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Source: Bulletin of the Wildlife Disease Association, 3(2) : 60-61

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-3.2.60

Free and Protein Amino Acid Composition of the Nematode (*Toxascaris leonina*) (Linstow, 1902) Occurring in the Red Fox (*Vulpes vulpes*)

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ABSTRACT

The free and protein amino acids of *Toxascaris leonina* occurring in the red fox (*Vulpes vulpes*) were tentatively identified by the method of descending two-dimensional paper chromatography. Lysine, histidine, arginine, glycine, serine, threonine, glutamic acid, alanine, proline, tyrosine, methionine, valine, phenylalanine, and leucine-isoleucine were found in the free amino acid fraction. The protein amino acid fraction revealed cystine, lysine, aspartic acid, histidine, arginine, glycine, serine, threonine, glutamic acid, alanine, proline, tyrosine, methionine, valine, phenylalanine and leucine-isoleucine. The presence of tryptophane was not determined as alkaline hydrolysates were not prepared.

INTRODUCTION

The necessity of acquiring basic data on the chemical composition of parasites is generally acknowledged. In some cases, biochemical similarities and differences have proved an effective tool in the investigation of problems in systematics. Several workers (too numerous to mention here) have shown the importance of determining the chemical composition of parasites as a precursor for the development of chemically defined diets for axenic cultivation[7].

The present work is concerned with the amino acid composition of the nematode, *Toxascaris leonina*.

MATERIALS AND METHODS

Adults of *T. leonina* were collected from the small intestine of naturally infected red foxes (*Vulpes vulpes*) trapped in Ward County, North Dakota. The parasites were cleaned of adhering debris in tap water, washed in five successive baths of sterile distilled water, followed by three baths of sterile distilled water, followed by three baths of sterile distilled water containing penicillin and streptomycin and rinsed in five changes of sterile distilled water. They were then blotted and weighed.

Three grams of adult worms were homogenized in 80 per cent ethanol, centrifuged and the supernatant removed. The residue was washed with 80 per cent ethanol and respun. This was repeated twice and the combined ethanolic solutions (containing free amino acids) were evaporated to near dryness over a steam bath. The solution was diluted with 10 ml of distilled water, decolorized with Aqua Nuchar A, filtered through Whatmann No. 1 paper and the filtrate concentrated by evaporation to 0.5 ml.

The proteinaceous residue (containing bound amino acids) was hydrolyzed for 5 hours at 120°C in 30 ml of peroxide-free 6N HCl and the amino acids recovered by the method described above.

A modification of the descending two-dimensional paper chromatography method of Herlich[2] was employed for analysis of free and bound amino acids. Twenty lambda of solution was spotted on Whatmann No. 3 MM paper. The chromatocab was equilibrated with butanol-saturated water for 2 hours prior to the addition of the first-dimensional solvent, butanol: acetic acid: water (4:1:5 v/v) (Partridge, 1948). The chromatogram was then run for 16 hours and air dried overnight. Phenol: water 4:1 v/v) was used as the second-dimensional solvent over a 14 hour period with 2 hour prior equilibration of phenol-saturated water.

Chromatograms were developed in modified ninhydrin solution[4] and compared with standard amino acid solutions tested in parallel.

Control chromatograms of hydrolyzed known amino acids were prepared under the same conditions.

RESULTS

Lysine, histidine, arginine, glycine, serine, threonine, glutamic acid, alanine, proline, tyrosine, methionine, valine, phenylalanine and leucine-isoleucine were found in the free amino acid fraction. The protein amino acid fraction revealed cystine, lysine, aspartic acid, histidine, arginine, glycine, serine, threonine, glutamic acid, alanine, proline, tyrosine, methionine, valine, phenylalanine and leucine-isoleucine.

Chromatograms of hydrolyzed standard solutions showed that tryptophane was destroyed during acid hydrolysis. Complete resolution of leucine-isoleucine was not possible although separation of these amino acids in a similar system was reported by Herlich[2].

DISCUSSION

These experiments have shown that aspartic acid and cystine in protein hydrolysates were not detected in ethanolic extracts. Such a difference could be an indication that these amino acids have been synthesized by the parasites. Since a study of the amino acids occurring in the tissues of the host was not undertaken further comparison was not possible.

The presence of tryptophane was not determined as alkaline hydrolysates of the proteinaceous residue were not prepared. It has been reported from larvae of *Ascaris suum* (Jaskoski, 1962), the excretions of *Nematodirus* spp. (Rogers, 1955) and suspected in larvae of *Nippostrongylus brasiliensis* (Friedman and Kagan, 1958).

Quantitative estimations were not possible, but a rough comparison of the colour densities produced by standard chromatograms indicated that alanine was present in much greater quantity than other free amino acids.

Although the chromatograms obtained agreed as to both location and Rf values of standard chromatograms, true confirmation would depend on identification by their chemical and physical properties.

ACKNOWLEDGEMENT

This study was supported by NSF Grant CY-30.

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