about 6 hr. against 500 ml. of the same buffer and then overnight against a further 500 ml.

Stage 3. The dialysed solution was warmed to 60° with rapid stirring in a water bath at 60°, held at this temperature for 5 min., rapidly cooled in an ice bath and the precipitate removed by centrifuging as above.

Stage 4. An equal volume of cold saturated ammonium sulphate, pH 8.5, was then added to the supernatant and the precipitate, collected by centrifuging after 30 min., was dissolved in not more than 10 ml. of tris-hydrochloric acid buffer, pH 7.5 and *I* 0.002 (obtained by diluting the stock buffer tenfold). The enzyme solution was dialysed for 6 hr. against 500 ml. of the same buffer and then overnight against a further 500 ml. under reduced pressure (550 mm. Hg) to concentrate the solution to approximately 1 ml.

Stage 5. Diethylaminoethylcellulose (DEAE-cellulose) was washed free from fines, packed in columns 40 cm. $\times$ 1 cm., and equilibrated with tris-hydrochloric acid buffer, pH 7.5 and *I* 0.002. The concentrated protein solution from stage 4 was applied to the top of the column and washed with the equilibrating buffer. Ornithine carbamoyltransferase was washed through, and was collected and concentrated under reduced pressure as above.

Stage 6. Carboxymethylcellulose (CM-cellulose) columns were prepared and equilibrated in the same way as for DEAE-cellulose. The concentrated protein solution was applied to the top of the column and washed with the equilibrating buffer. The ornithine carbamoyltransferase was again washed through. The active fractions were combined, divided into 1 ml. lots and stored at -10° .

The purification and recovery obtained by this fractionation scheme are shown in Table 1.

Purity of ornithine transcarbamoylase and further purification

The final preparation was subjected to horizontal starch-gel electrophoresis (Smithies, 1955), with the discontinuous buffer system of Poulik (1957). The electrophoretogram (Fig. 1) shows that the preparation is still heterogeneous. The gel was cut into transverse segments, the protein eluted with glycylglycine buffer, pH 8.3, and enzyme activity determined by the standard assay method. Enzyme activity appears to move as a diffuse band rather than as a discrete component. A similar electrophoretogram was obtained by electrophoresis on a starch block with the same buffer conditions. The specific activity of the most active fraction, representing 10% (w/w) of the total protein, had been increased fourfold. A cut representing 25% of the total protein showed a threefold increase in specific activity.

In all the following experiments the preparation obtained before electrophoresis was used.

RESULTS

Properties of ornithine carbamoyltransferase

Effect of ionic strength on activity. Variation of the ionic strength with sodium chloride has a significant effect on the initial velocity of the forward reaction under saturating conditions of substrate concentration (Fig. 2). For this reason all subse-

Table 1. *Purification of ornithine carbamoyltransferase*

Summary of the effects of the purification stages in the preparation of ornithine carbamoyltransferase from a cetyltrimethylammonium bromide (CTB) extract of ox-liver mitochondria as described in the text.

Stage	Protein (mg.)	100 $\times$ Total activity (μ moles of citrulline/min.)	Specific activity (μ moles of citrulline/ min./mg.)	Recovery (%)
1. CTB extract	15 400	730	4.75	100
2. (NH ₄) ₂ SO ₄ , pH 7.0, fraction	1 680	160	9.55	22
3. Heat treatment	298	44	14.8	6
4. (NH ₄) ₂ SO ₄ , pH 8.5, fraction	166	35	21	4.8
5. DEAE-cellulose column	42.5	34	79	4.6
6. CM-cellulose column	17	32.5	191	4.4

quent experiments were carried out at constant ionic strength.

Effects of pH and buffers on activity. Fig. 3 shows the pH-activity curve in tris-hydrochloric acid buffer with an optimum at about pH 6.9. In this experiment and in those with glycylglycine-sodium hydroxide buffer the pH of each reaction

mixture was measured at the end of the incubation period. This allowed for any effect of the substrates on the pH. Over the pH range the tris concentration varied from 0.4 to 0.42M. If the pH was maintained constant and the tris concentration varied over a wide range a marked effect on the initial velocity, under saturating substrate conditions, was observed (Fig. 4), with high concentrations of tris being strongly inhibitory.

Fig. 1. Starch-gel electrophoretogram of ornithine carbamoyltransferase after the final stage of purification. The histogram indicates the distribution of enzyme activity in arbitrary units. Electrophoresis was carried out at pH 8.9 for 15 hr. at 12 mA (constant current) at 4°.

Fig. 2. Effect of ionic strength on the initial velocity of citrulline synthesis by ornithine carbamoyltransferase at 37°. The reaction volume was 1 ml., containing: ornithine, 50 μmoles ; carbamoyl phosphate, 50 μmoles ; enzyme, 1 $\mu\text{g.}$; glycylglycine, pH 8.3, 100 μmoles . The ionic strength was adjusted with 1M-sodium chloride.

Fig. 3. Effect of pH on the initial velocity of citrulline synthesis by ornithine carbamoyltransferase in 0.4M-tris-hydrochloric acid buffer at 37°. The reaction volume was 1 ml., containing: ornithine, 50 μmoles ; carbamoyl phosphate, 50 μmoles ; enzyme, 4 $\mu\text{g.}$ The ionic strength was adjusted to 1.045 with 1M-sodium chloride. The initial velocity at the optimum pH was 0.4 $\mu\text{mole of citrulline/min.}$

Fig. 4. Effect of tris concentration on the initial velocity of citrulline synthesis by ornithine carbamoyltransferase at 37°. The reaction volume was 1 ml., containing: ornithine, 25 μmoles ; carbamoyl phosphate, 25 μmoles ; enzyme, 2 $\mu\text{g.}$; tris buffer, pH 7.0. The ionic strength was adjusted to 1.05 with 1M-sodium chloride.

With the same reaction mixture the pH optimum was then redetermined in glycylglycine-sodium hydroxide buffer. Fig. 5 shows the results plotted on the same relative-initial-velocity scale as in Fig. 3 so that the two curves are directly comparable: the relative initial velocity increased by about 80% and the optimum shifted from pH 6.9 to pH 7.9. Variation of the glycylglycine concentration at constant pH was without effect. The pH optimum in glycylglycine-sodium hydroxide buffer was also determined with low substrate concentrations (Fig. 5). Although placed on the same scale for comparison, the initial velocities of the reactions at high and low substrate concentrations are not directly comparable. With low substrate concentrations a small but significant shift in the optimum to higher pH occurs.

Effect of temperature on activity. Fig. 6 shows an Arrhenius plot for the effect of temperature from

Fig. 5. Effect of pH on the initial velocity of citrulline synthesis by ornithine carbamoyltransferase in 0.25M-glycylglycine-sodium hydroxide buffer at 37°. The reaction volume was 1 ml., containing: either (●) ornithine 50 μ moles, carbamoyl phosphate, 50 μ moles, or (○) ornithine, 10 μ moles, carbamoyl phosphate, 10 μ moles; both with enzyme, 3 μ g. The ionic strength was adjusted to 1.045 with 1M-sodium chloride. The relative-initial-velocity scale is the same as that in Fig. 3 for the experiment with high substrate concentrations. For the experiment with low substrate concentrations the initial velocity at the optimum pH was 0.25 μ mole/min.

5° to 40° on the initial velocities of the reaction. From these results the overall activation energy for this reaction was calculated to be 13.05 kcal./mole, which, after correction for the indirect effect of the temperature on the initial velocities, by altering the pH values of the buffers, became 13.3 kcal./mole.

Effect of temperature on stability. The effect of elevated temperatures on the stability of the purest carbamoyltransferase fraction (specific activity 191 μ moles of citrulline/min./mg. of protein) is shown in Fig. 7. The enzyme was first dialysed for 5 hr. in each of two changes of 100 ml. of glycylglycine buffer, pH 8.3 and 1.05 (prepared by diluting the buffer of 1.04 and readjusting the pH). Enzyme activity was determined by measuring the amount of citrulline synthesized in 5 min. under conditions where the formation of product was linear with enzyme concentration.

Effects of substrate concentration. Under equilibrium conditions, the velocity of an enzyme reaction converting two substrates into two products is most simply described by the kinetic equation (1):

$$v = \frac{V_{\max.}}{1 + \frac{K_a}{a} + \frac{K_b}{b} + \frac{K_a K_b}{ab}} \quad (1)$$

where for ornithine carbamoyltransferase K_a and K_b are the dissociation constants for ornithine and carbamoyl phosphate from the enzyme and K_a

Fig. 6. Effect of temperature on the initial velocity of citrulline synthesis by ornithine carbamoyltransferase in 0.1M-glycylglycine-sodium hydroxide buffer, pH 8.3 and 1.020. The reaction volume was 1 ml., containing: ornithine, 25 μ moles; carbamoyl phosphate, 25 μ moles; enzyme, 10 μ g.

and K_b , are the dissociation constants for ornithine from the enzyme-carbamoyl phosphate complex and carbamoyl phosphate from the enzyme-ornithine complex respectively. $V_{m,x}$ is the maximum velocity. The four constants are related by the equation:

$$K_a K_b = K_a' K_b'$$

Velocity measurements under various conditions of substrate concentrations were made in the forward direction to determine the four constants.

Fig. 7. Effect of temperature on the stability of ornithine carbamoyltransferase in 0.12M-glycylglycine-sodium hydroxide buffer, pH 8.3 and I 0.05. Enzyme samples (1 μ g. in 0.5 ml.) were incubated for the times shown at: $\circ$, 40°; $\bullet$, 50°; $\blacktriangle$, 55°; $\triangle$, 60°. Each sample was cooled in ice and the amount of citrulline produced in 5 min. at 37° measured in a reaction mixture of final volume 0.75 ml., containing: ornithine, 10 μ moles; carbamoyl phosphate, 10 μ moles.

Fig. 8. Lineweaver-Burk plots for ornithine at the following concentrations (the amounts specified were contained in 1 ml. of reaction mixture) of carbamoyl phosphate: $\bullet$, 5 μ moles; $\circ$, 2.5 μ moles; $\blacktriangle$, 1.66 μ moles; $\triangle$, 1.25 μ moles; $\blacksquare$, 1.0 μ mole. Each reaction mixture, of total volume 1 ml., also contained: glycylglycine-sodium hydroxide buffer, pH 8.3, 10 μ moles; enzyme, 5 μ g. The ionic strength was adjusted to I 0.025 with 1M-sodium chloride. Incubations were carried out at 37°.

The line of best fit was calculated for each plot and the mean value for each constant and its standard error determined. Fig. 8 shows Lineweaver-Burk plots for ornithine at several fixed concentrations of carbamoyl phosphate. The value for K_a obtained from these data was 1.45 ± 0.19 mM and shows no significant dependence on the concentration of carbamoyl phosphate.

Fig. 9 shows the data for the reciprocal experiment yielding, for carbamoyl phosphate,

$$K_b = 1.79 \pm 0.16 \text{ mM.}$$

As before, the concentration of second substrate had no significant effect.

Maximum velocities, measured from the intercepts at the $1/v$ axis, in Figs. 8 and 9 have been plotted against the reciprocals of the appropriate substrate concentration in Figs. 10 and 11 to give K_b' and K_a' , from the intercepts on the $1/s$ axis (Florini & Vestling, 1957). The values found were for K_a' , 1.55 ± 0.23 mM, and for K_b' , 1.81 ± 0.30 mM.

Product inhibition. Significant inhibition of the reaction by citrulline occurs only at very high concentrations (Burnett & Cohen, 1957) and for this reason was not studied. Inhibition by phosphate at various concentrations of carbamoyl phosphate is plotted in Fig. 12 to show classical competitive inhibition. Non-competitive inhibition occurred when the concentration of ornithine was varied (Fig. 13). The inhibitor constant, K_p , was

$$1.88 \pm 0.21 \text{ mM}$$

from Fig. 12 and 1.56 ± 0.30 mM from Fig. 13.

Fig. 9. Lineweaver-Burk plots for carbamoyl phosphate at the following concentrations (the amounts specified were contained in 1 ml. of reaction mixture) of ornithine: $\bullet$, 5 μ moles; $\circ$, 2.5 μ moles; $\blacktriangle$, 1.67 μ moles; $\triangle$, 1.25 μ moles; $\blacksquare$, 1 μ mole. Other conditions were as in Fig. 8.

Fig. 10. Determination of K_b by using the data obtained from Fig. 8. Details are given in the text.

Fig. 11. Determination of K_a by using the data obtained from Fig. 9. Details are given in the text.

General properties. Ornithine carbamoyltransferase was unaffected by dialysis against 1 mM-EDTA for 2 days, by the presence of iodoacetate, Mn^{2+} or Mg^{2+} ions up to 10 mM, or by SO_4^{2-} ions up to 50 mM.

DISCUSSION

The results of the present work are in general agreement with the findings of Burnett & Cohen (1957) and Reichard (1957), with ox- and rat-liver preparations respectively (Table 2). Since our preparation was obtained by a different isolation procedure the similarity of enzyme properties suggests that the contaminating protein is not having a significant effect.

Because of the differences in the method used for assay, direct comparison of the purity of the present preparation with those of other workers is not possible. However, the present preparation

Fig. 12. Competitive inhibition of ornithine carbamoyltransferase by inorganic phosphate at the following concentrations (the amounts specified were contained in 1 ml. of reaction mixture) at 37°: ▲, 5 μmoles ; ●, 2 μmoles ; ○, 1 μmole . The reaction volume was 1 ml., containing: carbamoyl phosphate, 50 μmoles ; ornithine, 50 μmoles ; glycylglycine-sodium hydroxide buffer, pH 8.3, 0.1 m-mole. The ionic strength was adjusted to 1.05 with 1M-sodium chloride.

Fig. 13. Non-competitive inhibition of ornithine carbamoyltransferase by inorganic phosphate at the following concentrations (the amounts specified were contained in 1 ml. of reaction mixture) at 37°: ▲, 2 μmoles ; ○, 1 μmole ; ●, phosphate absent. The reaction volume was 1 ml., containing: ornithine, 50 μmoles ; carbamoyl phosphate, 50 μmoles ; enzyme, 8 $\mu\text{g.}$; glycylglycine-sodium hydroxide buffer, pH 8.3, 0.1 m-mole. The ionic strength was adjusted to 1.05 with 1M-sodium chloride.

appears to be about 40% more active than that of Burnett & Cohen (1957), after the difference in the ionic strength of the assay system has been allowed for, and less pure than that of Reichard (1957). This purification can be increased a further three- to four-fold by starch-block electrophoresis, as a

result of which the overall purification is a considerable improvement on those previously obtained. This preparation, unlike those of previous workers, did not show any marked loss of stability after purification and did not require the addition of bovine albumin as a stabilizing agent (Fig. 7). This may result from the use of a detergent extract of fresh liver rather than an aqueous extract of an acetone-dried powder. The initial extract obtained by this procedure had a much higher specific activity.

The effect of ionic strength on the carbamoyl-transferase reaction cannot be interpreted simply in terms of the charges on the reacting groups (La Mer, 1932), since the slope of the log plot (Fig. 2) is not integral.

The difference of approximately 1 unit in the pH optima previously found for the rat and ox enzymes (Table 2) appears to result from the buffer used rather than from differences between the enzymes. The pH optima found in tris-hydrochloric acid buffer (Fig. 3) and glycylglycine-sodium hydroxide buffer (Fig. 5) under high substrate concentrations, when corrected for the effect of temperature on the pH of the buffer at 37° (Datta & Grzybowski, 1961), had values of 6.5₅ and 7.5₅ respectively. The inhibitory effect of tris and its effect in depressing the pH optimum can be explained if it is assumed that the neutral species of tris is the inhibitor. At constant total tris concentration (Fig. 3) an increase in the pH would increase the concentration of inhibitor and hence progressively depress the initial velocity and the right-hand limb of the pH-activity curve relative to that found in glycylglycine. The nature of the activating effect of low concentrations of tris relative to the activity in glycylglycine could not be established, since variation of the concentration of the latter had no effect on the activity of the enzyme.

At low substrate concentrations the pH optimum in glycylglycine buffer is shifted to a higher pH (Fig. 5). Such a finding has been interpreted to indicate the formation of a complex between one substrate and the basic group only on the enzyme (Laidler, 1958). It is doubtful whether a conclusion of this kind can be applied to ornithine carbamoyltransferase.

Figs. 8 and 9 show the independence between the dissociation constant of one substrate and the concentration of the other substrate for both substrates. Provided that the reaction agrees with equilibrium kinetics, this finding eliminates the possibility that the carbamoyltransferase reaction proceeds by a compulsory pathway in which the substrates are bound in a particular order before formation of an intermediary complex and conversion into products occurs. Steady-state kinetics probably do not apply, since $K_a = K_a'$ and $K_b = K_b'$, which would require a fortuitous relationship between the constants (Takenaka & Schwert, 1956). This argument has been presented in greater detail for an analogous situation occurring with pyruvate kinase (Reynard, Hass, Jacobsen & Boyer, 1961). In addition, the dissociation constant, K_p , for the product, phosphate (Fig. 12), is also unaffected significantly by ornithine concentration (Fig. 13). Then for ornithine carbamoyltransferase equation (1) simplifies to:

$$v = \frac{V_{\max} \cdot \frac{ab}{K_a K_b}}{\left(1 + \frac{a}{K_a}\right) \left(1 + \frac{b}{K_b}\right)}$$

which is the product of two Michaelis functions, one for each substrate. Thus these kinetic data indicate that ornithine and carbamoyl phosphate have separate binding sites on the enzyme. This is in complete agreement with the isotope-exchange data of Reichard (1957).

Table 2. Comparison of some properties of mammalian ornithine carbamoyltransferases

Property	Ox-liver enzyme		Rat-liver enzyme (Reichard, 1957)
	Present work	Burnett & Cohen (1957)	
pH optimum			
In tris buffer	6.9	—	7.3
In glycylglycine buffer	7.9 (high)* 8.1 (low)*	8.05 —	— —
Michaelis constant			
Ornithine	1.4 ₃ mM	3.0 mM	1.4 mM
Carbamoyl phosphate	1.7 ₉ mM (at <i>I</i> 0.025) 1.7 ₂ mM (at <i>I</i> 0.50)	1.2 mM —	0.4 ₁ mM —
Energy of activation	13.05 kcal./mole†	13.1 kcal./mole	—
Inhibition by phosphate	Yes	—	Yes

* Substrate concentrations both either 50 mM (high) or 10 mM (low).

† Uncorrected (see Discussion section).

The finding that $K_b = K_p$, within the limits of experimental error, suggests that the binding of carbamoyl phosphate to the enzyme can be attributed almost entirely to the phosphate group. This binding site must have a specific geometry, since arsenate is only 25 % as inhibitory as inorganic phosphate under the same conditions (Reichard, 1957), and sulphate does not inhibit at all. Identical binding constants for phosphate and carbamoyl phosphate also appear to occur in the enzyme from *S. lactis*. Ravel, Grona, Humphreys & Shive (1959) report a value of 3.7 mM for the Michaelis constant of carbamoyl phosphate. From their phosphate-inhibition data we have calculated K_p to be 3.65 mM (mean of two values).

The present work suggests that there are no fundamental differences between the ornithine carbamoyltransferases from ox and rat liver. The kinetic data and the general similarity between the properties of the bacterial and ox enzymes suggests that, despite these widely differing origins, the carbamoyltransferase reaction proceeds by a similar chemical path.

SUMMARY

1. A purification procedure for ornithine carbamoyltransferase from ox liver and some of the properties of the enzyme are described.

2. The initial velocity of the enzyme reaction is strongly inhibited by tris buffer, which also lowers the pH optimum. A marked dependence on ionic strength is also found.

3. The kinetics of substrate-binding and inhibition by phosphate suggest that each substrate is

bound by a separate site on the enzyme and that there is no compulsory order of binding.

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Organ Specificity and Lactate-Dehydrogenase Activity

1. THE RELATIVE ACTIVITIES WITH PYRUVATE AND 2-OXOBUTYRATE OF ELECTROPHORETICALLY SEPARATED FRACTIONS

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Lactate dehydrogenase is widely distributed throughout mammalian tissues and, though in all cases it catalyses the reversible reduction of pyruvate, the enzyme has certain properties characteristic of the tissue from which it is derived. Human and rabbit tissues have been shown to contain a number of fractions exhibiting lactate-

dehydrogenase activity that may be separated by electrophoresis on a suitable supporting medium, e.g. starch block (Vesell & Bearn, 1957, 1961), paper (Sayre & Hill, 1957; Wieland & Pfeleiderer, 1957; Hess, 1958), agar gel (Wieme, 1959*a, b*; Blanchaer, 1961) or starch gel (Plagemann, Gregory & Wróblewski, 1960*a*; Latner & Skillen, 1961).