

THE RESPIRATORY METABOLISM OF HELMINTHS

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[Manuscript received January 5, 1950]

Summary

The respiratory metabolism of the trematode *Paramphistomum cervi* and the nematodes *Haemonchus contortus*, *Ostertagia circumcincta*, *Syphacia* sp., *Heterakis spumosa*, *Strongylus equinus*, and *Strongylus vulgaris* have been examined.

The nematodes gave Q_{O_2} values ranging from -7.4 to -1.1 . When calculated on the basis of the relative surface areas of the parasites the differences in oxygen uptake were much smaller. Cyanide inhibited the uptake of oxygen; R.Q. values ranged from 0.8 to 3.3 . Nematodes inhabiting those parts of the gut in which bacterial fermentation is most active generally showed higher R.Q. values.

Paramphistomum cervi gave the low Q_{O_2} of -0.03 , and an R.Q. of 8 or over. Cyanide caused a pronounced increase in the uptake of oxygen. When the trematodes were incubated aerobically *in vitro* the R.Q. gradually fell and the activating effect of cyanide decreased. The cyanide activation of oxygen uptake of brei prepared from *Paramphistomum cervi* was not obtained when methylene blue was present. The present investigation has shown that the pattern of respiratory metabolism in helminths may vary, even among morphologically related forms. However, different species of parasites from regions of similar physiological activity in the host show some similarity in the nature of their respiratory activity.

I. INTRODUCTION

The respiratory metabolism of large helminths such as *Ascaris lumbricoides* and *Parascaris equorum* has been examined extensively (see Laser 1944 for references) but smaller parasites, particularly oxyurids, have not been examined to any extent. Further, most of the species already examined have been parasites of the small intestine. The present investigation includes species which, though not closely related taxonomically, come from somewhat similar physiological regions in the host's alimentary tract. Thus the oxyurid parasites *Heterakis spumosa* and *Syphacia* sp., and the strongylids *Strongylus equinus* and *S. vulgaris* are found in the colon of rats and mice and the caecum of horses, respectively, both of which are regions of active bacterial fermentation. The trematode *Paramphistomum cervi* was obtained from the reticulum of the ox, where bacterial fermentation takes place also. The other strongylids, *Haemonchus contortus* and *Ostertagia circumcincta*, were obtained from the abomasum of the sheep where bacterial fermentation does not usually occur.

The selection of parasites might be expected to show whether the pattern of their respiratory activity is determined more by taxonomic relationships based on morphological considerations, or by the nature of their environments.

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II. METHODS

Syphacia (probably *S. obvelata*) and *Heterakis spumosa* were collected from the colon of mice and rats which had been fasted overnight. The parasites were washed repeatedly in 0.9 per cent. saline till clean, the whole process taking 2-5 hours. *Haemonchus contortus* and *Ostertagia circumcincta* were recovered from the abomasum of sheep and used about three to six hours after the killing of the host. *Strongylus equinus* and *S. vulgaris* were collected from the caecum of horses about four hours after the death of the host and examined about $\frac{3}{4}$ hour later. The stomach fluke of cattle, *Paramphistomum cervi*, found adhering by the posterior sucker to small papillae on the reticulum, was removed two to five hours after the death of the host and used at periods of $\frac{1}{4}$ hour to six hours after transferring to 0.9 per cent. saline.

Respiration was studied in open-type Warburg manometers with vessels of about 5 ml. capacity, containing 0.9 per cent. saline medium at 38°C. Approximately equal numbers of male and female nematode parasites were used and fluid volumes were adjusted to allow for the volume of the parasites.

Q_{O_2} values, i.e. oxygen uptake in μ l/mg. dry weight of tissue/hour, were determined in air or, when specified, in oxygen. Oxygen consumption and carbon dioxide production were determined by the direct method of Warburg (1926). Anaerobic carbon dioxide production was examined in an atmosphere of nitrogen which had been passed over copper turnings at 400°C. to remove traces of oxygen.

Neutralized potassium cyanide, with a final concentration of 0.001M, was used in the determination of cyanide inhibition except when stated otherwise. It was shown by Riggs (1945) that the previously accepted methods (Krebs 1935; Umbreit, Burris, and Stauffer 1947) employed to prevent the loss of cyanide from the medium in Warburg vessels, due to absorption by the alkali in the centre wells, were unsatisfactory. Adjustments to compensate for the removal of cyanide were therefore made empirically.

Dry weights were determined after drying to constant weight at 100°C. The results given in the tables are averages calculated from the gas changes occurring during the first 30 minutes of the experiments.

III. RESULTS

The amount of dry matter found in the species which were studied is shown in Table 1. The average figures showing the character of the respiration of the nematode parasites are listed in Table 2.

Ostertagia circumcincta and *Haemonchus contortus* were the only two species with R.Q.'s. similar to those determined by Rogers (1948) who obtained R.Q.'s. ranging from 0.6 to 0.96 from four species of nematodes examined under similar experimental conditions.

Two experiments with *Heterakis spumosa* gave R.Q.'s. of 1.0 and 1.2. Similar figures were obtained several times when *Syphacia* sp. was examined, but on two occasions the species gave the unusually high quotient of 1.7 accompanied by a pronounced cyanide activation. In all experiments the R.Q.'s. of

both species of *Strongylus* were even higher, reaching a value of 3.3 during the first 30 minutes of the experiments. Cyanide sensitivity was examined in *Strongylus vulgaris* only, inhibitions of about 15 per cent. being obtained.

TABLE 1
DRY MATTER IN SEVERAL SPECIES OF PARASITES

Species	Per cent. Dry Matter
<i>Ostertagia circumcincta</i>	19 – 21
<i>Haemonchus contortus</i>	19 – 21
<i>Syphacia obvelata</i>	23
<i>Heterakis spumosa</i>	22
<i>Strongylus equinus</i>	15 – 16
<i>Strongylus vulgaris</i>	17 – 18
<i>Paramphistomum cervi</i>	23 – 30

The results with *Paramphistomum cervi* are summarized in Table 3. Depending on the treatment of the flukes before examination, the character of the respiration, in particular the $Q_{O_2}^{CN}$ and the R.Q., varied considerably.

Immediately after removal from the host tissues the flukes' oxygen consumption was very low, but nevertheless they produced considerable amounts of carbon dioxide; the longer these flukes were kept *in vitro* the higher their oxygen consumption rose and the R.Q. decreased finally to 1.7.

TABLE 2
RESPIRATORY ACTIVITY OF NEMATODE PARASITES

Species	No. of Observations	Q_{O_2}	Q_{CO_2}	R.Q.	$Q_{CO_2}^{N_2}$	$Q_{O_2}^{CN}$	Inhibition by 0.001M KCN (%)
<i>Ostertagia circumcincta</i>	1	-7.4	5.9	0.8	3.1	-3.6	50
<i>Haemonchus contortus</i>	2	-4.6	4.0	0.87	5.3	-2.3	50 (0.02M)
<i>Syphacia obvelata</i>	3	-4.4	4.7	1.1	2.5	-2.7	40
	2	-5.0	8.6	1.7	6.7	-8.5	*
<i>Heterakis spumosa</i>	2	-3.8	4.0	1.1	2.2	-1.9	50
<i>Strongylus equinus</i>	2	-1.1	3.3	3.0			
<i>Strongylus vulgaris</i>	2	-1.1	3.6	3.3		-0.92	15

* In this experiment cyanide caused an activation of 70 per cent.

Paramphistomum cervi has a low area/volume ratio. Hence it was considered that the high R.Q. might have resulted from poor oxygen penetration and these parasites were, therefore, re-examined in an atmosphere of oxygen. The Q_{O_2} was greatly increased under these conditions and an R.Q. of 1.7 was obtained, which was similar to that found in experiments with *Syphacia*.

The effect of cyanide on the respiration of the flukes was unusual. It caused an activation of the oxygen consumption in fresh flukes of, usually,

over 100 per cent. which decreased somewhat during the period of the experiment. The activation of oxygen consumption by cyanide decreased as the time the flukes were kept *in vitro*, before the experiment, was increased (see Table 3). Carbon dioxide production was also increased by cyanide so that the R.Q. of a given experiment was unaffected by the addition of cyanide. In an atmosphere of oxygen, cyanide either inhibited up to 30 per cent. or caused only a very slight activation.

TABLE 3
RESPIRATORY ACTIVITY OF *PARAMPHISTOMUM CERVİ*

Treatment of Trematodes Before Examination	Q_{O_2}	Q_{CO_2}	R.Q.	$Q_{CO_2}^{N_2}$	Effect of 0.001M KCN
Examination in air immediately after the death of the host	- 0.03	0.7	8 and over	0.7	Over 100% activation
Examined in oxygen immediately after the death of the host	- 0.71	1.2	1.7	-	0.30% activation
Left 6 hours on host tissue at 20°C.	- 0.17	0.87	5.6	0.7	30% activation
Left 6 hours in saline at 20°C.	- 0.23	0.55	2.4	0.28	Slight inhibition
Left 6 hours in saline at 38°C.	- 0.18	0.3	1.7	0.28	No effect

IV. DISCUSSION

Certain features of the techniques used require brief comment. Thus, as the unusual action of cyanide on *Paramphistomum cervi* may suggest that the technique was defective, it should be emphasized that the same procedures were used, without unusual results, when other helminths were examined. Laser (1942) has pointed out certain limitations of the direct method of Warburg in determining respiratory quotients which apply to the present work. Anaerobic carbon dioxide determinations were not carried out in an attempt to measure anaerobic glycolysis. As nematode parasites probably produce only small amounts of lactic acid (Rogers and Lazarus 1948) but may produce considerable amounts of other acids, classical methods of determining anaerobic glycolysis may be misleading when used with these organisms. Therefore anaerobic carbon dioxide determinations were used in this investigation to give rough indications of the relative activity of anaerobic catabolic mechanisms in the species examined. However, it can be seen from the results given in Table 2 that no clear distinctions could be drawn between the different parasites examined by this method.

The oxygen consumption of all the nematodes examined was found to be greater than that of *Ascaris lumbricoides* (Laser 1944) but similar to that of the parasites examined by Rogers (1948). The trematode, *Paramphistomum cervi*, had a much lower Q_{O_2} than the nematode parasites.

The organisms examined, with the exception of *Paramphistomum cervi*, were of a similar shape but differed in size. The surface area of a given weight of tissue, therefore, varied also, and respiratory activity might best be

examined per unit surface area rather than on a weight basis. Though the dry matter in the parasites varied slightly (see Table 1) the specific gravity of the parasite tissues has been taken as constant for the purpose of estimating the relative surface area. On this assumption the relative surface areas of the nematodes used was calculated. The diameter of each species was obtained by measuring three male and three female nematodes at several points along their length and the mean average was used in calculating surface area. The relative surface area for a given weight of parasite tissue and the relative oxygen consumptions per unit surface of each parasite are shown in Table 4. Though the distinction between the respiratory activity of the trematode *P. cervi* and the several nematodes is still evident, the oxygen consumption rates of the nematode parasites show a smaller range when calculated on a surface-area basis.

TABLE 4
RELATIVE OXYGEN UPTAKE PER UNIT SURFACE AREA OF THE PARASITES

Species	Relative Surface Area	Relative Oxygen Uptake	Oxygen Uptake per Unit Surface Area
<i>Paramphistomum cervi</i>	1	0.03	0.03
<i>Strongylus equinus</i>	2	1.1	0.5
<i>Strongylus vulgaris</i>	3	1.1	0.4
<i>Haemonchus contortus</i>	12	4.6	0.4
<i>Heterakis spumosa</i>	13	3.8	0.3
<i>Ostertagia circumcincta</i>	14	7.4	0.5
<i>Syphacia obvelata</i>	18	4.7	0.3

Among the parasites examined, *Haemonchus contortus*, *Ostertagia circumcincta*, and *Paramphistomum cervi* contain haemoglobin (Rogers 1949 and unpublished data) and such organisms might, therefore, be expected to give higher Q_{O_2} 's than those which lack haemoglobin. That no such distinction was found is in agreement with the observation that haemoglobin in nematode parasites does not function as an effective oxygen carrier (Rogers 1949b).

As the R.Q. results from a number of different simultaneous reactions and does not simply indicate the type of substrate metabolized, the value of such determinations might be doubted. However, one of the possible interpretations of the high R.Q. values obtained for *Syphacia*, *Strongylus*, and *Paramphistomum* is that active carbohydrate-to-fat conversions occur in the tissues of these parasites. It is perhaps significant that the organisms giving high R.Q. values came from those regions of the host where conditions were most suitable for bacterial fermentation, e.g. the caecum of the horse and rodent and the reticulum of the ruminant. Such organisms might be expected to show considerable variation in the R.Q. as the varying degrees of interconversion occurred. This variation in the R.Q. was found for *Syphacia* and *Paramphistomum*. A change from fermentative metabolism towards a more aerobic metabolism occurred in *Paramphistomum* as the period increased during which it was incubated under aerobic conditions (see Table 3).

Cyanide was an effective inhibitor of oxygen consumption in all species of parasites which had an R.Q. below about 1.1. Higher R.Q.'s. were associated with a lower sensitivity to cyanide until, in *Paramphistomum* and, on occasions, in *Syphacia*, cyanide caused a pronounced increase in the Q_{O_2} . Activation by cyanide was not obtained when *Paramphistomum brei* was examined in the presence of methylene blue. The activation of oxygen uptake by cyanide is not commonly found when animal tissues are examined. However, von Brand and Tobie (1948) have reported that oxygen uptake of trypanosomes is stimulated by cyanide, which also causes an increase in glucose utilization.

In general, the present work has shown that the pattern of respiratory metabolism in nematode parasites may vary greatly, even among morphologically related forms such as the strongylids *Haemonchus*, *Ostertagia*, and *Strongylus*. Indeed, the nature of the respiratory activity of *Strongylus* appears more like that of the oxyurids *Syphacia* and *Heterakis* than that of *Haemonchus* and *Ostertagia*. The respiratory metabolism of the one trematode examined, *Paramphistomum cervi*, was quite different from that of the nematodes.

V. ACKNOWLEDGMENT

The author is indebted to Dr. W. P. Rogers for his helpful suggestions and assistance in preparing the manuscript.

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