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Nitrogen Metabolism in *Ostertagia*
(*Teladorsagia*) *circumcincta*

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NOORZAID MUHAMAD
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Table of Contents

Table of Contents	i
Abstract	xii
Acknowledgements	xiv
List of Figures	xvi
List of Tables	xxiii
List of Abbreviations	xxviii
Introduction	xxxiv
CHAPTER 1 LITERATURE REVIEW	1
1.1 Sources of Nitrogen	2
1.2 Uptake of Nitrogenous Compounds	3
1.2.1 Uptake of nitrogenous compounds in helminths	5
1.3 Excretion of Nitrogenous Compounds	6
1.3.1 Ammonia excretion	6
1.3.2 Uric acid excretion	7
1.3.3 Urea excretion	8
1.3.4 Excretion of nitrogenous compounds in nematodes	8
1.4 Urea Metabolism	9
1.4.1 The Ornithine-Urea Cycle	9
1.4.2 Creatinase	10

1.4.3 Urease	11
1.5 Arginine Metabolism	12
1.5.1 Synthesis of ornithine by arginase	13
1.5.2 Synthesis of ornithine by arginine deiminase.....	14
1.5.3 Synthesis of nitric oxide	14
1.5.4 Synthesis of agmatine	15
1.5.5 Synthesis of polyamines	15
1.5.6 Synthesis of creatine	17
1.5.7 Synthesis of glutamate and proline.....	18
1.6 Alanine and Aspartate Metabolism: Transamination	20
1.6.1 Alanine racemase	21
1.6.2 L-Alanine dehydrogenase	21
1.6.3 Opine dehydrogenases.....	22
1.6.4 Pyruvate synthesis.....	22
1.6.5 Alanine synthesis by aspartate-4-carboxylase.....	23
1.6.6 β -Alanine synthesis	23
1.6.7 Asparagine synthesis and catabolism.....	24
1.6.8 Aspartate kinase	25
1.6.9 Purine salvage pathway.....	25
1.6.10 Aspartase	26
1.6.11 Transamination.....	26
1.6.11.1 Alanine aminotransferase	27
1.6.11.2 Aspartate aminotransferase	28

1.6.11.3 Other aminotransferases.....	29
1.7 Glutamate Metabolism	30
1.7.1 Glutamate dehydrogenase	32
1.7.2 Glutaminase	34
1.7.3 Glutamine synthetase	36
1.7.4 Glutamate synthase	36
1.7.5 Synthesis of N-acetylglutamate	37
1.7.6 Glutamate decarboxylase	38
1.8 Other Nematode Enzymes	38
1.8.1 Chitin metabolism	38
1.8.2 Amino acid catabolism.....	39
1.8.3 Shikimate pathway	40
1.8.4 Sulphur amino acid metabolism	40
1.8.5 Neurotransmitters	41
1.8.6 Transglutaminase	42
1.8.7 Glutathione S-transferase	42
1.9 Conclusions	43

Chapter 2 Uptake and Excretion of Nitrogenous Compounds by *Ostertagia circumcincta*

2.1 Introduction.....	44
2.1.1 Uptake of nitrogenous compounds	44
2.1.2 Excretion of nitrogenous compounds	45

2.2 Materials and Methods	46
2.2.1 Worm culture	46
2.2.2 Excretion of nitrogenous compounds	47
2.2.2.1 Ammonia excretion	47
2.2.2.2 Urea excretion	48
2.2.2.3 Uric acid excretion	48
2.2.2.4 Amino acid excretion	48
2.2.2.5 Protein excretion	48
2.2.3 Uptake of amino acids	49
2.2.3.1 Incubation media	49
2.2.3.2 Uptake in adult worms	49
2.2.3.3 Uptake in sheathed L3	50
2.2.3.4 Calculation of amino acid uptake	51
2.2.3.5 Statistics	51
2.3 Results	51
2.3.1 Ammonia excretion	51
2.3.1.1 pH of medium	51
2.3.1.2 Incubation temperature	51
2.3.1.3 Parasite density	52
2.3.1.4 Time of incubation	52
2.3.1.5 External ammonia concentration	52
2.3.1.6 Exsheathed L3	52
2.3.1.7 Adult worms	53

2.3.2 Urea excretion	53
2.3.3 Uric acid excretion	53
2.3.4 Amino acid excretion	53
2.3.5 Protein excretion.....	53
2.3.6 Amino acid uptake	53
2.4 Discussion.....	54
2.4.1 Amino acid uptake	55
2.4.2 Nitrogen excretion.....	56
2.4.3 Ammonia and urea production.....	59

Chapter 3 Metabolism of Arginine and Urea in *Ostertagia circumcincta*

3.1 Introduction.....	60
3.1.1 Arginase (EC 3.5.3.1)	61
3.1.2 Creatinase (EC 3.5.3.3)	62
3.1.3 Urease (EC 3.5.1.5).....	62
3.1.4 Δ^1 -pyrroline-5-carboxylic acid dehydrogenase (EC 1.5.1.12)	63
3.2 Materials and Methods	64
3.2.1 Homogenate preparation	64
3.2.2 Arginase	64
3.2.2.1 <i>Kinetic parameters</i>	65
3.2.2.2 <i>Effectors/inhibitors</i>	65

3.2.3 Creatinase	65
3.2.3.1 <i>Kinetic parameters</i>	66
3.2.3.2 <i>Effectors/inhibitors</i>	66
3.2.4 Urease	67
3.2.5 Pyrroline-5-carboxylate dehydrogenase	67
3.3 Results	68
3.3.1 Arginase	68
3.3.1.1 <i>Effect of pH</i>	68
3.3.1.2 <i>Kinetic parameters</i>	68
3.3.1.3 <i>Effectors/inhibitors</i>	68
3.3.2 Creatinase	69
3.3.2.1 <i>Effect of pH</i>	69
3.3.2.2 <i>Kinetic parameters</i>	69
3.3.2.3 <i>Effectors/inhibitors</i>	69
3.3.3 Urease	69
3.3.4 Pyrroline-5-carboxylate dehydrogenase	70
3.4 Discussion	70
3.4.1 Arginase	70
3.4.2 Creatinase	73
3.4.3 Urease	75
3.4.4 Pyrroline-5-carboxylate dehydrogenase	76
3.4.5 Urea and arginine metabolism.....	77

Chapter 4 Metabolism of Alanine and Aspartate in *Ostertagia circumcincta*

4.1 Introduction	79
4.1.1 Alanine aminotransferase (AlaAT) (EC 2.6.1.2).....	79
4.1.2 Aspartate aminotransferase (AspAT) (EC 2.6.1.1)	80
4.1.3 Aspartase (EC 4.3.1.1)	81
4.2 Materials and Methods	81
4.2.1 Homogenate preparation	81
4.2.2 Alanine aminotransferase	82
4.2.2.1 <i>Effect of pH and PLP concentration</i>	82
4.2.2.2 <i>Kinetic parameters in the direction of alanine utilisation</i>	83
4.2.2.3 <i>Kinetic parameters in the direction of alanine formation</i>	83
4.2.3 Aspartate aminotransferase	84
4.2.3.1 <i>Effect of pH, PLP concentration, ADP and ATP</i>	84
4.2.3.2 <i>Kinetic parameters in the direction of aspartate utilisation</i> ...	85
4.2.3.3 <i>Kinetic parameters in the direction of aspartate formation</i> ...	86
4.2.4 Aspartase	86
4.2.4.1 <i>Kinetic parameters in the direction of fumarate utilisation</i>	86
4.2.4.2 <i>Kinetic parameters in the direction of fumarate formation</i>	87
4.2.4.3 <i>Effect of pH</i>	88
4.2.4.4 <i>Effectors/inhibitors</i>	88
4.3 Results	88
4.3.1 Alanine aminotransferase	88

4.3.1.1	<i>Effect of pH and PLP concentration</i>	88
4.3.1.2	<i>Kinetic parameters in the direction of alanine utilisation</i>	89
4.3.1.3	<i>Kinetic parameters in the direction of alanine formation</i>	89
4.3.2	Aspartate aminotransferase.....	90
4.3.2.1	<i>Effect of pH, PLP concentration, ATP and ADP</i>	90
4.3.2.2	<i>Kinetic parameters in the direction of aspartate utilisation</i>	90
4.3.2.3	<i>Kinetic parameters in the direction of aspartate formation</i>	90
4.3.3	Aspartase	91
4.3.3.1	<i>Kinetic parameters in the direction of fumarate utilisation</i>	91
4.3.3.2	<i>Kinetic parameters in the direction of fumarate formation</i>	91
4.3.3.3	<i>Effect of pH</i>	91
4.3.3.3	<i>Effectors/inhibitors</i>	92
4.4	Discussion	92
4.4.1	Alanine aminotransferase (AlaAT)	92
4.4.2	Aspartate aminotransferase (AspAT).....	94
4.4.3	Aspartase	96
4.4.4	Alanine and aspartate metabolism	98

Chapter 5 Metabolism of Glutamate in *Ostertagia circumcincta*

5.1	Introduction	101
5.1.1	Glutamate dehydrogenase (GDH) (EC 1.4.1.2-4).....	101
5.1.2	Glutaminase (EC 3.5.1.2)	103
5.1.3	Glutamine synthetase (GS) (EC 6.3.1.2)	104

5.1.4	Glutamate synthase (GOGAT or GltS) (EC 1.4.1.14)	105
5.2	Materials and Methods	106
5.2.1	Homogenate preparation	106
5.2.2	Glutamate dehydrogenase	106
5.2.2.1	<i>Effect of pH</i>	106
5.2.2.2	<i>Effect of temperature</i>	107
5.2.2.3	<i>Effect of ATP and ADP</i>	107
5.2.2.4	<i>Kinetic parameters in the direction of glutamate utilisation</i>	108
5.2.2.5	<i>Kinetic parameters in the direction of glutamate formation</i>	108
5.2.3	Glutaminase	109
5.2.3.1	<i>Kinetic parameters</i>	109
5.2.3.2	<i>Effectors and inhibitors</i>	109
5.2.4	Glutamine synthetase	110
5.2.4.1	<i>Kinetic parameters</i>	110
5.2.5	Glutamate synthase	111
5.2.5.1	<i>Kinetic parameters</i>	111
5.2.5.2	<i>Comparison of GOGAT activities in sheep muscle and adult worms</i>	112
5.2.5.3	<i>Effect of azaserine</i>	112
5.2.5.4	<i>Comparison of GOGAT and GDH activities</i>	113
5.3	Results	113
5.3.1	Glutamate dehydrogenase	113
5.3.1.1	<i>Effect of pH</i>	113

5.3.1.2	<i>Effect of temperature</i>	113
5.3.1.3	<i>Effect of ATP and ADP</i>	114
5.3.1.4	<i>Kinetic parameters in the direction of glutamate utilisation</i> .	114
5.3.1.5	<i>Kinetic parameters in the direction of glutamate formation</i> .	114
5.3.2	Glutaminase	115
5.3.2.1	<i>Kinetic parameters</i>	115
5.3.2.2	<i>Effectors and inhibitors</i>	115
5.3.3	Glutamine synthetase	116
5.3.3.1	<i>Kinetic parameters</i>	116
5.3.4	Glutamate synthase.....	116
5.3.4.1	<i>Kinetic parameters</i>	116
5.3.4.2	<i>Comparison of GOGAT activities in sheep muscle and adult worms</i>	116
5.3.4.3	<i>Effect of azaserine</i>	117
5.3.4.4	<i>Comparison of GOGAT and GDH activities</i>	117
5.4	Discussion	117
5.4.1	Glutamate dehydrogenase	117
5.4.2	Glutaminase	121
5.4.3	Glutamine synthetase	122
5.4.4	Glutamate synthase (GOGAT)	123
5.4.5	Glutamate metabolism.....	125
Chapter 6	General Discussion	128
References	135

Appendix 1: Parasitology	183
1.1 Larval culture.....	183
1.2 Faecal egg counts.....	183
1.3 Exsheathing L3	183
1.4 Recovery of adult worms.....	184
1.5 Baermannisation and counting of larvae	184
Appendix 2: Assays	185
2.1 Ammonia assay.....	185
2.2 Urea assay	185
2.3 Protein microassay.....	186
2.4 Total amino acid assay	186
2.5 Uric acid assay.....	186
2.6 Preparation of homogenates	187
2.7 Continuous enzyme assays	187
2.8 Determination of extinction coefficient.....	189
2.9 Calculation of enzyme activity	190
2.10 Glutamine synthetase activity.....	190
Appendix 3: Solutions	192
3.1 Phosphate buffer.....	192
3.2 Tris buffer.....	192
3.3 Phosphate buffered saline.....	192

Abstract

The aim of the experiments was to investigate some key areas of nitrogen metabolism in adult and third-stage larval *Ostertagia (Teladorsagia) circumcincta*, to seek enzymes either not present in mammals or with distinctive kinetic properties, which clearly differentiated the nematode and host metabolic systems. The study encompassed excretion and uptake in intact worms and determining the kinetic properties of eleven enzymes involved in the metabolism of arginine, urea, alanine aspartate and glutamate.

The metabolism of *O. circumcincta* was different from that in mammals and more like that of microorganisms and plants. Ammonia was the main excretory product, with a little urea, both apparently crossing the cuticle through specific permeases. The excretion rate increased with temperature, but decreased as the external ammonia concentration increased, suggesting that ammonia may be a source of nitrogen additional to amino acids, which were taken up by adult worms. Ammonia could be incorporated directly into glutamate and other amino acids through the glutamine synthetase-glutamate synthase pathway, which was more active in adult worms. Glutamate dehydrogenase was able to use either NADH or NADP in the deaminating direction, which would be the predominant direction because of the low affinity of GDH for ammonia. In the aminating direction, there was greater activity with NADH than NADPH.

Creatinase and arginase were probably the sources of excreted urea. There was no urease activity to convert urea to ammonia. No role could be assigned to creatinase other than to degrade host creatine, perhaps to supply sarcosine for metabolism. The unusual feature of aspartate metabolism was aspartase activity in addition to aspartate aminotransferase, which, in larvae, had the highest activity of all enzymes studied. In adult worms, which are

believed to have a more anaerobic metabolism than larvae, aspartase would allow aspartate to be formed directly from fumarate in association with only a partial TCA cycle.

Perhaps the most important finding was the identification in the parasites of three enzymes, creatinase, aspartase and glutamate synthase, which are not believed to be expressed in the sheep host or other mammals, making them possible candidates for developing novel anthelmintic therapies.

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**"The man who removes a mountain begins by
carrying away small stones"**

List of Figures

	Facing page
Figure 1.1. The γ -glutamyl cycle which acts as an amino acid transporter.	4
Figure 1.2. Degradation of purines to uric acid and other excretory products.	7
Figure 1.3. Ornithine-Urea cycle.	9
Figure 1.4. Pathways by which creatinine and creatine may be degraded in microorganisms.	11
Figure 1.5. Arginine metabolism in mammals.	12
Figure 1.6. Invertebrate phosphagen precursors which form the corresponding phosphagen by covalent attachment of a phosphate group to the guanidino moiety at the left of the molecule.	14
Figure 1.7. Enzymes involved in the interconversion of arginine, glutamate and proline.	18
Figure 1.8. The major reactions and pathways for which alanine is a substrate.	20
Figure 1.9. The major reactions and pathways for which aspartate is a substrate.	21
Figure 1.10. Diagram of the malate-aspartate shuttle for the transport of reducing equivalents between the cytosol and mitochondria in the electron transport system of the mammalian cell.	28
Figure 1.11. The major reactions and pathways for which glutamate is a substrate.	30
Figure 1.12. The reactions and enzymes catalysing the interconversions of glutamine, glutamate and 2-oxoglutarate.	31
Figure 1.13. Generalised scheme of methionine metabolism in mammals and parasites.	40
Figure 2.1. Diagrammatic representation of the procedure for separating <i>O. circumcincta</i> adult worms from residual medium by centrifugation through a dibutyl pthalate solution.	50

- Figure 2.2.** Ammonia concentrations of the incubation media (mean \pm SEM, n = 3) during the incubation of 50,000 sheathed L3 in 1 ml 0.8 mM phosphate buffer of pH 6.0, 6.5, 7.0 and 7.5 at 37°C for 4 hours. 51
- Figure 2.3.** Ammonia concentrations of the incubation media (mean \pm SEM, n = 3) during the incubation of 50,000 sheathed L3 in 1 ml 0.8 mM phosphate buffer, pH 7.0 at 4°C or 20°C or 37°C for 4 hours. 51
- Figure 2.4.** Ammonia concentrations of the incubation media (mean \pm SEM, n = 3) after the incubation for 2.5 hours of 5,000, 10,000, 50,000, 70,000 and 100,000 sheathed L3 in 1 ml 0.8 mM phosphate buffer, pH 7.0 at 37°C. 52
- Figure 2.5.** Ammonia concentrations of the incubation media (mean \pm SEM, n = 3) during the incubation of 50,000 sheathed L3 in 1 ml 0.8mM phosphate buffer, pH 7.0 at 37°C. 52
- Figure 2.6.** Ammonia concentrations of the incubation media (mean \pm SEM, n = 3) during the incubation of 50,000 sheathed L3 in 1 ml 0.8 mM phosphate buffer, pH 7.0 at 37°C for 5 hours, with and without the addition of 60 μ M NH₄Cl. 52
- Figure 2.7.** Ammonia concentrations of the incubation media (mean \pm SEM, n = 3) during the incubation of 50,000 sheathed or exsheathed L3 in 1 ml 0.8 mM phosphate buffer, pH 7.0 at 37°C for 5 hours. 52
- Figure 2.8.** Ammonia concentrations of the incubation media (mean \pm SEM, n = 3) during the incubation of adult worms (~ 6 mg wet weight) in 1 ml 0.8 mM phosphate buffer, pH 7.0 at 37°C for 9 hours. 53
- Figure 2.9.** Urea concentrations in the incubation media (mean \pm SEM, n = 3) during the incubation of 50,000 sheathed or exsheathed L3 in 1ml 0.8 mM phosphate buffer, pH 7.0 at 37°C for 4 hours. 53
- Figure 2.10.** Protein concentrations in the incubation media (mean \pm SEM, n = 3) during the incubation of 50,000 sheathed L3 or adult worms (~6 mg wet weight) in 1ml 0.8 mM phosphate buffer, pH 7.0 at 37°C for 4 hours. 53
- Figure 3.1.** The reaction catalysed by arginase. 61
- Figure 3.2.** The reaction catalysed by creatinase. 62
- Figure 3.3.** Reaction catalysed by Δ^1 -pyrroline-5-carboxylate dehydrogenase (P5CDH). 63
- Figure 3.4.** Effect of pH on arginase activity (mean \pm SEM, n = 2) of sheathed L3 *O. circumcincta* homogenate SL1. 68

Figure 3.5. Arginase activity of sheathed L3 <i>O. circumcincta</i> homogenate SL2a with increasing concentration of arginine.	68
Figure 3.6. Arginase activity of adult <i>O. circumcincta</i> homogenate A1a with increasing concentration of arginine.	68
Figure 3.7. Effect of pH on creatinase activities (mean \pm SEM, n = 2) of sheathed L3 <i>O. circumcincta</i> homogenate SL5.	69
Figure 3.8. Creatinase activity of sheathed L3 <i>O. circumcincta</i> homogenate SL6 with increasing concentration of creatine.	69
Figure 3.9. Creatinase activity of adult <i>O. circumcincta</i> homogenate A2a with increasing concentration of creatine.	69
Figure 3.10. Pyrroline-5-carboxylate dehydrogenase (P5CDH) activity of sheathed L3 <i>O. circumcincta</i> homogenate SL13b with increasing concentration of 1-pyrroline-5-carboxylate.	70
Figure 3.11. Metabolic map of enzymes of urea and arginine metabolism identified in L3 or adult <i>O. circumcincta</i> homogenates.	77
Figure 4.1. Reaction catalysed by alanine aminotransferase.	79
Figure 4.2. Reaction catalysed by aspartate aminotransferase.	80
Figure 4.3. Reaction catalysed by aspartase.	81
Figure 4.4. Effect of pH on AlaAT activities of sheathed L3 <i>O. circumcincta</i> homogenates in the direction of alanine utilisation (SL15) (\blacktriangle) and formation (SL16) (\blacksquare).	89
Figure 4.5. Effect of PLP concentration on AlaAT activity (mean \pm SEM, n = 2) of sheathed L3 <i>O. circumcincta</i> homogenate SL17 in the direction of alanine utilisation.	89
Figure 4.6. AlaAT activity of sheathed L3 <i>O. circumcincta</i> homogenate SL20 monitored in the direction of alanine utilisation with increasing concentration of 2-oxoglutarate.	89
Figure 4.7. AlaAT activity of sheathed L3 <i>O. circumcincta</i> homogenate SL21b monitored in the direction of alanine utilisation with increasing concentration of alanine.	89
Figure 4.8. AlaAT activity of adult <i>O. circumcincta</i> homogenate A5a monitored in the direction of alanine utilisation with increasing concentration of alanine.	89
Figure 4.9. AlaAT activity of sheathed L3 <i>O. circumcincta</i> homogenate SL23 monitored in the direction of alanine formation with increasing concentration of glutamate.	89

- Figure 4.10.** AlaAT activity of sheathed L3 *O. circumcincta* homogenate SL28 monitored in the direction of alanine formation with increasing concentration of pyruvate. 89
- Figure 4.11.** AlaAT activity of adult *O. circumcincta* homogenate A6 monitored in the direction of alanine formation with increasing concentration of pyruvate. 89
- Figure 4.12.** Effect of pH on AspAT activities (mean \pm SEM, n = 2) of sheathed L3 *O. circumcincta* homogenate SL29 in the direction of aspartate utilisation. 90
- Figure 4.13.** Effect of PLP concentration on AspAT activities (mean \pm SEM, n = 2) of sheathed L3 *O. circumcincta* homogenate SL30 in the direction of aspartate utilisation. 90
- Figure 4.14.** AspAT activity of sheathed L3 *O. circumcincta* homogenate SL32 monitored in the direction of aspartate utilisation with increasing concentration of 2-oxoglutarate. 90
- Figure 4.15.** AspAT activity of adult *O. circumcincta* homogenate A7a monitored in the direction of aspartate utilisation with increasing concentration of 2-oxoglutarate. 90
- Figure 4.16.** AspAT activity of sheathed L3 *O. circumcincta* homogenate SL35 monitored in the direction of aspartate utilisation with increasing concentration of aspartate. 90
- Figure 4.17.** AspAT activity of adult *O. circumcincta* homogenate A8 monitored in the direction of aspartate utilisation with increasing concentration of aspartate. 90
- Figure 4.18.** AspAT activity of sheathed L3 *O. circumcincta* homogenate SL36 monitored in the direction of aspartate formation with increasing concentration of glutamate. 90
- Figure 4.19.** AspAT activity of sheathed L3 *O. circumcincta* homogenate SL37c monitored in the direction of aspartate formation with increasing concentration of oxaloacetate. 91
- Figure 4.20.** Aspartase activity of sheathed L3 *O. circumcincta* homogenate SL39 monitored in the direction of fumarate utilisation with increasing concentration of ammonia. 91
- Figure 4.21.** Aspartase activity of sheathed L3 *O. circumcincta* homogenate SL41 monitored in the direction of fumarate utilisation with increasing concentration of fumarate. 91
- Figure 4.22.** Aspartase activity of sheathed adult *O. circumcincta* homogenate A10 monitored in the direction of fumarate utilisation with increasing concentration of fumarate. 91

- Figure 4.23.** Aspartase activity of sheathed L3 *O. circumcincta* homogenate SL43 monitored in the direction of fumarate formation with increasing concentration of aspartate. 91
- Figure 4.24.** Effect of pH on aspartase activities of sheathed L3 *O. circumcincta* homogenate SL46 in the direction of fumarate formation. 91
- Figure 4.25.** Metabolic map of enzymes of alanine and aspartate metabolism identified in L3 or adult *O. circumcincta* homogenates. 98
- Figure 5.1.** The reaction catalysed by glutamate dehydrogenase (GDH) by which ammonia is reversibly incorporated into 2-oxoglutarate. 101
- Figure 5.2.** The reaction catalysed by glutaminase. 103
- Figure 5.3.** The reaction catalysed by glutamine synthetase. 104
- Figure 5.4.** The reaction catalysed by glutamate synthase (GOGAT or Glts). 105
- Figure 5.5.** Effects of pH on glutamate dehydrogenase (GDH) activities (mean \pm SEM, n = 2) at 30°C of sheathed L3 *O. circumcincta* homogenates in the direction of glutamate formation (SL48-49) (\blacktriangle) and glutamate utilisation (SL50-51) (\blacksquare). 113
- Figure 5.6.** Effects of temperature on glutamate dehydrogenase (GDH) activities (mean \pm SEM, n = 2) of sheathed L3 *O. circumcincta* homogenates SL52 (\blacktriangle) and SL53 (\blacksquare) in the direction of glutamate formation. 113
- Figure 5.7.** Glutamate dehydrogenase (GDH) activity at 30°C of sheathed L3 *O. circumcincta* homogenate SL56, monitored in the direction of glutamate utilisation, with increasing concentration of glutamate. 114
- Figure 5.8.** Glutamate dehydrogenase (GDH) activity at 30°C of adult *O. circumcincta* homogenate A9c, monitored in the direction of glutamate utilisation, with increasing concentration of glutamate. 114
- Figure 5.9.** Glutamate dehydrogenase (GDH) activities at 30°C of sheathed L3 *O. circumcincta* homogenate SL60, monitored in the direction of glutamate utilisation, with increasing concentration of NAD⁺ or NADP⁺. 114
- Figure 5.10.** Glutamate dehydrogenase (GDH) activity at 30°C of sheathed L3 *O. circumcincta* homogenate SL62a, monitored in the direction of glutamate formation, with increasing concentration of 2-oxoglutarate. 114

- Figure 5.11.** Glutamate dehydrogenase (GDH) activity at 30°C of adult *O. circumcincta* homogenate A1c, monitored in the direction of glutamate formation, with increasing concentration of 2-oxoglutarate. 114
- Figure 5.12.** Glutamate dehydrogenase (GDH) activity at 30°C of sheathed L3 *O. circumcincta* homogenate SL64, monitored in the direction of glutamate formation, with increasing concentration of ammonia. 115
- Figure 5.13.** Glutamate dehydrogenase (GDH) activity at 30°C of adult *O. circumcincta* homogenate A9d, monitored in the direction of glutamate formation, with increasing concentration of ammonia. 115
- Figure 5.14.** Glutamate dehydrogenase (GDH) activities at 30°C of sheathed L3 *O. circumcincta* homogenate SL68, monitored in the direction of glutamate formation, with increasing concentration of NADH or NADPH. 115
- Figure 5.15.** Glutaminase activity at 30°C of sheathed L3 *O. circumcincta* homogenate SL70 with increasing concentration of glutamine. 115
- Figure 5.16.** Glutaminase activity at 30°C of adult *O. circumcincta* homogenate A9e with increasing concentration of glutamine. 115
- Figure 5.17.** Glutamine synthetase (GS) activity of sheathed L3 *O. circumcincta* homogenate SL75a, monitored at 30°C in the direction of glutamate utilisation, with increasing concentration of ammonia. 116
- Figure 5.18.** Glutamine synthetase (GS) activity of sheathed L3 *O. circumcincta* homogenate SL77, monitored at 30°C in the direction of glutamate utilisation, with increasing concentration of glutamate. 116
- Figure 5.19.** Glutamate synthase (GOGAT) activity at 30°C of sheathed L3 *O. circumcincta* homogenate SL81 with increasing concentration of glutamine. 116
- Figure 5.20.** Glutamate synthase (GOGAT) activity at 30°C of adult *O. circumcincta* homogenate A12 with increasing concentration of glutamine. 116
- Figure 5.21.** Glutamate synthase (GOGAT) activity at 30°C of adult *O. circumcincta* homogenate A13 with increasing concentration of 2-oxoglutarate. 116

- Figure 5.22.** Assay of glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) activity at 30°C in a sheep muscle homogenate and adult *O. circumcincta* homogenate A14. 116
- Figure 5.23.** Assay of glutamate synthase (GOGAT) activity at 30°C of adult *O. circumcincta* homogenate A15 showing inhibition by 2 mM azaserine (added at D). 117
- Figure 5.24.** Experiment to distinguish activities of glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) at 30°C in adult *O. circumcincta* homogenate A16. 117
- Figure 5.25.** Metabolic map of enzymes of glutamate metabolism identified in L3 or adult *O. circumcincta* homogenates. 125
- Figure 6.1.** Metabolic map of enzymes of nitrogen metabolism identified in L3 or adult *O. circumcincta* homogenates. 128
- Figure A2.1.** Example of a continuous assay in which the rate of NADH utilisation was monitored spectrophotometrically at 340 nm. 188
- Figure A2.2.** The spectra of 1-7 mM phenylalanine in phosphate medium at 30°C. 189

List of Tables

	Facing page
Table 1.1. Classification of amino acid transport systems in the brush border membrane (top) and basolateral membrane (bottom) of mammalian enterocytes.	4
Table 2.1. Uptake of amino acids (mean \pm SEM, n), expressed as adjusted disintegrations per minute, by adult <i>O. circumcincta</i> in three experiments in which they were incubated with a [14 C]-protein hydrolysate in PBS.	54
Table 3.1. Arginase activities of sheathed L3 <i>O. circumcincta</i> homogenates with increasing concentration of arginine.	68
Table 3.2. Arginase activities of adult <i>O. circumcincta</i> homogenates with increasing concentration of arginine.	68
Table 3.3. Arginase activities (mean \pm SEM, n = 2) of sheathed L3 <i>O. circumcincta</i> homogenate SL4 in the presence of metal ions or EDTA.	68
Table 3.4. Creatinase activities of sheathed L3 <i>O. circumcincta</i> homogenates with increasing concentration of creatine.	69
Table 3.5. Creatinase activity of adult <i>O. circumcincta</i> homogenate A2 with increasing concentration of creatine.	69
Table 3.6. Creatinase activities (mean \pm SEM, n = 2) of sheathed L3 <i>O. circumcincta</i> homogenate SL9 in the presence of metal ions, ADP, ATP or EDTA.	69
Table 3.7. Pyrroline-5-carboxylate dehydrogenase activities of sheathed L3 <i>O. circumcincta</i> homogenates with increasing concentration of 1-pyrroline-5-carboxylate.	70
Table 3.8. K_m values for arginases of different organisms.	72
Table 3.9. Effects of various inhibitors on <i>P. putida</i> creatinase activity (Yoshimoto <i>et al.</i> , 1976).	73
Table 3.10. K_m values for pyrroline-5-carboxylate in the reaction catalysed by pyrroline-5-carboxylate dehydrogenase in different organisms.	76
Table 4.1. AlaAT activities of sheathed L3 <i>O. circumcincta</i> homogenates monitored in the direction of alanine utilisation with increasing concentration of 2-oxoglutarate.	89

Table 4.2. AlaAT activities of sheathed L3 <i>O. circumcincta</i> homogenates monitored in the direction of alanine utilisation with increasing concentration of alanine.	89
Table 4.3. AlaAT activities of adult <i>O. circumcincta</i> homogenates monitored in the direction of alanine utilisation with increasing concentration of alanine.	89
Table 4.4. AlaAT activities of sheathed L3 <i>O. circumcincta</i> homogenates monitored in the direction of alanine formation with increasing concentration of glutamate.	89
Table 4.5. AlaAT activities of sheathed L3 <i>O. circumcincta</i> homogenates monitored in the direction of alanine formation with increasing concentration of pyruvate.	89
Table 4.6. AlaAT activities of an adult <i>O. circumcincta</i> homogenate monitored in the direction of alanine formation with increasing concentration of pyruvate.	89
Table 4.7. Effects of 1 mM ATP or ADP on the activities of AspAT (mean \pm SEM, n = 3) of sheathed L3 <i>O. circumcincta</i> homogenate SL31 in the direction of aspartate utilisation.	90
Table 4.8. AspAT activities of sheathed L3 <i>O. circumcincta</i> homogenates monitored in the direction of aspartate utilisation with increasing concentration of 2-oxoglutarate.	90
Table 4.9. AspAT activities of adult <i>O. circumcincta</i> homogenates monitored in the direction of aspartate utilisation with increasing concentration of 2-oxoglutarate.	90
Table 4.10. AspAT activities of sheathed L3 <i>O. circumcincta</i> homogenates monitored in the direction of aspartate utilisation with increasing concentration of aspartate.	90
Table 4.11. AspAT activities of adult <i>O. circumcincta</i> homogenates monitored in the direction of aspartate utilisation with increasing concentration of aspartate.	90
Table 4.12. AspAT activities of sheathed L3 <i>O. circumcincta</i> homogenates monitored in the direction of aspartate formation with increasing concentration of glutamate.	90
Table 4.13. AspAT activities of sheathed L3 <i>O. circumcincta</i> homogenates monitored in the direction of aspartate formation with increasing concentration of oxaloacetate.	91
Table 4.14. Aspartase activities of L3 <i>O. circumcincta</i> sheathed L3 homogenates monitored in the direction of fumarate utilisation with increasing concentration of ammonia.	91
Table 4.15. Aspartase activities of L3 <i>O. circumcincta</i> sheathed L3 homogenates monitored in the direction of fumarate utilisation with increasing concentration of fumarate.	91

Table 4.16. Aspartase activity of adult <i>O. circumcincta</i> homogenate A10 monitored in the direction of fumarate utilisation with increasing concentration of fumarate.	91
Table 4.17. Aspartase activities of sheathed L3 <i>O. circumcincta</i> homogenates monitored in the direction of fumarate formation with increasing concentration of aspartate.	91
Table 4.18. Aspartase activities (mean \pm SEM, n = 2) in the direction of fumarate formation of sheathed L3 <i>O. circumcincta</i> homogenate SL47 in the presence of ions, ATP, ADP or EDTA.	92
Table 4.19. K_m values for the substrates alanine (Ala), 2-oxoglutarate (2-OG), pyruvate (Pyr) and glutamate (Glu) for the reactions catalysed by alanine aminotransferases of different organisms.	93
Table 4.20. K_m values for the substrates aspartate (Asp), 2-oxoglutarate (2-OG), oxaloacetate (OAA) and glutamate (Glu) for the reactions catalysed by aspartate aminotransferases of different organisms.	95
Table 4.21. K_m values for the substrate aspartate (Asp) for the reactions catalysed by aspartases of different organisms.	96
Table 4.22. Activities and substrate K_m of AlaAT, AspAt and aspartase compared with those of some TCA cycle enzymes in homogenates of L3 <i>O. circumcincta</i> .	99
Table 5.1. Glutamate dehydrogenase (GDH) activities (mean \pm SEM, n = 2) at 30°C of sheathed L3 <i>O. circumcincta</i> homogenates (SL 54-55) in the directions of glutamate formation and utilisation with 1 mM ATP or ADP added to the reaction mixture..	114
Table 5.2. Glutamate dehydrogenase (GDH) activities at 30°C of sheathed L3 <i>O. circumcincta</i> homogenates, monitored in the direction of glutamate utilisation, with increasing concentration of glutamate.	114
Table 5.3. Glutamate dehydrogenase (GDH) activities at 30°C of adult <i>O. circumcincta</i> homogenates, monitored in the direction of glutamate utilisation, with increasing concentration of glutamate.	114
Table 5.4. Glutamate dehydrogenase (GDH) activities at 30°C of sheathed L3 <i>O. circumcincta</i> homogenates, monitored in the direction of glutamate utilisation, with increasing concentration of NAD ⁺ or NADP ⁺ .	114
Table 5.5. Glutamate dehydrogenase (GDH) activities at 30°C of sheathed L3 <i>O. circumcincta</i> homogenates, monitored in the direction of glutamate formation, with increasing concentration of 2-oxoglutarate.	114

Table 5.6. Glutamate dehydrogenase (GDH) activities at 30°C of adult <i>O. circumcincta</i> homogenates, monitored in the direction of glutamate formation, with increasing concentration of 2-oxoglutarate.	114
Table 5.7. Glutamate dehydrogenase (GDH) activities at 30°C of sheathed L3 <i>O. circumcincta</i> homogenates, monitored in the direction of glutamate formation, with increasing concentration of ammonia.	115
Table 5.8. Glutamate dehydrogenase (GDH) activities at 30°C of adult <i>O. circumcincta</i> homogenates, monitored in the direction of glutamate formation, with increasing concentration of ammonia.	115
Table 5.9. Glutamate dehydrogenase (GDH) activities at 30°C of sheathed L3 <i>O. circumcincta</i> homogenates, monitored in the direction of glutamate formation, with increasing concentration of NADH or NADPH.	115
Table 5.10. Glutaminase activities at 30°C of sheathed L3 <i>O. circumcincta</i> homogenates with increasing concentration of glutamine.	115
Table 5.11. Glutaminase activity at 30°C of an adult <i>O. circumcincta</i> homogenate with increasing concentration of glutamine.	115
Table 5.12. Glutaminase activities (mean ± SEM, n = 2) at 30°C of sheathed L3 <i>O. circumcincta</i> homogenates (SL73-74) in the presence of metal ions, arginine or EDTA.	115
Table 5.13. Glutamine synthetase (GS) activity of sheathed L3 <i>O. circumcincta</i> homogenates monitored in the direction of glutamate utilisation with increasing concentration of ammonia.	116
Table 5.14. Glutamine synthetase (GS) activities of sheathed L3 <i>O. circumcincta</i> homogenates, monitored at 30°C in the direction of glutamate utilisation, with increasing concentration of glutamate.	116
Table 5.15. Glutamate synthase (GOGAT) activity at 30°C of sheathed L3 <i>O. circumcincta</i> homogenates with increasing concentration of glutamine.	116
Table 5.16. Glutamate synthase (GOGAT) activity at 30°C of adult <i>O. circumcincta</i> homogenate with increasing concentration of glutamine.	116
Table 5.17. Glutamate synthase (GOGAT) activity at 30°C of an adult <i>O. circumcincta</i> homogenate with increasing concentration of 2-oxoglutarate.	116
Table 5.18. K_m values for substrates of glutamate dehydrogenases from different organisms.	118

Table 5.19. K_m values for glutamine for the reaction catalysed by glutaminases in different organisms.	121
Table 5.20. K_m values for glutamate and ammonia for the reaction catalysed by glutamine synthetase in different organisms.	123
Table 5.21. K_m values for glutamine for the reaction catalysed by glutamate synthase (GOGAT) in different organisms.	124

List of Abbreviations

2D	two dimensional
<i>A. aegypti</i>	<i>Aedes aegypti</i>
<i>A. galli</i>	<i>Ascaridia galli</i>
<i>A. lumbricoides</i>	<i>Ascaris lumbricoides</i>
<i>A. marina</i>	<i>Arenicola marina</i>
<i>A. suum</i>	<i>Ascaris suum</i>
aa	amino acids
ADC	arginine decarboxylase
AGAT	arginine-glycine amidinotransferase
AK	arginine kinase
AlaAT	alanine aminotransferase
AMP	adenosine monophosphate
APC	acid-polyamine-choline
AS	asparagine synthetase
AspAT	aspartate aminotransferase
ATF1	amino acid transporter superfamily 1
<i>B. malayi</i>	<i>Brugia malayi</i>
<i>B. mori</i>	<i>Bombyx mori</i>
<i>B. pahangi</i>	<i>Brugia pahangi</i>
BCAT	branched chain aminotransferases
<i>C. briggsae</i>	<i>Caenorhabditis briggsae</i>
<i>C. elegans</i>	<i>Caenorhabditis elegans</i>
<i>C. emasculans</i>	<i>Cercaria emasculans</i>
<i>C. lingua</i>	<i>Cryptocotyle lingua</i>
<i>C. oncophora</i>	<i>Cooperia oncophora</i>
cAlaAT	cytosolic alanine aminotransferase

cAspAT	cytosolic aspartate aminotransferase
CCBL	cysteine S-conjugate β -lyase
cGDH	cytosolic glutamate dehydrogenase
Ci	curie
CK	creatine kinase
cNOS	constitutive nitric oxide synthase
CoA	Coenzyme A
CPS	carbamoyl phosphate synthetase
<i>D. immitis</i>	<i>Dirofilaria immitis</i>
<i>D. melanogaster</i>	<i>Drosophila melanogaster</i>
<i>D. polymorpha</i>	<i>Dreissena polymorpha</i>
DFMO	difluoromethylornithine
DNA	deoxyribonucleic acid
dpm	disintegrations per minute
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	Ethylene diamine tetra acetic acid
e.p.g.	eggs per gram
ES	excretory/secretory
Expt	experiment
<i>F. hepatica</i>	<i>Fasciola hepatica</i>
Fd	ferredoxin
g	gram
g	gravitational force
<i>G. intestinalis</i>	<i>Giardia intestinalis</i>
<i>G. lamblia</i>	<i>Giardia lamblia</i>
GABA	γ -aminobutyric acid
GABA-T	4-aminobutyrate:2-oxoglutarate aminotransferase
GAMT	S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase

GDH	glutamate dehydrogenase
GOGAT	glutamate synthase
GS	glutamine synthetase
GSH	glutathione
GST	glutathione S-transferase
GTP	guanosine triphosphate
h	hour
<i>H. alvei</i>	<i>Hafnia alvei</i>
<i>H. citelli</i>	<i>Hymenolepis citelli</i>
<i>H. contortus</i>	<i>Haemonchus contortus</i>
<i>H. diminuta</i>	<i>Hymenolepis diminuta</i>
<i>H. nana</i>	<i>Hymenolepis nana</i>
<i>H. polygyrus</i>	<i>Heligmosomoides polygyrus</i>
<i>H. pylori</i>	<i>Helicobacter pylori</i>
IMP	inosine monophosphate
iNOS	inducible nitric oxide synthase
kg	kilogram
<i>L. carinii</i>	<i>Litomosoides carinii</i>
L3	third stage larva
L4	fourth stage larva
LASPO	L-aspartate oxidase
M	molar
<i>M. expansa</i>	<i>Moniezia expansa</i>
<i>M. similis</i>	<i>Microphallus similis</i>
mAlaAT	mitochondrial alanine aminotransferase
mAspAT	mitochondrial aspartate aminotransferase
mCi	millicurie
MDH	mitochondrial malate dehydrogenase

MFS	major facilitator superfamily
mg	milligram
mGDH	mitochondrial glutamate dehydrogenase
min	minute
ml	millilitre
mM	millimolar
MW	molecular weight
n	number
<i>N. americanus</i>	<i>Necator americanus</i>
<i>N. brasiliensis</i>	<i>Nippostrongylus brasiliensis</i>
NAD ⁺	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NADP ⁺	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NAG	N-acetylglutamate
nl	nanolitre
nm	nanometre
nmole	nanomole
NOS	nitric oxide synthase
<i>O. circumcincta</i>	<i>Ostertagia circumcincta</i>
<i>O. cuniculi</i>	<i>Obeliscooides cuniculi</i>
<i>O. volvulus</i>	<i>Onchocerca volvulus</i>
OAA	oxaloacetate
OAT	ornithine aminotransferase
ODC	ornithine decarboxylase
OTCase	ornithine transcarbamylase
OUC	Ornithine-Urea Cycle
<i>p</i>	probability

<i>P. crassipalpis</i>	<i>Parasarcophaga crassipalpis</i>
<i>P. freudenreichii</i>	<i>Propionibacterium freudenreichii</i>
<i>P. islandicum</i>	<i>Pyrobaculum islandicum</i>
<i>P. pacifica</i>	<i>Pista pacifica</i>
<i>P. putida</i>	<i>Pseudomonas putida</i> .
<i>P. redivivus</i>	<i>Panagrellus redivivus</i>
P5C	Δ^1 -pyrroline-5-carboxylic acid
P5CDH	pyrroline-5-carboxylate dehydrogenase
P5CR	pyrroline-5-carboxylate reductase
P5CS	pyrroline-5-carboxylate synthase
RO	reverse osmosis
PBS	phosphate buffer saline
PC	pyruvate carboxylase
PEP	Phosphoenolpyruvate
PEPCK	phosphoenolpyruvate carboxykinase
PLP	pyridoxal 5'-phosphate
PMP	pyridoxamine 5'-phosphate
POT	proton oligopeptide transporter
RNA	ribonucleic acid
<i>S. sclerotiorum</i>	<i>Sclerotinia sclerotiorum</i>
<i>S. bibionis</i>	<i>Steinernema bibionis</i>
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
<i>S. cynthia ricini</i>	<i>Samia cynthia ricini</i>
<i>S. frugiperda</i>	<i>Spodoptera frugiperda</i>
<i>S. japonicum</i>	<i>Schistosoma japonicum</i>
<i>S. mansoni</i>	<i>Schistosoma mansoni</i>
<i>S. solida</i>	<i>Semele solida</i>
<i>S. typhimurium</i>	<i>Salmonella typhimurium</i>

SAMdc	S-adenosyl methionine decarboxylase
SDS	sodium-dicarboxylate symporters
SEM	standard error of the mean
SL	sheathed larva
<i>T. colubriformis</i>	<i>Trichostrongylus colubriformis</i>
<i>T. cruzi</i>	<i>Trypanosoma cruzi</i>
<i>T. spiralis</i>	<i>Trichinella spiralis</i>
TCA	tricarboxylic acid
μCi	microcurie
μg	microgram
μl	microlitre