J. Membrane Biol. 18, 231–242 (1974)

Carbamyl Phosphate and Glutamine Stimulation of the Gallbladder Salt Pump

Duncan W. Martin and Beth Murphy*

Department of Zoology, University of Arkansas, Fayetteville, Arkansas 72701

Received 2 October 1973; revised 6 May 1974

Summary. Carbamyl phosphate stimulates neutral salt transport in the *in vitro* gallbladder. Acetyl phosphate also stimulates transport. In addition, the metabolic precursors of carbamyl phosphate, glutamine and bicarbonate ion, stimulate transport of fluid across the gallbladder mucosa. These data suggest that the effect of glutamine in stimulating increases in transport is achieved via the synthesis of carbamyl phosphate which in turn reacts with the transport mechanism. Metabolic precursors of glutamine, glutamate plus a nitrogen source, when combined will produce an effect similar to glutamine alone. Fluid transport is also slowed by azaserine; thus, the stimulation effect appears to be restricted to glutamine.

Bicarbonate ion, when present in the bathing medium of isolated rabbit gallbladders, will increase the rate of fluid transport (Diamond, 1964). In a companion manuscript, Martin (1974) has described the bicarbonate effect in the gallbladder in greater detail. The bicarbonate effect has been investigated further under the assumption that carbon dioxide fixation was involved in the process. Some mechanisms of carbon dioxide fixation lead to metabolizable substrates which can restore metabolic activities (anaplerotic pathways, Mahler & Cordes, 1971). In addition, carbon dioxide fixation can also lead to the synthesis of carbamyl phosphate ($NH_2COOPO_3H_2$), an intermediate which functions in the urea cycle and in pyrimidine synthesis. Not only bicarbonate ion stimulates fluid transport in the gallbladder, but also glutamine as well as carbamyl phosphate itself. Moreover, carbamyl phosphate can be formed in the rabbit gallbladder from bicarbonate ion and glutamine (Murphy & Martin, 1972).

Carbamyl phosphate is formed from bicarbonate ion, a nitrogen source, and ATP (Jones, 1963; Grisolia & Raijman, 1964; Cohen, 1970); the

* *Present address:* Department of Chemistry, Phillips University, Enid, Oklahoma 73701.

synthesis may be summarized as follows:

$$NH_4^+ + HCO_3^- + 2\,ATP \rightarrow NH_2COOPO_3^{2-} + 2\,ADP + iP + 2\,H^+.$$

The *in vitro* rabbit gallbladder provides an excellent preparation for the study of the relationship between energy-yielding processes and the coupling of this energy to the NaCl pump. Transport is readily measured in the isolated rabbit gallbladder (Diamond, 1964) and the relationship of transport to overall energy utilization has been described previously (Martin & Diamond, 1966; Frederiksen & Leyssac, 1969). The present study was conducted to evaluate the effect of carbamyl phosphate, acetyl phosphate, glutamine, asparagine, and aspartate upon isotonic fluid transport.

Materials and Methods

Gallbladder preparations were made by procedures described by Diamond (1964) and Martin (1974), and the Q_{O_2} was determined according to Martin and Diamond (1966). Carbamyl phosphate, acetyl phosphate, glutamine, aspartate and asparagine-containing solutions were made up in phosphate Ringer's solution immediately prior to each experiment. The composition of the solutions employed is described elsewhere (Martin, 1974). Experimental errors are quoted as standard errors (SE) of the mean if the confidence measures were derived from the *t* test.

Results

Effect of Carbamyl Phosphate on Fluid Transport

A hypothesis that the bicarbonate effect results from endogenous carbamyl phosphate synthesis within the gallbladder mucosal cells requires that added carbamyl phosphate should increase fluid transport if the carbamyl phosphate is a permeant substance. That this condition is met is shown by the following results: Gallbladders bathed in phosphate Ringer's solution (0 mM glucose, 0 mM HCO_3^-) exposed to 1 mM carbamyl phosphate transported fluid faster after the addition of the carbamyl phosphate. The average rate and standard error was 15.21 ± 3.5 μliters of fluid/mg (dry wt) hr prior to the addition of carbamyl phosphate and 19.16 ± 3.39 afterwards. The *t* test was performed for paired variates ($n = 11$), thus, the effect of carbamyl phosphate was significant ($p < 0.05$) and the increase in fluid transport was 26%. Carbamyl phosphate is a very unstable compound and it decomposes into cyanate ion and phosphate at pH 6 to 8 with a half time of 18 hr at room temperature (Allen & Jones, 1964). It is possible that for

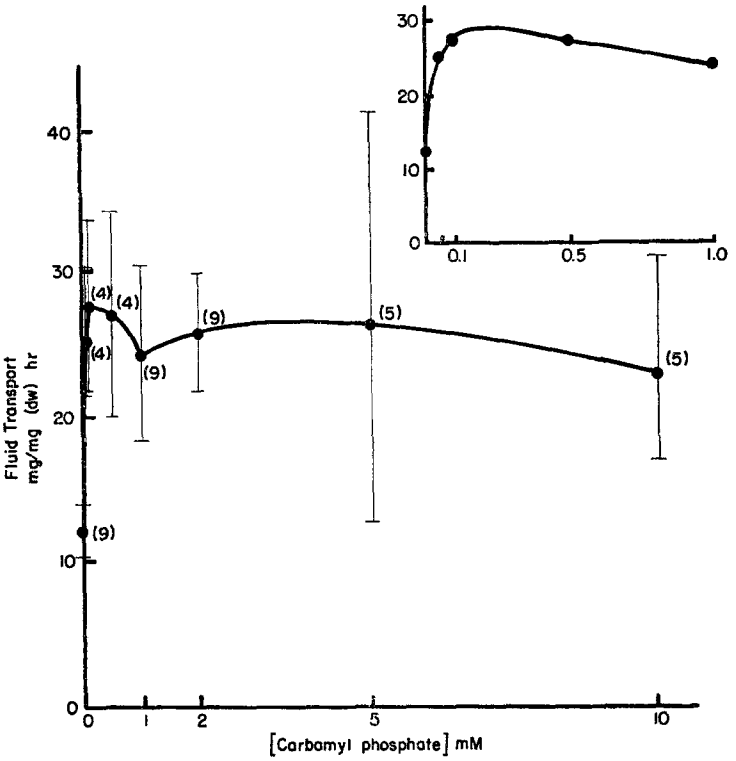

Fig. 1. Effect of different concentrations of carbamyl phosphate upon fluid transport in the isolated rabbit gallbladder. The rate at each concentration is represented by the arithmetic mean, $\bar{x}$, $\pm$ SE for the number of gallbladders indicated. *Insert:* Abscissa expanded 5 × to show low concentrations. Ordinate reduced by one-half. dw = dry weight

such an unstable compound the decomposition products may be the stimulatory components rather than the original compound. Experiments performed with carbamyl phosphate which had been stored at room temperature for various periods from a day to a week showed that 10 mM carbamyl phosphate was still effective for stimulation of transport after one day's time, but material which stood for a week at room temperature did not increase or decrease transport.

Fig. 1 shows the rate of fluid transport as a function of the concentration of carbamyl phosphate for a total of nine gallbladders. In these experiments the concentration of carbamyl phosphate was increased from zero to 0.05, 0.1, 0.5, 1.0, 2.0 mM in each of four gallbladders and to 1, 2, 5, and 10 mM in each of an additional five gallbladders. Increasing the concentration of carbamyl phosphate after the initial exposure to the compound does not produce additional increase in the rate of fluid transport. That is, there is

no significant increase in transport between 0.05 and 10 mM in the same gallbladder. A rigorous analysis of the kinetic data is unjustified since the endogenous level of carbamyl phosphate and the permeability of the membranes to the exogenous carbamyl phosphate are both unknown. However, one can readily see that maximum transport rates are achieved with concentrations of carbamyl phosphate of less than 0.1 mM.

Acetyl Phosphate

Acetyl phosphate, a substance of microbial origin, can interact with some of the same systems in which carbamyl phosphate and ATP can be established as reactants. Table 1 shows the response of three gallbladders to acetyl phosphate exposure. This substance was added to phosphate Ringer's solution (0 mM HCO_3^- and 0 mM glucose) after control measurements were obtained. Although this data set is not extensive, it is sufficient to show that acetyl phosphate will produce an effect similar to that of carbamyl phosphate in a mammalian system (Table 1).

The Relationship of Glutamine to Fluid Transport

Glutamine addition at concentrations from 1 to 10 mM to gallbladders in phosphate Ringer's solution increases transport an average of 58% ($n = 18$ for all experiments). At a concentration of 1 mM glutamine ($n = 6$) the rate of transport was 24.5 ± 6.1 μliters/mg(dry wt)hr ± SE while the rate without glutamine was 11.2 ± 1.5 ($n = 18$). This effect was highly significant ($p < 0.01$, t test). Fig. 2 shows the transport rate as a function of the concentration of glutamine. The data of 18 gallbladders are expressed in this figure. Four gallbladders were used to describe the first portion of the curve (0.05 to 1 mM). For the remaining points, a gallbladder was exposed only once to a concentration of glutamine greater than zero. These effects of glutamine upon transport are similar to those produced by carbamyl

Table 1. The effect of acetyl phosphate on fluid transport of the *in vitro* gallbladder

Exp.	Fluid transport (μliters/mg(dry wt)hr)		
	Control	Acetyl phosphate (1 mM)	Ratio (Exp./Control)
S_{240}	14.5	20.9	1.44
S_{241}	11.6	40.0	3.46
S_{242}	5.4	10.0	1.84

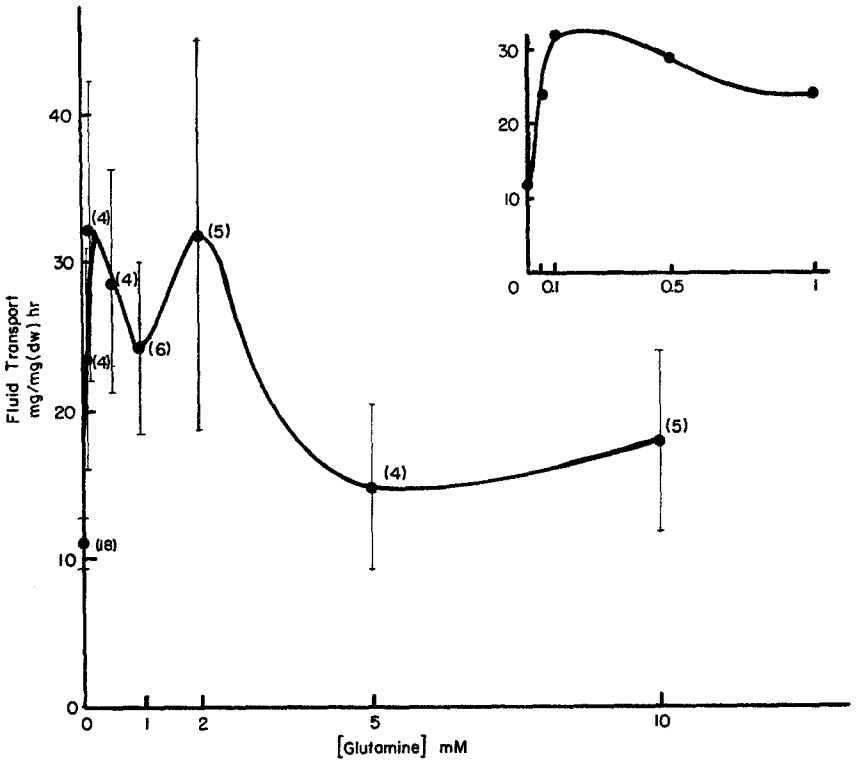

Fig. 2. Effect of glutamine upon fluid transport in the isolated rabbit gallbladder. The rate at each concentration is given as the arithmetic mean, $\bar{x}$, $\pm$ SE for the number of gallbladders indicated. *Insert:* Abscissa expanded 5× to show low concentrations. Ordinate reduced by one-half. dw = dry weight

phosphate in that added glutamine significantly elevates the rate of transport, but there is no significant difference observed between any pair of concentrations after the stimulating effect has been achieved. The apparent depression at 5 and 10 mM is not statistically significant. However, maximum stimulation occurs with concentrations of less than 0.1 mM glutamine.

Glutamine Specificity

The reaction bringing about the increase in transport of fluids with the addition of glutamine may be nonspecific. That is, other amino acids may bring about a similar result. Tables 2 and 3 illustrate that amino acids other than glutamine stimulate the gallbladder. Since the effect being assessed was a general one, i.e., any amino acid may provide the nitrogen source for glutamine synthesis, either aspartate or asparagine was added to individual

Table 2. The effect of 10 mM of amino acid metabolites, aspartate (asn), asparagine (asn^2) on fluid transport of the *in vitro* rabbit gallbladder

Control	Fluid transport (μliters/mg (dry wt) hr; $\bar{x} \pm$ SE (n))
0 mM amino acid	9.1 ± 2.8 (5)
asn or asn^2	15.5 ± 3.1

Experiments done in 0 mM HCO_3^-, no added substrates. $p < 0.02$; asn or asn^2 elevates transport rate; paired variates. For discussion of treatment *see text.*

Table 3. The effect of 10 mM aspartate (asn) or asparagine (asn^2) on fluid transport in gallbladders depleted of metabolites by storage at 4 °C in 25 mM HCO_3^- without added substrate for 20 to 24 hr

Control	Fluid transport (μliters/mg (dry wt) hr, $\bar{x} \pm$ SE (n))
0 mM amino acid	20.7 ± 3.8 (8)
asn or asn^2	8.8 ± 2.6 (8)

Transport measurements done at 38 °C in the presence of glucose (11 mM) and in the absence of bicarbonate ion (0 mM HCO_3^-). $p < 0.05$; treatment decreases transport; unpaired variates. For discussion of treatment *see text.*

gallbladders. Table 2 shows that these amino acids do increase transport. Moreover, if these amino acids were simply nitrogen sources for subsequent glutamine synthesis the possibility exists that the depletion of metabolites by storage at 4 °C for 20 to 24 hr would give a different result. Table 3 shows that aspartate or asparagine added after this metabolite depletion procedure actually slows the transport rate, a result which demonstrates that the metabolite depletion procedure was effective in altering the necessary conditions for the amino acid utilization for the stimulation of transport. However, glutamine should still stimulate the gallbladder to increase transport if the effect is indeed specific. In two sets of experiments this idea was tested directly and in a third set an indirect test was done. Table 4 shows the glutamine effect on three individual gallbladders which had been exposed to aspartate, asparagine and glutamate. These gallbladders had previously shown decreased transport resulting from the effects of added asparagine or aspartate. After a measurement without amino acids, the glutamine was added or the transport slowed with asparagine addition prior to the glutamine addition. The effect of glutamine on transport persists after these treatments. Glutamine utilization by systems requiring it may be inhibited by azaserine (Mahler & Cordes, 1971). A set of

Table 4. The effect of glutamine (gln^2) added to preparations previously exposed to an amino acid (asn, asn^2, or glutamate (gln))

Exp.	Fluid transport (μliters/mg(dry wt)hr)				
	0 mM amino acid	1 mM asn^2	10 mM gln^2	1 mM gln^2	Ratio b/a
S_{281}	2.6[a]		7.9[b]		3.0
S_{282}	1.4[a]		7.0[b]		5.3
S_{283}	12.7	8.35[a]		9.1[b]	1.1

Preparations were depleted of metabolites as in Table 3. Conditions of measurement were 11 mM glucose with 25 mM HCO_3^-. The ratio was obtained by dividing the rate in gln^2 (b) by the rate (a) immediately preceding the addition of gln^2.

$S_{281\,\&\,282}$ were previously exposed to asn, asn^2, and gln. S_{283} had not been previously exposed to amino acids prior to the exposure to the 1 mM asn^2 indicated here.

Table 5. Effect of azaserine and glutamine (gln^2) upon fluid transport in metabolite-depleted gallbladders

Exp.	Fluid transport (μliters/mg(dry wt)hr)				
	0 mM azaserine, 0 mM gln^2	3 mM azaserine	% change	3 mM azaserine, 1 mM gln^2	% change
S_{300}	8.2	5.7	−30	4.9	−14
S_{301}	8.9	6.0	−32	5.4	−10
S_{302}	10.9	9.7	−11	8.7	−10
S_{303}	22.7	15.6	−31	12.7	−18

Gallbladders were successively exposed to the azaserine, then azaserine + glutamine. Glucose (11 mM), 0 mM HCO_3^-.

experiments was done to test the effect of azaserine and glutamine to metabolite-depleted gallbladders (Table 5). These results show that azaserine slows transport and that added glutamine is ineffective when the azaserine is present. Glutamine synthesis requires glutamate and a nitrogen source. In a third set of experiments individual metabolite-depleted gallbladders were exposed to either glutamate or asparagine (nitrogen source) and then to glutamate and asparagine combined. In these experiments the first exposure to an amino acid was alternated between glutamate and asparagine for the different gallbladders. Table 6 shows that glutamate in combination with a nitrogen source results in an increase in transport. Thus, metabolic precursors of glutamine when combined will give an effect identical to that of glutamine. Glutamate (gln) alone does not increase transport [0 mM gln,

Table 6. The effect of glutamate (gln) or asparagine (asn[2]) on fluid transport in the rabbit gallbladder followed by glutamate plus asparagine

Amino acid	Fluid transport (μliters/mg(dry wt)hr, $\bar{x} \pm$ SE (n))
asn[2] or gln	9.5±3.2 (5)
asn[2] and gln	14.4±3.5

Metabolite-depleted gallbladders as in Table 3. Glucose (11 mM), without bicarbonate ion. $p < 0.05$; asn[2] or gln *vs.* asn[2] and gln; paired variates.

no added substrate, transport was 9.4 ± 1.3(± SE), 10 mM gln, transport was 9.8 ± 0.7 μliters/mg(dry wt)hr, n = 12]. In the metabolite-depleted gallbladders it slows transport as do the other amino acids (Table 4). Thus, the glutamine effect can be blocked by withholding the appropriate metabolic precursors or by the addition of azaserine.

The relationship of the metabolites, glutamine and carbamyl phosphate, was also tested with respect to their capacity to affect the metabolism of the gallbladder. Carbamyl phosphate had no effect upon oxygen consumption. With the carbamyl phosphate present the average Q_{O_2} (μliters O_2 mg^{-1} hr^{-1}) was 6.7 ± 0.7 (± SE, n = 6) and the average of the paired controls was 7.1 ± 0.7. Glutamine depressed the Q_{O_2}, as the average for the paired control was 7.5 ± 0.6, and with glutamine present the average Q_{O_2} was 6.7 ± 0.6 (n = 6, *t* test for paired variates, $p < 0.05$). This result represents a 10% depression in the oxygen consumption of the gallbladder. Clearly, glutamine cannot be regarded as having its effect on transport by way of a direct entry into the tricarboxylic acid cycle (TCA) to support metabolism.

Discussion

The fact that carbamyl phosphate and acetyl phosphate can stimulate transport is not particularly surprising in view of the data available regarding the effects of these two compounds upon the ($Na^+ + K^+$)-activated ATPase systems studied by others (Bond, Bader & Post, 1971). However, the demonstration that the metabolic precursors of carbamyl phosphate, bicarbonate ion and glutamine, enhance transport allows the speculation that carbamyl phosphate may have a physiological role in transport. The biosynthesis of carbamyl phosphate requires a nitrogen source. The ornithine cycle carbamyl phosphate synthetase (CP synthetase I) utilizes ammonia or amino nitrogen from transaminase reactions and it requires the co-factor N-acetyl-

glutamate (Jones, 1963; Cohen, 1970). In pyrimidine synthesis the amide nitrogen of glutamine provides the nitrogen source for carbamyl phosphate. Ammonia can also provide the required nitrogen for CP synthetase II, but glutamine is regarded as the physiological substrate as it is effective in much lower concentrations (Cohen, 1970). The distinction in substrate and co-factor requirements distinguishes these two carbamyl phosphate synthetase enzymes (Tatibana & Ito, 1969; Cohen, 1970). Bovine and rabbit gallbladder mucosae do not contain CP synthetase I, but they have a carbamyl phosphate synthetase which is similar to CP synthetase II in that it utilizes glutamine and is sensitive to azaserine (Murphy & Martin, 1972). If the concentrations of endogenous glutamine and intracellular bicarbonate could be influenced, either one or the other could be rate-limiting and thereby function as a control over the rate of fluid transport. Unfortunately, it is difficult to directly test which of these two substrates could be limiting in an experimental test as the intracellular bicarbonate ion is a function of cellular respiration as well as pH and carbonic anhydrase activity. Since respiration is a constant feature of cells doing biological work, it would be tempting to assume that the intracellular distribution of bicarbonate could be expected to be uniform if carbonic anhydrase were present. However, there is no unequivocal way of controlling the intracellular bicarbonate ion concentration.

Just as there is no certain method of knowing the intracellular bicarbonate concentration, the endogenous concentration of glutamine is also difficult to estimate. In demonstrating the specificity of this system to glutamine, we provide evidence which suggests that glutamine may be readily synthesized for this reaction by the presence of glutamate and general sources of nitrogen. The glutamate of course may take its origin from the amination of α-keto glutarate. The amino acid pool is apparently limiting for the synthesis and subsequent utilization of glutamine in the increase of fluid transport. The fact that the amino acid pool is limiting in the metabolite-depleted gallbladder is indicated by the data of Tables 3 and 4, which show that transport is slowed by exogenous amino acid, whereas the tests prior to the depletion procedure show an increase in transport (Table 2). The fact that glutamine, or a metabolic precursor of it (Tables 4 and 6), does increase transport after the depletion procedure shows that glutamine is limiting. The depletion procedure then renders the gallbladder nonresponsive to the exogenous amino acids used except glutamine. A reasonable conclusion is that the increase in transport observed with glutamine is specific and is glutamine-limiting. The glutamine effect is also blocked with the presence of azaserine (Table 5). Bicarbonate on the other hand does not

exhibit any analogous limiting phenomenon as the addition of bicarbonate ion to metabolite-depleted gallbladders produces an increase in transport (Martin, 1974, Table 4). Thus, the fundamental effects of glutamine and bicarbonate ion may be independent of one another.

The direct effect of carbamyl phosphate in stimulating active fluid transport in the gallbladder can be compared to the effects of other high energy phosphorylated compounds (ATP, *p*-nitrophenylphosphate, acetyl phosphate) upon the $(Na^+ + K^+)$-activated ATPase associated with the gallbladder and other tissues. The transport enzyme (ATP phosphohydrolase, EC 3.6.1.3.) occurs in many tissues and is characterized by the hydrolysis of a phosphate from ATP in two steps. Na^+ and Mg^{++} must be present in the first step for the formation of the phosphorylenzyme and the hydrolysis of a phosphoenzyme occurs in the presence of potassium (Post *et al.*, 1969). The $(Na^+ + K^+)$-dependent enzyme was recently described for the gallbladders of rabbits and guinea pigs; it requires Mg^{++} and is ouabain-sensitive (Van Os & Slegers, 1971). They report that the enzyme activity correlates well with the transport activity of the gallbladder and they regard this enzyme as being linked to the transport phenomena of the gallbladder. In the guinea pig kidney the $(Na^+ + K^+)$-activated ATPase will utilize acetyl phosphate as a substrate (Bond *et al.*, 1971). The acetyl phosphate apparently behaves as a competitive inhibitor to ATP. Carbamyl phosphate has been documented to be a substitute for ATP in the K^+-dependent phosphatase activity obtained from guinea pig brain microsomes (Izumi, Nagai & Yoshida, 1966). This phosphatase enzyme was shown to be identical with the transport ATPase by these workers. The substrate specificity was greatest for ATP, then carbamyl phosphate, and followed by *p*-nitrophenyl-phosphate and acetyl phosphate, in that order. In addition, the potassium sensitivity was greatest for carbamyl phosphate as opposed to acetyl phosphate. These mammalian systems, rabbit and guinea pig gallbladder, guinea pig kidney and brain are probably sufficiently similar to warrant regarding them as being representative of a general biochemical pattern. In contrast to these systems, acetyl phosphate is not an effective alternate substrate for ATP in the extrusion of Na^+ from the squid axon (Brinley & Mullins, 1968). Our results demonstrating that carbamyl phosphate increases fluid transport might be linked to the effect of the carbamyl phosphate upon the $(Na^+ + K^+)$-activated ATPase present in the gallbladder tissue. As yet, we have no direct evidence that this is actually the case as carbamyl phosphate is yet to be tested upon gallbladder ATPase by us. However, the finding that acetyl phosphate also stimulates transport in the gallbladder supports the idea that the $(Na^+ + K^+)$-activated ATPase is the system which is affected.

Kaye, Maenza and Lane (1966) demonstrated that cell renewal occurs in the rabbit gallbladder. This of course means that pyrimidine synthesis occurs in the tissue and one could expect to find CP synthetase II. However, new cells appear very slowly and the regions performing transport appear to have only mature cells. We regard the participation of the pathway for the biosynthesis of pyrimidines to be an unlikely event to be coupled to transport phenomena, and we tentatively consider these CP synthetase phenomena to be independent of one another. However, adequate characterization of the transport-associated CP synthetase is required.

Carbamyl phosphate formed by the pyrimidine biosynthesis pathway could be utilized by the transport system if the activity of that pathway were to exceed its normal demands. That is, carbamyl phosphate synthesized as a consequence of increased glutamine availability would spill over into other channels of high energy phosphate utilization. A model for such a mechanism can be found in the carbamyl phosphate metabolism of *Neurospora* (Davis, 1972). Wild type *Neurospora* synthesize carbamyl phosphate via two distinct pathways to provide arginine and pyrimidines. Mutant forms exhibit the property of metabolizing carbamyl phosphate provided by the alternate path if the enzymes are lacking for the normal pathway. Normally the carbamyl phosphate appears to be channelled within its own pathway and only in high concentrations does the overflow to the second path occur (Davis, 1972). A similar phenomenon may occur in the gallbladder in that the glutamine stimulation may provide an excess of carbamyl phosphate which spills over and is utilized by the transport system. Although the utilization of carbamyl phosphate to drive transport would be metabolically inefficient since carbamyl phosphate synthesis requires two moles of ATP, it may provide an energy source for transport parallel to the mitochondrial source by the mechanism proposed above. This notion assumes that the ATP for the CP synthetase originates from glycolysis, an assumption which is reasonable as the CP synthetase system is also an extramitochondrial system (Murphy & Martin, 1972).

Glutamine stimulation of the transport system in the gallbladder may then be a consequence of increased carbamyl phosphate synthesis. The bicarbonate effect may also contribute to this effect, but since glutamine absence is not limiting to the bicarbonate effect, it is likely that these effects are independent of one another. The bicarbonate ion appears to affect basic metabolic events leading to the mobilization of energy for transport (Martin, 1974).

The authors are indebted to the excellent technical assistance of Diana K. Horton. This work was supported by PHS Grant No. NIH AM12832 to D. W. M. and by the

Graduate School, University of Arkansas. The authors are also pleased to acknowledge the comments of Dr. P. F. Curran and Dr. J. M. Diamond who read an early version of the manuscript.

References

Allen, C. M., Jones, M. E. 1964. Decomposition of carbamyl phosphate in aqueous solutions. *Biochemistry* **3**:1238

Bond, G. H., Bader, H., Post, R. L. 1971. Acetyl phosphate as a substitute for ATP in (Na + K)-dependent ATPase. *Biochim. Biophys. Acta* **241**:57

Brinley, F. J., Mullins, L. J. 1968. Sodium fluxes in internally dialized squid axons. *J. Gen. Physiol.* **52**:181

Cohen, P. P. 1970. Biochemical differentiations during amphibian metamorphosis. *Science* **186**:533

Davis, R. H. 1972. Metabolite distribution in cells. *Science* **178**:835

Diamond, J. M. 1964. Transport of salt and water in rabbit and guinea pig gall bladder. *J. Gen. Physiol.* **48**:1

Frederiksen, O., Leyssac, P. P. 1969. Transcellular transport of isosmotic volume by the rabbit gall bladder *in vitro*. *J. Physiol.* **201**:201

Grisolia, S., Raijman, L. 1964. Biosynthesis and utilization of acetyl phosphate, formyl phosphate, and carbamyl phosphate and their relations to the urea cycle. *Advanc. Chem. Ser.* **44**:128

Izumi, F., Nagai, K., Yoshida, H. 1966. Studies on potassium dependent phosphatase. II. Substrate specificity of the enzyme. *J. Biochem.* **60**:533

Jones, M. E. 1963. Carbamyl phosphate. *Science* **140**:1373

Kaye, G. I., Maenza, R. M., Lane, N. 1966. Cell replication in rabbit gall bladder: An autoradiographic study of epithelial and associated fibroblast renewal *in vivo* and *in vitro*. *Gastroenterology* **51**:670

Mahler, H. R., Cordes, E. H. 1971. Biological Chemistry. 2nd Edition. Harper and Row, New York

Martin, D. W. 1974. The effect of the bicarbonate ion on the gallbladder salt pump. *J. Membrane Biol.* **18**:219

Martin, D. W., Diamond, J. M. 1966. Energetics of coupled active transport of sodium and chloride. *J. Gen. Physiol.* **50**:295

Murphy, B., Martin, D. W. 1972. Carbamyl phosphate synthetase II in the mucosal cells of gall bladders. *Biochem. Biophys. Res. Commun.* **49**:121

Post, R. L., Kume, S., Tobin, T., Orcutt, B., Sen, A. K. 1969. Flexibility of an active center in sodium-plus-potassium adenosine-triphosphatase. *J. Gen. Physiol.* **54**:306s

Tatibana, M., Ito, K. 1969. Control of pyrimidine biosynthesis in mammalian tissues. Partial purification and characterization of glutamine utilizing carbamyl phosphate synthetase of mouse spleen and its tissue distribution. *J. Biol. Chem.* **244**:5403

van Os, C. H., Slegers, J. F. G. 1971. Correlation between (Na + K)-activated ATPase activities and the rate of isotonic fluid transport of gall bladder epithelium. *Biochim. Biophys. Acta* **241**:89