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Studies on transaminase reactions in some parasitic helminths

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Introduction

In recent years a rather large amount of work has been done on the metabolism and nutrition of endoparasites. But little progress has been made far in the field of nitrogen metabolism.

Cheng(1964) stated that the interrelationship between carbohydrate and protein metabolisms is probably very close in certain helminths.
It is considered that in this interrelationship the enzymes called transaminases are very important links between the metabolism of amino acids, carbohydrates and even in fats. Yoon(1964) indicated that this transaminase activity seemed to play an important role on the synthesis of protein in some helminth parasites.

It is well known that animal tissues contain transaminases which catalyze reactions such as the following:

α-ketoglutarate + aspartate $\rightleftarrows$ oxaloacetate + glutamate and

α-ketoglutarate + alanine $\rightleftarrows$ pyruvate + glutamate.

The enzyme involved in the first reaction is conveniently abbreviated "GOT" and the transaminase of the second is abbreviated "GPT".

Although transaminases were identified in animal tissues as early as 1937, very few experiments have been performed on these enzyme systems in parastic helminths. Daugherty(1952) found that homogenates of the liver fluke, *Fasciola hepatica*, contained very high transaminase activity. Aldrich, Chandler and Daugherty(1954) reported the transaminase activity in *Hymenolepis diminuta*, and Foster and Daugherty(1959) made a comparison of the transaminase activity between *Raillietina cesticillus* and *Hymenolepis diminuta*. Also transaminase activity of three species of *Hymenolepis* were detected by Wertheim, Zeldon, and Read(1960).

In this study, a comparison is made on the transaminase activities (GOT and GPT) of some parasitic helminths, i.e., 2 kinds of nematodes, *Ascaris lumbricoides* and *Ascaridia galli*; 5 kinds of trematodes, *Clonorchis sinensis*, *Paragonimus wetermani*, *Fasciola hepatica*, *Eurytrema pancreaticum* and *Paramphistomum cervi*; and 5 kinds of cestodes including 2 kinds of larval forms; *Diphyllobothrium mansoni*, *Dipylidium caninum* *Taenia pisiformis*, *Cysticercus cellulosae* and *Cysticercus pisiformis*.

Materials and Methods

Collection of worms:

Specimens of *A. lumbricoides*, *F. hepatica*,

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E. pancreaticum and *P. cervi* were obtained alive from an abattoir in Seoul, and brought to laboratory in normal saline. The worms of *A. galli* were collected from the infected hen, *C pisiformis* from infected rabbits. The adult flukes of *C. sinensis* were obtained from the liver of rabbit, also the worms of *P. westermani* were from the lungs of dog. These hosts had been already infected with each of metacercariae of *Clonorchis* or *Paragonimus* in the laboratory. Living worms of *D. caninum* were collected from the intestine of naturally infected dog. *D.mansoni* and *T. pisiformis* were obtained from the intestine of dog previously infected with plerocercoids and cysticerci. From the subcutaneous tissue of a cysticercosis patient, *C. cellulosae* were obtained by surgical removal.

Preparation of testing materials:

The most active and intact living worms were selected and immediately washed thoroughly several times in sterile saline solution. The body of *A.lumbricoides* was dissected into muscular tissue and male or female genital organs, and body fluid was collected, in order to determine each transaminase activity separately. Two kinds of cycticerci were isolated from their worm capsules and cystic fluids were also collected for the test of their enzymatic reactions.

Then the worms were weighed and transferred with 1.0 ml of 0.1 M phosphate buffer (pH 7.5) to a chilled ground glass homogenizer, and homogenized in the cold. The homogenate was brought to 10 ml with phosphate buffer, centrifuged at low speed for 5 min. to remove large fragments, and the cell-free supernatant was pipetted off for determination of transaminase activity. Fluid specimens such as body fluid of *A. lumbricoides*, cystic fluids of cysticerci were immediately used without further treatment. And nitrogen contents of all fluid specimens were measured by micro-Kjehldahl technique (Frankel and Reitman, 1963).

Incubation procedure:

Transaminase activities were determined by Sigma-Frankel method (Reitman and Frankel, 1957; Frankel and Reitman, 1963). The incubation mixure was essentially the same that of Frankel and Reitman (1963) and was composed of the following materials:

1) 0.5 ml of GOT substrate (mixture of α-ketoglutaric acid 2mM/L, and dl-aspartate 200 mM/L) or GPT substrate (α-ketoglutaric acid 2mM/L and dl-alanine 200mM/L)

2) 0.1 ml of tissue homogenates or body fluid or cystic fluid. These mixtures were incubated for exactly 60 min for GOT or 30 min for GPT at 37°C water bath.

Spectrophotometry:

After the incubation, 0.5 ml. of 1mM 2,4-dinitrophenylhydrazine reagent was added, there by stopping the reaction and this was set for 20 min at room temperature. After then, 5.0 ml of 0.4N NaOH was added and mixed by inversion. The absorbance or transmittance of mixture was determined after a minimum of 5 min. at 505 mμ against a distilled water blank. Calibration curve was prepared by the standardization of 2mM/L pyruvate solution. For the spectrometry, "Spectronic 20" spectrophotometer was utilized.

Results

The results are calculated in terms of micromoles of amino acid formed per gram of wet weight of worm tissue per hour by tissue homogenates, or in terms micromoles of amino acid formed per mg of nitrogen per hour by fluid specimens. And the figures are the average of determinations from six experiments and many of the duplicate measurements.

The present data showed highly variable transaminase activities which have been witnessed

by previous experimenters (Daugherty, 1957; Read and Rothman, 1956; Foster and Daugherty, 1959) when using invertebrate material.

The data on transaminations obtained with homogenates of the two species of nematodes are presented in Table 1. In these data, it is obvious that these nematodes are very restricted in their capacity to carry out transaminations. But it may be noted that the transaminase activity for *A. lumbricoides* was in general higher than that for *A. galli* and in case of *A. lumbricoides* the activity in genital organs was higher than in muscular tissue. It is also noted that there is no significant difference between GPT and GOT activity in both worms.

Table 1. Transaminase activity of 2 species of nematodes. Values are micromole of amino acid formed per gram wet weight of worms per hour.

	α-ketoglutaric-glutamic			
	A. lumbricoides			*A. galli*
Amino donor	male genital organ	female genital organ	muscle	
alanine	26.7	21.6	10.9	10.2
aspartic acid	25.7	19.0	8.9	17.5

A comparison of the transaminase activity in five species of trematodes is given in Table 2. In trematodes, these enzymatic activities are much higher than those of nematodes. The activity of GOT was consistently higher than that of GPT in all five species of flukes. The highest GOT activity was observed by a tissue parasite, *P. westermani* and the liver fluke, *F. hepatica* showed the highest GPT activity. It is of interest that the rates of transamination are nearly same in *C. sinensis* and *F. hepatica*. In *P. cervi*, a lumen dwelling trematodes of cattle, the lowest activity of these reactions was shown in this experiment. These values obtained from *P. cervi* are scarcely different from those of nematodes.

Table 2. Transaminase activity of 5 species of trematodes. Values are micromole of amino acid formed per gram per hour.

	α-ketoglutaric-glutamic				
amino donor	*C. sinensis*	*F. hepatica*	*E. pancreaticum*	*P. cervi*	*P. westermani*
alanine	174.6	178.5	103.4	13.1	162.0
aspartic acid	220.6	228.6	141.4	19.2	233.6

The higher metabolic activity of several tapeworms has been indicated in previous experiments (Aldrich et al., 1954; Foster and Daugherty, 1959, Wertheim et al., 1960). In this study three species of adult tapeworms and two larval cestodes were utilized, and the results from these parasites are presented in Table 3. In adult worms, it is also noted that GOT activity was higher than GPT activity. Larval cestode showed very restricted activity of transamination in comparison with that of adult worms, and certain degree of difference in transamination capacity was observed between two cysticerci. Among the five species of ces-

Table 3. Transaminase activity of 5 species of cestodes. Values are micromole of amino acid formed per gram wet weight of worm per hour.

	α-ketoglutaric-glutamic				
amino donor	*D. mansoni*	*D. caninum*	*T. pisiformis*	*D. cellulosae*	*C. pisiformis*
alanine	101.2	173.4	125.8	14.8	31.8
aspartic acid	123.9	214.5	129.2	14.3	41.2

todes, *D. caninum* utilized the highest amount of amino donors for transamination and in *C. cellulosae*, the least transaminase activity was observed.

The results of the survey on fluid specimens from three kinds of parasites for transaminase activity are presented in Table 4. The highest

Table 4. Transaminase activity in the fluid specimens of 3 kinds of helminths. Values are micromoles per mg nitrogen per hour

amino donor	α-ketoglutaric-glutamic		
	A. lumbricoides	*C. cellulosae*	*C. pisiformis*
alanine	7.7	2.4	2.6
aspartic acid	11.0	3.2	4.5

enzymatic activity was observed in the body fluid of *A. lumbricoides*. The cystic fluids from two larval worms showed nearly same degree of transamination reaction and it is also declared that GOT is higher than GPT activity. Unfortunately, these data could not be used for comparison with these systems in their corresponding tissue homogenates of cysticerci because the methods for analysis were different.

The stability of the transaminases in these helminths was examined briefly. The results obtained are similar to that of previous experiment (Daugherty, 1952). The transamination reaction remained active for several weeks by the homogenates which were allowed to remain for a period at cold room temperature (4°C) or by the homogenates which were prepared by freezing the fresh tissue for a period before homogenization. And unfrozen worms had full activity if they were homogenized before the death of the worm.

Discussion

Many isolated reactions involving transamination have been described for mammalian tissues (Awapara and Seale, 1952; Conrad, 1957) and for microorganisms, but few of these reactions have been reported in parasitic helminths. The demonstration of transaminase systems probably provides an open wedge into the problem concerning the intermediary metabolism of protein in helminths. For the interconversion of amino acids, the conversion of amino acids to ketoacids, and synthesis and oxidative deamination of amino acid by coupled reactions are all among the potential functions of transamination. By interconversion, amino acids less essential to the metabolism of the parasite can be exchanged, in the presence of proper ketoacid, for those more metabolically significant in the synthesis of protein by the worm. Von Brand(1952) pointed out that nonessential amino acids presumably can be synthesized by the parasites and one mechanism involved in often that of transamination in which an amino group is transferred by means of enzymatic activity to a ketoacid.

Aldrich et al.(1954) stated that transamination thus provides a means whereby amino groups may be removed from amino acid present in excess amounts in the host gut or tissues and attached to certain carbon fragments of carbohydrate degradation in the worm. And this is essentially the mechanism by which interconversion of protein with carbohydrate and fat occurs.

In the present experiment, the nematodes showed very restricted activity in transamination. The very limited capacity of these worms to carry out transaminations suggests that they may utilize synthesis to a relatively minor extent in satisfying amino acid requirements. Cheng(1964) described that the uptake of amino acids had to be rapid and in relatively large quantities. These facts suggest that Ascarid may derive its required amino acids from the host tissues with which it is in close contact or directly from materials passing into the host's intestine.

In trematode, the enzymatic activities are higher in tissue parasites than in lumen dwe-

llors. It is considered that tissue parasites cannot absorb the requisite amount of amino acid from surrounding tissues while intestinal parasites may derive it from the host tissues or directly from the food stuffs. In this experiment, almostly the same degree of enzymatic activity was observed in *C. sinensis*, *F. hepatica* and *P. westermani*. In comparison with the previous report of Daugherty(1952) in *F. hepatica*, these values showed slightly higher activity.

Because of the great variability found between separate experiments on the same animal species and on the same substrate, it is extremely difficult to compare the works done by different investigators. There are probably dietary effects, age effects, and effects resulting from reproductive phases of worms, among many others that are responsible for the variation in activity. In mammals, the transaminase activity varies greatly according to the organs (Cammarata and Cohen, 1950; Awapara and Seale, 1952). However in the homogenates of entire worm, like in this experiment, it should be considered the composites of the total activities of different tissues of the worm.

Aldrich et al. (1954) reported that four transaminase systems were found in *H. diminuta* and castration of the host markedly reduced the rate of activity of the transaminase system. Foster and Daugherty(1959) investigated the transaminase system of *H. diminuta* and *R. cesticillus*, and discussed the significance of the transaminase reactions to cestodes. Wertheim et al.(1960) declared that very few compounds serve as effective amino donors in three species of *Hymenolepis* and those closely related species can be distinguished by the presence or absence of transaminase. In the present experiment, *D. caninum* showed the highest, *D. mansoni* and *T. pisiformis* the moderate, and larval cestodes the lowest activity. Compared with the works of others, there are no appreciable differences among the adult tapeworms. The restricted transamination reaction in larval tapeworm can be interpreted to be the result of limited growth and reproductive phonomena in the intermediate hosts.

In comparison with the vertebrate (Awapara et al., 1952), and insects (Kilby and Neville, 1957), the helminths examined in the present study have somewhat lower capacity for transamination.

Chandler(1943) found that when rats were placed on a protein free diet, normal worms of *H.diminuta* or sometimes larger than normal were found at the end of 30 days feeding with protein free diet. These worms were apparently able to survive independent of protein in the host diet. From the work of Read(1950 and 1955), it would appear that intestinal parasites are not dependent entirely upon the dietary food in the intestine for nutrients. They are capable absorbing amino acids and other materials which diffuse through the mucosa of the host gut into the lumen. In such a situation, it can be expected that the transamination reaction plays an important role in the parasites.

The transamination of many amino acids to form glutamate provides an important link with the amino acid oxidase systems. L-proline l-glutamic acid have been shown to be oxidized to completion through the Krebs cycle by way of α-ketoglutaric acid (Taggart and Krakauer, 1949). It is significant that glutamic acid is consistently found in large amounts in both free amino acid and protein fraction of certain parasites(Lee et al., 1964). Greater importance is associated with its ability to serve not only as substrate but also as end product in the most active transaminase enzyme systems. Through the analyses of the protein and non-protein fractions of parasites, it was found that there are various amino acids to be able to function in certain transaminase systems, however it is

thought that the α-ketoglutaric acid-glutamic acid mechanism is most fundamental, at this time.

Summary

By an application of Sigma-Frankel methods, two transaminase systems, glutamic-pyruvic transaminase and glutamic-oxalocetic transaminase, were found to operate at a measurable rate in 2 species of nematodes(*Ascaris lumbricoides* and *Ascaridia galli*), 5 species of trematodes (*Clonorchis sinensis*, *Fasciola hepatica*, *Eurytrema pancreaticum*, *Paramphistomum cervi* and *Paragonimus westermani*) and 5 kinds of cestodes (*Diphyllobothrium mansoni*, *Dipylidium caninum*, *Taenia pisiformis*, *Cysticercus cellulosae* and *Cysticercus pisiformis*).

A comparison was made of the transamination reactions in nematodes and those of trematodes and cestodes. And the significance of transaminase in these parasites is discussed in relation to protein synthesis and its utilization.

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≫國文抄錄≪

蠕虫類 Transaminase 作用에 關한 硏究

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寄生蠕虫類에 있어 蛋白質代謝過程에 널리 關與하는 Transaminase 作用을 알기 爲하여 線虫類 2種 (*Ascaris lumbricoides, Ascaridia galli*) 및 吸虫類 5種 (*Clonorchis sinensis, Fasciola hepatica, Eurytrema pancreaticum, Paramphistomum cervi, Paragonimus westermani*) 條虫 5種類 (*Diphyllobothrium mansoni, Dipylidium caninum, Taenia pisiformis, Cysticercus cellulosae, Cysticercus pisiformis*)를 擇하여 虫體 homogenate 를 만들어 그 GPT, GOT 를 測定하였다.

蛔虫을 비롯한 네가지 寄生虫은 屠殺場에서 산채로 採集하여 實驗室까지 保溫生理的 食鹽水에 넣어 運搬하였고 鷄蛔虫의 2種의 虫體는 自然感染된 各動物宿主에서 採取하였다. 特히 肺吸虫, 肝吸虫 및 2種의 條虫類 成虫 및 幼虫은 各各 家兎 또는 개에게 實驗感染시키고 一定時日를 經過한 後 各各 屠殺宿主에서 採取한 것이다. 또 一種의 囊尾虫은 囊尾虫症 患者로 부터 外科的으로 摘出한 것을 即時實驗에 使用하였다.

各虫體는 0.1M Phosphate 緩衝液에서 homogenate 를 만들어 遠心沈澱하여 그上淸液을 實驗에 使用하였다. 蛔虫은 雌雄生殖器 筋肉組織 및 體液을 分離實驗하였고 囊尾虫에서는 囊尾虫體液을 注射器로 吸引採取하고 그대로 實驗하였다.

GOT 및 GPT 의 活性度는 Sigma-Frankel 法으로 "Spectronic 20" spectrophotometer 를 使用 測定하였다. 活性度는 組織 homogenate 에 있어서는 每虫體 組織 每 g (Wet weight) 當 每時間 形成된 아미노酸量 (micromole)으로 表示하였고 虫體液에 있어서는 mg nitrogen 當 每時間 形成된 아미노酸量으로 表示하였다.

一般으로 線虫類에 있어서는 닭蛔虫보다 人蛔虫에 依한 Transaminase 活性度가 높았고 人蛔虫 雌雄生殖器에 있어서의 活性度는 筋肉組織에 있어서 보다 높았다. 吸虫類에 있어서는 *P.cervi*를 除外하고 全部 線虫類보다 훨씬 活性度가 높았으며 모든 吸虫類에 있어서 GOT 는 GPT 보다 높은 값을 나타 내었다.

GOT 는 肺吸虫에서 가장 높았고 GPT 는 肝蛭에서 가장 높았으며 一般으로 吸虫類 寄生 棲息狀況과 이들 活性度와의 關係를 볼때 組織寄生型이 腸腔寄生型보다 顯著히 높은 Transaminase 作用을 가졌음을 推測케 하였다.

條虫類 成虫3種에 있어서도 一般으로 線虫類보다 높은 活性度를 表示하였으며 GOT 는 GPT 보다 높았다. 成虫에서는 幼虫에서 보다 活性度가 顯著히 높았다. 囊尾虫體液과 蛔虫體液 活性度를 比較한바 後者에서 數倍의 높은 값을 나타내었다.

以上의 成績으로 볼때 一般으로 組織寄生 蠕虫特히 吸虫에 있어 Transaminase 活性度가 높은 理由를 다음과 같이 說明하였다. 即 이들 寄生虫體는 終宿主組織으로 부터 必要한 量의 아미노酸을 攝取하지 못하는데 基因할것이라 하였고 또 條虫類幼虫이 活性度가 낮은 것은 中間宿主組織內에서 成長이 抑制되며 生殖作用이 없는 것이 原因일 것이라 하였다.