NITROGEN CATABOLISM IN NEMATODE PARASITES

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[*Manuscript received August* 7, 1951]

Summary

Nematodirus filicollis, Nematodirus spathiger, and *Ascaridia galli* were maintained for periods of 24 hours in a non-nutrient medium containing streptomycin and penicillin to prevent bacterial growth. Under aerobic conditions the total soluble nitrogenous material excreted by *Nematodirus* spp. in three experiments was 1.23-1.59 mg. nitrogen per g. dry wt. of tissue. Peptide nitrogen accounted for 33-37 per cent. of the total soluble nitrogen excreted, ammonia nitrogen 40-42 per cent., and urea nitrogen 11-17 per cent. Small amounts of uric acid were found. Under similar conditions the total soluble nitrogen excreted by *Ascaridia galli* was 0.29-0.41 mg. per g. dry wt.; of this, 14-15 per cent. was due to peptide nitrogen, and 8 per cent. to urea nitrogen. No uric acid was found.

Under anaerobic conditions, excretion of soluble nitrogenous material by *Nematodirus* spp. was increased 40-42 per cent.; *Ascaridia galli* was not appreciably affected.

Ammonia was formed in brei prepared from *Nematodirus* spp. and *Ascaridia galli*; the amounts found were increased in the presence of added urea, alanine, aspartic acid, and glutamic acid. Urea production was greatly increased by adding citrulline, ornithine, and arginine. Cobalt ions, 10^{-3}M, increased urea production in the presence of arginine. It was concluded that urea formation in the tissues of nematode parasites took place via a citrulline cycle similar to that described by Krebs and Henseleit (1932).

Urease and arginase activity in homogenates prepared from the intestine of *Ascaris lumbricoides* were very much greater than in homogenates prepared from the ovary, or muscle, which included the lateral line organs.

Of the purine derivatives examined, only muscle adenylic acid, and, to a less extent, adenine, caused increased ammonia formation in brei prepared from mature *Ascaridia galli, Nematodirus* spp., or the muscle of *Ascaris lumbricoides.* Young adult *Ascaridia galli,* from infestations 4-5 weeks old, formed large quantities of urea and ammonia from added xanthine, uric acid, and allantoin. Guanase activity was absent. It was concluded that the breakdown of purines in nematode parasites, when it occurs, takes place by the action of uricase, allantoinase, allantoicase, and urease.

I. Introduction

In nematode parasites, where the major metabolic activities are directed towards egg production, the emphasis on protein and nucleic acid synthesis must be considerable, and nitrogen metabolism generally might be expected to be rapid. The catabolic processes of nitrogen metabolism, however, appear

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to be relatively slow, and most workers agree that the products usually associated with the breakdown of protein in other animals have not been found in appreciable quantities in media in which adult parasites have been maintained (Lapage 1937). Thus the total nitrogen excreted by *Ascaris lumbricoides* in 24 hours ranges from 15 to 29 mg. per 100 g. wet weight (Weinland 1904; von Brand 1934). About one-third of the excreted nitrogen is ammonia (Weinland 1904). The remainder consists of substances precipitated by phosphotungstic acid, amino acids, small amounts of amines, and mercaptan (Flury 1912, Weinland 1904). Chitwood (1938) found a small quantity of urea in fluid collected from the excretory pore of *Ascaris* but considered that it probably came from the host. About a quarter of the excreted nitrogen is in the eggs discharged by the parasites (von Brand 1934).

There are a number of difficulties in determining the nature of the excretory metabolism of nematode parasites. The most important are those due to the inability to study the parasites under the physiological conditions in which they exist in host animals, and inability to avoid the misleading results that might be caused by the presence of bacteria in the medium. The results reported in this paper were obtained with parasites kept in non-nutrient and glucose-containing media, to which antibiotics had been added to limit the growth of bacteria. The results, therefore, apply to parasites living under abnormal conditions. However, the possible errors in earlier work, arising from bacterial contamination, have been lessened.

Most of the nematodes examined were parasites of the small intestine. They were *Ascaridia galli*, from the chicken, *Nematodirus filicollis* and *N. spathiger* from the sheep, and *Ascaris lumbricoides* from the pig. The nature of the chief nitrogenous excretory products, and some of the biological processes leading to the formation of these materials have been examined.

II. Methods

(*a*) *Biological Materials*

Nematodirus filicollis and *N. spathiger*, which were not separated for use, and *Haemonchus contortus*, were obtained from naturally infested sheep; *Ascaris lumbricoides* from naturally infested pigs, and *Ascaridia galli* from experimentally infected chickens.

The parasites were washed in saline till free of debris, and then washed several times in sterile saline if they were to be used in "culture" experiments. *Ascaridia galli* was cleaned and placed in media within 30 minutes of the death of the host; the cleaning of *Nematodirus* spp. took up to 2 hours. *Ascaris lumbricoides* was not used for "culture" experiments.

(*b*) *Culture Experiments*

The basal medium contained 8.8 g. NaCl, 0.42 g. KCl, and 0.48 g. $CaCl_2.6H_2O$ per litre. To this was added 100 ml. of 0.15M phosphate buffer at pH 6.8. When glucose medium was used, 50 ml. of isotonic glucose solu-

tion was added per litre of the basal medium. Streptomycin and penicillin, in saline, were added in various amounts to the basal medium. Solutions were sterilized by filtering through sintered-glass bacterial filters. Air was used as a gas phase for aerobic preparations; anaerobiosis was obtained by passing nitrogen, which had been freed of oxygen over copper turnings at 400°C., through the medium containing the parasites.

The "cultures" were set up in small Erlenmeyer flasks in pairs so that the results obtained in aerobic or anaerobic containers, or in the presence or absence of glucose, could be compared with parasites from the same source. The flasks were provided with stopcocks, which were closed after gassing. For *Ascaridia galli*, 8 ml. of medium was used for 10 parasites of total weight about 0.5 g. The same proportion of males and females, usually 1 to 10, was used in each pair of flasks. For *Nematodirus* spp. 250 mg. of mixed males and females were used in 10 ml. of medium.

After incubation at 37°C. for 24 hours, a sample, 0.5 ml. of the medium, was taken for bacteriological examination. The parasites were taken from the medium and washed with 2 ml. of fresh medium. The washings were added to the original medium which was then centrifuged. The supernatant solution was used for chemical analysis; the analytical results given in this paper thus refer to soluble nitrogen compounds only. The solid material, which consisted largely of eggs, was suspended in water and the eggs in suitable samples were counted. The parasites were freed of excess moisture with filter paper and weighed.

Samples for bacteriological examination were diluted and cultured on serum agar anaerobically and aerobically. *Nematodirus* spp. maintained under aerobic conditions were frequently contaminated with bacteria, even when high concentrations of antibiotic were present. Only those that were sterile, or contained less than 100 organisms per ml., were used for analysis.

Brei or mince, prepared by the method of Seevers and Shideman (1941), was brought to the appropriate pH and suspended in $^{M}/_{15}$ phosphate buffer or Krebs-Ringer phosphate without calcium chloride.

(*c*) *Chemical Methods*

Ammonia was estimated by a modification of the method of Conway and Byrne (1933). After distillation, the ammonia was estimated in a Beckman spectrophotometer with Nessler reagent at 530 mμ. Urea was estimated manometrically (Krebs and Henseleit 1932; Krebs 1942) and by the method of Conway (1933). The urease was prepared from soy beans. Uric acid was estimated colorimetrically, with and without treatment with uricase (Buchanan, Block, and Christman 1945). Owing to the presence of peptides in the media, phosphotungstic acid was used as a precipitant instead of sodium tungstate. Total nitrogen was estimated by the micro-Kjeldahl method, and protein and polypeptide nitrogen were estimated similarly after precipitation with trichloracetic acid and phosphotungstic acid (Gotfried 1939).

The *L*-(+)citrulline was prepared from arginine by the method of Gornall and Hunter (1939) using arginase from ox liver (Hunter and Dauphinee 1930). The other substrates were commercial products of high purity. Adenosine-triphosphate was prepared as the barium salt by the method described by Umbreit, Burris, and Stauffer (1946). It was converted to the sodium salt immediately before use.

III. Procedure and Results

(*a*) *Behaviour of the Parasites in Artificial Media*

When penicillin, about 1000 units per ml., and streptomycin, 0.4 mg. per ml., were present in the medium, the anaerobic preparations of *Nematodirus* spp. were found to be sterile, but only about 15 per cent. of the aerobic preparations were suitable for analysis. Contamination at high antibiotic concentrations was due to *Serratia* sp. The aerobic preparations of *Ascaridia galli* which contained antibiotics were usually sterile, but the anaerobic ones were frequently contaminated with *Clostridium* sp.

Before examining the nature of the nitrogen compounds excreted by the parasites, it was necessary to examine the suitability of the medium. This was done by observing the egg production, activity, and gross morphological changes of the parasites while they were kept in the medium.

For this purpose, 50 female *Nematodirus* spp. or two female *Ascaridia galli* were maintained in 10 ml. of medium, which was replaced every 24 hours. Under aerobic conditions the parasites remained active and appeared healthy for 9-14 days; under anaerobic conditions the parasites soon became inactive but became active again on exposure to air up to the fifth day. No obvious morphological changes occurred in *Ascaridia galli* but in old "cultures" the cuticle of *Nematodirus* spp. became inflated in about 30 per cent. of the parasites examined. Egg production by both parasites fell rapidly until the third day after which no eggs were produced. Under anaerobic conditions, egg production was about a half to a third of that found under aerobic conditions. The addition of glucose to the medium, or the use of bicarbonate-carbon dioxide buffers, did not appreciably affect the egg-laying capacity of the parasites. Except for the addition of glucose to the medium, no attempt was made to provide food for the parasites. It is considered that their behaviour indicated that the medium was not toxic and that it was suitable for the study of the excretion of nitrogenous products by fasting parasites for the first 24 hours.

(*b*) *The Nitrogenous Compounds Excreted*

The results of three analyses carried out with several lots of *Nematodirus* spp. and *Ascaridia galli* under aerobic and anaerobic conditions are shown in Table 1. The nitrogen in the streptomycin and penicillin was estimated, and corrections for its presence in the medium were made. *Nematodirus* spp. excreted about five times as much soluble nitrogenous material as *Ascaridia galli*. In aerobic cultures of *Nematodirus* spp., polypeptide nitrogen accounted

for 33-37 per cent. of the total nitrogen excreted; ammonia nitrogen 40-42 per cent.; urea nitrogen 11-17 per cent., and uric acid 2-4 per cent. These components accounted for about 90 per cent. of the total soluble nitrogen excreted. In aerobic cultures of *Ascaridia galli*, polypeptide nitrogen accounted for 14-15 per cent.; ammonia nitrogen 52-58 per cent., and urea nitrogen 9-18 per cent. Uric acid was not found in any of the *Ascaridia galli* "cultures." The pattern of nitrogen excretion in *Ascaridia galli* did not alter much in changing from aerobic to anaerobic conditions, though the total excretion was increased by 3-13 per cent. The nitrogen excretion of *Nematodirus* spp. was increased by 20-40 per cent. under anaerobic conditions; the polypeptide nitrogen was increased in amount though it still formed 32-39 per cent. of the total soluble nitrogen excreted. The amount and proportion of ammonia nitrogen, 27-31 per cent., and urea nitrogen, less than 8 per cent., were decreased and it appears that the increased nitrogen excretion was mainly due to unidentified material.

TABLE 1

NATURE AND AMOUNT OF SOLUBLE NITROGEN COMPOUNDS EXCRETED BY FASTING *NEMATODIRUS* SPP. AND *ASCARIDIA GALLI* IN AEROBIC AND ANAEROBIC MEDIA (MG./G. WET WT./24 HR.)

The figures between brackets show the percentage partition of the nitrogen in the excreta of the parasites

Culture	Total N	Poly-peptide N	Ammonia N	Urea N	Uric Acid N	N Accounted For (%)
Nematodirus spp.						
Aerobic 1	1.29	0.43 (34)	0.54 (42)	0.20 (15)	0.06 (5)	96
Aerobic 2	1.57	0.58 (37)	0.66 (42)	0.17 (11)	0.04 (2)	92
Aerobic 3	1.23	0.41 (33)	0.49 (40)	0.21 (17)	0.11 (9)	99
Anaerobic 1	1.76	0.56 (32)	0.48 (27)	0.01 (1)	0.03 (2)	62
Anaerobic 2	1.93	0.76 (39)	0.59 (30)	0.08 (4)	0.07 (4)	78
Anaerobic 3	1.48	0.49 (33)	0.42 (29)	0.10 (7)	0.10 (7)	75
Ascaridia galli						
Aerobic 1	0.29	— —	0.18 (52)	0.04 (15)	Nil (Nil)	—
Aerobic 2	0.35	0.05 (14)	0.20 (57)	0.03 (9)	Nil (Nil)	80
Aerobic 3	0.41	0.06 (15)	0.24 (58)	Nil (Nil)	Nil (Nil)	73
Anaerobic 1	0.32	— —	0.16 (50)	0.06 (19)	Nil (Nil)	—
Anaerobic 2	0.37	0.06 (16)	0.21 (56)	0.04 (10)	Nil (Nil)	82
Anaerobic 3	0.42	0.06 (14)	0.30 (72)	Nil (Nil)	Nil (Nil)	86

(*c*) *Formation of Ammonia*

Mince prepared from *Ascaridia galli*, *Nematodirus* spp., and *Haemonchus contortus*, suspended in $^{M}/_{15}$ phosphate buffer at pH 7.3, was incubated at 37°C. At intervals, samples were taken for the estimation of ammonia. In different lots of *Nematodirus* spp. ammonia production ranged from 0.3 to 1.1 mg. per g. dry wt. of tissue per hour. Somewhat similar figures were obtained with *Ascaridia galli*. The one experiment carried out with *Haemonchus contortus* gave a very low ammonia production.

(*d*) *Source of the Excreted Ammonia*

The possibility that the ammonia found in the medium was the result of urease activity in the tissues of the parasite was first examined. The activity of urease was estimated manometrically at pH 5 with mince prepared from *Ascaridia galli* and *Nematodirus* spp. In the presence of added urea, carbon dioxide production was increased, but the amount was so small that it could not be measured conveniently in semi-micro Warburg vessels. Urease activity was therefore examined by measuring the ammonia formed in the presence of added substrate, in Conway microdiffusion vessels. The results of one experiment with *Nematodirus* spp. are shown in Figure 1. Urea, 2 mg., in 1 ml. $^{M}/_{15}$ phosphate buffer at pH 7.3, or phosphate buffer alone, was added to 3 ml. of a suspension of mince in Krebs-Ringer phosphate. At intervals, enzyme activity was stopped by adding glacial acetic acid and boiling, and the ammonia was determined. The addition of urea caused a large increase in ammonia production. *Ascaridia galli* showed a slightly lower urease activity than did *Nematodirus* spp.; *Haemonchus contortus* showed about one-tenth of the activity of the other species.

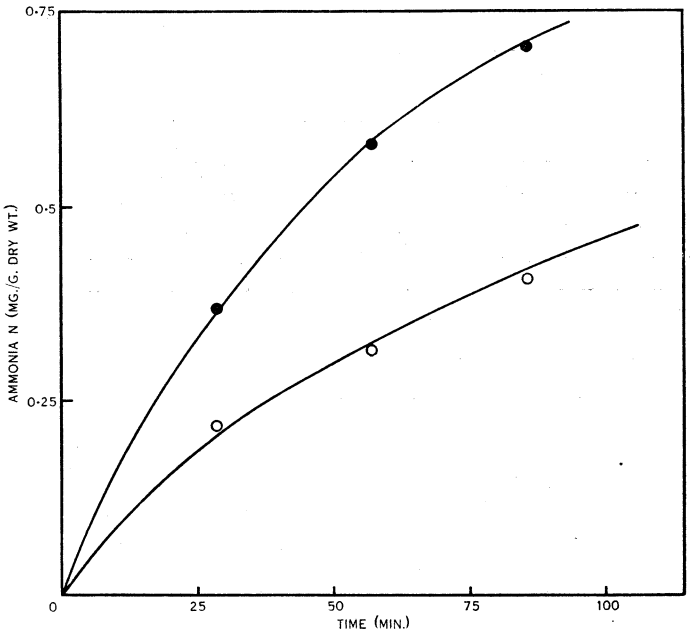

Fig. 1.—Urease activity in the tissues of *Nematodirus* spp.; ●, ammonia production in the presence of added urea; O, ammonia production in the control preparations.

Urease, of activity approaching that of the whole tissue of *Nematodirus* spp., could be extracted from brei with $^{M}/_{15}$ phosphate buffer at pH 7.5 in about 18 hours at 5°C.

(e) *Formation of Ammonia from Amino Acids*

The amino acids *DL*-aspartic acid, *L*-alanine, *L*-arginine, and *L*-glutamic acid, were added at pH 7.3 to brei prepared in Krebs-Ringer phosphate solution to give a final concentration of 0.01M of the active isomer. The ammonia found in experiments with *Nematodirus* spp. is shown in Table 2. In other similar experiments the increase in ammonia production due to the amino acids varied greatly, but in all instances the ammonia produced in the presence of alanine was less than that found when the other amino acids were used. Somewhat similar results were obtained with brei prepared from *Ascaridia galli*.

TABLE 2

FORMATION OF AMMONIA IN BREI OF *NEMATODIRUS* SPP. IN THE PRESENCE OF AMINO ACIDS

Amino Acid Added	Ammonia N (mg./g. dry wt./hr.)
None	0.21
Alanine	0.43
Aspartic acid	0.63
Glutamic acid	0.61
Arginine	0.64

(f) *Source of the Excreted Urea*

The occurrence of urea in the medium in which the parasites had been maintained, and the increased ammonia production in brei, known to contain urease, when certain amino acids were added, suggested that the ornithine cycle of Krebs and Henseleit (1932) might function in the tissues of the parasites. Arginase activity in the parasites was therefore examined.

TABLE 3

EFFECT OF Co^{++} ON ARGINASE IN *ASCARIDIA GALLI*

Concentration of Co^{++}	Urea N + Ammonia N (mg./g. dry wt./hr.)
Nil	0.61
10^{-5}M	0.63
10^{-4}M	0.79
10^{-3}M	0.83
10^{-2}M	0.77

Brei was prepared, brought to pH 9.5, and glycine buffer of pH 9.5 was added. Arginine buffered to pH 9.5 was added to a final concentration of 0.01M. All these preparations were carried out with chilled materials and vessels. The mixture was then incubated at 37°C., and at intervals, samples were taken for the estimation of ammonia and urea. One set of results ob-

tained with *Ascaridia galli* is shown in Figure 2. Somewhat similar results were obtained with *Nematodirus* spp. and *Ascaris lumbricoides* muscle. Urea production was always greatly increased in the presence of added arginine.

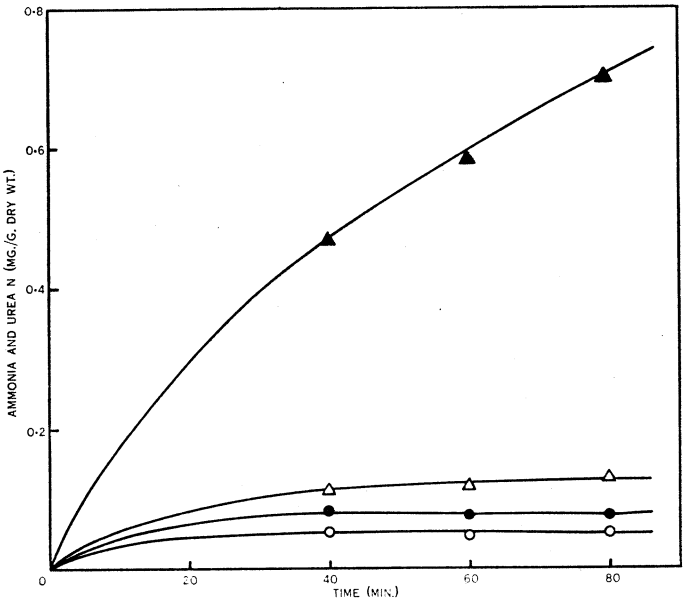

Fig. 2.—Arginase activity in the tissues of *Ascaridia galli*, ▲, urea production in the presence of added arginine; △, ammonia production in the presence of added arginine; ●, urea production in the control preparations; O, ammonia production in the control preparations.

The action of cobalt ions in activating the arginase was studied. Urea and ammonia formation was examined over the period of one hour in brei containing arginine and cobaltous chloride at concentrations of 10^{-5}M, 10^{-4}M, 10^{-3}M, and 10^{-2}M. The results obtained with *Ascaridia galli* are shown in Table 3. A concentration of cobalt ions at about 10^{-3}M caused a considerable increase in the urea and ammonia formed. Somewhat similar results were obtained with brei from *Nematodirus* spp.

The production of urea from the intermediates of the Krebs and Henseleit cycle was examined in the presence of added oxaloacetic acid. Adenosinetriphosphate was not added because it was rapidly deaminated in the brei and the high ammonia production masked the results obtained when citrulline and ornithine were added. Brei was prepared in Krebs-Ringer phosphate and freshly prepared, neutralized oxaloacetic acid, ornithine, and citrulline were added. The mixtures, in Erlenmeyer flasks, were shaken in an atmosphere of air for one hour at 37°C. after which the ammonia and urea formed were determined. The results obtained with brei prepared from *Ascaridia galli* and

the intestine of *Ascaris lumbricoides* are shown in Table 4. The increased production of urea and ammonia when ornithine and particularly when citrulline were added to the brei suggests that some form of the Krebs and Henseleit cycle was responsible for the synthesis of urea in the tissues of the parasites.

(*g*) *Distribution of Urease and Arginase in the Tissues of* Ascaris lumbricoides

Intestine, muscle, and ovary were dissected from *Ascaris lumbricoides,* washed in chilled saline, and weighed after removing excess fluid with filter paper. The materials were homogenized in a small glass mill of the type described by Potter and Elvehjem (1936) with two volumes of chilled phosphate buffer at pH 7.4. The "muscle" included a small amount of cuticle and a large part of the lateral line organs. Only the fine, convoluted tubules of the ovary were used. The substrates were added in neutral solution and the arginase and urease activity were determined over a period of one hour. Two sets of experiments were carried out. Enzyme activity in the ovary and muscle was very low, but in the intestine both arginase and urease were present. Thus, when urea was added, ammonia production was increased by 68-100 μg. ammonia nitrogen per g. dry wt. per hour, and, when arginine was added, the urea plus ammonia nitrogen was increased by 160-250 μg. per g. dry wt. per hour.

TABLE 4

FORMATION OF UREA AND AMMONIA FROM THE INTERMEDIATES OF THE ORNITHINE CYCLE

Substrate Added	Ammonia plus Urea N (mg./g. dry wt.)	
	Ascaris Intestine	*Ascaridia galli*
None	0.26	0.36
Oxaloacetate	0.25	0.36
Oxaloacetate + ornithine	0.26	0.44
Oxaloacetate + citrulline	0.74	0.50
Oxaloacetate + arginine	0,78	0.68

(*h*) *The Catabolism of Purines and Related Compounds*

The effects of muscle adenylic acid, adenine, guanine, xanthine, uric acid, and allantoin on ammonia and urea production were examined. Of these compounds, only adenylic acid appreciably increased ammonia production in brei prepared from the muscle of *Ascaris lumbricoides.* Brei prepared from large *Ascaridia galli,* taken from birds that had been infected eight weeks previously, produced large amounts of ammonia from adenylic acid and smaller amounts from adenine. The other compounds were not attacked. Small *Ascaridia galli,* from five-week-old infestations, gave different results. With the exception of guanine all the compounds caused an increase in ammonia production;

uric acid and allantoin caused an increase in urea formation (see Table 5). The magnitude of activity, particularly in respect to urea formation, varied considerably in different lots of *Ascaridia galli,* but in all experiments activity was greatest when young parasites were used, and the relative activity of the different substrates was consistent.

TABLE 5

AMMONIA AND UREA PRODUCTION (MG./G. DRY WT.) FROM PURINES AND THEIR DERIVATIVES

	Large *A. galli*		Small *A. galli*	
Substrate Added	Ammonia N	Urea N	Ammonia N	Urea N
None	0.16	Trace	0.19	0.04
Guanine	0.16	Trace	0.19	0.03
Adenine	0.22	Trace	0.21	0.04
Adenylic acid	0.40	Trace	1.58	0.05
Xanthine	0.16	Trace	0.36	0.06
Uric acid	0.16	Trace	0.37	0.68
Allantoin	0.16	Trace	0.51	0.91

IV. DISCUSSION

The partition of nitrogen in the excreta of *Nematodirus* spp. and *Ascaridia galli* is shown in Table 1. The predominance of ammonia over urea and uric acid as the chief excretory product is similar to that found in annelids (Delaunay 1931; Bahl 1947; Cohen and Lewis 1949), echinoderms (Delaunay 1931), Crustacea (Delaunay 1931; Dresel and Moyle 1950), teliosts, and Dipnoi (Scheer 1948), protozoa (Nardone and Wilber 1950), and aquatic reptiles (Coulson, Hernandez, and Brazda 1950; Khalil 1947). Urea forms the major excretory product of mammals and Amphibia; uric acid predominates in the excreta of birds, terrestrial reptiles, and insects.

The nature of the end products of nitrogen metabolism is of considerable biological significance (Delaunay 1931; Needham 1931, 1942). Though a large part of the excreted nitrogen in all animals has a common origin, namely the α-amino acid nitrogen of the food proteins, the excretory catabolism may take any of several different forms. Thus, as indicated above, uricotelism is a characteristic of terrestrial animals that live under conditions of water shortage and have cleidoic eggs; ureotelism is found among animals that do not suffer acute water shortage and are viviparous or produce non-cleidoic eggs laid in humid environments; ammonotelic organisms live in environments providing sufficient water to allow the ammonia concentration in their tissues to be kept below a toxic level.

The ammonotelic character of the nitrogen catabolism of nematode parasites of the alimentary canal is in accordance with the fact that water is freely available from the environment. Though the osmotic pressure of the contents of the digestive tracts of most animals is high compared to serum and tissue, the osmotic pressure of the fluids and tissues of the parasites is even higher

(Schopfer 1932) and there is, therefore, an osmotic gradient tending to drive water into the parasites. Ammonia is, however, highly toxic to nematode parasites (von Brand and Simpson 1947) and it is probable that a lowering of the rate of water exchange between the parasites and the environment would soon lead to the accumulation of toxic amounts of ammonia in the tissues. Some free-living stages of nematodes, e.g. third-stage larvae of *Trichostrongylus*, probably live under conditions of water shortage and it would be expected that uric acid would form the major nitrogenous excretory product.

The production of urea, which formed an appreciable proportion of the total nitrogen excreted (see Table 1), was much decreased when *Nematodirus* spp. were kept under anaerobic conditions. It is known that the synthesis of urea in mammalian tissues is an endergonic process and that the amounts produced are increased in the presence of lactate, or adenosinetriphosphate, and oxygen (Krebs and Henseleit 1932; Cohen and Hayano 1948). If an apprecible proportion of the energy requirements of *Nematodirus* spp. is satisfied by aerobic mechanisms (Rogers 1949) it would be expected that the production of urea might decrease under anaerobic conditions. On the other hand *Ascaridia galli*, in which urea production was not reduced in the absence of oxygen, is largely anaerobic *in vivo* (Rogers 1949) and is probably better equipped to obtain energy from anaerobic mechanisms.

The material precipitated by phosphotungstic acid, but not by trichloracetic acid, forms one of the most interesting components found in the excreta of the parasites. This material has not been examined as yet, but in view of the possibility that toxins and antigens secreted by the parasites would be included in this fraction, its composition will be described in detail in a later publication. It might appear that this material was obtained from the host ingesta and represents the undigested residue from the alimentary tracts of the parasites. However, when the parasites were kept in artificial media for periods greater than 24 hours this fraction tended to increase rather than to decrease. It is probable then, that it was a true excretory product, perhaps arising from the cells of the intestines of the parasites, or from the reproductive organs.

The total amount of the soluble nitrogen compounds excreted by *Nematodirus* spp. was large compared with that of many invertebrates. For instance, the nitrogen excreted by fasting Crustacea examined by Dresel and Moyle (1950) ranged from the low level of the terrestrial *Oniscus asellus*, 0.03 mg. per g. wet wt. per 24 hours, to that of the estuarine form *Gammarus zaddachi*, 0.60 mg. per g. per 24 hours. The level of nitrogen excretion in *Ascaridia galli* was a third to a sixth of that of *Nematodirus* spp. and was nearer to that of *Ascaris lumbricoides*, 0.15-0.29 mg. per g. wet wt. per 24 hours (Weinland 1904; von Brand 1934). The high level of nitrogen excretion in *Nematodirus* spp., compared with *Ascaridia galli*, suggests that it may have utilized protein as a source of energy to a greater extent. The respiratory quotient of *Ascaridia galli* indicates that carbohydrate is the chief source of its energy; the low respiratory quotient of *Nematodirus* spp. (Rogers 1948) indicates that important substrates besides carbohydrate may be oxidized to provide energy.

The ammonia found in the media in which parasites had been kept may have been due to the action of urease or deaminases. The results obtained indicate that adenylic deaminase and adenase were present in the parasites' tissues. No guanase activity was found. It is possible that some ammonia was produced directly by the action of amino acid deaminases. Urease was present in all the parasites examined. *Haemonchus contortus* was examined especially for urease activity because Glick, Zak, and von Korff (1950) suggest that the production of ammonia by urease in the mucosa of the stomach may assist in protecting the tissue from acid and from pepsin. This parasite lives in the abomasum of the sheep and would need some mechanism to protect it from the host's gastric juices. However, the results obtained indicate that urease was not present in sufficient amounts to warrant the suggestion that it has a protective function in *Haemonchus contortus*.

The substrate for urease may have been provided from ingested urea, urea formed by the ornithine cycle, or from the catabolism of purines. It is possible that the urea found in the culture media could have come from any of these sources. The catalytic action of ornithine, citrulline, and arginine on urea production suggests that ammonia arising from amino acid deamination was probably captured in the cyclical process, ornithine + carbamyl glutamate → citrulline → arginine → ornithine + urea (Ratner 1949). This ornithine cycle, or something similar to it, has been found to function in the liver of the rat and other mammals, and in the tortoise and the frog. Recent work (Cohen and Lewis 1950) has shown that the ornithine cycle also functions in the tissues of the intestine of *Lumbricus terrestris*. This mechanism of urea synthesis is not found in the livers of birds or snakes.

The results shown in Table 5 show that urea was formed in the parasites by the breakdown of purines. It is probable that the route taken was similar to that suggested by Florkin and Duchateau (1943), and involved the successive action of urico-oxidase, allantoinase, and allantoicase. The conversion of small amounts of xanthine to uric acid by the action of xanthine oxidase would account for the urea formed from this substrate.

The complete catabolism of purines to urea, and finally to ammonia, as probably occurs in the tissues of some nematode parasites, has been found in marine lamellibranchs, sipunculids, and Crustacea (Florkin 1949). In other animals the breakdown may stop at urea, allantoic acid, or allantoin; or uric acid may not be attacked at all.

The difference in the rate of purine catabolism of tissue preparations from young and old *Ascaridia galli* was pronounced and it seems that certain metabolic activities of the parasites are suppressed or lost as they grow larger. It is possible that this represents a simplification of metabolism, which took place as the parasitic mode of life became more firmly established.

V. Acknowledgments

The author is indebted to Mr. R. Simmonds and Mr. M. White for technical assistance, and to Mr. G. Merritt, who carried out the bacteriological examinations.

VI. References

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