The Oxidative Metabolism of *Ascaris suis*

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Bunge (1883–4) stated that *Ascaris mystax* is able to live for 5–6 days under anaerobic conditions in 1% saline solution. At that time the intestinal gases were considered to be entirely free of oxygen. Bunge, although inclined to attribute to *Ascaris* a strictly anaerobic mode of life, observed that worms kept in air lived, as a rule, longer than those under anaerobic conditions. He therefore concluded that *Ascaris* was probably unable to live in complete absence of O_2, but that the O_2 requirement was very small. Bunge later (1890) confirmed these results with *A. acus* and *A. lumbricoides*. His results, however, have constantly been misquoted (Weinland, 1901; Schulte, 1917; Krummacher, 1919 and Jordan, 1929) as having proved the obligatory anaerobiosis of *Ascaris*. Weinland goes so far as to dismiss some of his own results which are not in accordance with strict anaerobiosis; having found that the CO_2 production of worms was much greater under aerobic than under anaerobic conditions, he rejected the possibility that this increase might have been due to respiratory CO_2 but attributed it to the formation of eggs or bacterial action. Jordan even quotes the authority of Bunge for stating that 'O_2 is a poison for *Ascaris* and cannot be utilized by them for oxidative processes'. Slater (1925), however, drew attention to the facts that the physiology of digestion and the anatomical picture inside the gut, especially in proximity to the portal vein, do not justify the assumption of complete absence of O_2 in the intestine. Long & Fenger (1917) had already demonstrated that the intestinal gases contain on an average about 5% O_2 (by vol.). Slater (1925) speaks of a 'suppressed animation' of *Ascaris* in absence of O_2, the avoidance of movements enabling the worms to live for some time in inert gases. He showed that *Ascaris* exposed to repeated electric stimuli soon lose their ability to react when they are maintained in an atmosphere of H_2 but not in air. He is of the opinion that O_2 is necessary for the normal life of *Ascaris*.

Later workers (Adam, 1932; Harnisch, 1932 *a*, *b*, 1933; v. Brand, 1934–5; Friedheim & Baer, 1933; Krüger, 1935) have determined by direct measurement and with different methods the O_2 consumption of *Ascaris*. Although individual differences are considerable, all the determinations agree on a mean O_2 uptake of about 80 μl./g. wet wt./hr. at 39° in air (range 65–100 μl.). Harnisch (1933), who determined the O_2 consumption of 1 cm. lengths of the anterior end of worms at O_2 tensions varying from 0·8 to 95% O_2, found that the magnitude of O_2 uptake depends on the O_2 tension, rising considerably with increasing O_2 tension and being about four times as great in 95% O_2 as in air.

Harnisch also observed that the CO_2 output at different O_2 tensions remains constant although the magnitude of O_2 uptake varies considerably. He maintained that no respiratory CO_2 is formed (respiratory quotient (R.Q.) = 0), but v. Brand found the R.Q. = 0·9.

In this paper an attempt is made to analyze the ability of *A. suis* to utilize O_2.

METHODS

The O_2 uptake and, in some cases, the CO_2 output of whole worms or muscle pulp were measured in open-type Warburg manometers at 39°. The media used were either 0·05M-phosphate buffer, pH 7·3, or Ringer phosphate solution. No difference was found attributable to these different media. For work with whole worms small specimens, usually males, were chosen. For worms of 1–2 g. wet wt. flasks of 18–20 ml. capacity and for larger worms (3–4 g.) flasks of about 28 ml. were used. For the calculation of the vessel constants the weight of the worms was considered as extra liquid, and added to the volume of the fluid, since determination of the density of the worms showed that this was virtually 1.

When substrates were added to the medium the worms were first injected with an amount of substrate sufficient to establish within them the same substrate concentration as in the outside medium. In this way no complication due to the rate of diffusion of substrate into the worm arose. A small amount of body fluid was withdrawn from the worm by means of a syringe and either reinjected into the worm after the substrate had been dissolved in it, or the body fluid of another worm, suitably adjusted with substrate, was injected.

Muscle pulp was prepared in the following way. Larger worms, of about 6 g., were cut open longitudinally and freed from intestine, eggs, etc. The muscular layer was then scraped from the cuticle and ground with a little sand in 6 ml. medium per worm. The sand was removed by slight centrifugation. The supernatant pinkish, opaque fluid contained the finely dispersed muscle pulp. 0·5–1·0 ml. of this was used per vessel and made up to 3·0 ml. with buffer, substrate and other reagents. The muscle pulp keeps fairly well in the ice-chest for several days with only slight loss of its oxidative capacity.

Coenzyme-free muscle pulp. 10 ml. of muscle pulp prepared as before were diluted with buffer or water to 100 ml. and brought to pH 5 with acetic acid. A slight precipitate

which formed within ½ hr. at room temperature was centrifuged, resuspended in 100 ml. medium and precipitated a second time at pH 5. This was again centrifuged and was suspended in 10 ml. of neutral buffer solution. Its aerobic oxidative power was lost within 2–3 days, even when kept in the cold, but its power to reduce methylene blue (M.B.) anaerobically in the presence of sodium succinate remained unimpaired much longer.

RESULTS

O_2 uptake of whole worms and of muscle pulp. In agreement with the earlier results recorded in the introduction, the O_2 uptake of whole worms in air was found to be about 80 µl./g. wet wt./hr. at 39°. The O_2 uptake of the pulp, calculated/1 mg. dry wt. of the pulp/hr. (Q_{O_2}), was about 1·3.

Addition of substrates. Substrates, such as glucose (0·2 %), sodium lactate (0·02 M) and sodium succinate (0·02 M), added to the muscle pulp or injected into worms, failed to increase their O_2 uptake.

Influence of O_2 tension. The magnitude of the O_2 uptake of whole worms is dependent on the O_2 tension. In 5 % O_2 the O_2 uptake is half that in air, and in 100 % O_2 it is about three times higher than that in air (Fig. 1). High O_2 tension, however, is

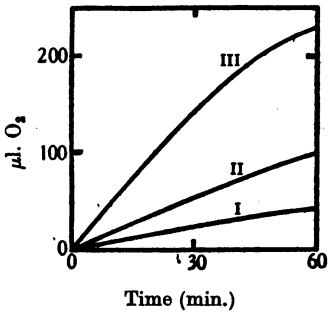

Fig. 1. O_2 uptake of whole worm (µl./g.) at different O_2 tensions. I, 5 % O_2, 95 % N_2; II, air; III, 100 % O_2.

toxic to the worms, causing their death within about 1 hr. The reasons for this effect will be discussed later.

O_2 debt of whole worms. Worms brought after 17–20 hr. anaerobiosis into 5 % O_2 in N_2 or into air showed an increase of the O_2 uptake by 60–100 % when compared with worms of the same batch which had previously been kept in air (Fig. 2). This increase in O_2 uptake develops during the first ½ hr., remains for about 2 hr. and then gradually decreases to the values of the controls.

Addition of M.B. and the succinic system. 0·5 ml. of muscle pulp made up with buffer to 3·0 ml. reduces 0·3 ml. M.B. (0·4 mg./ml.) anaerobically within 3–4 min. The addition of succinate shortens the reduction time to 1–1½ min. The addition of M.B. to muscle pulp in the absence of added substrate raises the O_2 uptake: thus M.B. is here acting as an O_2 carrier in the oxidation of some undetermined substrate present in the muscle pulp.

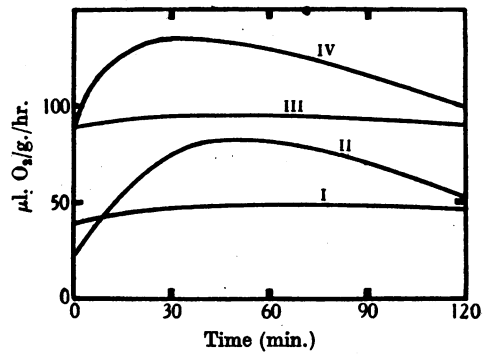

Fig. 2. Effect of anaerobiosis on subsequent O_2 uptake of whole worms (µl./g./hr.) at different O_2 tensions. I and II, 5 % O_2, 95 % N_2; III and IV, air; II and IV, following 18 hr. anaerobiosis; I and III had been kept in air.

While the addition of succinate to muscle pulp does not raise the O_2 uptake, the simultaneous presence of succinate and M.B. produces a considerable increase. This shows that part of the succinic system is present in muscle pulp but that an available O_2 carrier is lacking. The addition of cytochrome *c* or of riboflavin to muscle pulp in the presence of succinate does not, however, increase the O_2 uptake (Fig. 3).

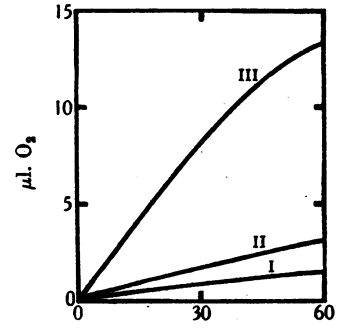

Fig. 3. O_2 uptake (µl./mg. dry wt.) of muscle pulp in air. Additions: I, none and sodium succinate only; II, M.B. only; III, sodium succinate and M.B. together.

Coenzyme-free muscle pulp. Oxaloacetic acid is a strong inhibitor of succinic dehydrogenase (Das, 1937). It is a normal intermediate in the oxidation of succinic acid by higher animals. Keilin & Hartree (1940) have shown that the inhibition of the cytochrome-succinic system by coenzyme I is due to the formation of oxaloacetic acid from fumaric via malic acids. A coenzyme-free muscle pulp was therefore prepared, which had lost the ability to form oxaloacetic acid. In the presence of M.B. and succinate,

the O_2 uptake (Q_{O_2}) of this preparation was twice as high as that of untreated muscle pulp (Fig. 4).

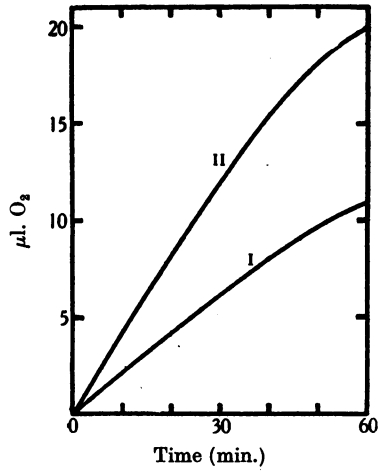

Fig. 4. O_2 uptake (μl./mg. dry wt.) of muscle pulp (I) and of coenzyme-free muscle pulp (II) in presence of sodium succinate and M.B. in air.

Influence of catalase. The addition of catalase to muscle pulp considerably raised the O_2 uptake. A still further increase was obtained when catalase and M.B. were added together (Fig. 5). *Ascaris* is

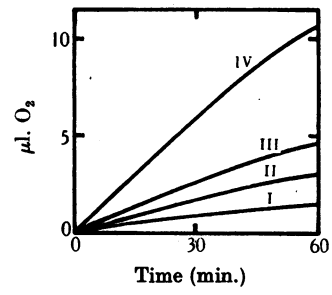

Fig. 5. O_2 uptake of muscle pulp (μl./mg. dry wt.) in air. Additions: I, none; II, M.B.; III, catalase; IV, M.B. and catalase.

known to contain very little catalase. The catalase content of the body of *Ascaris* was found to be only 1/500th of that of the same weight of mammalian blood corpuscles. The fact that the addition of catalase to muscle pulp increased the O_2 uptake suggested that H_2O_2 may have been formed during the oxidation of some substrate, and in the absence of catalase may have destroyed the enzyme concerned.

The formation of H_2O_2 in muscle pulp. The formation of H_2O_2 in a primary oxidation reaction can be detected easily by its utilization in a coupled oxidation of ethanol to aldehyde catalyzed by catalase (Keilin & Hartree, 1935–6). Fig. 6 shows that the addition of either ethanol or catalase alone to muscle pulp in air and in the presence of M.B. and sodium succinate has practically no effect on the rate of O_2 uptake, while the addition of both catalase and ethanol doubles the O_2 uptake of the primary oxida-

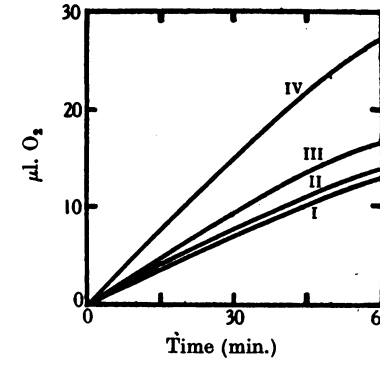

Fig. 6. O_2 uptake of muscle pulp (μl./mg. dry wt.) in air. Sodium succinate and M.B. were present in all samples. Further additions: I, none; II, ethanol; III, catalase; IV, ethanol and catalase.

tion reaction. Similar results with regard to coupled oxidation were obtained when coenzyme-free muscle pulp was used instead of untreated muscle pulp. Catalase, however, produced here a greater increase in O_2 uptake than with ordinary pulp, owing to the fact that coenzyme-free muscle pulp is practically free of catalase (Fig. 7).

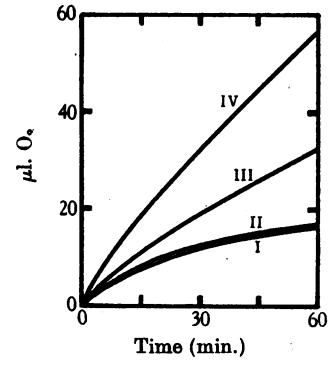

Fig. 7. O_2 uptake of coenzyme-free muscle pulp (μl./mg. dry wt.) in air. Sodium succinate and M.B. were present in all samples. Further additions: I, none; II, ethanol; III, catalase; IV, ethanol and catalase.

The addition of the unphysiological O_2 carrier, such as M.B., might be held responsible for the formation of H_2O_2; hence it was determined if coupled oxidation occurs also in the absence of M.B. No effect was obtained when catalase and ethanol were added to muscle pulp at the O_2 tension of air.

In 100% O_2, however, which raised the O_2 uptake of the controls by 80% as compared with that in air, coupled oxidation took place, as was shown not only by the increased O_2 uptake (Fig. 8) but also by a brownish coloration of the KOH-soaked filter

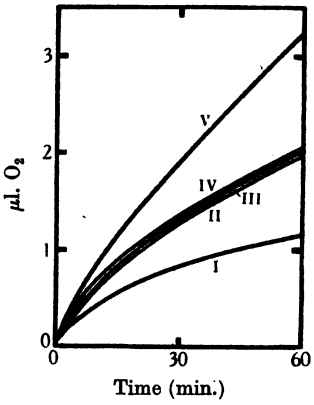

Fig. 8. O_2 uptake of muscle pulp (μl./mg. dry wt.) in air and in 100% O_2. I, air, no addition; II–V, 100% O_2. Additions: II, none; III, ethanol; IV, catalase; V, ethanol and catalase.

paper used for absorption of CO_2. This discoloration is due to polymerization of aldehyde, and proves that ethanol has been oxidized by peroxide in a secondary oxidation.

Formation of H_2O_2 *in the whole worm.* The fact that the raising of the oxidation rate of muscle pulp by the addition of M.B., or by an increase of the O_2 tension, caused the formation of H_2O_2, seemed to offer an explanation for the observation that worms die fairly quickly when brought into an atmosphere of pure O_2. Death appears with characteristic symptoms, the worms becoming very active, often bending their bodies in vertical loops. After about 30–60 min. they seem to be in a state of rigid spasm, with their loops, especially at the posterior end, well maintained. They then die in this spasm, which persists for some time after death. In addition, they quickly change colour from pink to yellowish white.

Experiments were undertaken to see whether the formation of H_2O_2 could be proved in such worms. This was done in two ways: (1) by peroxidatic oxidation of *p*-phenylenediamine, (2) by the oxidation of oxyhaemoglobin to methaemoglobin.

Worms were suspended in Erlenmeyer flasks in 20 ml. buffer per worm, to which a small amount of *p*-phenylenediamine and 1–2 drops of a purified peroxidase preparation were added. The flasks were then filled with O_2 and incubated at 39°. After ½–1 hr. a blue colour began gradually to develop in the medium, turning to dark blue within 2–3 hr. This indicates the presence of H_2O_2 and its gradual and increasing appearance in the medium by diffusion out of the dying or dead worm. The blue colour is quite different from the spontaneous darkening of the medium to a burgundy shade which appears in the control flasks, containing the same medium but no worms. The blue colour did not develop when worms were kept in the same medium under anaerobic conditions.

That the colour change from pink to whitish yellow of the worms when exposed to O_2 is due to the oxidation of haemoglobin to methaemoglobin by H_2O_2 was demonstrated in the following way. Worms were injected with chicken oxyhaemoglobin, which contains only a little catalase, and were exposed to O_2 hypertension. The colour of the worms changed rapidly from red to brown because of the complete oxidation of the injected oxyhaemoglobin to methaemoglobin.

Respiratory quotient (R.Q.) *of whole worms in air and* 100% O_2. From a large batch of worms two pairs of equal weight were chosen. Two Warburg vessels containing one worm each of a pair were filled with air, and two vessels, containing the other two, with O_2. One vessel of each pair contained KOH for the measurement of the O_2 uptake, while in the others KOH was omitted. On the assumption that the O_2 uptake was equal in both vessels of each pair, the R.Q. can be calculated. In experiments of 1 hr. duration the R.Q. was found to be 1·1–1·2 in air and 0·5–0·6 in O_2.

The O_2 uptake in O_2, as already stated, is at the beginning of the experiment about 300% of that in air and it falls within 2 hr. to zero or to a very low level. It continues, however, in all cases, and is sometimes quite large, after the apparent death of the worm. This is most probably due to post-mortem autolytic oxidative reactions and not to respiration. The worm's death which, as stated in the literature, is sometimes difficult to determine, was assumed to have occurred when rapid warming of the worm from room temperature to 39° or 42° failed to induce any movements.

Influence of KCN *and* NaN_3. Neither KCN (0·002–0·01 M) nor sodium azide (0·001–0·004 M) affects the O_2 uptake of muscle pulp with or without addition of substrate, nor do they prevent the rise of O_2 uptake on addition of M.B. (Fig. 9). On the contrary, high concentrations of KCN (0·01 M) in the presence of M.B. increase the O_2 uptake still further than M.B. alone. This must be considered due to the fact that KCN, as a ketone fixative, combines with oxaloacetic acid, rendering it incapable of inhibiting succinic dehydrogenase (Green & Dewan, 1937). This case is comparable to the increased O_2 uptake by coenzyme-free muscle pulp, described above.

The respiration of whole worms is also unaffected by KCN in as high a concentration as 0·01 M and,

to a lesser degree, by NaN_3 (0·004 M). In air the worms show, in the presence of KCN, an O_2 uptake which remains normal for a long time and they, as a rule, remain alive for at least 24 hr. With NaN_3

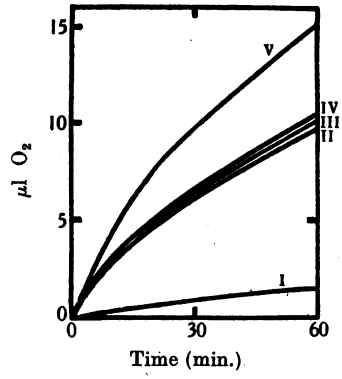

Fig. 9. O_2 uptake of muscle pulp (μl./mg. dry wt.) in air. Sodium succinate was present in all samples. Further additions: II, M.B.; III, M.B. and NaN_3 (0·004 M); IV, M.B. and KCN (0·002 M); V, M.B. and KCN (0·01 M).

worms die within about 3 hr. For the first 60–90 min. their O_2 uptake is very little affected, but later drops rapidly to zero (Fig. 10). The fact that even after injection into the worm of NaN_3 (final concentration in the worm 0·004 M) there is no immediate inhibition

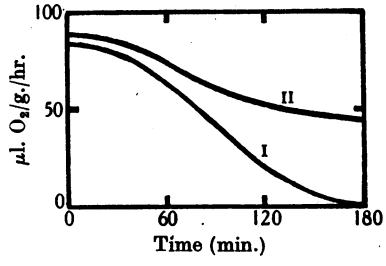

Fig. 10. O_2 uptake of whole worm (μl./g. wet wt./hr.) in air. I, NaN_3 (0·004 M); II, KCN (0·01 M).

of respiration seems to suggest that death is not due to the poisoning of the respiratory system but to other secondary effects, e.g. paralysis of the nervous system. The same applies to a lesser extent to the influence of KCN.

DISCUSSION

The O_2 uptake in air of the whole worm is, on an average, 80 μl./g. wet wt./hr. and the Q_{O_2} of the worm muscle pulp is 1·3. The addition of substrates, such as glucose, lactate or succinate does not increase these figures. Although, in view of the great activity of the worms at 39°, this appears to be a small rate of O_2 uptake, it is nevertheless of the same magnitude as that of the human body. The O_2 uptake of man at rest is of the order of 200 μl./g./hr., while that of the worms in air is as much as $\frac{2}{5}$–$\frac{1}{2}$ of this rate. The low Q_{O_2} (1·3) of muscle pulp hardly differs from that of mammalian muscle pulp. On the other hand, the rate of O_2 uptake of the worms is strictly dependent on the O_2 tension, which is not the case with mammalian tissues. Normally at the low O_2 tension in the intestine of the host, the O_2 uptake of the worm will be much lower than 80 μl./g./hr., and this seems to justify Bunge's conclusion that the O_2 requirement of *Ascaris* is very small. Is the worm a facultative aerobic organism, or is the O_2 uptake, however small, necessary? The second viewpoint, i.e. the necessity of aerobiosis, is supported by the evidence that the O_2 uptake is quite considerable even at the low O_2 tension of 5% by vol. and also by the appearance of an O_2 debt after prolonged anaerobiosis. Although the magnitude of the O_2 debt does not seem to be impressive, it must be considered in relation to the very small O_2 requirement.

The low O_2 requirement seems to be conditioned by, or to have conditioned, a system which is particularly adapted to allow only low rates of oxidation. Very little cytochrome is present. The fact that the addition of cytochrome *c* or of riboflavin to pulp did not increase the O_2 uptake may be explained by the large molecular size of cytochrome which does not permit its entry into the cells and by the assumption that riboflavin also does not readily enter. Methylene blue, which is readily diffusible, acts as an effective O_2 carrier and raises the O_2 consumption. Another brake on the O_2 consumption is probably due to the apparent inability of *Ascaris* to decompose oxaloacetic acid which, when formed, poisons succinic dehydrogenase and in this way indirectly depresses the oxidation of succinic acid. This was proved by two facts: (1) a coenzyme-free muscle pulp, which is unable to form oxaloacetic acid, shows a much increased O_2 uptake, and (2) if oxaloacetic acid is removed by combination with KCN the O_2 uptake rises considerably.

Every increase of the O_2 uptake, whether brought about by M.B. in air or by an increase of the O_2 tension, causes the formation of H_2O_2, which is a powerful cell poison. Its presence was proved in pulp by coupled oxidation on addition of catalase and ethanol, and in whole worms by the oxidation of *p*-phenylenediamine in the presence of peroxidase and the oxidation of injected oxyhaemoglobin to methaemoglobin. Owing to the fact that the worms contain very little catalase, they are unable to decompose H_2O_2 sufficiently rapidly to render it harmless. Whether at their normal low oxidation rate no H_2O_2 at all is formed, or only so little as can be dealt with by the worms, cannot be decided. It seems unlikely, however, that by simply increasing the O_2 tension a type of respiration whereby O_2 is re-

duced to H_2O should be changed to another type whereby O_2 is reduced to H_2O_2. The fact, however, that methaemoglobin, which is a weak peroxidase, is formed under the influence of H_2O_2, makes it feasible that some H_2O_2, if produced at low oxidation rates, may be eliminated by secondary peroxidatic oxidations catalyzed by methaemoglobin.

The death and change of colour of worms exposed to pure O_2 are assumed to be due to the formation of H_2O_2, the colour change being due to the formation of methaemoglobin.

The determinations of the R.Q. in air and in O_2 are open to criticism. The rate of respiration of two worms of equal weight is not necessarily the same, and still less so as one worm respires in almost complete absence of CO_2, while the other worm is in an atmosphere of increasing CO_2 tension. Differences in the oxidative rate with differing CO_2 tensions exist with other tissues (Laser, 1942; Craig & Beecher, 1943). It is furthermore possible that some of the CO_2 evolved is not respiratory CO_2 but is expelled by fixed acids from the bicarbonate present in the worm. The bicarbonate content of the worm, as used under the conditions of these experiments, was found to be very low, 30 μl./ml. body fluid, or per g. wet wt. As the worm produces constantly comparatively large amounts of fixed acid, it must be assumed to possess a fairly efficient buffer system of which bicarbonate is only a minor part. It seems unlikely, therefore, with the small initial amount of bicarbonate present, that sufficiently large amounts of CO_2 have been evolved from the bicarbonate to affect materially the relative values of the two sets of R.Q. measurements. The figures obtained, i.e. an apparent R.Q. of slightly above 1 in air and of 0·5–0·6 in O_2, can therefore be taken as an indication that in high O_2 tension a large proportion of the oxidation proceeds with the formation of very little or no CO_2.

Neither cyanide nor azide affect the O_2 uptake of muscle pulp with or without addition of substrate and M.B. The respiration of whole worms is also unaffected for long periods by cyanide, while azide kills the worms within a few hours; the initial respiratory rate, however, is unaffected. Death is assumed not to be due to the poisoning of the oxidative system, but to other secondary effects.

The pattern of the oxidative enzymic system of a parasitic worm, *A. suis*, as described in this paper, seems to show a perfect adaptation to its surroundings, i.e. to the low O_2 tension; or vice versa, the low O_2 requirement may be considered as conditioned by the peculiar enzymatic system.

SUMMARY

The oxidative metabolism of a parasitic helminth, *Ascaris suis*, has been studied.

1. The O_2 uptake of muscle pulp and of whole worms is dependent on the O_2 tension, rising with increasing tension.

2. Prolonged anaerobiosis causes an O_2 debt.

3. The normal rate of oxidation is low. To this end the following factors contribute, in addition to the low O_2 tension in the intestine of the host:

(*a*) the low concentration of cytochrome in spite of the presence of succinic dehydrogenase,

(*b*) the apparent inability of the worm to deal with accumulated oxaloacetic acid, which decreases the activity of succinic dehydrogenase.

4. Increased O_2 uptake, whether caused by the addition of methylene blue or by an increase of the O_2 tension, is accompanied by the formation of H_2O_2 which is not rapidly decomposed, owing to the very low concentration of catalase.

5. Worms in an atmosphere of pure O_2 die fairly quickly with characteristic symptoms. H_2O_2 is formed and is assumed to be the cause of death.

6. In O_2 hypertension oxyhaemoglobin is oxidized by H_2O_2 to methaemoglobin. This can act as a weak peroxidase. It is thought possible that small amounts of H_2O_2, if formed at low oxidation rates and low O_2 tension, may be eliminated by secondary peroxidatic oxidation reactions catalyzed by methaemoglobin.

I wish to express my thanks to Prof. D. Keilin for many helpful suggestions during the course of these experiments.

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