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

I. OXYGEN TENSIONS IN THE NORMAL ENVIRONMENT OF THE PARASITES

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Summary

As a preliminary to the study of the relative importance of aerobic mechanisms in the metabolism of *Nippostrongylus muris, Nematodirus spathiger, Nematodirus filicollis,* and *Haemonchus contortus* at oxygen pressures of the normal environmental fluids of these parasites, the determination of oxygen in the contents of the small intestine of the rat and sheep, and the abomasum of the sheep, has been carried out. The method which was used allowed measurements to be made close to the mucosa of the alimentary canal of anaesthetized animals in which the circulation was left intact, and the procedure ensured that conditions in the normal gut were very little disturbed.

In the small intestine of the rat, oxygen tensions were found to vary from 30.2 to 8.9 mm. of mercury; these readings were usually lower as distances from the pylorus were increased. Oxygen was always present in the contents of the small intestine of the sheep close to the mucosa, but in smaller amounts than those found under similar conditions in the rat. As the oxygen tensions in regions very close to the intestinal mucosa of rats were influenced by the nature of the gases inspired by the animals, it is apparent that some oxygen diffused into the intestinal contents from the blood stream.

I. INTRODUCTION

A knowledge of the relative importance of aerobic and anaerobic mechanisms in the metabolism of nematode parasites of the alimentary canal is a necessary fundamental to most studies on the physiology of these animals. A direct experimental examination of the problem has not, as yet, been made *in vivo* nor, indeed, have satisfactory *in vitro* observations been made. The work of Slater (1925) and Davey (1938), who studied certain nematode parasites *in vitro,* must be considered inconclusive. Slater showed that starving *Ascaris lumbricoides,* when stimulated to activity under aerobic conditions, maintained movement longer than parasites in an oxygen-free medium, but it is doubtful if these results have much significance concerning the normal physiology of the actively feeding, largely motionless parasites *in vivo* (Archer and Peterson 1930). Davey found that several different trichostrongyle parasites of sheep, when placed in non-sterile media, survived longer under aerobic conditions. These results must be interpreted with caution because the growth of bacteria in the medium markedly influences the survival of nematode parasites *in vitro,* and the nature of the gas phase may have affected the parasites indirectly by influencing

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the nature and degree of bacterial growth. Davey, however, was aware of this complication and it is probable that bacterial growth, at least in his non-nutritive media, was slight.

The determination of the relative importance of aerobic and anaerobic metabolism in nematode parasites has been approached from another point of view by von Brand and Weise (1932) and Toryu (1934), who have shown that oxygen was either absent from the gut fluids of several host species, or present only in small amounts, thus indicating the unlikelihood of active aerobic mechanisms in parasites inhabiting those fluids. However, it might be expected on physico-chemical grounds that oxygen would diffuse from arterial blood, through the thin intestinal mucosa, into the lumen of the intestine; indeed, the permeability of the gut wall to gases has been demonstrated by McIver, Redfield, and Benedict (1926). It might be expected, then, that oxygen pressures, though very low in the bulk of the gut fluids, might be quite appreciable close to the gut mucosa. Methods needing relatively large samples of gut contents taken from dead animals might not indicate the true amounts of oxygen available to small parasites of the alimentary tract which usually live close to the mucosa or even between the villi (Porter 1935). It is clear then that the determination of the oxygen available to small nematode parasites *in vivo* should be carried out by the use of animals with an intact circulatory system, and the method should allow measurements to be made close to the mucosa of the alimentary tract without disturbing the milieu which exists in the normal animal.

The present work aims to define the limits of the oxygen pressures available to parasites in the alimentary tract of the rat and of the sheep. The ability of parasites to utilize oxygen at the pressures at which it occurs in their normal environments will be dealt with in a later publication.

II. Physical and Chemical Methods

The electrometric method of Brink and Davies (1942) was considered suitable for the determination of dissolved oxygen in fluids of the alimentary tract. The use of this method allowed the measurements to be carried out in animals with the blood supply to the alimentary canal intact. Further, oxygen tensions in localized regions close to the intestinal and abomasal mucosa could be measured. Because results obtained with the "oxygen" electrode are sometimes variable (Brink and Davies 1942), the characteristics of the electrodes used in the present experiments will be described in detail to indicate the degree of accuracy which may be accorded to the results obtained.

(i) *The Electrodes.*—It was usually necessary to follow changes in oxygen pressures occurring over short periods of time, and open electrodes were therefore frequently used. However, the results were checked, whenever possible, by the use of recessed electrodes. The dimensions of typical electrodes were as follows:

Open electrode No. 2, outside diameter at tip, 0.96 mm., diameter of platinum at tip, 0.40 mm.

Recessed electrode No. 4, outside diameter at tip, 0.91 mm., diameter of platinum at tip, 0.65 mm., depth of recess, 0.56 mm.

The electrodes were mounted in 20-cm. glass holders; leakage to earth was reduced by coating the surface of the shaft of the holders with methylchlorosilane mixture.

The characteristics of the current-voltage and time-current relationships of open electrode No. 2 are shown in Figures 1 and 2. Open electrodes were

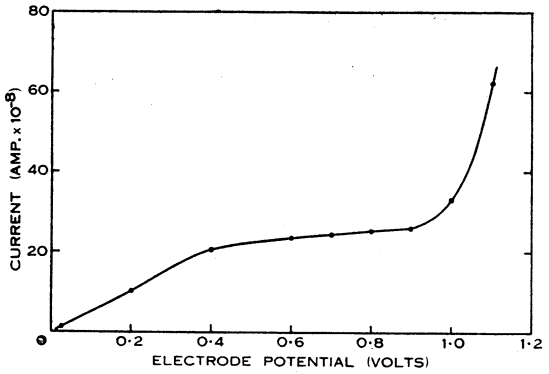

Fig. 1.—The current produced from open electrode No. 2 in air-saturated 0.15M NaCl at 24°C. at different potentials *v.* 0.15M calomel half-cell. Readings were taken 40 sec. after closing the switch.

calibrated before and after each set of experiments (Fig. 3). It was found that the change in the response of open-type electrodes over an experimental period of two hours might be as great as 20 per cent., but as a rule it was in the region of 10 per cent. Recessed electrodes were found to be more stable than open electrodes but not to the extent indicated by Brink and Davies (1942).

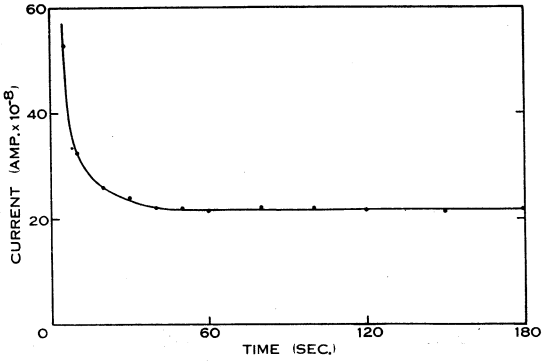

Fig. 2.—Time-current relationships of open electrode No. 2 in air-saturated 0.15M NaCl at 24°C. Electrode potential 0.8 volt *v.* 0.15M calomel half-cell.

When not in use, open electrodes were stored with tips in 0.9 per cent. saline; recessed electrodes were stored dry. Before each experiment the shafts

of the electrodes were wiped with a dry cloth. Before calibrating an electrode for a day's work, it was "run in" at a potential of 1.1 volts for 1 to 2 minutes. All these procedures increased the stability of the electrodes.

(ii) *The Measurement of Electrode Currents.*—Electrode currents were measured with a valve meter (Roberts 1939) calibrated from 5×10^{-5} to 1×10^{-9} amperes over 5 scales of 100 divisions. The electrodes were connected with the meter as shown in Figure 4. A 0.15M NaCl calomel half-cell was used to complete the circuit through a salt bridge. Electrodes and the half-cell were held in clamps arranged to give a high resistance to earth.

(iii) *The Calibration of the Electrodes.*—Physiological saline, warmed to 37°C., in which the oxygen content was lowered by means of a stream of nitrogen, was used to calibrate the electrodes. Dissolved oxygen was estimated

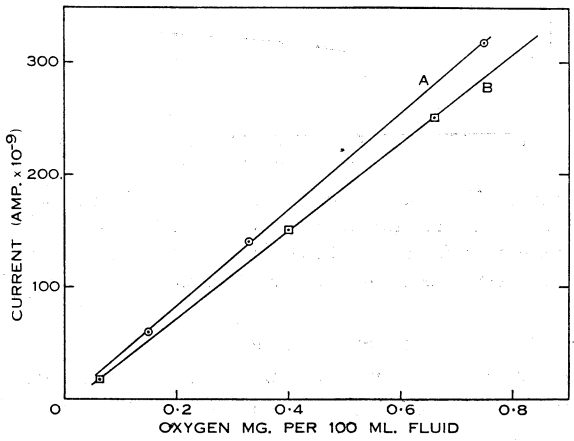

Fig. 3.—Current-oxygen tension curves of open electrode No. 2. Calibration was carried out using 0.15M NaCl at 37°C. containing known amounts of oxygen. Electrode potential 0.8 volt *v.* 0.15M calomel half-cell. Readings were taken 40 sec. after closing the switch.

chemically by Winkler's method, corrections being made for the addition of oxygen in the reagents (Krogh 1935). Calibration curves of open electrode No. 2 are shown in Figure 3.

III. Biological Methods

Experiments with rats were carried out with animals of about 100 g. weight which had been starved for several hours before use. "Nembutal," 40 mg. per kg., given subcutaneously, was used as an anaesthetic. After being anaesthetized, the rats were tied to a small operating board equipped with clamps for the controlled positioning of electrodes.

The sheep were anaesthetized with "Kemithal" after atropine.

The surgical methods were simple and are described when reference is made to specific experiments.

IV. Procedure and Results

The abdomen of an anaesthetized rat was shaved and a 2-cm. incision made in the body wall over the section of the small intestine to be examined. Bleeding of the cut surfaces was checked with 1/5000 adrenalin in saline. About 2 cm. of the small intestine was lifted gently through the incision and supported on warm saline-damped gauze. A small purse-string suture (000 silk) was placed in position on the intestine and a stab wound made in the centre of the suture. The intestine was gently pressed to expel a little of the intestinal contents, and any blood that was present, through the wound. The tip of an electrode which had just been calibrated was inserted in the stab wound, care being taken to avoid the introduction of air into the intestine. The suture was pulled tight around the shank of the electrode and the intestine was lowered into the body

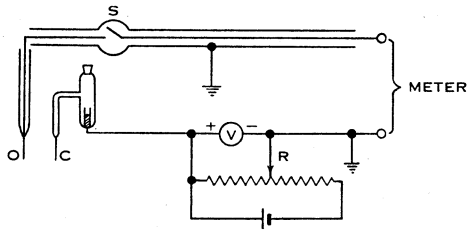

Fig. 4.—Method of connecting the electrodes to the meter. The switch, *S*, and the connection of the "oxygen" electrode, *O*, to the meter were covered by an earthed screen. The "oxygen" electrode and the calomel half-cell, *C*, were connected through a 0.15M NaCl bridge. A 300-ohm wire-wound potentiometer, *R*, and a 1½-volt dry cell were used.

cavity. The side of the electrode was then pressed gently against the intestinal mucosa, the calomel half-cell was lowered to make contact, through a fine capillary, with the surface of the intestine near the "oxygen" electrode, and the body cavity was closed with a loose suture. Ten minutes after inserting the electrode the meter was connected and the current readings were taken with the animal breathing either air, or 95 per cent. oxygen-5 per cent. carbon dioxide, or 95 per cent. nitrogen-5 per cent. carbon dioxide. At the end of the experiment the animal was killed with chloroform and the position of the electrode, in relation to the mucosa, and its distance from the pylorus were determined. If any blood was seen in the lumen of the intestine the result was discarded. Immediately the experiment was concluded, the "oxygen" electrode was re-calibrated; if the current-dissolved oxygen calibration curves obtained before and after the experiment differed by more than 15 per cent., which was seldom, the results were considered too unreliable to use. In this manner, oxygen pressures in gut fluids near the mucosa of the small intestine were determined at different distances from the pylorus in a number of rats. Both normal rats and rats infected with *Nippostrongylus muris* were used.

The respiratory rate of the rats before they were anaesthetized was usually about 80-120 per minute. When oxygen pressures were being taken the rate was about 40-50 per minute, though it changed when the animals were allowed to breathe the different gas mixtures.

The results of these experiments are listed in Table 1. It can be seen that small amounts of oxygen were always present in the small intestinal contents of the rat close to the gut mucosa; the amounts found usually grew smaller as the distance from the pylorus was increased. On occasions, and especially when the electrode tip was pressed against the gut mucosa, the oxygen tensions recorded were markedly affected by the nature of the gas inspired by the experimental animals (see Fig. 5).

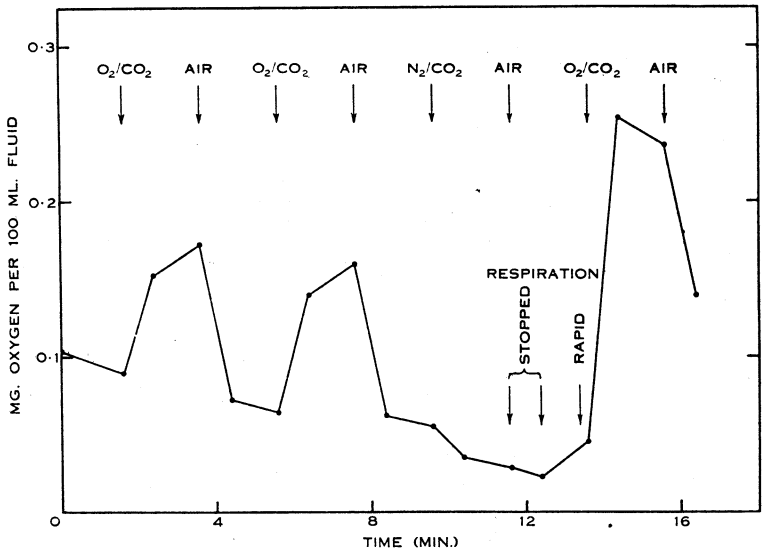

Fig. 5.—The effects of the nature of inspired gas mixtures on the oxygen content of the fluid very close to the mucosa of the rat small intestine. For further explanation, see text.

In the examination of the alimentary tract of the sheep, the cardiac end of the abomasum was first exposed and the electrode inserted into the fundus through a purse-string suture as before. After taking readings, the stab wound in the fundus was closed and a numbered tag fastened at the site. Readings were taken with the electrode inserted at the pyloric end of the abomasum and at several positions along the small intestine. Owing to the thickness of the wall of the abomasum, the position of the electrode tip in relation to the mucosa was difficult to assess. Also, it was difficult to avoid the introduction of air into the abomasal contents when placing the electrode in position. Other difficulties were caused by the long leads to the electrodes necessitated by the size of the animal. The results obtained from examination of the abomasum may thus have little significance. The effect on oxygen tensions in the gut fluids of allowing the animal to breathe nitrogen-carbon dioxide gas mixture was not examined because of the large number of determinations made on the one sheep.

At the conclusion of the experiment the animal was killed with chloroform, the alimentary tract removed, and the positions of the numbered tags marking the sites where determinations had been made were noted. Two animals were examined in this manner. The results obtained are given in Table 2. On two occasions when the "oxygen" electrode was pressed against the intestinal mucosa, a rhythmic rise and fall in the electrode current was noted. The readings indicated that the partial oxygen pressure rose as high as 17 mm. of mercury.

TABLE 1

OXYGEN TENSIONS IN THE CONTENTS OF THE SMALL INTESTINE OF THE RAT CLOSE TO THE MUCOSA AT DIFFERENT DISTANCES FROM THE PYLORUS

Position of Electrode: Distance from Pylorus (cm.)	Partial Pressure of Oxygen (mm. Hg at 16°C.)	Number of Parasites in Rat Intestine
2	23.5	None
5	14.3	None
6	21.8	None
12	30.2	Moderate
13	28.6	None
13	19.0	Moderate
15	23.5	None
19	19.0	Few
20	28.2	Few
25	22.2	Some solid food present
30	7.9	None
30	18.2	Moderate
35	17.5	None
43	19.0	None
75	11.2	None

During the experiments the respiratory rate of the sheep usually fell to between 8 and 10 per minute, less than half the rate noted before anaesthesia.

V. DISCUSSION

Apart from the inaccuracy of the actual method of measuring oxygen tensions as used in the present experiments, other factors influenced the reliability of the results obtained. Thus, although care was taken to avoid the introduction of air into the intestine when electrodes were inserted, the possibility that contamination with atmospheric oxygen gave rise to elevated oxygen tensions must be considered. However, ten minutes were allowed to pass after closing the intestine before the readings were taken. During this time, normal conditions might well have been established if contamination was slight. The observation that the pressure of oxygen recorded was sometimes influenced by the nature of the gas mixture inspired by the animal suggests that the oxygen reached the fluids of the gut by diffusion from tissues supplied with arterial blood. Hence it is probable that the lowered respiratory rates prevailing during experiments with anaesthetized animals led to abnormally low tensions in the alimentary

tract, for Campbell (1925) has shown that certain anaesthetics lower the pressure of oxygen in animal tissues. It would appear, then, that though some features of the present experiments may have led to increased oxygen tensions above the normal, other features, equally important, would reduce this error.

Heavy infestations with *Nippostrongylus muris* lead to a dilation of the blood vessels of the intestine where the parasites are localized in the region of 6 to 28 cm. from the pylorus. It is to be expected that the dilation of the blood vessels would cause an increase in the partial pressures of oxygen in the contents of the gut in this region. On the other hand, the utilization of oxygen by the parasites themselves, and the pathological enlargement of the lumen of the infected intestine, would reduce the oxygen tensions (Rogers 1948). The present experiments (see Table 1) did not reveal any difference between the infected and uninfected intestines.

TABLE 2

OXYGEN TENSIONS IN THE CONTENTS OF THE SMALL INTESTINE AND ABOMASUM OF THE SHEEP CLOSE TO THE MUCOSA AT DIFFERENT DISTANCES FROM THE PYLORUS

	Position of Electrode: Distance from Pylorus (cm.)	Oxygen Tension (mm. Hg)
Sheep 1	22	approx. 4.3
Abomasum	5	17.5
Sheep 1	52	12.7
Small intestine	114	4.7
	397	approx. 4.2
	804	approx. 4.0
Sheep 2	122	9.5
Small intestine	251	8.0
	336	approx. 4.1
	604	approx. 4.4

The results obtained in the examination of the small intestine of the sheep (see Table 2) were frequently two or three times as great as those obtained by von Brand and Weise (1932). This difference may well be due to the fact that, in the present experiments, the oxygen tensions relate to the thin layers of gut fluid adjacent to mucous membranes which retained an intact blood supply. The oxygen pressures found in fluids of the small intestine of the rat (see Table 1) were much higher than those found in that of the sheep. In the sheep the relatively smaller surface area per unit volume of the intestinal lumen would cause a more rapid fall in oxygen tension as distances from the mucosa towards the centre of the lumen of the intestine were increased. The intestines of the sheep used in these experiments contained considerable amounts of ingesta; the rat intestines, however, contained relatively little. These differences would again lead to lower oxygen tensions in the sheep intestine.

The tension of oxygen in the mucous membrane of the empty small intestine of the cat has been reported to be 35-40 mm. of mercury (McIver, Redfield, and Benedict 1926; Campbell 1932). It is not surprising, therefore, that oxygen tensions of the order indicated in Tables 1 and 2 were found close to the mucous membrane of the small intestine of the rat and sheep, even when ingesta were present. Further, oxygen tensions of 10-20 mm. of mercury (McIver, Redfield, and Benedict 1926; Campbell 1932) are found in the mucous membrane of the stomach of the cat, which again suggests that appreciable amounts of oxygen may be present in fluids close to the mucosa of the sheep abomasum. However, though there is little doubt that some oxygen was present at the sites examined in the present experiments, the amounts were low compared to those found in the usual aerobic environments. It remains to determine, therefore, whether the small nematode parasites found on the walls of the sheep abomasum or the rat and sheep small intestine are capable of utilizing oxygen when it is present in such small amounts. The function of haemoglobin in these parasites as a carrier of oxygen at low partial pressures of oxygen will be discussed in a later publication.

VI. Acknowledgments

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