ON THE RELATIVE IMPORTANCE OF AEROBIC METABOLISM IN SMALL NEMATODE PARASITES OF THE ALIMENTARY TRACT

II. THE UTILIZATION OF OXYGEN AT LOW PARTIAL PRESSURES BY SMALL NEMATODE PARASITES OF THE ALIMENTARY TRACT

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Summary

Nippostrongylus muris, Haemonchus contortus, Nematodirus spathiger, and *N. filicollis* are all capable of utilizing oxygen for respiratory purposes even when it is present at very low oxygen tensions. Thus with a partial pressure as low as 5 mm. of mercury the respiration of *Nippostrongylus muris* may reach 40 per cent. of its maximum rate, whereas *Nematodirus* spp. and *H. contortus* may respire at 25 and 12 per cent. of their maximum rates respectively. Further, the results indicate that *in vivo* the oxygen consumption rates may sometimes reach 80 per cent. of the maximum *in vitro* rate in the case of *Nippostrongylus muris* in the small intestine of the rat and 40 per cent. in the case of *Nematodirus* spp. in the small intestine of the sheep. *Haemonchus contortus* in the sheep abomasum probably respires at a relatively lower rate than either of the intestinal parasites *in vivo.*

Evidence which indicates that the maximum uptake of oxygen by the parasites *in vitro* may be much higher than *in vivo* has been presented. It is concluded that the oxygen tensions of the host gut fluids surrounding the parasites in their normal habitat may not greatly limit oxygen uptake *in vivo,* especially in the case of *Nippostrongylus muris.*

The oxygen consumption – oxygen tension curve for *Nippostrongylus muris* followed a hyperbolic course. When the results were treated according to the equation

$$A = \frac{P}{K_1 + K_2 P},$$

where A represents oxygen uptake, P is the oxygen pressure, and K_1 and K_2 are constants, P/A plotted against P gave a straight line with K_1, the intercept, of 1.2, and K_2, the slope, of 0.14. The sheep parasites gave similar results, with K_1 and K_2 values of 2.6 and 0.15 for *Nematodirus* spp., and 10 and 0.14 for *Haemonchus contortus.*

Mechanisms of oxygen transport used by the parasites are discussed.

I. Introduction

Previous work has shown (Rogers 1949) that oxygen is present in the contents of the alimentary tract of the rat and of the sheep in appreciable amounts when determinations are made close to the mucosa in animals which have an intact alimentary circulation. In the present paper the results of experiments designed to determine the ability of certain nematode parasites to utilize

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oxygen at the partial pressures found in their normal environments are discussed. The animals examined were *Nippostrongylus muris* from the rat small intestine, *Haemonchus contortus* from the sheep abomasum, and *Nematodirus filicollis* and *N. spathiger* from the sheep small intestine. These parasites were selected because it was thought that they might be small enough to allow oxygen to penetrate to their central tissues in the absence of a circulatory system, and because all these parasites contain haemoglobins which have very low loading tensions (Rogers, unpublished data). The functions of the haemoglobins of the parasites in the transport of oxygen will be discussed in a later publication.

Nematode parasites have been shown to utilize oxygen from saline solutions in equilibrium with air at 38°C. (Laser 1944; von Brand 1934; Rogers 1948). The animals used in the present investigation all consume oxygen at a relatively high rate (Rogers 1948; Lazarus, unpublished data). The oxygen consumption of larger parasites such as *Ascaris lumbricoides* or even *Ascaridia galli* increases when an oxygen atmosphere is used instead of air (Laser 1944; Rogers 1948). High oxygen tensions are, however, toxic to *Ascaris lumbricoides;* the low concentrations of catalase present in this animal's tissues apparently allow fatal concentrations of hydrogen peroxide to accumulate (Laser 1944). However, Laser states that the pattern of the oxidative enzyme system of *Ascaris* seems to show a perfect adaptation for functioning at low oxygen tensions. If even a minor part of the metabolism of the large *A. lumbricoides* in its natural habitat can be considered to be aerobic, the importance of aerobic mechanisms in small nematode parasites may be considerable.

II. Methods

(i) *Biological Materials.*—*Nippostrongylus muris* was obtained from rats which had been infected experimentally. The rats were starved overnight, then killed, and the parasites were washed from the intestine with saline; the contaminating material was removed with a Pasteur pipette. The parasites were used within a few hours of the death of the host.

Nematodirus filicollis, N. spathiger, and *Haemonchus contortus* were obtained from naturally infested sheep. The parasites were removed from ingesta with light forceps. The cleaned worms were ready for use about four hours after the death of the host animals. The two species of *Nematodirus* were not separated for use.

(ii) *Manometric Methods.*—Oxygen uptakes were determined in saline at 38°C. in small Warburg flasks with K_{O_2}'s of the order of 0.3. With such vessels, using the "direct" method of Warburg (1926), adequate readings could be obtained with 50 mg. wet weight of material at oxygen partial pressures above 38 mm. of mercury. At oxygen pressures of 4 to 8 mm. of mercury, even 100 mg. of tissue, the largest amount which could be safely used in the vessels, did not give large enough readings to furnish accurate results. It is considered that measurements carried out manometrically at the lower pressures of oxygen may have errors as much as ± 15 per cent.

Gas mixtures were prepared with cylinder nitrogen and air over saturated sodium chloride solutions. The measurement of the volumes of the gases used in the mixtures was accurate to within ± 7 per cent. Each Warburg vessel was gassed with 2½ litres of gas mixture at room temperature. Q_{O_2} values (µl. of gas taken up per hour per mg. dry weight of respiring material) were calculated from readings taken over the first 30 minutes.

At the end of each experiment *Nematodirus* spp. and *H. contortus* were taken from the Warburg vessels, dried on filter paper, and weighed. The small *Nippostrongylus muris* were counted. The dry matter content as related to wet weight or to the number of parasites was obtained on several occasions, giving factors that were subsequently used for converting results to a dry weight basis in the calculation of Q_{O_2} values.

TABLE 1

THE EFFECT OF WARBURG SHAKING RATE ON THE X_{O_2} OF *NEMATODIRUS* SPP. FOR PERIODS OF 15 MIN. AT AN OXYGEN PARTIAL PRESSURE OF 3.8 MM. OF MERCURY AT 38°C.

Period	X_{O_2} (µl./100 mg. wet tissue)	Shaking Rate (7 cm. swing) per Minute
1	− 2.96	70
2	− 4.25	95
3	− 4.40	105
4	− 4.32	120
5	− 2.81	70

III. PROCEDURE AND RESULTS

Oxygen uptakes were determined manometrically at oxygen partial pressures of 3.8 to 152 mm. of mercury. In the lower range of oxygen tensions and with a V_F of 0.5 ml., the oxygen uptake was independent of the Warburg shaking rate above 105 per minute through a travel of 7 cm. (Table 1). When the speed of shaking was adequate at a given oxygen tension the oxygen uptake was directly proportional to the amount of respiring tissue within the limits of the amounts suitable for the particular Warburg vessels used (50 to 100 mg. wet weight). With oxygen partial pressures of 15 mm. of mercury and more, a shaking speed of 95 per minute was adequate.

The results varied somewhat with different lots of the same species of parasite. The variation, which was greatest at low partial pressures of oxygen, was probably partly due to the fact that the animals tended to get twisted into clumps in the Warburg vessels, especially when the units were shaken rapidly.

Results obtained with *Nippostrongylus muris, Haemonchus contortus,* and *Nematodirus* spp. are shown in Figures 1 and 2. The maximum pressures of oxygen found in the host fluids normally inhabited by *Nematodirus* spp. and *Nippostrongylus muris* are also shown. The oxygen tension of the contents of the sheep abomasum is not given, as the results reported previously (Rogers 1949) were taken from one animal only and so may possibly be misleading.

In *N. muris,* it would appear that even if the oxygen requirements of the parasite were as great *in vivo* as *in vitro,* enough oxygen would sometimes be

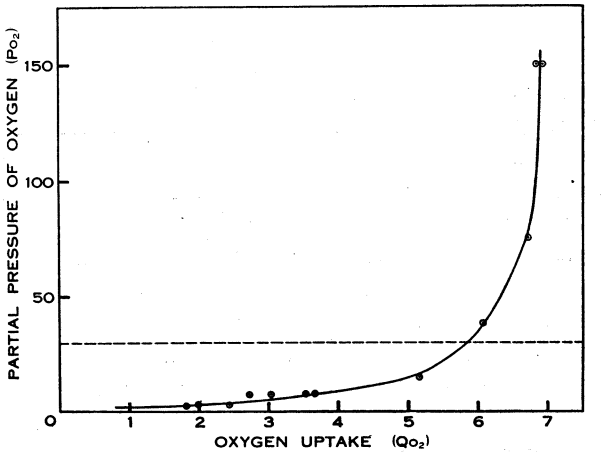

Fig. 1.—The relationship between the partial pressure of oxygen (mm. of mercury) and the oxygen consumption (µl./hr./mg. dry weight) of *Nippostrongylus muris.* The broken line indicates the upper level of the oxygen tensions found close to the mucosa in the small intestine of the rat.

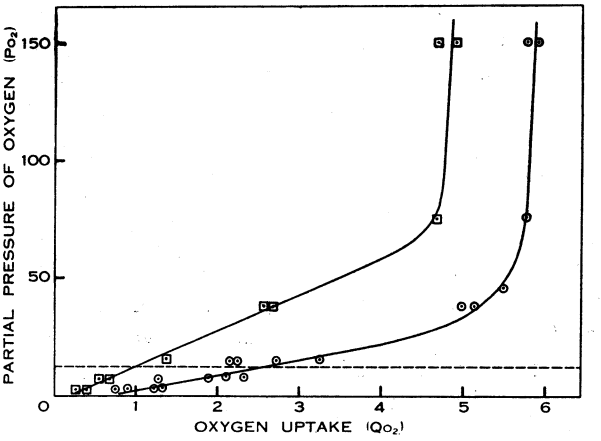

Fig. 2.—The relationship between the partial pressure of oxygen (mm. of mercury) and the oxygen consumption (µl./hr./mg. dry weight) of *Nematodirus* spp. (circles) and *Haemonchus contortus* (squares). The broken line indicates the upper level of the oxygen tension close to the mucosa in the small intestine of the sheep.

available in the normal environment to allow respiration to reach 80 per cent. of the maximum. *Nematodirus* spp. may be expected to have, on some occasions

at least, enough oxygen to allow 50 per cent. of the maximum respiratory activity. *H. contortus,* in the sheep abomasum, probably respires at a relatively lower rate than either of the intestinal parasites.

IV. Discussion

In interpreting the results obtained in the present investigation, consideration must be given not only to the reliability of the determination of oxygen tensions in host gut-fluids (see Rogers 1948), but also to the rate of diffusion of oxygen through the fluids surrounding the parasites *in vivo.* Clearly, the rate of movement of gut contents over the surface supplying the oxygen, which may be taken to be the intestinal mucosa, will be small compared to the rate of mixing of the gas phase with the fluid phases in the Warburg vessels. However, it is probable that the nematode species examined in the present work live close to the mucosa of the host's alimentary tract, in which case even a slow rate of diffusion would not necessarily limit the oxygen available for the parasites.

The relative ability of the three species of nematode parasites to use oxygen at low partial pressures is clearly shown by comparing the oxygen uptake at a partial pressure of 5 mm. of mercury. In such an environment the respiration of *Nippostrongylus muris* would reach 40 per cent. of the maximum *in vitro* rate, and that of *Nematodirus* spp. and of *Haemonchus contortus* 25 and 12 per cent. respectively. The amount of oxygen found in host fluids (Rogers 1949) would indicate the oxygen consumption *in vivo* may sometimes reach 80 per cent. of the maximum rate *in vitro* in the case of *Nippostrongylus muris* and 40 per cent. in the case of *Nematodirus* spp. *Haemonchus contortus* in the sheep abomasum probably respires at a relatively lower rate than either of the intestinal parasites. It must be emphasized, however, that these figures relate to the maximum *in vitro* rate of oxygen consumption. Even at low oxygen tensions all the species of nematodes used in the present investigation made intensely active movements *in vitro* at 38°C. This activity was not apparent *in vivo;* at least, it was not evident in parasites in anaesthetized or freshly killed animals. Brody (1945) states that the ratio of sustained hard work to rest energy is probably "independent of size or species as such." In man or the horse, energy requirements for highly active movements as compared to basal metabolism energy may vary from 10 or even 20 to 1 (Dill 1936; Robinson, Edwards, and Dill 1937). If it is reasonable to apply these results to nematode parasites, it would appear that the maximum oxygen consumption of the highly active parasites *in vitro* may be as much as twenty times as great as the maximum consumption of the sluggishly motile parasites *in vivo.* It is quite possible, then, that conditions *in vivo,* especially for *Nippostrongylus muris* and *Nematodirus* spp., may allow oxygen consumption rates to approach the maximum *in vivo* rate.

A factor which has not been considered in this argument is that concerned with egg production by the parasites. Egg production falls very rapidly *in vitro,* though it may be quite high for short periods after the parasites are taken from the host. It is reasonable to suppose, then, that oxygen requirements for the energy of egg production by nematode parasites may be greater *in vivo* than

in vitro. The importance of this in assessing the relationship between maximum oxygen uptake *in vivo* and *in vitro* is difficult to determine. However, egg production may be classed, like other productive processes, as a growth process (Brody 1945) and so the oxygen requirements for the growth of *Tubifex tubifex* may give some indication of the order of the oxygen requirements for nematode egg production. Brody (1945, quoting Collier 1942) states that the oxygen uptake at the peak of regeneration in *Tubifex tubifex* rose 85 per cent. above the normal level. This suggests that the correction for the lowered egg output of the nematode parasite *in vitro* may not be very great and does not seriously invalidate the suggestion that oxygen may sometimes be available in such amounts *in vivo* as to allow small parasites of the alimentary tract to respire quite actively.

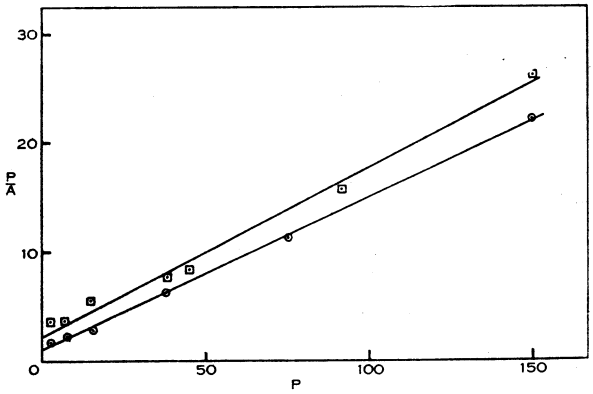

Fig. 3.—The relationship between P and P/A where P, the partial pressure of oxygen, is given in mm. of mercury, and A, the oxygen uptake of *Nippostrongylus muris* (circles) and *Nematodirus* spp. (squares) is given in µl./hr./mg. dry weight. The intercepts and slopes of the curves give the respective values of K_1 and K_2 for the equation $A = \frac{P}{K_1 + K_2 P}$.

The results obtained in the study of the oxygen consumption of *Nippostrongylus muris* and *Nematodirus* spp., when treated according to the relation

$$A = \frac{P}{K_1 + K_2 P} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where A = oxygen uptake in ml. per hour per g. dry weight, P = oxygen pressure in mm. of mercury, and K_1 and K_2 are constants, show a linear relationship (Fig. 3) in which the intercept, K_1, = 1.2 for *Nippostrongylus muris* and 2.6 for *Nematodirus* spp., and the slope, K_2, = 0.14 for *Nippostrongylus muris*, and 0.15 for *Nematodirus* spp. The results obtained with *Haemonchus contortus* were more scattered than those for the other species, but indicated that K_1 was approximately 10 and K_2 about 0.14. These results are compared with some of those given by Tang (1933) in Table 2.

Equation (1) is similar to that developed by Langmuir (1918) to account for the absorption of gases on a solid surface and that describing the dissociation curve of oxyhaemoglobin (Barcroft 1928). Gerard (1931), assuming that oxygen was transported by an intermediary carrier or enzyme, also derived a similar type of equation for cell respiration. Working with yeast, Winzler (1941) found that at very low oxygen tensions the combination of oxygen with an oxygen-transferring enzyme was the process which determined the shape and constants of the curve relating oxygen uptake with oxygen pressure.

TABLE 2

THE VALUES OF K_1 AND K_2 FOR THE EQUATION $A = \frac{P}{K_1 + K_2 P}$ FOR SEVERAL DIFFERENT SPECIES (FOR FURTHER EXPLANATION, SEE TEXT)

Material Used	Temperature (°C.)	K_1	K_2
Nippostrongylus muris	38	1.2	0.14
Nematodirus spp.	38	2.6	0.15
Haemonchus contortus	38	10	0.14
Planaria agilis	20	137.0	7.4
Unfertilized eggs of *Arbacia punctulata*	25	6.0	2.10
Fertilized eggs of *Arbacia punctulata*	25	6.5	0.55
Fragments of *Chironomus thummi* larvae	16.5-23.0	42.5	2.02
Earthworm	25	28.0	5.25
Termopsis nevadensis	20	0.06	0.0043
Yeast	37	0.2	0.02

The relationship between the tension of oxygen on the surface of a nematode parasite and the oxygen tensions at the central tissues of the parasite resulting from diffusion processes may be approximately determined by a treatment similar to that devised by Fenn (1928) for the study of the penetration of oxygen into nerves. The parasites may be considered to be cylinders of tissue of radius a consuming A ml. of oxygen per hour per g. dry weight, at an oxygen pressure on the surface of P_0 cm. of mercury. The diffusion constant, D, may be taken as that assessed for muscle by Krogh (1919), 8.4×10^{-4} ml. of oxygen diffusing across a surface area of 1 cm.^{2} per hour under a pressure of 76 cm. of mercury. In any concentric cylindrical layer of tissue of radii r and $r\text{-}dr$ within the parasite, the oxygen consumption in time, dt, is equal to the amount of oxygen diffusing into the layer minus the amount leaving the layer in the same time. Under these circumstances an equation

$$rDd^2c/dr^2 + Ddc/dr = Ar \quad \ldots\ldots\ldots\ldots \quad (2)$$

can be derived if the passage of oxygen in and out of the ends of the cylinder is neglected. This equation is similar to that obtained by Fenn (1928) except for the additional factor r in the first term. A solution of the differential equation (2) is

$$P = P_0 - A/4D(a^2 - r^2) \quad \ldots\ldots\ldots\ldots \quad (3)$$

where P is the oxygen tension in cm. of mercury at a distance r cm. from the

centre of the cylinder. If $r = 0$, P gives the oxygen tension at the centre of the parasite of radius a. By using appropriate values of P_0, A, D, and a, the oxygen concentrations in the central tissues of *Nippostrongylus muris*, *Nematodirus* spp., and *Haemonchus contortus* can be calculated. Table 3 lists the radii of the nematodes examined and the oxygen pressures in the medium necessary to give zero oxygen tensions in the central tissues. The radii given are average values obtained by measuring ten mixed males and females of each species at

TABLE 3

OXYGEN TENSIONS AT THE SURFACES OF NEMATODE PARASITES OF DIFFERENT SIZES NECESSARY TO GIVE ZERO OXYGEN TENSIONS IN THE CENTRAL TISSUES

Species of Parasite	Radius of Parasite (mm.)	Surface Oxygen Tension Giving Zero Tensions at the Centre (mm. of Hg)
Nippostrongylus muris	0.051	16
Nematodirus spp.	0.077	21
Haemonchus contortus	0.14	32

five points along their length. The results obtained by means of equation (3) are merely first approximations, for the equation makes no allowance for variations in A and D in different tissues of the parasites. However, the method is sufficient to show that at low partial pressures of oxygen the fall in oxygen consumption by the parasites is much slower than would be expected from the rate, according to the equation, at which the anaerobic region in the central tissues increases. Thus, without some other form of physical or chemical oxygen-transporting system, diffusion would not be sufficiently rapid to allow the parasites to respire actively at low partial pressures of oxygen. There may be several different oxygen-transporting systems assisting the diffusion of oxygen in the parasites. *In vitro*, the parasites' alimentary tracts make sluggish movements, which, in disturbing the perienteric fluid, would assist oxygen transport. Further, the presence of haemoglobins of low loading tensions and cytochrome in the parasites (Rogers, unpublished data) suggests that oxygen transport other than diffusion is of importance in the species of nematodes which have been examined.

Though nematode parasites do not appear to take up fluids *per os* under the conditions used in the present experiments *in vitro*, those species which have been examined *in vivo* appear to feed very rapidly (Wells 1931; Nishi 1933; Rogers and Lazarus 1949*a*). It would appear, then, that the ingestion of host tissues or fluids from the surface of the host gut mucosa might provide oxygen which could be absorbed through the alimentary tracts of the parasites. Calculation shows that this source of oxygen would be inadequate in the case of *Ascaridia galli*. However, in smaller parasites the result might be quite different, especially if the rate of passage of materials along the parasite alimentary tract was comparable to that of *Ancylostoma* (Wells 1933). In the absence of precise knowledge concerning the feeding habits of the animals used in the

present investigation no definite statement can be made concerning alimentary oxygenation. In *Nippostrongylus muris,* which feeds on host tissue and blood (Rogers and Lazarus 1949*a*; Rogers, unpublished data), it is probable that some oxygen is delivered to the central tissues via its alimentary tract.

Consideration of the results obtained in the present investigation leads to the suggestion that aerobic mechanisms of certain *trichostrongyle* parasites may be important in the economy of these animals. As yet, studies on the intermediary metabolism of these organisms have been confined to anaerobic processes of energy production arising from the breakdown of glycogen to pyruvate and lactate (Rogers and Lazarus 1949*b*). It is quite probable that such processes are preliminary to the more efficient oxidative processes of energy production such as the tricarboxylic acid cycle. The nature of such processes in nematode parasites is being examined.

V. Acknowledgments

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