

## Studies on metabolism of lung flukes, genus *Paragonimus*

### I. Paper chromatographic analyses of free amino acids and aminosugar in uterine eggs, larvae and adults

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Lung flukes, genus *Paragonimus*, have three different hosts during their life cycle. Biochemical studies on the flukes are of great interest in comparative biochemistry, and important for the rational diagnosis and medical treatment of paragonimiasis. To this end the author has studied the metabolism of the flukes. Detection and estimation of amino acids and aminosugar in some helminths have been performed by Flury (1912), Yoshimura (1930), Wakabayashi (1941), Kajihara and Hashimoto (1952), Kreuzer (1953), Cavier and Savel (1954), Ogi-moto (1956), Ando (1957), Ueno (1960), Robinson (1961), Jaskoski (1962) and so on. However, biochemical studies of metabolism of free amino acids and aminosugar in uterine eggs, rediae, cercariae, metacercariae and adults are scanty. Therefore, in this paper the author has reported on the detection and estimation of free amino acids and aminosugar in the uterine eggs, larvae and adults as the first report of his studies on the metabolism of lung flukes.

#### Materials and Methods

##### *Preparation of Samples:*

Adult lung flukes were obtained from lungs of freshly killed dogs which had been sacrificed 6 months after infection with *Paragonimus westermani* (Kerbert, 1878). They were immersed at 37°C in Ringer's solution to wash the surface of their bodies and the inside of

their intestines for 12 hours. Fluid filling the excretory bladder and any uterine eggs were removed from the body. Uterine eggs were prepared from resected uteri of the adult *P. westermani*. Mature rediae and cercariae were liberated from livers of naturally infected snails, *Semisulcospira bensoni* (Philippi), with *P. westermani*. Metacercariae including the inner cyst wall were isolated from naturally infected crabs, *Potamon dehaani* (White), with *Paragonimus miyazakii* Kamo, Nishida, Hatsushika et Tomimura, 1961. These materials were washed at least five times with water (purified water), centrifuged at very low speed and placed on filter papers to absorb excess moisture. They were weighed precisely, and a 50 to 500 mg-portion of the materials was treated in a boiling water bath for a few minutes. This was then homogenized thoroughly in 1 to 10 ml of cold water with the aid of a motor driven glass homogenizer.

##### *Analyses of Free Amino Acid and Aminosugar:*

The homogenate was treated in a tube with an equal volume of 10% trichloroacetic acid (TCA) in an ice bath with occasional stirring. After 10 minutes the tube was centrifuged at 5,000 r.p.m. for 10 minutes. The supernatant was decanted, and the precipitate was treated twice with an equal volume of 5% TCA. TCA from the combined supernatant was removed five times with equal volumes of ether. After

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that, the combined extract was evaporated to dryness in vacuo at 65°C and the residue was dissolved in a known volume (0.05–0.5 ml) of water. Using a standardized micropipette, a 20 to 30  $\mu$ l-portion was spotted on Toyo Roshi No. 51-A filter paper for one- and two-dimensional chromatographic separation.

Solvent used for one-dimensional ascending chromatography was a mixture of m-cresol and phenol (1:1, v/v) with borate buffer at pH 9.3 by Levey (1953). For two dimensional ascending chromatography the upper layer of a mixture of n-butanol, acetic acid and water (4:1:5, v/v) and phenol saturated with water was used. Chromatography was carried out at room temperature. The solvent was removed by drying the paper in an air current at 40°C for 3 hours. Color was developed with spray of 0.1% ninhydrin acetone solution. Spots corresponding to amino acids and aminosugar from the samples were detected with authentic samples. The presence of aminosugar was detected with glucosamine by using anilinhydrogenphthalate. In addition to the above analyses, the amounts of free amino acids and aminosugar in the adult flukes on chromatogram were determined by comparison with standard optical density values in Beckman spectrophotometer at 440 and 570  $m\mu$  according to the method reported by Awapara (1949).

## Results and Discussion

### Detection of Free Amino Acids and Aminosugar:

Figure 1 shows representative paper chromatographic patterns of the free amino acids and aminosugar in the adult lung flukes. These positive spots on the chromatogram were detected with the authentic samples. In Figure 1, eighteen amino acids and one aminosugar corresponding to their respective sites were detectable, i. e. alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, hydroxyproline, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine and aminosugar. One unidentified spot was visible in an upper side of the

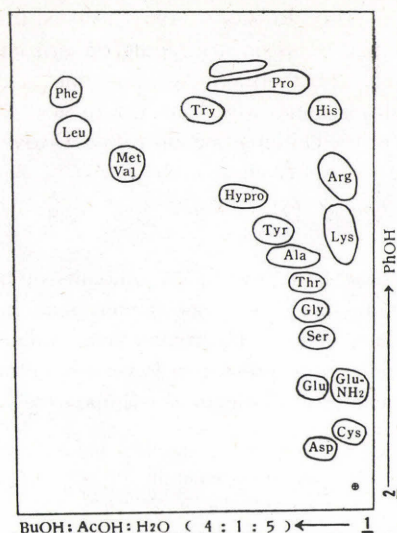


Fig. 1. Two-dimensional chromatogram of free amino acids and aminosugar in the adult *Paragonimus westermani*

proline. On the other hand, in the case of the uterine eggs nine spots corresponding to locations of alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, leucine, lysine and aminosugar were detected with the authentic samples. Moreover, in the case of the rediae and cercariae sixteen spots corresponding to spots of alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, leucine, lysine, methionine, phenylalanine, serine, tryptophan, tyrosine, valine and aminosugar were detected. Furthermore, in the case of the metacercariae thirteen amino acids corresponding to these spots were detectable, i. e. alanine, arginine, aspartic acid, glutamic acid, glycine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine and valine. In the solvents for two dimensional ascending chromatography methionine runs together with valine so that a spot for methionine plus valine is obtained. However, one-dimensional chromatography with buffered m-cresol and phenol gave evidence of valine and a trace of methionine. Kajihara and Hashimoto (1952) reported that eight free amino acids were detected in coelomic fluid of *Ascaris*. Cavier and Savel (1954) found thirteen free amino acids in the fluid and Ando (1957) reported six free amino acids in the adult of *Gnathostoma spini-*

gerum. Also, Robinson (1961) isolated thirteen free amino acids in the adult of *Schistosoma mansoni*. Furthermore, glucosamine and N-acetylglucosamine were detected in acid hydrolysates of the chitinous membrane of *Ascaris lumbricoides* eggs (Kreuzer, 1953).

*Estimation of Free Amino Acids and Aminosugar :*

In Table 1 are shown the amounts of fifteen free amino acids and one aminosugar in the adult lung fluke. Hydroxyproline, valine and methionine were present in lower concentrations and a quantitative estimate was impossible. The

Table 1. Amount of eighteen amino acids and one aminosugar in the adult *Paragonimus westermani*

	Milligrams per gram of fresh tissue**
Alanine	0.27
Arginine	0.55
Aspartic acid	0.20
Cystine	0.33
Glutamic acid	0.15
Glycine	0.10
Histidine	0.50
Leucine	0.43
Lysine	0.27
Phenylalanine	0.33
Proline	0.80
Serine	0.47
Threonine	0.20
Tryptophan	0.25
Tyrosine	0.37
Methionine+Valine	trace
Hydroxyproline	trace
Aminosugar*	0.23

\* Aminosugar was calculated as glucosamine.

\*\* The values presented are the averages of three determinations.

presence of taurine was not detectable. However, it is uncertain as yet whether this substance is present or not in the adult lung fluke. The largest quantity of any single amino acid was 0.80 mg of proline per gram of fresh tissue. This was followed by 0.55 mg of arginine. Alanine, aspartic acid and glutamic acid were

of lower concentration than proline and serine. These three amino acids are substances associated closely with the Krebs' cycle, and the presence of the cycle in the adult lung flukes was demonstrated by Tada et al. (1961) and Hamajima (1964, 1965). Consequently, their concentration could be regulated by the cycle. On the other hand, in coelomic fluid of *Ascaris*, Wakabayashi (1941) reported tryptophan and tyrosine in high amounts. On the contrary, Ueno (1960) found twelve amino acids in amounts ranging between 0.03-0.17 mg per millilitre.

Friedl (1961 a, b) observed survival time of rediae of *Fascioloides magna* in amino acids, and reported that proline and serine generally gave longer survival times than controls. From his information, it is noteworthy that in the adult lung flukes these two amino acids were present in higher concentration than other amino acids. This is felt to be in connection with the problem involving growth requirements of *Paragonimus*.

### Summary

The detection and estimation of the free amino acids and aminosugar in the uterine eggs, larvae and adults of the lung fluke, genus *Paragonimus*, were performed by means of paper chromatography. Nine spots corresponding to amino acids and aminosugar were detected in the uterine eggs; fifteen spots in the rediae and cercariae; thirteen spots in the metacercariae; and nineteen spots in the adults. In addition, in the adults, the quantities of fifteen free amino acids and one aminosugar were measured. These amounts ranged between 0.10-0.80 mg per gram of fresh tissue. The free amino acid in the highest concentration was proline, and this was followed by serine.

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## 肺吸虫の代謝に関する研究

I. 子宮卵, 幼虫, 成虫における遊離アミノ酸およびアミノ糖の  
ペーパークロマトグラフによる分析

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肺吸虫はその生活現象を異にする宿主を必要としており, その生化学的研究は比較生化学のうできわめて興味深く, また肺吸虫症の合理的な診断および治療をなすためにも重要な意義をもっている。寄生虫におけるアミノ酸およびアミノ糖の検出は多くの研究者によつて明らかにされているが, 肺吸虫のそれは少ない。そこで, ここでは肺吸虫の代謝に関する研究の一つとして虫体にお

ける遊離アミノ酸およびアミノ糖のペーパークロマトグラフによる分析について調べた。その結果, 遊離アミノ酸およびアミノ糖に相当するスポットは子宮卵で 9, イデア・セルカリアで 15, メタセルカリアで 13, 成虫で 19 が検出された。一方, 成虫における遊離アミノ酸およびアミノ糖の量は新鮮組織重グラム当り 0.10~0.80 mg であつた。