Rev. Biol. Trop., 10(2): 149-159, 1962

# Nematode metabolism with special reference to Ancylostoma caninum*

by

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(Received for publication on September 5 1962)

Although the title of this paper is rather broad, the subject matter shall be limited to a review of our knowledge of the energy metabolism of adult Strongylata, and an attempt to draw some conclusions concerning group characteristics from the data now available. Furthermore, some recent studies carried out in our laboratories on *Ancylostoma caninum* will be compared with data obtained on other Strongylata. As intestinal helminths this group is probably of the greatest medical and veterinary significance, since hookworms are still major disease producers among humans in the tropics, and the various ruminant Strongylata are still a great problem for the agriculturist.

Review papers on the carbohydrate metabolism of nematodes have been sufficiently abundant (VON BRAND, 28, BUEDING and MOST, 5, FAIRBAIRN, 8) to keep workers in this field abreast of current developments. The last review in this field by von Brand covering the period 1951-1959 was of considerable interest to a worker in the field of metabolism of Strongylata since it did not include a single study of a member of this order. This latter regrettable situation was not the fault of the reviewer in omitting pertinent data, but simply the fact that there weren't any available. Admittedly extensive contributions to our knowledge of respiration and energy metabolism in Strongylata inhabiting the digestive tract were made by Rogers and his co-workers as late as 1950. None-

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* Manuscript read at round table on intestinal helminthiases during Second Latin American and First National Congress of Microbiology San José, Costa Rica, 1961.

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theless, a decade has passed without renewed interest in the biochemistry of this group of bionomically important helminths.

In spite of the fact that Ancylostomidae have not been adequately studied with respect to respiration and carbohydrate metabolism as a group, more species of Strongylata have been investigated than in any other order of nematodes, the reason for this being the availability of equine and ruminant species of *Strongylus, Haemonchus, Ostertagia,* and *Nematodirus.* Furthermore, the Heligmosomid, *Nippostrongylus braziliensis* is readily maintained in laboratory rats. All the members of the group which have been studied exhibit high rates of oxygen consumption *in vitro* (table 1). The respiratory quotients observed for endogenous respiration are rather variable, and the high values reported by LAZARUS (12) for species of *Strongylus* should be reexamined to determine the sites of this high decarboxylative activity in the absence of oxidation. In table 1 values are given also for *Ascaris lumbricoides* and *Litomosoides carinii* as examples of the type of *in vitro* respiration observed for a species which can and cannot, respectively, survive at low levels of oxygen tension (BUEDING, 3, SLATER, 25). All of the species of Strongylata listed in the table exhibit a respiration sensitive to cyanide.

TABLE 1

*Oxygen consumption of adult Strongylata*

| Species | $Q0^2$ µl/mg dry wt/hr | R.Q. | Authority |
|---|---|---|---|
| *Strongylus equinus* | 3.3 | 3.0 | Lazarus, 1950 (12) |
| *S. vulgaris* | 3.6 | 3.3 | Lazarus, 1950 (12) |
| *Haemonchus contortus* | 4.8 | | Rogers, 1949 (20) |
| *Nematodirus sp.* | 5.1 | 0.66 | Rogers, 1948 (19) |
| *Nippostrongylus muris* | 6.8 | 0.69 | Rogers, 1948 (19) |
| *Ostertagia circumcincta* | 7.4 | 0.69 | Lazarus, 1950 (12) |
| *Ascaris lumbricoides* | 0.3 | 4 (appr.) | von Brand, 1934 (27) |
| *Litomosoides carinii* | 0.8* | | Bueding, 1949 ( 3) |
| *N. muris* | 1.4* | | Rogers, 1948 (19) |

* $Q0^2$ (wet weight).

ROGERS (20) has studied the effects of oxygen tension on the respiration of *Haemonchus, Nematodirus,* and *Nippostrongylus.* This author also examined the oxygen tensions prevailing in the immediate habitat of these parasites. From these analyses Rogers concluded that *Nippostrongylus* and *Nematodirus* are capable of respiring at a significant rate *in vivo,* but the oxygen response of *Haemonchus* was too weak to be of any significance under *in vivo* conditions characteristic of its environment in the host. However, the observed $QO_2$ of 1 is still three times that reported for *Ascaris in vitro.* Furthermore, READ (16)

has pointed out that the gradients of oxygen between the intestinal circulation and the lumen side of the mucosa are so large that even small oxygen tensions should be maintained continously.

ROGERS and LAZARUS (21) found that *Nematodirus* respiration was not stimulated when glucose was presented to intact worms. However fermentation of glucose to lactic acid could be observed in extracts of worms. Evidence for phosphorylation and energy transfer similar to that which occurs in yeast and mammalian muscle was reported.

MASSEY and ROGERS (13) found that the oxygen consumption of breis prepared from *Nematodirus fillicolis* and *N. spathiger* were stimulated by pyruvate, alpha-ketoglutarate, succinate, fumarate, malate, and oxalacetate, but not citrate. Malonate, arsenate, arsenite, pyrophosphate, and azide inhibited the respiration. Inhibition by malonate could be relieved by adding intermediates of the tricarboxylic acid cycle. Accumulation of succinate in the presence of malonate was increased when fumarate, fumarate plus pyruvate, or citrate was added. The mechanism by which citrate produces the latter effect without stimulating respiration should be investigated. Thus the presence of some form of the tricarboxylic acid cycle has been fairly well demonstrated in these two species.

Although hookworms are among the best understood helminths in terms of life cycle, epidemiology, diagnosis, and immunology, they are among the least understood in terms of biochemistry. A survey of the literature indicates a paucity of biochemical studies on Ancylostomidae. In 1938 VON BRAND and OTTO (29) studied the glycogen content of *A. caninum* in fed and fasting dogs. From this study it is evident that a substantial portion of the dry weight of the total worm consists of polysaccharide (5 per cent; based on analysis of dry weight in our laboratory). Energy metabolism has been virtually unexplored in adult *A. caninum*. HARWOOD and BROWN (11) reported in abstract that respiration of *A. caninum* was at least ten times that of *Ascaris*. The work to be reported here is an initial exploration of the energy requirements and oxidative capacities of this species, which lives with its body in one of the most anaerobic environments known (the vertebrate intestine), and its mouth in an aerobic environments (mammalian blood). This unique combination suggests an aerobic biochemical solution to living in a relatively anaerobic environment.

## MATERIALS AND METHODS

Adult *A. caninum* of both sexes were obtained from naturally infected dogs found in the city of Caracas or dogs infected in the laboratory. Although we have never encountered any *A. braziliense* this species does occur in about 30 per cent of the dogs in Caracas, and always in mixed infections, being less than 3 per cent of the total worm burden. Nonetheless, it should be emphasized here that we have not checked all worms used, but have only made spot checks for possible occurrence of *A. braziliense*. Dogs were sacrificed by administering anesthesia and hypertonic KCl intravenously, and worms were removed from

the intestine and transferred to isotonic saline at 37°C. After several washings worms were then transferred to Krebs-Ringer phosphate solution (pH= 7.2) in conventional 13 ml Warburg flasks for manometric studies on endogenous oxygen consumption, or to a mixture of the buffered Krebs-Ringer solution (50%) and dog serum (50%) for studies carried out in special chambers designed to measure the feeding rate of hookworms (ROCHE and MARTÍNEZ, 17). In the case of manometric studies changes in gas volumes in excess of 10 $\mu$l per hour were considered valid results. Activity less than 10 $\mu$l per hour was considered to indicate insufficient quantities of tissue.

The studies carried out in the special chambers were done by determining the concentrations of glucose, lactate, and pyruvate in the anterior chamber in the serum Ringer's solution present to the worms, and also in the Krebs-Ringer-phosphate solution in the posterior chamber after the period of incubation. Prior to incubation the serum in the anterior chamber was supplemented with $Cr^{51}$ sodium chromate. The isotope served as a marker for the volume of fluid which passed through the worm from the anterior chamber to the posterior chamber during the experiments (ROCHE and MARTÍNEZ, loc. cit.). The expected quantities of glucose, lactate, and pyruvate were estimated from the amount of isotope transported to the posterior chamber. Comparison of theoretical and observed chemical levels indicated minimal efficiencies of glucose utilization and acid production.

A more refined and controlled approach to the study of glucose utilization and acid production was made by washing the worms in three changes of 5 ml sterile Krebs-Ringer phosphate solution containing 50 units of crystalline potassium penicillin, and 100 $\mu$g dihydrostreptomycin per ml respectively. The worms were then transferred to 5 ml of a sterile mixture of 50% dog serum and 50% Krebs-Ringer phosphate solution containing antibiotic as previously described. After twelve hours the worms were then transferred to fresh serum-Ringer's-antibiotic mixtures and glucose was measured initially and after periods ranging from 12-18 hours incubation. All experiments involving the metabolism of *A. caninum* were carried out at 37°C.

Glucose was determined by the method of NELSON (14); pyruvate by the method of FRIEDMAN and HAUGEN (9), and lactate by the method of BARKER and SUMMERSON (2).

## RESULTS

### RESPIRATION OF INTACT ADULT A. CANINUM.

*A. caninum* adults endogenously consumed large quantities of oxygen when compared with other nematodes of the vertebrate intestine. Males consumed somewhat higher quantities of oxygen (2200 $\mu$l/ gm. wet wt./hr) than females (1600 $\mu$l/gm. wet wt./hr). Respiratory quotients determined by the direct method of Warburg as described by UMBREIT *et al.* (26), were quite low, and suggest that this organism may fix carbon dioxide. Respiration of males and females

was not stimulated by the addition of 0.01 M glucose; 0.01 M maltose did not stimulate the respiration of female worms. The latter substrate was not checked against male worms. The respiration experiments are summarized in table 2.

TABLE 2

*Respiration of* Ancylostoma caninum *in the absence of added substrate*

| Sex | $Q0_2$ $\mu l/g$ wet wt/hr | Range | n | S.E. | R.Q. | n |
|---|---|---|---|---|---|---|
| Females | 1600 | 1200-1900 | 6* | ±30 | 0.43 | 3** |
| Males | 2200 | 1900-2600 | 4* | ±30 | 0.56 | 4* |

* 10-20 worms per determination
** 20 worms per determination

UTILIZATION OF GLUCOSE FROM A SERUM-RINGER'S MIXTURE.

Although glucose failed to stimulate the endogenous respiration of male and female worms maintained in buffered Krebs-Ringer solution, it was possible to demonstrate the uptake of this substrate in Krebs-Ringer solution with added serum. Two types of experiments were carried out. One, utilizing the *in vitro* chambers of ROCHE and MARTÍNEZ (loc. cit.).

This technique has the advantage of eliminating the possible influence of end products formed during the experimental period since the anterior end of the worm is in the feeding solution and the remainder of the worm's body is in a separate chamber containing a physiological solution. Thus, adult female worms feeding on a Krebs-Ringer phosphate-serum mixture containing 50-100 mg% glucose (supplemented with $Cr^{51}$ as a marker for the volume of Ringer-serum solution which passed through the worm) metabolized glucose from the medium. Evidence was also obtained for the accumulation of small amounts of pyruvate in excess of that expected from the transport of the medium through the worm into the posterior chamber. The results of these experiments are summarized in table 3. In order to eliminate the possible role of contaminating bacteria, a similar short term experiment was done with worms which had previously been freed of their bacteria by antibiotic treatment. The results of the latter experiments are summarized in table 4. No significant quantities of lactate were detected.

TABLE 3

*Excretion of pyruvate and utilization of glucose by* Ancylostoma caninum *(females) in hookworm chambers without previous antibiotic treatment*.*

| Chamber | Time (hrs) | mg worms | Gamma per mg per hour excreted in posterior chamber | | | |
|---|---|---|---|---|---|---|
| | | | Glucose | | Pyruvate | |
| | | | expected | observed | expected | observed |
| 1 | | 20 | Mean: 0.63 | —— | 0.04 | 0.40 |
| 2 | 6 | 20 | 0.30 | none | 0.05 | 0.10 |
| 3 | 6 | 12 | 0.33 | none | 0.02 | 0.16 |
| 4 | 16 | 17 | 1.25 | none | 0.01 | 0.17 |
| | 16 | | ** | —— | 0.03 | 0.21 |

* 10 worms per chamber.
** Glucose not determined.

TABLE 4

*Excretion of pyruvate and utilization of glucose by* Ancylostoma caninum *(females) in hookworm chambers after antibiotic teatment*.*

| Chamber | mg worms | Gamma per mg per hour excreted in posterior chamber | | | |
|---|---|---|---|---|---|
| | | Glucose | | Pyruvate | |
| | | expected | observed | expected | observed |
| 1 | 14 | 0.83 | none | 0.02 | 0.07 |
| 2 | 16 | 1.63 | none | 0.03 | 0.11 |
| 3 | 24 | 0.66 | none | 0.01 | 0.06 |
| | | Mean: 1.04 | —— | 0.02 | 0.08 |

* 6 hours incubation; 15 worms per chamber.

When antibiotic treated worms were incubated in 125 ml erlenmeyer flasks (under an atmosphere of air) a different pattern of metabolism appeared. Glucose consumption could still be demonstrated, but the production of pyruvate decreased to insignificant quantities. Furthermore, the rate of utilization was about three times the amount expected from feeding experiments. The results of these experiments are shown in table 5. Under an atmosphere of nitrogen, glucose utilization was greater than that observed in controls incubated in air (table 6).

TABLE 5

*Aerobic glucose utilization by* Ancylostoma caninum *(females) after antibiotic treatment*

| Exp. | Period incubation | mg worms | Glucose consumed gamma/mg/hour |
|---|---|---|---|
| 1 | 12 hours | 86 | 2.04 |
| | 18 " | | 1.78 |
| 2 | 13 hours | 84 | 1.09 |
| | 13 " | | 1.56 |
| 3 | 12 hours | 45 | 2.46 |
| | 12 " | | 1.93 |
| | | 35 | |
| | 12 " | | 1.03 |
| | | | 1.85 |
| | | | Mean: 1.71 - S.E. 0.17 |

TABLE 6

*Anaerobic glucose utilization by* Ancylostoma caninum *(females) after antibiotic treatment*

| Gas phase | Period incubation | mg worms | Glucose consumed gamma/mg/hour |
|---|---|---|---|
| Air | 12 hours | 82 | 1.94 |
| | 14 " | | 1.72 |
| Nitrogen | 12 hours | 91 | 2.61 |
| | 14 " | | 2.44 |

There seems to be a factor in dog serum which stimulates the hookworm to ingest the ambient medium. The evidence we have for this is twofold: 1) Although we have demonstrated glucose consumption *in vitro* in the presence of serum, glucose has no effect upon the respiration of intact worms suspended in Krebs-Ringer solution, even when starved worms are used; 2) Worms maintained in feeding chambers in Ringer's solution which contained radiochromate in the anterior compartment do not transport the isotope to the posterior compartment as they do when serum is present.

## DISCUSSION

*A. caninum* follows the general pattern of strong respiratory activity exhibited by other Strongylata studied. The respiration is sensitive to cyanide

at concentrations of the order magnittude of $10^{-4}$ M (WARREN *et al.*, 30). It differs from *Nematodirus* in that lactic acid has not been observed as a major end product of fermentation, and that some pyruvic acid is produced. However, our studies reflect the activity of the intact worms; whereas, the work done on *Nematodirus* represents the glycolytic activity of worm extracts. It is possible that intac *Nematodirus* would not produce lactic acid in significant quantities in spite of having a lactic dehydrogenase, as is the case in *Trichinella spiralis* (GOLDBERG, 10; AGOSIN and ARAVENA, 1) and *A. lumbricoides* (RATHBONE and REES, 15; BUEDING, 4). The discrepancies observed between glucose metabolism within the *in vitro* chambers and the erlenmeyer flask arrangement may be the result of having conditions fairly anaerobic in the chamber since there is very little air space above the serum under these conditions. Analysis of anaerobic incubates in erlenmeyer flaks for pyruvate will be necessary before any final conclusion can be made concerning the conditions which promote pyruvate production.

The low respiratory quotients observed in *A. caninum* may indicate a fixation of carbon dioxide. Fixation of carbon dioxide into pyruvic acid has been demonstrated in *Heterakis* and *Ascaris* (FAIRBAIRN, 7; SAZ and VIDRINE, 24). It would be tempting to speculate that a similar sequence of reaction leading to the formation of propionic acid occurs in *A. caninum*, but we have been unable to detect the production of propionic acid by intact worms. The fixation of carbon dioxide may be related to the recently reported tropism of third stage *A. caninum* larvae to carbon dioxide (SASA *et al.*, 23). This would be an interesting adaptation of a biochemical phenomenon to the ecology of the parasite.

The requirement of a serum factor for esophageal activity in *A. caninum* is supported by recent studies (ROCHE, MARTÍNEZ, and MACPHERSON, 18) on the electrical activity associated with esophageal contractions. The contractions produce a characteristic wave potential, but worms maintained in Ringer's solution produce atypical potentials, at irregular intervals, or the worms fail to contract after a short period of time. Other investigators (CAVIER and SAVEL, 6; ROGERS and LAZARUS, 22) have found the cuticle of adult nematodes to be impermeable to substrates.

The dependence of hookworms upon a tissue stimulus for feeding could prove useful in a systemic approach to prophylaxis of low-level hookworm infestation.

## CONCLUSIONS

From the species of Strongylata studied to date we may draw the following conclusions:

1. Intestinal Strongylata respire at high rates when compared to other species of vertebrate nematodes.

2. The respiration of Strongylata involves heavy metal catalysis as indicated by the inhibitory effect of cyanide.

3. Oxidation of carbohydrates appears to follow the patterns characteristic of mammalian tissue.

4. The cuticle of some Strongylata is impermeable to glucose, but is permeable to gases.

5. *A. caninum* excretes pyruvic acid as an end product of carbohydrate metabolism under certain *in vitro* conditions, but this does not account for more than a small percentage of the total end products. An increased glycolysis under anaerobic condition has been observed for this organism.

6. *A. caninum* and three other species of Strongylata exhibit a low endogenous respiratory quotient.

7. *A. caninum* requires a factor or factors present in serum for normal feeding behavior.

## RESUMEN Y CONCLUSIONES

De las especies de Strongylata estudiadas hasta la fecha, podemos derivar las siguientes conclusiones:

1. Las especies intestinales de Strongylata poseen una tasa de respiración alta, cuando se comparan con otras especies de nemátodos de vertebrados.

2. En la respiración de los Strongylata interviene la catálisis por metales pesados, a juzgar por la acción inhibidora del cianuro.

3. La oxidación de los carbohidratos parece seguir el camino característico de los tejidos de mamíferos.

4. La cutícula de algunos Strongylata es impermeable a la glucosa, pero es permeable a gases.

5. El *A. caninum* excreta ácido pirúvico como un producto final del metabolismo de carbohidratos, bajo ciertas condiciones *in vitro;* este ácido representa una pequeña porción del total de todos los productos finales. La glucolisis de este nemátodo aumenta en condiciones anaeróbicas.

6. El *A. caninum,* al igual que otras tres especies de Strongylata, posee un cociente respiratorio bajo.

7. El *A. caninum* necesita de algún factor o factores presentes en el suero, para llevar a cabo su alimentación normal.

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