

THE RESPIRATION OF NEMATODES OF THE ALIMENTARY TRACT

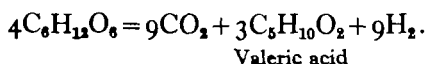
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(With One Text-figure)

BECAUSE of their peculiar habitat, tacitly assumed to have an oxygen content small enough to be negligible, the metazoan parasites living in the alimentary canal of various animals have long been the subjects of respiratory studies. The work of the earlier investigators reached its culmination in Weinland's animal fermentation theory, put forward to explain the anaerobic existence of *Ascaris lumbricoides* and *A. megaloccephala*. The fundamentals of the theory are embodied in the well-known equation



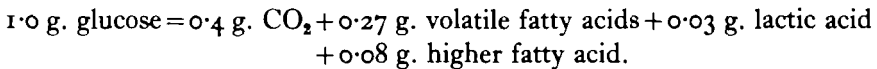
For more than 20 years Weinland's work was accepted without question—indeed his theory is still quoted, unimpeached, in many of the standard textbooks of the biological sciences—and it was not until 1924 that some of his experiments were repeated. Where Weinland had found only valeric acid, either in the saline in which ascarids had been kept, or in the products of the fermentation of glucose by extracts of the worms, Fischer now found lactic and phosphoric acids as well. The finality of Weinland's results thus became doubtful, and it was suggested that the valeric acid might be the outcome of the activity of bacteria contaminating his material. Unfortunately, the actual part played by bacteria in the experiments that have been done by the various workers has never been clearly elucidated. The background of experimental evidence relating to their importance, or otherwise, is so meagre that the question is inevitably controversial, and the reader is referred to Slater (1928) and von Brand (1934) for the views of the two schools of thought. A close study of the question will reveal some fallacies in the reasoning of both.

Leaving aside the unsolved problem of what are, and what are not the final products of the breakdown of glycogen by nematodes, we may go back to what is, after all, the fundamental problem: Are nematodes anaerobic, that is to say, can they continue to live and perform muscular movement without oxygen?

During the experiments which led the earlier observers to believe that the intestinal nematodes were anaerobic, the ascarids existed in a state approaching one of suspended animation. Slater (1925), remembering that the normal environment

of the worms called for little energy production apart from that required for movement, saw the significance of this, and undertook the repetition of the experiments with *Ascaris*, but with the important difference that he forced the worms to move by periodically stimulating them with mild induction shocks. Under such conditions they died within 48 hr., while controls, exposed to air and subjected to the same treatment, continued to live. Slater concluded that "intestinal worms of this type could make use of oxygen when it was available and further . . . with a fixed amount of medium into which the end products could diffuse, they could not live anaerobically and perform muscular work for more than a limited time".

It must be admitted that Slater's work has not received the consideration due to it, and it is probably no exaggeration to say that most helminthologists and most biologists regard the intestinal nematodes as being really anaerobic. The current conception of their energy production is still that of Weinland, even though some alterations of his original equation have been suggested (see Schulte, 1917; von Brand, 1934). For example, von Brand thinks the most likely one to be:



He does not believe, however, that all the end-products have yet been found.

The controversial state of the problem of the respiration of nematodes of the alimentary tract will be apparent from the above short discussion of the literature, and there is an obvious necessity for more experimental work, especially work concerned with the primary issue—the need of intestinal nematodes for oxygen.

MATERIAL

The material used in the present experiments were nematodes from the alimentary canal of sheep. The method of collecting them has already been described (Davey, 1936). They were kept in a simple Ringer solution having the composition NaCl 0.9%, KCl 0.042%, CaCl₂ 0.024%. The worms were considered dead when all movement had ceased; touching with a blunt needle was the method adopted in trying to elicit some movement.

EXPERIMENTAL

At the outset it must be stated that I have been fortunate in my choice of material. *Ascaris* is an exasperating inert creature, and its large size is the only good quality it can be said to have for respiration experiments. Slater had to stimulate it to make it move, while the subjects of my experiments would swim about unaided in their medium as long as they were able. For most of the experiments the oxygen-free atmosphere was attained with a Macintosh and Fildes' jar. The worms were kept in Ringer in a pyrex Erlenmeyer flask, and placed in the jar at laboratory temperature. They are apparently unharmed by some hours' exposure (at least 3) to room temperature, and the half-an-hour or so required for

the completion of a hydrogen atmosphere in the jar is well within the bounds of safety.

When all the oxygen had been removed the jar was incubated, and the worms were observed at intervals as well as was possible. Movement remained normal for some time, but after 2 or 3 hr. without oxygen it became distinctly slow and spasmodic; most specimens of *Ostertagia circumcincta* and *Trichostrongylus* sp. had ceased completely to move after 4 hr. If air were now admitted, the power of normal movement was quickly regained. Even after 6 hr. without oxygen *Ostertagia* began to move again within 15 min. of exposure to the air, but in one experiment, in which ten worms were kept in the jar for 15 hr., the three that recovered did not begin to move for 5 hr. after releasing the hydrogen, and for the duration of their life their movements were very slow and infrequent. In the experiments in which this species was kept under anaerobic conditions for 24 hr. there was no recovery. Generally speaking, the longer the period without oxygen, the longer was the time needed for recovery, until eventually the oxygen lack had been maintained so long that death resulted.

These reactions of *Ostertagia* to oxygen-free conditions may be taken as indicative of the reactions of all the nematodes that were studied. The results of the experiments on the various nematodes are summarized in Table I.

Table I. *Effect of anaerobiosis on nematodes from sheep*

Nematode	Maximum period of anaerobiosis from which complete recovery was observed	Maximum period of anaerobiosis from which partial recovery was observed	Minimum period of anaerobiosis from which no recovery was observed
<i>Ostertagia circumcincta</i>	7 hr.	15 hr. (3 of 10 recovered)	24 hr.
<i>Trichostrongylus colubriformis</i> , <i>T. vitrinus</i>	9 hr.	15 hr. (9 of 25 recovered)	24 hr.
<i>Cooperia curticei</i>	9 hr.	15 hr. (4 of 10 recovered)	24 hr.
<i>Cooperia oncophora</i>	24 hr.	40 hr. (3 of 10 recovered)	48 hr.
<i>Nematodirus filicollis</i>	24 hr.	40 hr. (5 of 50 recovered)	48 hr.

Note. The use of the word "observed" in the subheadings to this table is important. Many experiments were done and the table represents a summary of them, but it is not suggested that the figures given are absolute. Quite possibly, complete recovery might take place after longer periods of oxygen lack than those indicated, and possibly the figures given in the third column are too high, although in view of the large number of worms used in the experiments, it is improbable that they are too low.

It will be seen that there is some degree of variation in the period of anaerobiosis necessary to kill the different species, but in all cases it is a short time compared with the 4-12 days which the controls lived when kept in Ringer exposed to the air. Further, and an important point, the period during which movement is possible is much shorter, and intestinal nematodes, even though they lead a parasitic

existence, cannot live continuously in their normal habitat without moving. They must move, either to the mucous membrane in search of food, or to resist the tendency to be carried down the alimentary canal under the influence of peristalsis. It will be seen, too, that the gross effect of the oxygen lack on the nematodes is much like its effect on isolated vertebrate muscle. There is first a period of contraction, but, because oxygen is absent, "fatigue" more or less quickly sets in, and finally, if fatigue is made to continue long enough, death results.

It would seem from these experiments that the nematodes of the sheep, at least, have an aerobic metabolism, and that prolonged anaerobiosis is fatal. Slater, however, on the basis of Fischer's observation that the medium in which ascarids have been kept contains an appreciable amount of lactic acid, made the interesting suggestion that normally the lactic acid resulting from the breakdown of glycogen permeates the body wall into the intestinal contents of the host and, with the continual passage onwards of these, is removed from the neighbourhood of the worms. Because the lactic acid would be got rid of in this way, the worms would be freed of any necessity to oxidize it. It will be apparent that Slater's suggestion makes nematodes virtually anaerobic, but because the mechanism of their energy production is that which is involved in the anaerobic phase of the lactic acid cycle, they are brought into line with the general theory governing energy production in all animals. Admittedly, if no resynthesis of glycogen occurred, the process would be wasteful, but no more wasteful than Weinland's fermentation hypothesis, and as the nematodes are parasites, and are surrounded by a continuous supply of food material, it might not be important that energy should be conserved within their tissues. The idea was definitely interesting and within the bounds of possibility. It was therefore investigated.

The apparatus that was developed to give a continually changing environment under anaerobic conditions is illustrated in the diagram. A 2 litre flask is connected by means of a siphon arrangement with a valve, the outlet of which dips into the tube holding the worms. The valve is of the screw-pin type and actually is the kind used on the Macintosh and Fildes' jars supplied by Baird and Tatlock. The screw-pin makes it reasonably easy to adjust the flow of liquid to any desired rate. The tube for the worms carries a two-way bung, through one hole of which the outflow tube is fitted, while the outlet from the valve can be inserted through the other.

In the setting up of the apparatus, the flask is nearly completely filled with Ringer, and the bung, carrying the siphon, a tube for connexion with a hydrogen Kipp, and a third tube closed with rubber tubing and a screw clip, is fitted. The valve being kept closed, the Ringer within the flask is boiled for some 10-15 min., allowance having been made for this by appropriate dilution with water. While the contents of the flask are still boiling its outlets are closed, the valve is opened, and under the head of steam so developed the siphon is filled, and the valve is closed again. The apparatus is quickly transferred to the incubator, and the tube serving as a lead to the Kipp is pushed through the "thermometer hole" in the roof of the incubator. A certain degree of cooling will have taken place during the transfer, and to ensure against this the Ringer is again brought to boiling for a short while. With steam issuing from the Kipp connexion, and hydrogen from the Kipp, the two are joined. Immediately, the screw clip closing the third entrance to the flask is opened and hydrogen rushed through.

Both the Kipp and the third tube were found to be essential if anaerobic conditions were to prevail in the apparatus. Boiling the Ringer and covering with liquid paraffin before cooling had not proved satisfactory, nor, when the Kipp was incorporated, was mere reliance on the issuing steam to remove the air above the boiling Ringer efficacious. The third tube was therefore introduced, and with the aid of this hydrogen was rushed through to remove the mixture of steam and air within. The requisite anaerobic conditions could be shown to be present by testing for hydrogen at the screw-clip exit.

When the temperature of the apparatus had fallen to that of the incubator, the tube containing a small quantity of Ringer with the worms was connected up. It will be noticed that the outlet from the valve leads to the bottom of the tube, and that the outflow rises above the entrance of the siphon into the valve. By manœuvring the tube all air bubbles can be removed from valve and tube when the flow is commenced.

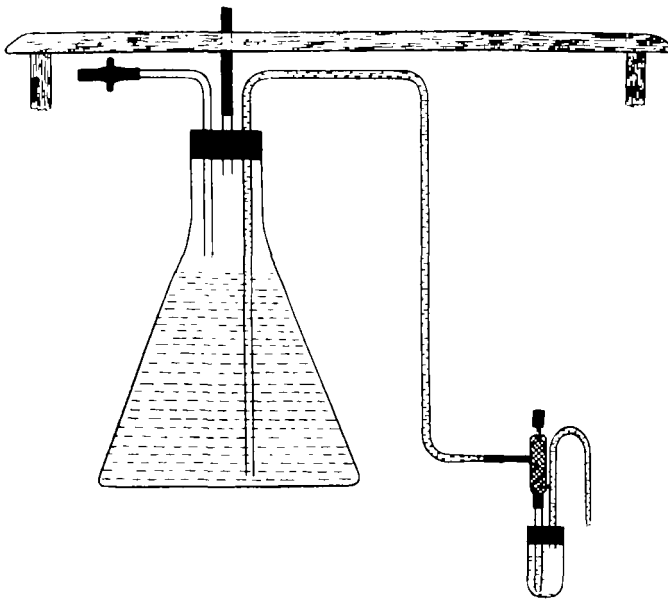


Fig. 1.

The worms used in the experiment were *Ostertagia circumcincta* and *Trichostrongylus vitrinus*. These two species were chosen because they withstand only 24 hr. absence of oxygen. Others, like *Nematodirus*, require 48 hr. anaerobiosis to kill them, and the technical difficulties involved in catering for a copious continuous flow of Ringer for 48 hr. discouraged their use in the experiments. However, *Ostertagia* and *Trichostrongylus* may be considered as typical representatives of the abomasal and intestinal faunas, respectively, of the sheep. The worms had not been removed from the slaughtered host more than 1½ hr. before they were subjected to experimentation. The valve was so arranged that a drop issued from the out-flow about once every 2 sec. Thus, in one experiment 1200 ml. flowed over the worms in 24 hr. Because the tube, when the bung was fitted, held 7 ml. the medium was changed just over seven times every hour, although, because the valve outlet dipped to the bottom of the tube, and this was where the worms remained, the changing of the medium in the immediate vicinity of the worms was far more frequent. In any event, it is believed that Slater's suggestion received a fair trial.

The results of the experiments showed that the percolation made no difference to the time during which the worms could live under anaerobic conditions. The exact time at which anaerobiosis was complete in the tube cannot, of course, be gauged; but, in the quoted experiment, 5 hr. after the valve had been opened only two *Ostertagia* were moving and these only spasmodically. The percolation was kept up for 24 hr. and the ten *Ostertagia* and ten *Trichostrongylus* were then transferred to Ringer exposed to the air. Twelve hours later one *Ostertagia* recovered and moved slowly for a further 2 days, but ceased to move before the controls, kept in Ringer which had passed through the apparatus, commenced to die. It may be said, therefore, that these nematodes cannot continue to live in the absence of oxygen even in a constantly changing medium, and what is true of these two typical members of the Trichostrongylidae is probably true of other intestinal nematodes.

So far there are very close and very obvious resemblances between the behaviour of these parasitic worms under anaerobic conditions, and the behaviour of free-living animals in similar circumstances. Such various types as leeches, earthworms, and snails can live for quite long periods without oxygen, and Lesser (1907-8), in his experiments with earthworms, noticed that after the worms had been in hydrogen for some time, they behaved as though they were narcotized. Even so highly evolved an animal as the frog can resist oxygen lack for as long as 17 hr. (Lesser, 1908). The capacity of these animals to live even a short anaerobic life constitutes, however, a marked contrast with the inability of mammals to live anaerobically. A man, for example, would die if he were subjected to complete absence of oxygen for longer than about 3 min., although the explanation of this difference between him and other animals has not yet been given. At first it was thought that the capacity of some animals to live anaerobically for a while depended on stored oxygen carried in their haemoglobin, but Leitch (1916; see Barcroft, 1934, p. 116) showed that the oxygen carried by *Planorbis* in this manner would serve its ordinary needs for about 3 min. only, and that the haemoglobin of the larva of *Chironomus*, which can live for 5 days in water freed from oxygen, is nearly half saturated when roughly only 0.001 ml. of oxygen per litre of water is present. As Barcroft says (p. 133): "It is difficult to believe that oxygen is stored for any length of time in low forms of life."

CONCLUSION

In the light of the above experiments it may be said that, while the nematodes of the alimentary canal of sheep are capable of a limited anaerobic existence, they are killed by prolonged periods without oxygen, and that their reactions to a period of limited anaerobiosis are not significantly different from those of the other animals which have been studied, some of which are as far removed from nematodes as the earthworm and the frog. It was hoped that quantitative experiments might have been done on certain aspects of the chemistry of the metabolism of the nematodes, but so far the difficulties resulting from their extremely small size have not been surmounted. Some thousands of individual *Ostertagias* are required to give a dry weight of as little as 0.05 g., and to obtain an idea of the mechanism of

the glycogen breakdown many series of experiments would be required. It is therefore suggested that the results of the experiments on the sheep nematodes which have been described above should be extended to other intestinal nematodes more suited to quantitative chemical study. The problem of the respiration of intestinal nematodes will not be near a final solution until such a quantitative study is made.

In view of the controversies being waged around this problem of the respiration of nematodes, and of the conflicting results of different observers, a summary of the evidence in favour of the hypothesis that the metabolism of intestinal nematodes is aerobic may be instructive. Such a summary is given below:

1. Prolonged anaerobiosis (24-48 hr.) kills *Ostertagia circumcincta*, *Trichostrongylus colubriformis*, *T. vitrinus*, *Cooperia curticei*, *C. oncophora*, and *Nematodirus filicollis*, all of which are nematodes parasitic in the alimentary canal of sheep. It is also fatal to *Ascaris*, on the condition that the worms are made to move.

2. Anaerobiosis is still fatal to the nematodes from the sheep even when the medium around them is continually changing and percolating over them, so that any by-products of their metabolism which may have permeated their cuticle are constantly being removed from their environment.

3. It has been shown from measurements of the oxygen consumption that both *Ascaris* (Harwood & Brown, 1933), and *Ancylostoma caninum* (Harwood & Brown, 1933) can utilize oxygen when it is available. These measurements are, however, difficult to interpret, because no relationship can be demonstrated as yet between the oxygen uptake and the carbon dioxide output, nor did *Ascaris*, after a period without oxygen, show an oxygen debt.

4. In a paper to be published shortly it will be shown that there is much evidence that intestinal nematodes synthesize oxyhaemoglobin. Whether they require to obtain from the host's blood the primary constituents for this synthesis is immaterial at this stage; what is important is the fact that a synthesis, however simple, almost certainly occurs. Oxyhaemoglobin is the most important respiratory pigment that is known, and the fact that nematodes parasitic in the alimentary canal of various vertebrates probably synthesize this substance must be regarded as significant. Apparently the amount of oxygen present in the contents of the alimentary canal is so small (Tappeiner, 1883; Long & Fenger, 1917; von Brand & Weise, 1932) that haemoglobin might be a necessity for the intestinal nematodes to lead an aerobic life. In any event, it would be remarkable if the nematodes of the alimentary canal synthesized haemoglobin and made no use of it.

5. Keilin in 1925 described a new respiratory pigment which he named cytochrome. It was present in the animal tissues he examined, including those of *Ascaris*; it was also present in aerobic bacteria, but *not* in anaerobic bacteria.

SUMMARY

1. An investigation has been made of the dependence on oxygen of some nematodes parasitic in the alimentary canal of sheep. Contrary to current conceptions it is shown that they are not anaerobic, and that all die within 48 hr.

in the complete absence of oxygen. This is true of *Ostertagia circumcincta* and *Trichostrongylus vitrinus* even when arrangements are made—the apparatus is described—for any by-products of their metabolism which may permeate their cuticle to be removed from their environment.

2. A summary is given of the evidence in favour of the view that the metabolism of nematodes of the alimentary tract is aerobic.

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