XC. THE NATURE OF THE METABOLIC PROCESSES IN *ASCARIS LUMBRICOIDES*.

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Introduction.

It is generally accepted that the oxidation of part of the lactic acid produced in muscular contraction and the simultaneous conversion of the rest of the lactic acid into glycogen form an essential part of muscular metabolism. If these conclusions are correct the existence of an organism which requires no oxygen for its metabolism, and yet is supplied with muscles having a normal contractile mechanism, is unlikely, unless by some means the oxidative recovery phase is dispensed with and the lactic acid removed by diffusion into the vascular system and ultimately excreted. The latter process would be a costly one, liberating less than a tenth of the total energy in the original glycogen.

Weinland [1901], however, maintains that the intestinal worm *Ascaris lumbricoides* does not require oxygen for its metabolism and lives equally well in atmospheres of inert gases, whilst on the other hand, it is well known that this worm is capable of marked muscular movement. In explanation of the source from which the energy is derived, Weinland puts forward the suggestion that a process nearly allied to fermentation takes the place of the ordinary aerobic metabolism.

The present work was undertaken in the first place to test the accuracy of Weinland's conclusions in respect to the anaerobiosis of *Ascaris*, and in the second place if the worm proved to be truly anaerobic to investigate the nature of the muscular process involved. The investigation was not carried beyond the first stage as evidence was forthcoming which rendered doubtful the anaerobiosis of the worms, and precluded entirely the possibility of work in the absence of oxygen or its equivalent.

It seems advisable before proceeding to the description of the experimental work to recapitulate briefly the evidence on which the anaerobiosis is based.

Bunge [1884, 1890] states that various types of intestinal worms can live for periods of 5–6 days in the absence of air, but that they become dormant and refuse to move. Weinland [1901] repeated these experiments using a variety of gases, and states that the worms remain alive for periods up to 9 days quite independent of the surrounding gas, except that in carbon

dioxide the life period is a little longer than the average. It is, however, unfortunate in view of Bunge's observations that Weinland gives no criterion by which he judged the worms to be alive, as some state akin to hibernation at once suggests itself. If the worms can, by remaining still, reduce their energy requirement to a very small amount, it is possible that there is a sufficient store of material capable of carrying out oxidation present in their bodies to maintain the necessary life processes for considerable periods, or that in these circumstances the energy is supplied by some purely temporary anaerobic process, which would be entirely inadequate for the normal energy requirements.

Other evidence advanced by Weinland consists of analyses of the body contents of the worms made at the beginning and the end of periods during which they were kept in 1 % sodium chloride solution; and further of analyses of the surrounding fluids at the end of these periods. By such means Weinland hoped to show what substances were metabolised by the worms, and what were the products of metabolism. The chief differences found by Weinland after a period of starvation in sodium chloride solution were a large fall in the glycogen content and a still larger rise in the water content. There were also small losses in the glucose and protein but not of the same order as in the case of the glycogen. The surrounding solution Weinland found to be acid and evil smelling. This he claimed to be due to the presence of valeric acid, but there is some doubt as to the validity of his findings owing to the inherent difficulty of his analytical methods.

The other product of metabolism identified by Weinland was carbon dioxide, and he explained the process involved by the following equation although he did not identify the hydrogen.

$$\underset{\text{glycogen}}{4C_6H_{10}O_5.H_2O} = 9CO_2 + \underset{\text{valeric acid}}{3C_5H_{10}O_2} + 9H_2.$$

The tentative suggestion put forward by Weinland that the hydrogen reduced some other body constituent is only another way of postulating a hydrogen acceptor, which must be used up in the process and whose absence would probably cause death.

In order to meet the criticism that acids similar to valeric (acetic, butyric, lactic and succinic) are produced by intestinal bacteria, Weinland [1902] pressed the worms and obtained a juice which he sterilised by the addition of disinfectants such as sodium fluoride, toluene, etc. This sterile juice he added to solutions of glucose and glycogen and obtained results similar to those which were given by the worms, *i.e.* valeric acid formed in the solution and carbon dioxide given off. In a more recent paper Anton Fischer [1924] does not agree with these findings. Fischer estimated the total acidity in a solution in which *Ascaris megalocephala* had lived, and also the lactic acid, and found that the lactic acid amounted to about 10 % of the total acidity. If, however, the worms were first ground up and the pulp sterilised with

toluene, the only products of autolysis were lactic and phosphoric acids. In view of this work it would appear that Weinland had not succeeded in rendering the pressed-out juice free from bacteria, and hence there is no evidence that the volatile fatty acids found in connection with the worms are not the products of bacterial action.

The present work divides itself into two sections:

(1) an attempt to repeat Weinland's experiment on the length of life of *Ascaris lumbricoides* in hydrogen, but at the same time using means to ensure their continued activity, and

(2) an investigation of the products of the activity of the bacterial organisms found in conjunction with the worms.

Duration of activity in hydrogen.

A number of worms (*Ascaris lumbricoides*) freshly taken from the gut of a pig, were washed in warm Ringer solution, and placed in bottles containing a 1 % solution of sodium chloride boiled to expel air.

Two worms of equal size were placed in each bottle, and the bottles closed by means of a cork through which inlet and exit tubes were passed. In this and subsequent experiments great difficulty was encountered in keeping the inlet tubes clear, as, unless care was taken to prevent it, the worms climbed up the tube until in the end one became firmly fixed in it. This is an interesting example of what appears to be a definite purpose in the movements of these creatures. A slow stream of hydrogen was passed continually through the solutions, and the temperature maintained at 37°. The hydrogen after passing through the bottles was passed through a wash bottle containing baryta.

After the worms had become accustomed to their surroundings, they ceased to be active, and except for slight occasional movements remained so for 5–6 days, the only definite movements being those in connection with climbing the inlet tubes. At the end of this time the bottles were opened and allowed to stand in air, when within a short time the worms began to move freely, and this movement was increased if they were transferred to clean saline. The wash bottles containing baryta showed that carbon dioxide had been produced in considerable quantities. The sodium chloride solution in which the worms had lived had a characteristic unpleasant smell resembling that of the lower saturated fatty acids. The solution also gave qualitative tests for lactic acid.

These experiments confirmed in the first place Bunge's observations that the worms became dormant, and also in general the observations of Weinland.

It remained now to test whether if the worms were stimulated to continuous activity, they would live as well in hydrogen as in air. In order to do this two electrodes were introduced into the bottles and connected with the secondary circuit of an induction coil. By this means an interrupted current was passed through the solution from time to time, as often as was found necessary in order to keep the worms in movement. The circuit was arranged so that as nearly as possible equal stimulation was given in each containing vessel.

Three bottles were used each containing two worms. A continuous stream of hydrogen was passed through two of the bottles, whilst through the third a similar stream of air was drawn by means of a filter pump. The liquid used in the bottles was mammalian Ringer solution. All the experiments gave similar results, the following being a typical example of the findings:

Date 1924	Time	Remarks	Bottle 1 Worms in air	Bottle 2 Worms in hydrogen	Bottle 3 Worms in hydrogen
24. iii.	3 p.m.	Experiment started	All worms moving freely but slowly without stimulation		
	3.45	Continuous stimulation for 5 mins.	Violent movement	Responded to stimulus, but not so violently as worms in air	
	3.50–4.40	No further stimulus	Continued violent movement	Little further movement	
	4.40	Stimulation for 2 mins.	Very violent movement	Moving slowly	
	5.15	,,	,,	,,	
	5.45	,,	,,	,,	
	6.30	,,	,,	,,	
25. iii.	10 a.m.		All worms quite still		
	10.15	Stimulation for 5 mins.	Fairly active	Only a very slight movement	
	11.15 and every hour to 6 p.m.	Stimulation for 2 mins.	,,	Slightly more movement but very weak and jerky	
26. iii.	10 a.m.		All worms quite still		
	10.30	Stimulation for 5 mins.	Considerable movement increasing with stimulation	No movement	A few slight tremors
	10.40	Air admitted to bottle 2			
	10.45		Moving easily	Slight movement	Quite still
	10.50	Stimulation for 2 mins.	,,	Slight movement confirmed	,,
	11.30	,,	,,	Movement increased	,,
	12 noon	,,	,,	,,	,,
	1 p.m.	,,	,,	,,	,,
	2	,,	,,	Movement less marked	,,
	3	,,	,,	Movement entirely stopped	,,
				It appears that the worms in both these bottles were dead	
27. iii.	10 a.m.	Stimulation for 2 mins.	Moving easily	No response whatever in either bottle	

Three other experiments were carried out, the result of one being identical with that given above, whilst in the other two where stimulation in hydrogen was not carried so far before the admission of air, the worms to which the air was admitted recovered completely. In no case did the worms which had a continuous supply of air cease to move, and in every case those which were entirely deprived of air became motionless in times varying from 24–48 hours.

From these experiments it appears reasonable to conclude that the worms are incapable of continuous movement in the absence of oxygen or its equivalent, and that it is only by avoiding movement that they are able to live for long periods in atmospheres of inert gases.

One criticism which might be made of these experiments is that the oxygen is necessary only for the excitation mechanism which is stimulated by the

current, and not for the actual muscular movement. If this were the case, however, it would be expected that the worms would commence to move freely almost as soon as the oxygen was admitted, whereas they recover only very slowly.

It might also be suggested that the stimulating current by becoming partially rectified may have introduced small quantities of electrolytic oxygen which might have the effect of rendering possible movement in the anaerobic vessels longer than would otherwise be possible. This does not appear to be likely however as the stream of hydrogen would rapidly wash away any oxygen formed.

Examination of the products of bacterial action.

Several attempts were made to obtain the worms in a bacteria-free condition in order that they might be examined without the confusion consequent upon bacterial activity. All common antiseptics in concentration sufficient to render the worms free from bacteria had at the same time a lethal effect on them. Proflavine was tried in concentrations necessary for effective sterilisation (1 in 10,000), but the worms died in 1–2 days showing marked staining by the antiseptic. It was therefore decided to prepare a culture of the bacteria and to test this alone, as the worms could not be tested without the bacteria.

A peptone culture was prepared of the fluid in which the worms had lived. A number of mixed colonies resulted, and a solution containing salt, peptone and glucose was inoculated with bacteria from as many of these colonies as possible. After 2 days at 37° the solution was examined and found to smell unpleasantly. A part of the acid in the solution was distilled in steam and on examination appeared to be a mixture of saturated fatty acids (acetic, butyric, etc.) such as those which are characteristic of the solution in which the worms have lived.

The further examination of these acids was not attempted as it seemed that no useful purpose would be served by this investigation unless pure cultures were first obtained; it had been established that the products of the bacterial action were at least very similar to those of the worms plus bacteria.

Discussion and conclusions.

The general conclusion to be drawn from the experiments described appears to be, that although the worms are capable of prolonged existence in the absence of air, they achieve this only by cutting down their movements to a minimum, and that for their normal metabolism they require a supply of oxygen or its equivalent. Weinland's observations in themselves are confirmed, but he was misled by this faculty of "suppressed animation" into attributing to the worms an anaerobic mechanism. The metabolism of the worms is in general normal and the presence of the volatile fatty acids is due to bacterial activity.

This conclusion involves the assumption that the worms have the power of reducing their rate of metabolism to a very small value at 37°. Although reductions in metabolic rate are common in many animals at low temperatures during periods of hibernation, the present case appears to be without precedent. This may be due to the fact that the *Ascaris* has no circulatory system and is maintained at a constant temperature in the gut of its host.

It was hoped that calorimetric measurements of the metabolic rate in oxygen and in hydrogen could be made in order to confirm this point, but owing to the accompanying bacteria this was found to be impossible. It is proposed at a future date to carry out some measurements on the respiratory exchanges of thin strips of muscle tissue from the worms, which have been rendered bacteria-free, and thus to establish certainly whether oxygen is metabolised or not.

There are two further points which require comment. The first of these is the question of the oxygen supply to such an organism living in the gut of a mammalian host. It has been maintained that the oxygen pressure in the gut is almost negligible, and it has been generally assumed in consequence that there is no oxygen available for any organism living in the gut. A more careful consideration of the circumstances suggests however that this view may not be wholly correct. The gut is in intimate contact with the portal circulation, which during the period of digestion is highly oxygenated (the epithelial cells of the gut wall must themselves use oxygen), and hence it is to be expected that the gut and the neighbouring gut contents will have available an appreciable oxygen supply. The strongly reducing character of the gut contents is sufficient to explain the difficulty of finding oxygen in the intestinal gases. That molecules much larger than oxygen can be absorbed from the blood stream by parasites living in the gut is illustrated by an observation made by Dr Wollard (appended as a note to this paper), in which a tapeworm was found to have absorbed methylene blue, which had been injected intravenously into its host.

The other point requiring comment is the utilisation by the bacteria in the saline of carbohydrate derived from the bodies of the worms. The only conclusion which seems possible is that the carbohydrate (possibly as lactic acid) is excreted by the worms through their simple gut wall into the saline. The absorption of water from 1 % saline, and the extreme sensitivity of the worms to lower concentrations, suggests that the gut contents of the host rarely fall below that concentration under normal conditions. Hence if the gut wall of the worms is permeable in both directions, the conditions would be favourable to the excretion of the carbohydrates which are present in the body cavity of the worms in high concentration.

In conclusion the author wishes to acknowledge his thanks to Prof. H. S. Raper for the interest which he took in the work, the major part of which was carried out in his laboratory at Manchester.

NOTE BY H. WOLLARD.

On the absorption and subsequent reduction by a tapeworm of methylene blue from the gut of its host.

A rat which had been injected for another purpose with 0·05 % methylene blue in normal saline at body temperature and immediately opened up, was found to contain a large tapeworm in its small intestine. The intestines had rapidly become blue. (Structures such as the nerve endings in muscle had been readily stained.) Portions of the tapeworm which was perfectly colourless were withdrawn and placed in air. These portions showed slight contractions and relaxations, and after they had been exposed to air for some time they exhibited a uniform blue colour of moderate density. They were not so deeply stained as the intestine, but the colour was approximately the same as that shown by the surface of the striated muscle. Several proglottides were placed on a glass slide and covered by a cover slip. The segments began immediately to decolorise and were soon quite colourless. This process of coloration and decoloration could be watched under the microscope by raising and lowering the coverslip, and it was observed to be taking place in the cytoplasm of the cells. The particles of the dye accumulated as perinuclear rings, and there underwent decoloration.

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