THE INTERMEDIARY METABOLISM OF NEMATODE PARASITES

I. THE GENERAL REACTIONS OF THE TRICARBOXYLIC ACID CYCLE

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Summary

The oxygen consumption of brei or mince prepared from *Nematodirus filicollis, N. spathiger, Ascaridia galli,* and *Neoaplectana glaseri* was stimulated by the addition of pyruvate, α-ketoglutarate, succinate, fumarate, malate, and oxaloacetate. Citrate did not stimulate respiration. Malonate, arsenate, arsenite, pyrophosphate, and azide inhibited respiration. When brei from *Nematodirus* spp. and *Ascaridia galli* were fortified with coenzymes, the inhibition due to malonate was decreased by adding intermediates of the tricarboxylic acid cycle, and the accumulation of succinate in the malonate-poisoned brei was increased when fumarate, fumarate plus pyruvate, or citrate was added.

When calcium ions were present in the medium in which the brei was suspended, the results indicated that only isolated reactions of the tricarboxylic acid cycle were taking place because succinate failed to decrease malonate inhibition and did not accumulate even when fumarate or pyruvate was added. The characteristic reactions of the whole cycle could be obtained by omitting calcium chloride from the medium or by adding adenosinetriphosphate, diphosphopyridine nucleotide, and triphosphopyridine nucleotide.

On the basis of these results, it is suggested that some form of the tricarboxylic acid cycle functions in the tissues of the parasites.

I. INTRODUCTION

The nature of the processes in the metabolism of nematode parasites by which energy is obtained has been examined by a number of workers and generally it has been agreed that glycogen is the chief energy reserve (for references see von Brand and Jahn 1940). The anaerobic breakdown of glycogen, through hexose phosphates and triose phosphates to lactate, follows a course which is, superficially at least, similar to that in yeast and mammalian muscle and leads to the accumulation of energy in the labile phosphate bonds of adenosinetriphosphate (Rogers and Lazarus 1949). This route probably provides only a part of the anaerobic energy available to the parasites, because von Brand (1934), in a series of careful analyses, showed that the disappearance of glycogen in *Ascaris lumbricoides,* maintained *in vitro* under anaerobic conditions, was coupled with the production of lower fatty acids, chiefly valeric acid. A number of different routes have been suggested for the formation of

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valeric acid from glycogen in the tissues of nematode parasites (for references see von Brand and Jahn 1940) but there is no experimental evidence to support these hypotheses.

Whether the energy from anaerobic sources is supplemented by that obtained from oxidative mechanisms *in vivo* is not clear. There is no doubt that nematode parasites can utilize oxygen when it is available to them *in vitro* (Laser 1944; Rogers 1948); whether oxygen is always available to parasites of the alimentary tract *in vivo* is uncertain, though it does appear that conditions in the small intestine of some animals are such as to allow certain small parasites to respire quite actively (Rogers 1949*a*, 1949*b*). Some workers (Harnish 1933, 1935; Krüger 1937) are of the opinion that, whatever aerobic mechanisms exist in *Ascaris lumbricoides*, they cannot be regarded as processes which yield energy, or that the energy so formed could not be used. However, Slater (1925) has shown that *Ascaris lumbricoides*, when continuously stimulated to activity *in vitro*, lives longer if oxygen is available; and von Brand (1937), using the same parasite, found that after a period of anaerobiosis, glycogen resynthesis occurs when oxygen becomes available. These results suggest that oxidative mechanisms can be used by these parasites to provide useful energy.

Very little is known of the routes of aerobic catabolism in the tissues of nematode parasites. The amounts of glycogen used and the amounts of valeric acid and carbon dioxide produced by *Ascaris lumbricoides* under aerobic and anaerobic conditions were compared by von Brand (1934). Less glycogen was used when oxygen was available to the parasites but the results indicated that the oxidation of carbohydrates was partial and that probably only a small proportion was completely oxidized to carbon dioxide and water. Harnish (1935) has suggested that the aerobic route might involve the oxidation of isovaleric acid to acetoacetic acid, or to β-hydroxybutyric acid, with subsequent formation of acetone and carbon dioxide. Without denying the value of previous work and the hypotheses that have been formulated, it seems that the rational approach to the determination of oxidative routes in the tissues of nematode parasites is first to seek information on the importance of accepted sources of energy such as the tricarboxylic acid cycle (Krebs 1943). Though the respiratory quotients of large parasites respiring in air-saturated saline are high, the quotients of smaller parasites (Rogers 1948; Lazarus 1950) are not incompatible with the suggestion that some carbohydrate oxidation takes place through a mechanism of the nature of the tricarboxylic acid cycle.

This paper describes experiments designed to show whether cyclic oxidations similar to those of the tricarboxylic acid cycle occur in the tissues of three different nematode parasites. The results of a detailed examination of specific reactions of the cycle will be described in a later publication.

II. Biological Materials

Neoaplectana glaseri, which is naturally a parasite of the Japanese beetle, was grown *in vitro* on sterile liver slices supported on neutral agar. The mixed populations of males, females, and larvae were washed from the cultures with

0.5 per cent. saline, concentrated by centrifuging and washed several times before use. *Nematodirus filicollis* and *N. spathiger,* which were not separated for use, were obtained from naturally infested sheep. *Ascaridia galli* was obtained from experimentally infected chickens.

Brei was prepared by grinding the parasites in a chilled mortar with quartz sand. The machine described by Seevers and Shideman (1941) was used for mincing tissues. Pigeon breast muscle, which was used for comparison in some experiments, was prepared similarly. Krebs-Ringer phosphate, at pH 7.3, with or without calcium chloride, or 0.01M phosphate buffer, was used as a medium, 9 volumes to 1 volume of brei or mince. In some instances cofactors such as nicotinamide were ground with the tissues.

III. Methods

Oxygen uptakes were determined at 37°C. by the direct method of Warburg (1926). Substrates, cofactors, and inhibitors were dissolved in Krebs-Ringer phosphate and neutralized before being added to the brei in the flasks. As a rule, oxygen consumption was calculated from results obtained during the first 30 minutes after equilibration. Oxygen uptake was not limited by the rate of shaking of the Warburg manometers.

Succinate was extracted from brei or mince by continuous extraction with ether (Krebs 1937) and determined manometrically with succinoxidase from pig's heart (Cohen 1940). Citric acid was determined colorimetrically by Krebs and Eggleston's (1944) modification of the method of Pucher, Sherman, and Vickery (1936). Cytochrome *c* was prepared from ox heart by the method of Keilin and Hartree (1937) and estimated spectrophotometrically; coenzymes I and II were prepared by the methods of Williamson and Green (1940) and Warburg, Christian, and Griese (1935) respectively. Adenosinetriphosphate was prepared from rabbit muscle as the barium salt, from which the sodium salt was obtained as required immediately before an experiment.

IV. Procedure and Results

(*a*) *Effects of Added Oxygen Carriers on Respiration Rates*

The addition of methylene blue to brei prepared from *Ascaridia galli* or *Nematodirus* spp. up to a final concentration of M/1000 caused, at times, an increased oxygen consumption up to 25 per cent. above that in control flasks. The stimulating effect of methylene blue was more consistent and greater in the presence of added substrates such as succinate. No consistent differences were noted between the tissues of the two parasites.

The addition of cytochrome *c* to an approximate concentration of 2×10^{-5}M gave results similar to those obtained with methylene blue. In the experiments described in the following parts of this paper, methylene blue, usually at a final concentration of 0.0003M, was added as a routine measure.

(*b*) *Effects of Added Cofactors on Respiration Rates*

Cocarboxylase, riboflavin, nicotinamide, pyridoxin, diphosphopyridine nucleotide, and triphosphopyridine nucleotide had no consistent effect on the endogenous respiration of brei in Krebs-Ringer phosphate. The coenzymes, however, had a pronounced and consistent effect on oxygen uptake in the

TABLE 1

EFFECT OF COFACTORS ON SUBSTRATE-INHIBITOR RELATIONSHIPS

System	*Nematodirus* spp.		*Ascaridia galli*	
	Oxygen Uptake (µl.)	Change (%)	Oxygen Uptake (µl.)	Change (%)
No additions	92		141	
+ Malonate 0.01M	54	− 41	104	− 26
+ Succinate 0.01M	207	+ 125	236	+ 67
+ Malonate 0.01M + succinate 0.01M	75	− 64	129	− 45
+ α-Ketoglutarate 0.01M	131	+ 42	208	+ 47
+ α-Ketoglutarate 0.01M + malonate 0.01M	80	− 39	142	− 32
+ Cofactors	145	+ 58	185	+ 31
+ Cofactors + malonate 0.01M	62	− 57	125	− 35
+ Cofactors + succinate 0.01M	214	+ 47	222	+ 20
+ Cofactors + succinate 0.01M + malonate 0.01M	153	− 28	213	− 5
+ Cofactors + α-ketoglutarate 0.01M	161	+ 11	209	+ 13
+ Cofactors + α-ketoglutarate 0.01M + malonate 0.01M	98	− 39	194	− 7

The results were obtained from the first 20 minutes of the experiments. Each flask contained 1 ml. of parasite mince and methylene blue to a concentration of 0.0003M. The cofactor mixture, which was added from the side-arm of the Warburg flask after equilibration at 37°C., contained 1 mg. of coenzyme I (purity 40 per cent.), 0.1 mg. of coenzyme II (purity 15 per cent.), and 2.5 mg. of adenosinetriphosphate. The whole system was buffered with Krebs-Ringer phosphate solution at pH 7.3.

presence of malonate and added succinate. Without the addition of coenzymes, succinate added to malonate-inhibited brei failed to lift the inhibition; indeed, the inhibition was increased (Massey and Rogers 1949). When the brei or

mince was fortified with coenzymes I and II, the addition of succinate or α-ketoglutarate led to decreased malonate inhibition in the accepted manner. Typical results obtained from brei prepared from *Ascaridia galli* and *Nematodirus* spp. are shown in Table 1.

The effect of calcium ions on respiration was determined by comparing the oxygen consumption of brei prepared in Krebs-Ringer phosphate, with and without calcium chloride. Typical results are shown in Table 2. Endogenous respiration was decreased when calcium ions were present but succinate gave a greater stimulation. In the absence of calcium ions succinate was more effective in lifting malonate inhibition. Similar results were obtained from preparations of *Nematodirus* spp. and *Ascaridia galli.*

TABLE 2

EFFECT OF CALCIUM IONS ON SUCCINATE-MALONATE RELATIONSHIPS IN *NEMATODIRUS* SPP.

System		Oxygen Uptake (μl.)	Change (%)
Medium containing calcium	No additions	165	
	+ Malonate 0.01M	71	− 57
	+ Succinate 0.01M	233	+ 41
	+ Succinate 0.01M + malonate 0.01M	117	− 50
Medium without calcium	No additions	180	−
	+ Malonate 0.01M	91	− 50
	+ Succinate 0.01M	208	+ 15
	+ Succinate 0.01M + malonate 0.01M	151	− 27

Each flask contained 1 ml. of brei, methylene blue to a final concentration of 0.0003M, and was buffered with Krebs-Ringer phosphate solution of pH 7.3, with or without calcium chloride according to the particular experiment.

(*c*) *Effect of Added Substrates on Respiration Rates*

The effects of intermediates of the tricarboxylic acid cycle on the respiration of parasite brei in Krebs-Ringer phosphate were determined during the first 30 minutes of the experiment after equilibration. The percentage stimulation arising from the addition of the substrates is shown in Table 3. Though the degree of stimulation varied greatly in different preparations, it is clear that all the metabolites added, except citrate, actively stimulated respiration. The failure of citrate to stimulate respiration is difficult to explain in view of the fact that it was rapidly metabolized (see later in this paper). The effect may be partly attributed to its action in competing with its oxidation products, succinate and malate (Krebs 1943).

The general implication of the results shown in Table 3 is that some form of the tricarboxylic acid cycle functions in the tissues of the three nematode parasites examined.

TABLE 3
EFFECT OF ADDED SUBSTRATES ON RESPIRATION

Substrate	*Nematodirus* spp.	*Ascaridia galli*	*Neoaplectana glaseri*
Pyruvate 0.01M	0-45	20-30	–
Mean	25 (6)	25 (5)	
Citrate 0.01M	No effect (6)	–	–
α-Ketoglutarate 0.01M	0-45	20-120	–
Mean	25 (9)	60 (4)	
Succinate 0.01M	50-140	50-135	45-110
Mean	90 (10)	100 (4)	60 (3)
Fumarate 0.01M	0-60	15-90	25-35
Mean	40 (8)	60 (6)	30 (2)
Malate 0.01M	20-35	15-70	10-80
Mean	25 (4)	40 (3)	40 (3)
Oxaloacetate 0.01M	0-50	20-25	–
Mean	15 (6)	25 (3)	

The results refer to the percentage stimulation of respiration over the first 30 minutes of the experiments. The figures within brackets give the number of experiments from which the results were taken.

(*d*) *Effect of Inhibitors on Respiratory Rates*

Inhibitors were dissolved in Krebs-Ringer phosphate and neutralized with sodium hydroxide before being added to brei or mince prepared in Krebs-Ringer phosphate. The degree of inhibition was calculated as the percentage fall in oxygen uptake below that in the control vessels during a period of 30 minutes after equilibration. The results are shown in Table 4.

The effect of malonate on respiration varied with different lots of material, and only at higher concentrations, 0.01M and 0.02M, was inhibition consistently obtained. Pyrophosphate, arsenite, arsenate, and azide were all effective inhibitors of respiration in preparations from *Ascaridia galli* and *Nematodirus* spp.

The action of succinate in lifting malonate inhibition in brei fortified with coenzymes I and II and adenosinetriphosphate has already been described. The effect of other metabolites of the tricarboxylic acid cycle on malonate inhibition is shown by the results of typical experiments set out in Tables 5 and 6. Malate, fumarate, pyruvate and fumarate were all effective in reducing malonate inhibition in brei prepared from *Nematodirus* spp. and *Ascaridia galli*.

(*e*) *Formation of Succinate in Malonate-Poisoned Brei*

The succinate concentration in brei suspended in Krebs-Ringer phosphate containing 0.0003M methylene blue was determined before and after shaking for 2 hours at 37°C. Under these conditions succinate formation was negligible, even when both fumarate, 0.01M, and malonate, 0.01M, were present (Massey and Rogers 1949). However, in similar experiments carried out with pigeon breast muscle an appreciable accumulation of succinate was recorded.

TABLE 4
EFFECT OF INHIBITORS ON RESPIRATION

Inhibitor	*Nematodirus* spp.	*Ascaridia galli*	*Neoaplectana glaseri*
Malonate 0.001M	0-5	0-30	No effect (2)
Mean	1 (5)	10 (5)	
Malonate 0.01M	25-50	20-4	10-20
Mean	40 (11)	30 (7)	15 (2)
Malonate 0.02M	40-55	35-45	–
Mean	50 (4)	40 (2)	
Pyrophosphate 0.01M	40-50	15 (1)	–
Mean	45 (2)		
Arsenite 0.001M	70 (1)	50 (1)	–
Arsenite 0.01M	75 (1)	55 (1)	–
Arsenate 0.01M	30 (1)	30 (1)	–
Azide 0.01M	50 (1)	45 (1)	–

The figures given refer to the percentage inhibition of respiration during the first 30 minutes of the experiments. The figures within brackets give the number of experiments from which the results were taken.

The addition of coenzymes I and II and of adenosinetriphosphate to brei prepared from *Nematodirus* spp. had a pronounced effect on the accumulation of succinate, which was appreciable even in the absence of malonate. Succinate accumulation in brei from *Ascaridia galli* could also be shown when the brei was fortified with the nucleotides. Typical results are shown in Table 7. Succinate accumulation in pigeon breast muscle, examined under the same conditions as the preparations from the parasites, is shown in the same table for comparison.

(*f*) *Utilization of Citrate by the Tissues of the Parasites*

The utilization of citrate in the presence and absence of malonate with the subsequent formation of succinate was examined in brei prepared in Krebs-Ringer phosphate. The concentrations of citrate and succinate were determined immediately after adding the substrates to the brei and after the mixtures

had been shaken for 2 hours at 37°C. The results of a typical experiment are shown in Table 8; the percentage conversion of succinate equivalents of citrate to succinate have been calculated. Under the conditions of these experiments, when coenzymes were not added, the accumulation of succinate in the absence

TABLE 5
EFFECT OF SUBSTRATES ON MALONATE INHIBITION IN *NEMATODIRUS* SPP.

System	Oxygen Uptake (μl.)	Inhibition Due to Malonate (%)
No additions	155	
+ Malonate 0.005M	131	16
+ Fumarate 0.01M + pyruvate 0.01M	197	
+ Fumarate 0.01M + pyruvate 0.01M + malonate 0.005M	194	2
No additions	102	
+ Malonate 0.01M	63	39
+ Fumarate 0.01M	139	
+ Fumarate 0.01M + malonate 0.01M	137	2
No additions	190	
+ Malonate 0.02M	113	40
+ Malate 0.01M	242	
+ Malate 0.01M + Malonate 0.02M	196	20

Each flask contained 1 ml. of parasite brei and methylene blue to a final concentration of 0.0003M, and was buffered with Krebs-Ringer phosphate solution pH 7.3. The results given above were obtained from the first 30 minutes of the experiments.

of citrate was negligible even when malonate was present (Massey and Rogers 1949). In spite of the fact that citrate failed to stimulate respiration in brei it would appear that it was utilized with the formation of α-ketoglutarate and succinate. The interpretation of the results of these experiments is complicated by the fact that citrate may inhibit the further oxidation of its primary oxidation product, α-ketoglutaric acid, and because malonate may increase the utilization of added citrate (Stone, Lipton, and Goldinger 1941). In the absence of malonate, citrate competes with its oxidation products; malonate increases the

utilization of citrate by reducing this competition (Krebs 1943). It should be pointed out that the method used for the estimation of succinate really determines succinate plus α-ketoglutarate.

TABLE 6
EFFECT OF SUBSTRATES ON MALONATE INHIBITION IN *ASCARIDIA GALLI*

System	Oxygen Uptake (μl.)	Inhibition Due to Malonate (%)
No additions	153	
+ Malonate 0.02M	100	35
+ Fumarate 0.01M	204	
+ Fumarate 0.01M + malonate 0.02M	198	3
No additions	96	
+ Malonate 0.001M	71	26
+ Pyruvate 0.01M + fumarate 0.01M	143	
+ Pyruvate 0.01M + fumarate 0.01M + malonate 0.001M	150	No inhibition
No additions	96	
+ Malonate 0.01M	73	24
+ Malate 0.01M	110	
+ Malate 0.01M + malonate 0.01M	102	7

Each flask contained 1 ml. of parasite brei and methylene blue to a final concentration of 0.003M, and was buffered with Krebs-Ringer phosphate solution pH 7.3. The results given above were obtained from the first 30 minutes of the experiments.

V. DISCUSSION

It is probable that the action of calcium ions on the respiration of the tissue preparations of the parasites was due to the activation of phosphatases (Dubois and Potter 1943) which led to the rapid destruction of diphosphopyridine nucleotide, triphosphopyridine nucleotide, and adenosinetriphosphate. This action could be overcome by omitting calcium chloride from the medium or by the addition of relatively large amounts of the coenzymes. The suggestion that the action of calcium ions was due to the activation of phosphatases

is supported by the results obtained with brei which was prepared by grinding the parasites with a little phenothiazine. Phenothiazine is an inhibitor of phosphatases (Lazarus, unpublished data), and phenothiazine-poisoned brei gave results similar to those obtained when calcium chloride was omitted from the medium (see Table 9).

TABLE 7
EFFECT OF MALONATE ON SUCCINATE FORMATION

System	*Nematodirus* spp.	*Ascaridia galli*	Pigeon Breast Muscle
Fumarate 0.01M + pyruvate 0.01M	0.30	0.34	0.28
Fumarate 0.01M + pyruvate 0.01M + malonate 0.01M	0.30	0.36	1.33
Fumarate 0.01M + pyruvate 0.01M + cofactors	0.40	0.51	–
Fumarate 0.01M + pyruvate 0.01M + cofactors + malonate 0.01M	1.25	1.03	–

The figures represent milligrams of succinate which accumulated during shaking at 37°C. for 2 hours. Each flask contained 10 ml. of brei and methylene blue to a final concentration of 0.0005M. Each flask was buffered with Krebs-Ringer phosphate solution without calcium chloride of pH 7.3. The cofactor mixture which was added contained 10 mg. coenzyme I (purity 40 per cent.), 0.2 mg. coenzyme II (purity 15 per cent.), and 10 mg. adenosinetriphosphate.

TABLE 8
EFFECT OF MALONATE ON CITRATE UTILIZATION IN *NEMATODIRUS* SPP.

System	Initial Citrate	Citrate after 2 Hours	Initial Succinate	Succinate after 2 Hours	Conversion of Citrate to Succinate
+ Citrate	2.5 mg.	1.3 mg.	0.20 mg.	0.51 mg.	42%
+ Citrate + malonate 0.02M	2.5 mg.	2.0 mg.	0.20 mg.	0.42 mg.	71%

Each flask contained 5 ml. of parasite brei, citrate to a final concentration of 1 mg. per ml., methylene blue to a final concentration of 0.0002M, and was buffered with Krebs-Ringer phosphate solution pH 7.3. Samples for analysis were taken immediately after the addition of brei, and after 2 hours' shaking at 37°C.

When the coenzyme concentration of the brei was very low, oxidative activity would be restricted to isolated oxidations of individual intermediates of the tricarboxylic acid cycle; coenzyme-linked reactions would be weak and

the cycle would not function as a whole. Under such circumstances the failure of succinate to accumulate in malonate-poisoned brei to which fumarate and pyruvate had been added would be expected.

TABLE 9
EFFECT OF PHENOTHIAZINE ON MALONATE-SUCCINATE RELATIONSHIPS

	System	*Nematodirus* spp.		*Ascaridia galli*	
		Oxygen Uptake (μl.)	Change (%)	Oxygen Uptake (μl.)	Change (%)
Brei without phenothiazine	No additions	109		43	
	+ Malonate 0.01M	61	−44	33	−23
	+ Succinate 0.01M	200	+83	83	+93
	+ Malonate 0.01M + succinate 0.01M	101	−49	50	−44
Brei ground up with phenothiazine	No additions	85		60	
	+ Malonate 0.01M	58	−32	49	−18
	+ Succinate 0.01M	91	+7	76	+27
	+ Malonate 0.01M + succinate 0.01M	85	−7	78	+3

Each flask contained 1 ml. of brei, methylene blue to a final concentration of 0.0003M, and was buffered with Krebs-Ringer phosphate solution of pH 7.3. The results given above were obtained from the first 20 minutes of the experiments.

When conditions were such that an adequate concentration of coenzymes was maintained in the parasite brei, several characteristic reactions of the tricarboxylic acid cycle were demonstrated:

(1) The addition of fumarate, malate, oxaloacetate, pyruvate, α-ketoglutarate, or succinate, all of which are intermediates in the cycle, caused an increase in respiration.

(2) In the presence of malonate, succinate accumulated in respiring brei, and the amount formed was increased when fumarate or pyruvate was added.

(3) Malonate inhibited respiration and this inhibition was lessened when intermediates of the tricarboxylic acid cycle were added.

(4) Added citrate was utilized by the parasite brei, and utilization was inhibited by malonate. In the presence of citrate, succinate accumulation was increased, not only because citrate or its products inhibited succinic dehydrogenase, but also because citrate was apparently converted to succinate.

(5) It has been shown (Massey and Rogers, unpublished data) that citrate was formed in parasite brei as a result of the condensation of oxaloacetate and acetate or its products.

(6) The results obtained with inhibitors were largely in accordance with the view that the tricarboxylic acid cycle was functioning in the parasite brei. Thus pyrophosphate, which is considered to be an inhibitor of succinic dehydrogenase (Leloir and Dixon 1937) though it does not cause an accumulation of succinate (Krebs 1943), and arsenite, which inhibits the oxidative decarboxylation of α-ketoglutarate (Krebs and Johnson 1937), were effective inhibitors of the respiration of the brei. Malonate, however, was often a poor inhibitor when used in low concentrations. Though azide and arsenate chiefly inhibit systems outside the tricarboxylic acid cycle, their action would generally lead to lessened activity within the cycle.

Some of the results obtained in the present investigation are not completely in accord with the hypothesis that the tricarboxylic acid cycle was functioning in the parasite brei. Thus, though citrate was actively utilized by the brei, on no occasion did it cause an increase in respiration and it is difficult to agree that this was due to the fact that the inhibition of succinic dehydrogenase was always such that it exactly negated the stimulation produced by the increase in the concentration of substrates. Again, malonate was a relatively poor inhibitor of the respiration of parasite brei. In low concentrations, 0.004M, malonate is a specific inhibitor of succinic dehydrogenase; at higher concentrations, 0.02M, magnesium-activated enzymes of the tricarboxylic acid cycle are also inhibited (Pardee and Potter 1949). The results obtained when the tissues of the parasites were examined suggests that either the succinic dehydrogenase was relatively insensitive to malonate, or that the dehydrogenase was relatively less important in the respiratory mechanisms of the parasite than in mammalian tissue, or that some component in the parasite brei lessened the efficiency of malonate as an inhibitor. However, in view of the positive evidence already given, which indicated that the tricarboxylic acid cycle was functioning in the parasite brei, these inconsistencies are not serious.

The tricarboxylic acid cycle has been shown to function in the tissues of a wide variety of mammals. It also functions in certain protozoa (Evans 1946) and insects (Barron and Tahmisian 1948). The precise details of the cycle, however, are not known and it would be unprofitable to discuss the apparent differences in the cycle as it occurs in the tissues of different animals.

The parasites used in this investigation probably differed greatly in the nature of their metabolism *in vivo*. *Neoaplectana glaseri* is an obligate aerobe when cultured *in vitro* in a medium which is adequate for continued growth and reproduction (Massey and Rogers, unpublished data) and is probably essentially aerobic *in vivo* also. Though some oxygen is present in the contents of the small intestine of sheep, close to the mucosa, *Nematodirus* spp. are probably partially anaerobic *in vivo*, and the large *Ascaridia galli* in the small intestine of chickens, is probably largely anaerobic (Rogers 1949*a*, 1949*b*).

Yet it would appear from the results given in this paper that all these forms possess mechanisms whereby carbohydrate is oxidized. And under certain circumstances at least, the parasites can usefully utilize the energy from oxidative mechanisms. Thus, when *Ascaridia galli* and *Nematodirus* spp. are maintained *in vitro* in media containing sufficient penicillin and streptomycin to limit bacterial growth, egg production is greatly reduced when oxygen is excluded from the medium though the parasites live for a considerable period (Rogers, unpublished data). It would appear that the metabolic routes which can function in *Ascaridia galli* and *Nematodirus* spp. may be very similar, but under the conditions prevailing *in vivo,* the particular mechanisms used to obtain energy may vary considerably.

Neoaplectana glaseri is similar, from many points of view, to free-living nematodes, and it would appear that adaptation to parasitism of the alimentary tract may involve the development of mechanisms for the anaerobic catabolism of pyruvate.

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