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Enzymes of *de novo* Pyrimidine Biosynthesis in *Paragonimus ohirai*

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Introduction

A previous publication (Kobayashi *et al.*, 1978) indicated that the lung fluke, *Paragonimus ohirai* could synthesize pyrimidine nucleotide by *de novo* pathway. In that investigation the enzymes of pyrimidine biosynthesis *de novo*, carbamyl phosphate synthetase, aspartate transcarbamylase, dihydroorotase, and phosphoribosyl transferase-orotidylate decaboxylase were conclusively shown to be present and the actual operation of the pathway was demonstrated in *P. ohirai*.

Recently, Hill *et al.* (1981) enumerated the enzyme activities of pyrimidine *de novo* pathway detected in some parasitic helminths. However, the detailed catalytic properties of such enzymes have remained unknown, except for *P.ohirai* carbamyl phosphate synthetase (Kobayashi *et al.*, 1978) which may play a critical role in the regulation of the pathway in a similar manner as does the synthetase from *Ascaris* ovary (Aoki *et al.*, 1975).

On the other hand, enzymic studies of pyrimidine biosynthesis were carried out on a few parasitic protozoa (O' Sullivan *et al.*, 1981; Holland *et al.*, 1983; Asai *et al.*, 1983) and each enzymes of the pathway were characterized.

The present paper deals with the detection of dihydroorotate dehydrogenase and orotidylate decarboxylase in *P. ohirai* which were not described in our privious report, some properties of these and other enzymes and the effect of certain inhibitors on the enzymes.

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Materials and Methods

Materials. ATP and 5-phosphoribosyl-1-pyrophosphate (PP-ribose-P) were obtained from Kyowa Hakko Kogyo, Tokyo, and other nucleotides were obtained from Sigma. [carboxy-^{14}C]orotic acid (41.25 Ci/mol) were obtained from Nuclear Corp. L-5, 6-Dihydro-[carboxy-^{14}C]orotic acid (L-[carboxy-^{14}C]-DHO) and[carboxy-^{14}C] orotidine-5-phosphate ([carboxy-^{14}C]OMP) were kindly furnished by Dr. W. J. O'Sullivan, School of Biochemistry, University of New South Wales, Austraria. $KH^{14}CO_3$ was prepared from $Ba^{14}CO_3$ as described previously (Tatibana and Ito, 1969). [^{14}C]Carbamylphosphate was prepared from [^{14}C]cyanate as described previously (Mori *et al.*, 1975). Ornithine transcarbamylase was purified from bovine liver by the methods of Marshall and Cohen (1972). Prior to use, it was dialyzed against 20 mM Tris-HCl (pH 7.2) containing 0.5 mM L-ornithine to free it from ammonia. A enzyme mixture of orotate phosphoribosyltransferase (EC 2. 4. 2. 10) and orotidine-5'-phosphate decarboxylase (EC 4. 1. 1. 23), partially purified from yeast, were obtained from Sigma. *N*-(phosphonacetyl)-L-aspartate (PALA) was synthesized as described by Collins and Stark (1971).

Adult worms of *P. ohirai* were obtained from the lungs of Wistar rats which had been given 20 metacercariae of *P. ohirai*

6–7 weeks prior to harvesting. The metacercariae of *P. ohirai* were obtained from naturally infected *Sesarma dehaani* collected in Chiba prefecture, Japan.

Enzyme preparations. The worms were homogenized as descrived priviously (Kobayashi *et al.*, 1978). The homogenate was centrifuged at 105,000×*g* for 30 min at 4C and the supernatant was used for enzyme studies except for dihydroorotate dehydrogenase assay. Futher purification by ammonium sulfate fractionation employed in some enzyme studies was performed as described previously (Kobayashi *et al.*, 1978).

Enzyme assays. Except where otherwise indicated, the following assay procedures were used.

Carbamyl phosphate synthetase (CPSase). CPSase activity was assayed with $H^{14}CO_3$ as a substrate in the presence of excess ornithine and ornithine transcarbamylase by the procedure as described previously (Tatibana and Shigesada, 1972). [^{14}C]-Citrulline formed was decomposed to $^{14}CO_2$ with $NaNO_2$ according to the method of Stevens and Stoken (1963), $^{14}CO_2$ was collected quantitatively as decribed previously (Kobayashi *et al.*, 1978). Radioactivity was counted in a Beckman LS-235 spectrometer.

Aspartate transcarbamylase (ATCase). ATCase was measured by the incorporation of the radiolabel from [^{14}C]-carbamyl phosphate (1 mM ; 0.09 Ci/mol) into carbamylaspartate by the method of Mori *et al.*, (1975), except that 0.1 M potasium phosphate (pH 7.5) was replaced by 0.1 M Tris-HCl (pH 8.5).

Dihydroorotase (DHOase). The activity of DHOase in the nonbiosynthetic direction was determined by measuring the conversion of L-dihydroorotate into carbamylaspartate (Mori *et al.*, 1975). Carbamylaspartate was determined by the method of Prescott and Jones (1969).

Dihydroorotate dehydrogenase (DHO-DHase). The precipitate of 105,000×*g* resuspended in 50 mM potasium phosphate buffer (pH 7.0), containing 30% (W/V) glycerol and 1 mM dithiothreitol was used as enzyme preperation. DHO-DHase activity was assayed by following the conversion of L-[carboxy-^{14}C]dihydroorotate into UMP and $^{14}CO_2$ using a coupled enzyme assay (Smithers *et al.*, 1978).

Orotate phospho ribosyltransferase (OPRTase). The activity of OPRTase was assayed by measuring the conversion of [carboxy^{14}C]-orotic acid into UMP and $^{14}CO_2$, according to the procedure of Fox *et al.* (1971). Sufficient endogenous orotidylate decarboxylase was present to convert all the OMP formed into UMP.

Orotidylate decarboxylase (ODCase). ODCase activity was assayed by the release of $^{14}CO_2$ from [carboxy-^{14}C]OMP (Fox *et al.*, 1971).

Results

Activities of pyrimidine nucleotide biosynthetic enzymes. The relative activities of the six enzymes, of which four were shown in the previous publication, of *de novo* pyrimidine pathway are summarized in Table 1, in order to facilitate the comparison among each activity. While activity of orotidylate decarboxylase was found in the supernatant following high speed centrifugation, activity of dihydroorotate dehydrogenase was found in precipitate fraction as is usually observed in eukaryotic systems (Jones, 1980). Dihydroorotase, the third enzymes of the pyrimidine biosynthetic pathway was precipitated by ammonium sulfate at 39% saturation alike carbamyl phosphate synthetase and aspartate transcarbamylase (Kobayashi *et al.*, 1978). It seems that dihydroorotase of *P. ohirai* exists as a multienzyme complex with carbamyl phosphate synthetase and aspartate transcarbamylase, as reported in higher animals (Hoogenraad *et al.*, 1971 ; Shoaf and Jones, 1971 ; Mori and Tatibana, 1973, 1975 ; Kent *et al.*, 1975).

Requirements of carbamylphosphate synthetase reaction and substrate specificity. The requirements for carbamyl phosphate synthetase activity in *P. ohirai* are shown in Table 2. Maximal activity was dependent upon the addition of L-glutamine or ammonia, MgATP, ornithine and ornithine transcarbamylase. Either L-glutamine or ammo-

Table 1 Summary of enzyme activities of pyrimidine nucleotide biosynthesis in *Paragonimus ohirai*

Enzymes	Enzyme activities (Units*/g worms wet weight)		
	Homogenate Sup.	Ppt.	$(NH_4)_2SO_4$ Fraction
Carbamyl phosphate synthetase	27.0		57.0
Aspartate transcarbamylase	6,480		7,800
Dihydroorotase	2,630		5,100†
Dihydroorotate dehydrogenase	0	726†	
Orotate phosphoribosyltransferase	462		
Orotidylate decarboxylase	1,200†		

* One unit of these enzymes was defined as the amount of activity which produces nmol of product per hour under the standard conditions.

† The data were newly obtained by this study.

Table 2 Requirements for carbamyl phosphate synthetase activity

System	(^{14}C) Citrulline formed (dpm)
Complete	595
Plus 20 mM NH_4Cl	620
Plus 5 mM *N*-acetyl-L-glutamate	520
Minus enzyme	0
Minus L-glutamine ; Plus 20 mM NH_4Cl	640
Minus L-glutamine ; Plus 20 mM NH_4Cl and 5 mM *N*-acetyl-L-glutamate	531
Minus Mg-ATP	30
Minus ornithine and ornithinetranscarbamylase*	8

The table shows a typical result of one experiment. Qualitatively identical results were obtained in separate experiments with different enzyme preparations.

The complete reaction mixture (0.3ml) contained 10 mM ATP, 15 mM $MgCl_2$, 3.3 mM L-glutamine, 16.7 mM $KH^{14}CO_3$, 50 mM potassium-HEPES pH 7.0, 0.5mM ornithine, 4units (μmol/min) of ornithine transcarbamylase, 1mM dithiothreitol, 7.5% DMSO and 2.5% glycerol.

* The reaction mixture was stopped with 0.1 ml of 3N HCOOH and heated at 80 C for 5 min to decompose the [^{14}C]-carbamyl phosphate formed.

nia was utilized as a nitrogen donor in the standard assay system. However, when both L-glutamine and ammonia were incubated in the incubation system, there was no summation of the activity observed with the either subtrate alone, indicating that a single enzyme utilized either one of the two substrates. Addition of *N*-acetyl-L-glutamate, the essential activator for the hepatic carbamyl phosphate synthetase involved in urea biosynthesis, or the enzymes from the hepatopancereas of the land snail *Strophocheilus oblongus* (Tramell and Campbell, 1970) and the liver of the teleost fish *Micropterus salmoides* (Anderson, 1976), did not increase the activity.

Aspartate transcarbamylase. *Km* values of 2.32 ± 0.35 mM and 88.0 ± 0.4 μM for aspartate and carbamyl phosphate respectively, were determined. Though the *Km* for carbamyl phosphate was rather high, the value for aspartate was close upon those of these enzymes from other eukaryotes. In comparison, the values for the enzyme purified from mouse spleen were 8.4 mM and 1.8 μM, respectively (Hoogenraad *et al.*, 1971). PALA, a very potent inhibitor of the enzymes from many sources, showed a strong inhibition. A Dixon plot was used to determine the *Ki* for PALA, with carbamyl phosphate and aspartate held at 170 μM and 4.5 mM, respectively. A value of 0.611 μM was obtained (Fig. 1), compared to 0.25 μM and 26 nM for the *Toxoplasma gondii* and mouse spleen enzymes, respectively (Asai *et al.*, 1983 ; Hoogenraad *et al.*, 1971).

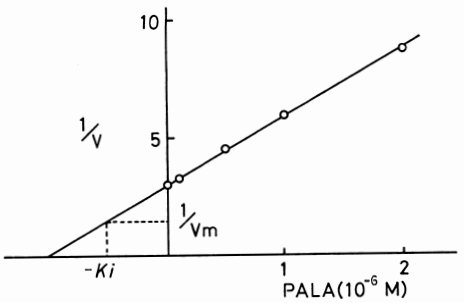

Fig. 1 Kinetic analysis of inhibition by PALA. The mean of duplication are protted. The reaction mixture (0.2 ml) contained 170 μM carbamyl phosphate, 4.5 mM aspartate, 0.1 mM Tris-HCl pH 8.5 and varied concentration of PALA as indicated. The enzyme was patially purified by ammonium sulfate precipitation and dialysed.

Dihydroorotase. Activity of DHOase, demonstrated in cytosol fraction previously (Kobayashi *et al.*, 1978), was precipitated by ammonium sulfate at 39 % saturation. Most activity of the enzyme in the homogenate was recovered in the supernatant fraction of 2,630 units and activity in the precipitate fraction of 538 units was much lower than that in the supernatant fraction. Essentially the same distribution was observed with CPSase and ATPase, except that the latter activity in the precipitate was fairly high (Kobayashi *et al.*, 1978).

Dihydroorotate dehydrogenase. As noted above, the DHO-DHase was associated with the particulate fraction following ultra high speed centrifugation (105,000×g for 30 min). The *Km* for dihydroorotate was determined as 1.22±0.29 μM. The activity was inhibited by cyanide (90% at 5 mM KCN); sodium azide (38% at 1 mM) and antimycin A (72% at 0.2 mM). NAD^+ (1 mM) had no effect on the reaction with L-DHO at 5 μM though $NADP^+$ (1 mM) appeared to produce a moderate degree (22%) of inhibition under the same conditions.

Orotate phosphoribosyltransferase. The *Km* for orotate in the OPRTase reaction was 0.454±0.04 μM at pH 8.0. A *Km* for PP-ribose-P was not determined but a concentration of 50 μM appeared to be saturated. A number of substrate analogs of orotate were tested as inhibitors of OPRTase. All compounds were added at a final concentration of 1 mM, with orotate at 1 μM. Under these conditions, the percentage inhibition observed was 5-methylorotate, 32; 5-aminoorotate, 35; 5-nitrobarbiturate, 35; 5-bromoorotate, 41. However, substantial inhibition (92%) was observed with barbiturate under these coditions. The hypoxanthine analog, allopurinol and the xanthine analog, oxipurinol were effective inhibitor, producing 100% and 91% inhibition at a concentration of 1 mM, repectively.

Orotidylate decarboxylase. The *Km* for OMP was determined to be 1.74±0.48 μM. As observed for the enzyme from other sources, 6-aza-UMP and XMP were effective inhibitors (Brown and O'Sullivan, 1977). With OMP at 3.5 μM, 6-aza-UMP produced 95% inhibiton at a concentration of 1 mM and XMP produced 85% inhibition at 1 mM. *Ki* values for 6-aza-UMP and XM Pwere determined as approximately 12.6 nM and 25.2 μM, respectively. No effect on the reaction were obser ved with allopurinol (1 mM) and oxipurinol (1 mM).

Discussion

Pyrimidine nucleotides are synthesized by salvage pathway and/or by *de novo* pathway in eukaryotes. Some enzyme activities of *de novo* pathway were detected in *P. ohirai* previously (Kobayashi *et al.*, 1978). Futhermore, [^{14}C]bicarbonate was actively incorporated into uracil nucleotide but a relatively little accumulation of the intermediates of the orotic pathway was observed in *P. ohirai* (Kobayashi *et al.*, 1978).

It is generally accepted that there exist three types of carbamyl phosphate synthetase in nature. Carbamyl phosphate synthetase I utilizes ammonia as nitrogen donor and requires *N*-acetyl-L-glutamate as an essential cofactor. Carbamyl phosphate synthetase II utilizes L-glutamine and does not require the cofactor. Carbamyl phosphate synthetase III utilizes L-gluatmine and shows a requirement for *N*-acetyl-L-glutamate. The synthetase I

is localized in mitochondria while the synthetase II is present in the cytosol fraction. The synthetase III from hepatopancreas of *Strophocheilus oblongus* (Tramell and Campbell, 1970) and from liver of *Squalus acanthias* (Anderson, 1981) has been shown in mitochondria.

Carbamyl phosphate synthetase of *P. ohirai* was revealed previously to be localized in cytosol fraction and to be subject to specific feedbak inhibition by pyrimidine nucleotides (Kobayashi *et al.*, 1978). It is clear that the synthetase shares the same properties of carbamyl phosphate synthetase II in that, in addition to the feature described above, a) it utilizes L-glutamine as well as ammonia although it has higher affinity for L-glutamine than for ammonia ; b) it does not require *N*-acetyl-L-glutamate. Furthermore, as in higher animals, the synthetase seems to exist as a multienzyme complex with aspartate transcarbamylase and dihydroorotase (Hoogenraad *et al.*, 1971 ; Shoaf and Jones, 1971 ; Mori and Tatibana, 1973, 1975 ; Kent *et al.*, 1975), judged by similar subcellular distribution and coprecipitation by ammonium sulfate of the three enzymes. Such a multienzyme complex of the synthetase was shown in *Ascaris* ovary (Aoki *et al.*, 1975, 1980).

As reported for all other sources, the high specific activity was observed for aspartate transcarbamylase. Its kinetic behavior appears to be somehow different from the enzymes of other systems. Since a *Km* for carbamyl phosphate in the aspartate transcarbamylase reaction was significantly higher than those from other sources although a *Km* for aspartate was close to that of aspartate transcarbamylase found in other systems. Such a high *Km* value, when low activity of carbamyl phosphate synthetase is taken into consideration, might be regarded inefficient system. There is some indication that specific regulatory mechanisms may be present in parasitic helminth. The aspartate transcarbamylase of *P. ohirai* differed with respect to the relatively high *Ki* value obtained for PALA. PALA has previously been shown to be a potent and specific inhibitor of the enzymes from *E. coli* (Clolins and Stark, 1971), mouse spleen enzyme (Hoogenraad, 1974) and C13/SV cultured hamster cells Swyryd, 1974). The *Ki* values reported for the enzymes from these sources are of the order of 1–10 nM, 2–3 orders of magnitude lower than those obtained for the *P. ohirai* enzyme (0.6 μM). PALA is still, however, an effective inhibitor of *P. ohirai* aspartate transcarbamylase and may be worthy of consideration as a controlling drug in *Paragonimus* infections.

Dihydroorotate dehydrogenase appeared to similar in its properties to the enzymes in other eukaryotic sources (Jones, 1980). The enzyme from *P. ohirai* was particulate and had a specific activity of a half of that found in *Toxoplasma gondii*. The enzyme from *Toxoplasma gondii* was shown to be particulate with a specific activity of 60 nmol/h/mg protein (Asai *et al.*, 1983). *P. ohirai* enzyme was inhibited by respiratory chain inhibitors, indicating that it was linked to oxygen utilization via respiratory chain, though the nature of the linkage was not investigated in detail.

The apparent *Km* for orotic acid of 0.454 μM in the orotate phosphoribosyltransferase reaction was lower than that of 2 μM reported for Ehrlich ascites cell (Shoaf and Jones, 1978). For comparison, the value for the enzyme form parasitic protozoa, *Babesia rodohaini* and *Toxoplasma gondii* were 3.8 μM and 1.6 μM, respectively (Holland *et al.*, 1983 ; Asai *et al.*, 1983). Of the orotate analogs tested as inhibitors of orotate phosphoribosyltransferase, only the substrate analog barbiturate, was an effective inhibitor, indicating that control at this step through inhibition by substrate was different. Both allopurinol and oxipurinol which, appart from being inhibitors of xanthine oxidase, were shown to be potent inhibitors of *P. ohirai* transferase, however, no effects were observed with allopurinol on the reaction of both *Toxoplasma* and *Babesia* (Asai *et al.*, 1983 ; Holland *et al.*, 1983).

Orotidylate decarboxylase in *P. ohirai* has similar kinetic properties to the enzymes in

other systems. The activity was found to be tree-fold higher than the orotate phosphoribosyltransferase activities. Similar results have been reported for two enzymes from human erythrocytes and Ehrlich ascites cells (Fox *et al.*, 1971; Shoaf *et al.*, 1973). The apparent *Km* for OMP of 1.74 μM was comparable to reported values from human tissue (Brown *et al.*, 1975). Substrate analog inhibition by 6-aza-UMP which has been widely noted in other systems (Brown and O'Sullivan, 1975; Asai *et al.*, 1983) was also observed for *P. ohirai* enzyme. On the other hand, 6-aza-uridine was been shown to have little effect on uridine nucleotide synthesis of *P. ohirai* maintained *in vitro* (Kobayashi *et al.*, 1978). This fact could indicate that *P. ohirai* uridine kinase lacks the ability to convert 6-aza-uridine to 6-aza-UMP. Futher observations are required before any conclusions are reached with regard to these mechanisms.

Summary

The pathway of *de novo* pyrimidine biosynthesis in *Paragonimus ohirai* has been investigated. The first three enzymes, carbamyl phosphate synthetase, aspartate transcarbamylase and dihydroorotase seems to exist as a multienzyme complex. All enzyme activities were found in cytosol fraction, with the exception of dihiydroorotate dehydrogenase of which activity was found in paticulate fraction.

Apparent *Km* values for the respective enzymes were: aspartate transcarbamylase, L-aspartate (2.32±0.35 mM), carbamyl phosphate (88.0±0.4 μM); dihydoorotate dehydrogenase, dihydroorotate (1.22±0.29 μM); orotate phosphoribosyltransferase, orotate (0.45 ±0.04 μM); orotidylate decarboxylase, orotidine 5′-monophosphate (1.74±0.48 μM).

The effect of some inhibitors, including pyrimidine and purine nucleotides and analogs and respiratory chain inhibitors, was also determined for the enzymes of the pathway.

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References

1) Anderson, P. M. (1976): A glutamine and *N*-acetyl-L-glutamate-dependent carbamyl phosphate synthetase activity in the teleost *Micropterus salmoides*. Comp. Biochem. Physiol., 54 B, 261–263.
2) Anderson, P. M. (1981): Purification and properties of the glutamine and *N*-acetyl-L-glutamate-dependent carbamyl phosphate synthetase from liver of *Squalus acanthias*. J. Biol. Chem., 256, 12228–12238.
3) Aoki, T., Oya, H., Mori, M. and Tatibana, M. (1975): Glutamine-dependent carbamyl phosphate synthetase in *Ascaris* ovary and its regulatory properties. Proc. Jpn. Acad., 51, 733–736.
4) Aoki, T., Oya, H., Mori, M. and Tatibana, M. (1980): Control of pyrimidine biosynthesis in the *Ascaris* ovary; Regulatory properties of glutamine-dependent carbamoyl phosphate synthetase and copurification of the enzyme with aspartate carbamoyltransferase and dihydroorotase. Biochem. Parasitol., 1, 55–68.
5) Asai, T., O'Sullivan, W. J., Kobayashi, M., Gero, A. M., Yokogawa, M. and Tatibana, M. (1983): Enzymes of the de novo primidine biosynthetic patway in *Toxoplasma gondii*. Mol. Biochem. Parasitol., 7, 89–100.
6) Brown, G. K., Fox, R. M. and O'Sullivan, W. J. (1975): Interconversion of different molecular weight forms of human erythrocyte orotidylate decarboxylase. J. Biol. Chem., 250, 7352–7358.
7) Brown, G. K. and O'Sullivan, W. J. (1977): Inhibition of human erythrocyte orotidylate decarboxylase. Biochem. Pharmacol., 26, 1947–1950

8) Collins, K. D. and Stark, G. R. (1971): Aspartate transcarbamylase. Interaction with the transition states analogue *N*-(phosphonacetyl)-L-aspartate J. Biol. Chem., 246, 6599-6605.
9) Fox, R. M., Wood, M. H. and O'Sullivan, W. J. (1971): Studies of coordinate activity and lability of orotidylate phosphoribosyltransferase and decarboxylase in human erythrocytes and the effect of allopurinol administration. J. Clin. Inv., 50, 1050-1060
10) Hill, B., Kilsby, J., Rogerson, G. W., McIntosh, R. T. and Ginger, C. D. (1981): The enzyme of pyrimidine biosynthesis in a range of parasitic protozoa and helminths Mol. Biochem. Parasitol., 2, 123-134.
11) Hoogenraad, N. (1974): Reaction mechanisms of aspartate transcarbamylase from mouse spleen. Arch. Biochem. Biophys., 161, 76-82.
12) Hoogenraad, N., Levine, R. L. and Kretchmer, N. (1971): Copurification of carbamyl phosphate synthetase and aspartate transcarbamylase from mouse spleen. Biol. Biophys. Res. Commun., 45, 796-802.
13) Hooland, J. W., Gero, A. M. and O'Sullivan, W. J. (1983): Enzymes of de novo pyrimidine biosynthesis in *Babesia rodhaini*. J. Protozool., 30, 36-40.
14) Jones, M. E. (1980): Pyrimidine nucleotide biosynthesis in animals: Genes, enzymes and regulation of UMP biosynthesis. Ann. Rev. Biochem. 49, 253-279.
15) Kent, R. J., Lin, R., Sallach, H. J. and Cohen, P. P. (1975): Riversible dissociation of a carbamyl phosphate synthetase-aspartate transcarbamylase-dihydroorotase complex from ovarian eggs from *Rana catesbeiana*: Effect of uridine triphosphate and other modifiers. Proc. Natl. Acad. Sci. U. S. A., 72, 1712-1716.
16) Kobayashi, M., Yokogawa, M., Mori, M. and Tatibana, M. (1978): Pyrimidine nucleotide biosynthesis in *Clonorchis sinensis* and *Paragonimus ohirai*. Int. J. Parasitol., 8, 471-477.
17) Marshal, M. and Cohen, P. P. (1972): Ornithine transcarbamylase from *Streptococcus faecalis* and bovine liver. J. Biol. Chem., 247, 1641-1653.
18) Mori, M. and Tatibana, M. (1973): Dissociation by elastase digetion of enzyme complex catalyzing the initial steps of pyrimidine biosynthesis in rat liver. Biochem. Biophys. Res. Comm., 54, 1525-1531.
19) Mori, M. and Tatibana, M. (1975): Purification of homogeneous glutamine-dependent carbamyl phosphate synthetase from ascites hepatoma cells as a complex with aspartate transcarbamylase and dihydroorotatase. J. Biochem., 78, 239-242.
20) Mori, M., Ishida, H. and Tatibana, M. (1975): Aggregation states and catalitic properties of the multienzyme complex catalyzing the initial steps of pyrimidine biosynthesis in rat liver. Biochem., 14, 2662-2630.
21) O'Sullivan, W. J., Johnson, A. M., Finney, K. G., Gero, A. M., Hagon, E., Holland, J. W. and Smithers, G. W. (1981): Pyrimidine and purine enzymes in *Toxoplasma gondii*. Aust. J. Exp. Biol. Med. Sci. 59, 763-767.
22) Prescott, L. M. and Jones, M. E. (1969): Modified method for the determination of carbamyl aspartate. Anal. Biochem., 32, 408-419.
23) Shoaf, W. T. and Jones, M. E. (1971): Initial steps in pyrimidine synthesis in Ehrlich ascites cartinoma. Biochem. Biophys. Res. Comm., 45, 796-802.
24) Smithers, G. W., Gero, A. M. and O'Sullivan, W. J. (1978): A simple radioassay for dihydroorotate dehydrogenase. Anal. Biochem., 88, 93-103.
25) Stevens, L. and Stocken, L. A. (1963): Studies on enzymes involved in nucleic acid metabolism in foetal, young and regenerating rat liver. Biochem. J., 87, 12-15.
26) Swyryd, E, A., Seaver, S. S. and Stark, G. R. (1974): *N*-(phosphonacetyl)-L-aspartate, a transition state anlog inhibitor of aspartate transcarbamylase, blocks proliferation of mammalian cells in culture. J. Biol. Chem., 294, 6945-6950.
27) Tatibana, M. and Ito, K. (1969): Control of pyrimidine biosynthesis in mammalian tissue. J. Biol. Chem., 244, 5403-5413.
28) Tatibana, M. and Shigesada, K. (1972): Control of pyrimidine biosynthesis in mammalian tissues. V. Reguration of glutamine dependent carbamyl phosphate: Activation by 5-phosphoribosyl 1-pyrophosphate and inhibition by uridine triphosphate. J. Biochem., 72, 549-560.
29) Tramell, P. R. and Campbell, J. W. (1970): Carbamyl phosphate synthetase in a land snail, *Strophocheilus oblongus*. J. Biol. Chem., 245, 6634-6641.

大平肺吸虫のピリミジン新生合成経路酵素

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大平肺吸虫のピリミジン新生合成経路の酵素について研究を行つた．同経路の最初の三つの酵素，カルバミルリン酸合成酵素，アスパラギン酸トランスカルバミラーゼおよびジヒドロオロターゼは酵素複合体を形成していると考えられる．ジヒドロオロット酸脱水素酵素は顆粒画分に，その他の五つの酵素は可溶性画分に活性が見出された．

それぞれの酵素に対応する基質のみかけ上のミカエリス定数は，アスパラギン酸トランスカルバミラーゼ，L-アスパラギン酸 (2.32±0.35mM)，カルバミルリン酸 (88.0±0.4μM)；ジヒドロオロット酸脱水素酵素，ジヒドロオロット酸 (1.22±0.29μM)；オロット酸ホスホリボシルトランスフェラーゼ，オロット酸 (0.45±0.04μM)；オロチジン-5′-リン酸脱炭酸酵素，オロチジン-5′-リン酸 (1.74±0.48μM) であつた．

またピリミジンおよびコリンヌクレオチドの類似物や，呼吸鎖阻害物質による各酵素の阻害作用についても検討した．