PAPER CHROMATOGRAPHIC STUDY ON THE AMINO ACIDS OF SOME PARASITIC HELMINTHS

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INTRODUCTION

Little is known about protein synthesis, intermediary metabolism or composition in helminths. Furthermore, nothing is known concerning the gross protein requirements or what amino acids are essential.

In recent years, some radioactive isotope tracer works on the protein metabolism of helminths have been reported (Stoner & Hanks, 1955 & 1958; Hanks & Stoner, 1956 & 1958; Daugherty, 1957; Daugherty & Foster, 1958; Prescott & Voge, 1959; Read et al, 1960). But the protein constituent of helminths is very imperfectly known, and no detailed analysis of the amino acid composition of these proteins has been made.

In previous works, reviewed by von Brand (1952), a total of 14 amino acids had been reported from nematode helminths and from tapeworms. Aldrich, Chandler and Daugherty (1954) subjected acid protein hydrolysates of *Hymenolepis diminuta* to chromatographic analysis, and they identified 14 amino acids. Sixteen amino acids had been detected in the hydrolysed cuticles of *Ascaris lumbricoides*, *Toxocara mystax* and *Strongylus equinus* (Bird, 1956 & 1957). Twenty different amino acids had been recovered chromatographically from acid protein hydrolysates of various tissues of adult and larval *H. diminuta*, and from the tissues and materials of the rat and *Tribolium confusum* hosts (Goodchild & Wells, 1957).

Fourteen amino acids were detected as the components of larval and adult *Gnathostoma spinigerum* (Ando, 1957). The amino acid constitution of 9 different trypanosomes and trypanosomid flagellate (Williamson, 1961), 18 amino acids from normal and decorticated embryonated eggs of *Ascaris suum* (Jaskoski, 1962), the free amino acid content of protoplasmic extracts of *Trichomonas foetus* (Johnson,

1962), and 7 free amino acids and 17 amino acids from *Clonorchis sinensis* (Ma, 1963) have been identified by means of chromatography.

This report presents a study of the constituent amino acids of some parasitic helminths, i.e., 2 kinds of nematodes, *Ascaris lumbricoides* var. *suum*, *Ascaridia galli*; 5 kinds of trematodes, *Clonorchis sinensis*, *Paragonimus westermani*, *Fasciola hepatica*, *Eurytrema pancreaticum*, *Paramphistomum cervi*; and 3 kinds of cestodes, *Taenia solium*, *Moniezia expansa*, *Hymenolepis nana*. Their amino acid constitutions of protein have been determined, by means of paper chromatography, after acid hydrolysis of worm tissues.

MATERIALS AND METHODS

Specimen collection: Specimens of *A. lumbricoides*, *F. hepatica*, *E. pancreaticum*, *P. cervi*, and *M. expansa* were obtained alive from an abattoir in Seoul and brought to the laboratory in normal saline solution at 38°C. The worms of *A. galli* were collected from the infected hen, and *H. nana* from infected house rat. The adult flukes of *C. sinensis* were obtained from rabbit liver according to the procedure of Hahn et al. (1961). Also the specimens of *P. westermani* were from lung of the dog which had been already infected with metacercariae in the laboratory. The whole specimen of *T. solium* was obtained from the human host with vermifuge, male fern extract.

The most active and intact living worms were selected and immediately washed several times in saline solution. The body of *A. lumbricoides* was divided into cuticle, muscular tissue, female genital organs and intestinal tract, in order to hydrolyse each separately. The bodies of *T. solium* and *M. expansa* were divided into 3 portions, i.e., anterior, middle and posterior one third. Other worms

were hydrolysed as a whole worm.

Hydrolysis: Protein hydrolysis of the worm tissue was carried out in 3 ml of 30% sulfuric acid per gram of parasite tissue at 105°C for 24 hours. Thereafter, the protein hydrolysate solution was neutralized with barium hydroxide. Solid material remaining in the hydrolysate was removed by filtration through a Büchner funnel in the presence of vacuum pump and the acidity of the clear filtrate was adjusted to pH 6.0-6.5. Finally, this filtrate in which the amino acids were suspended was condensed to 5 ml in volume. This solution constituted the sample to be analysed.

Chromatographic technique: The sample to be chromatographed was applied to a spot 0.3 cm in diameter, 4 cm from the bottom and right edge of 40 x 40 cm Whatman No. 1 filter paper by means of a micropipette, dried rapidly in a current of warm air and run ascendingly in two dimensions (Williams & Kirby, 1948; Satake, 1960; Lederer & Lederer, 1961). The following solvents were used in the order given at 25°C and run for 24 hours: butanol/acetic acid/water (4:1:4), and 12% water saturated phenol. Each solvent was allowed to flow for a distance of 30 cm. Eighty per cent aqueous ethanol was also used as a solvent in place of butanol/acetic acid/water, to verify the positions of certain amino acids.

The solvent was removed by drying in a current of warm air. After removal of the solvent, color development

was effected by spraying the chromatograms with 0.2% ninhydrin in water saturated n-butanol followed by drying at 105°C for 5 minutes. To preserve the color spots of chromatograms, 0.8% cupric acetate alcohol solution was additionally sprayed.

This chromatogram was compared with a map of known amino acids run under exactly similar condition. Standard solutions of known amino acids were prepared with *Collection amino acid Merck* by the method according to Levy and Chung (1953). The identity of the several equivocal amino acids was further verified by the following procedure: proline was easily detected by its yellow color reaction to ninhydrin, tyrosine by cutting out the area on the paper where the suspected spot occurred, eluting this in distilled water, concentrating it and applying it as a spot to filter paper which was then run unidimensional chromatography in butanol/acetic acid/water. Glycine, which occurred in close proximity to serine in the known map, was shown to be present by cutting out the spot area, eluting and running ascendingly in 80% aqueous ethanol with known glycine and serine. Valine was similarly shown to be present as distinct from methionine.

RESULTS

In the present investigation only 14 amino acids, the fundamental constituents of proteins, were qualitatively

Table 1. *Amino acids isolated from various worms.*

Worms	<i>A. lumbricoïdes</i>				<i>A. galli</i>	<i>C. sinensis</i>	<i>F. hepatica</i>	<i>E. pancreaticum</i>	<i>P. cervi</i>	<i>P. westermani</i>	<i>T. solium</i>			<i>M. expansa</i>			<i>H. nana</i>
	Cut.	Mus.	F.G.	Int.							Ant.	Mid.	Post.	Ant.	Mid.	Post.	
Glycine	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+	+	?
Alanine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serine	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+	+	?
Threonine	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+	+	?
Methionine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?
Valine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?
Leucine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?
Aspartic acid	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+	+	+
Glutamic acid	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+	+	?
Lysine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tyrosine	+	+	+	?	+	+	+	+	+	+	+	+	+	+	+	+	?
Proline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histidine	+	+	?	?	+	?	+	+	+	+	+	+	+	+	+	+	+

+: identified, ?: doubtful or absent.

Cut: cuticle, Mus: Muscle, F.G.: Female genital tract, Int.: Intestine,

Ant: Anterior one third, Mid: Middle one third, Post: Posterior one third.

studied. Identification of these amino acids was based upon the consistency with which the spots appeared at their respective locations and close agreement of their *R_f* value with those of known amino acids, as well as their color reaction to ninhydrin.

The results obtained were as follows (Table 1):

Fourteen colored spots were identified from the acid hydrolysed tissue proteins of *A. lumbricoides* (cuticle and musculature), *A. galli*, *F. hepatica*, *E. pancreaticum*, *P. cervi*, *T. solium* and *M. expansa*. These spots corresponded to the following amino acids on the known map: glycine, alanine, valine, methionine, serine, glutamic acid, threonine, leucine, lysine, tyrosine, aspartic acid, arginine, histidine and proline (Fig. 1), though slight difference in relative size and color intensity of spots was observed.

Fig. 1. Position of 14 amino acids identified from the acid hydrolysed tissue protein of *F. hepatica* after two dimensional chromatography.

Asp: Aspartic acid, Arg: Arginine, Ala: Alanine, Glu: Glutamic acid, Gly: Glycine, His: Histidine, Leu: Leucine, Lys: Lysine, Met: Methionine, Pro: Proline, Ser: Serine, Thr: Threonine, Tyr: Tyrosine, Val: Valine.

The presence of histidine was not obvious in female genital tract and intestinal tract hydrolysate of *A. lumbricoides*. Indistinct also was the tyrosine spot in intestinal tract hydrolysate of *A. lumbricoides*.

In the tissue hydrolysate of *C. sinensis*, 13 amino acids were detected. Following hydrolysis of lung fluke, *P. westermani*, alanine, methionine, valine, leucine, lysine,

arginine, tyrosine, proline, and histidine appeared. The presence of 6 amino acids was confirmed in the hydrolysate of *H. nana*; they were alanine, aspartic acid, lysine, arginine, proline and histidine.

Tryptophan, which may be present in tissue hydrolysates of various worms, was absent from the developed chromatograms owing to its destruction by acid hydrolysis.

DISCUSSION

The protein constituents of helminths are very imperfectly known. T. von Brand (1952) reported that 48 or 54% of dry substance was the protein fraction in *A. lumbricoides*. Smyth (1962) declared that the total protein in *Ascaris* made up 8~9% of the wet weight and the amino acid composition generally resembled that of its host. In the hydrolysed cuticles of *A. lumbricoides*, *T. mystax* and *S. equinus*, 16 amino acids had been detected in each and they were similar for all three worms except for slight quantitative difference (Bird, 1956 & 1957). Present work shows similarities in amino acid composition to them. But the nature of the cuticle is still obscure, and complete agreement has not been reached on amino acid composition. Amino acid composition of musculature was not different from that of cuticle in this experiment. Simple and two dimensional paper chromatograms were prepared of normal and decorticated embryonated eggs of *A. suum*, vitelline membrane, egg fluid and larvae (Jaskoski, 1962). A total of 18 amino acids were identified in larvae and embryonated eggs, i.e., 9 amino acids from vitelline membrane, 7 from a fraction in the middle coat, 16 were from the outer protein coat. In this experiment, the hydrolysate of female genital organs revealed 13 amino acid spots, and intestinal tract 12 spots. According to the review of von Brand (1952), *A. lumbricoides* had 14 amino acids. Yoshizawa (1954) detected 9 amino acids in the medium in which *A. lumbricoides* were kept alive.

Rogers (1955) noted that a similar pattern of amino acid and peptide excretion was found in *A. lumbricoides* and *A. galli*. In this experiment, a total of 14 amino acids were isolated in the tissue hydrolysate of *A. galli*. The free amino acids in Rogers (1955) experiment were almost identical those excreted by *Trichinella spiralis*. Under the aerobically incubated conditions, 28.5% of nitrogenous products of *T. spiralis* larvae was amino acid nitrogen, and 10 amino acids were identified from the in-

cubates of *T. spiralis* larvae (Haskins & Weinstein, 1957 a,b). Ando (1957) claimed that 14 amino acids were detected as the components of larval and adult *G. spinigerum* and were identical with those of the host.

The protein constituent of trematodes is very imperfectly known. In *F. hepatica* 58% of dry weight is protein fraction, but no detailed analyses of amino acid composition of these proteins have been made. Previously, 14 amino acids were identified from the tissue hydrolysates of *F. hepatica* and *E. pancreaticum* (Lee, 1963). In present experiment, 14 amino acids were detected from the tissue hydrolysates of *F. hepatica*, *E. pancreaticum*, *P. cervi*. Unknown amino acids on the chromatograms were similar for all three worms, although slight quantitative differences were observed. Watson (1960) stated that biochemical investigation of antigen from *F. hepatica* had revealed the presence of 15 different amino acids. While, it has been suggested that *F. hepatica* feeds exclusively on blood (Baer, 1952; Jennings et al., 1956). Rohrbacher (1957) was able to keep this fluke alive in basic saline solution containing liver extract. The amino acid constitution of *F. hepatica*, *E. pancreaticum* and *P. cervi* protein conforms to the general pattern of the majority of known proteins and is closely similar to bovine serum and liver. These may indicate a parallel amino acid requirement of the host and parasite. Only 9 amino acids were found in the tissue hydrolysate of *P. westermani*. In previous work, 13 amino acids were detected from the cyst content and body constituents of *P. ohirai*.

Except histidine, all the amino acids detected in present work were also recovered in the hydrolysate of *C. sinensis*. Ma (1963) detected 7 free amino acids and 17 amino acids from tissue hydrolysates of *C. sinensis* by means of chromatography, but he couldn't confirm the presence of histidine. Baer (1952) declared, survival of *C. sinensis* was due to the fact that all of its essential food requirements were furnished by the serum. In fact, constituent amino acids of *C. sinensis* include all the essential amino acids of human host, and furthermore, the similarity of amino acid constitution is observed between this fluke and human plasma. The similar result was also noted in the human serum and hemoglobin (Schroeder et al., 1950; and Yegna, 1954). But there is no assurance that indispensable amino acids of vertebrate hosts are indispensable one for parasites. Essential amino acids have not yet been determined for

any helminths parasite.

Cestodes are unique in the animal kingdom in that their protein content is less than the sum of glycogen and fat. *T. solium* had 46% of dry substance as protein content, while *M. expansa* had 30~36% of dry substance as protein (von Brand, 1952). The proteins of *M. expansa* and *H. diminuta* have been subjected to amino acid analysis, but this has revealed nothing unusual. The free and bound amino acids in *Railletina cesticillus* and *H. diminuta* were studied by Foster and Daugherty (1959). Aldrich et al. (1954) separated 22 discrete spots from acid protein hydrolysates of *H. diminuta*, and identified 14 amino acids. Kent (1957) isolated 4 different protein complexes from frozen worms of *H. diminuta* which were freed of lipids and amino acid composition of the complexes was established by paper chromatography and electrochromatography. Goodchild and Wells (1957) recovered 20 different amino acids chromatographically from various tissues of adult and larval *H. diminuta*. They noted an interesting parallel between relative abundance and scarcity of certain amino acids in parasite and the closely associated host tissue. This may indicate that these parasites are capable of extracting needed protein precursors from the mucosa of the intestine while in intimate contact with it. In present experiment, a total of 14 amino acids were recovered in the protein hydrolysates of *T. solium* and *M. expansa*, and no significant difference was noted among anterior, middle and posterior portion of the worm bodies. Nineteen amino acids were identified from the protein fraction of *M. expansa*, and possible role of these amino acids in the metabolism of tapeworm was discussed by Campbell (1960). Only 6 amino acids were isolated from *H. nana*.

The authors believe that the value of present experiment lies mainly in confirming the amino acid constitutions of certain helminths. In their continuing investigation, the effort would be directed to identifying entire amino acid constituents of the wider varieties of helminth other than the presently known 14 amino acids.

SUMMARY

- 1) Unidimensional and two dimensional paper chromatograms were prepared of 10 kinds of parasitic helminths.
- 2) Fourteen amino acids were identified from the acid

hydrolysed tissue proteins of *A. lumbricoides* (cuticle and musculature), *A. galli*, *F. hepatica*, *E. pancreaticum*, *P. cervi*, *T. solium*, and *M. expansa*. They were glycine, alanine, serine, threonine, methionine, valine, leucine, aspartic acid, lysine, arginine, tyrosine, proline and histidine. In hydrolysates of *A. lumbricoides* (female genital organ) and *C. sinensis*, 13 amino acids were recovered. Twelve amino acid from *A. lumbricoides* (intestinal tract), 9 from *P. westermani*, and 6 from *H. nana* were also identified in the tissue hydrolysates.

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—抄 錄—

蠕蟲類 蟲體構成아미노酸에 관한 研究

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寄生蟲 生理代謝研究에 基礎가 되는 蟲體의 生化學的인 組成에 關係서는 알려진바가 많지 않다. 特히 蠕蟲類 蟲體蛋白質을 構成하고 있는 아미노酸의 組成에 關한 研究는 極히 稀少하다.

著者等은 *A. lumbricoides*, *A. galli*, *C. sinensis*, *F. hepatica*, *E. pancreaticum*, *P. cervi*, *P. westermani*, *T. solium*, *M. expansa*, 및 *H. nana* 등 10 種의 寄生蠕蟲類 蟲體蛋白質을 構成하고 있는 아미노酸의 組成을 比較 觀察하였다.

A. lumbricoides, *F. hepatica*, *E. pancreaticum*, *P. cervi*, *M. expansa*의 成蟲體는 서울屠獸場에서 採取하였고 *C. sinensis* 및 *P. westermani*는 被囊幼蟲으로 感染시킨 實驗動物에서, *A. galli* 및 *H. nana*는 各各 自然感染된 닭 및 쥐에서 얻었으며 有鈎條蟲은 患者로부터 驅蟲하여 얻었다. 이들 蟲體 그람當 30% 黃酸 3ml를 加하고 105°C에서 24時間 加水分解한 다음 水酸化바리움을 適量 넣어 pH. 6.0~6.5 까지 中和하였다. 이것을 다시 濾過, 固形物質을 除去하여 아미노酸 抽出液을 얻었다. 이 抽出液을 5ml 까지 濃縮한 다음 0.02ml를 取하여 Whatman No.1 濾紙에 附着하고 25°C에서 24時間 二次元 paper chromatography를 施行하였다. 이때 展開溶媒로는 一次에 Butanol: 醋酸: 蒸溜水(4:1:4), 二次에 12% 水飽和 石灰酸을 使用하였다. 이 濾紙를 105°C에서 5分間 乾燥하여 溶媒를 除去한 後 0.2% Ninhydrin butanol溶液을 噴霧하여 濾紙에 分散되어 있는 아미노酸의 斑點을 呈色시켰다. 各 呈色斑點을 保存하기 爲하여 0.8% 醋酸銅 알콜溶液을 噴霧하여 乾燥시켰다.

같은 方法으로 同條件下에서 同時에 14 種의 既知 아미노酸의 二次元 paper chromatography를 施行하여 이를 對照群으로 採擇하고 蟲體에서 抽出한 未知 아미노酸과 比較 同定한 結果는 다음과 같다.

A. lumbricoides(角皮及 筋肉組織), *A. galli*, *F. hepatica*, *E. pancreaticum*, *P. cervi*, *T. solium*, *M. expansa*의 蟲體蛋白質을 加水分解하여 얻은 抽出物로부터 14 種의 아미노酸을 分離, 同定하였다. 卽 Glycine, alanine, serine, threonine, methionine, valine, leucine, aspartic acid, glutamic acid, lysine, arginine, tyrosine, proline, histidine 이 發見되었다. *A. lumbricoides*(雌性生殖器官), *C. sinensis*에서는 13 種, *A. lumbricoides*(腸管)에서는 12 種, *P. westermani*에서는 9 種, 그리고 6 種의 아미노酸이 *H. nana*에서 發見되었다.