STUDIES ON THE METABOLISM OF THE FILARIAL WORM, LITOMOSOIDES CARINII*

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During the miltiary operations in the Pacific Theatre members of the Armed Forces not infrequently were exposed to filariasis. This circumstance resulted in the initiation of a series of investigations of the chemotherapy of this disease (1–7). For a better understanding of the mechanism of action of available, and for the potential development of new, antifilarial drugs it was considered desirable to obtain information about the metabolism of filariae. Since the human forms of the filarial worm, *Wuchereria bancrofti* and *Wucheria malayi* as yet cannot be obtained for biochemical investigations, it was decided to study the metabolism of a morphologically closely related filarial worm, *Litomosoides carinii* (2). This organism is a common inhabitant of the pleural cavity of the wild cotton rat.

Methods

The organisms were removed from the pleural cavity of infested cotton rats and were placed in a salt medium. The medium used in the experiments described below, and referred to as a "basic filarial medium," had the following composition: 0.137 M NaCl; 0.0027 M KCl; 0.0003 M $CaCl_2$; 0.001 M $MgCl_2$; 0.06 M sodium phosphate buffer (pH 7.6). After repeated washings with this medium, the filariae were blotted with No. 50 Whatman filter paper, weighed on a torsion balance, and transferred to Warburg respirometer vessels. Unless otherwise stated, 15 to 25 mg. of worms were placed into 0.8 ml. of medium in each vessel; the total volume of the vessels varied between 4 and 5 ml. The center cup contained a roll of filter paper moistened with 0.1 ml. of 40 per cent KOH. Incubation and measurement of the oxygen uptake were carried out at 37.5°C. in the conventional Warburg apparatus in an atmosphere of air. Carbon dioxide production was measured by the direct method of Warburg (8). Glucose (9), lactic acid (10), pyruvic acid (11), and steam-volatile acids were determined in aliquots of the medium, before and after incubation of the filariae.

Steam-volatile acids were determined in an aliquot of the medium placed in the distilling flask of an all glass micro steam-distilling apparatus. An amount of molar phosphate buffer (2 volumes M H_3PO_4; 1 volume M NaH_2PO_4; pH 1.8) equivalent to one-fifth of the volume of the sample was then added to the latter. The steam distillate was collected in a layer of ice cold distilled water into which the lower end of the condensing tube was immersed. Steam

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distillation was carried out until the volume of distillate was twelve times greater than that of the sample to be analyzed. During the distillation sufficient heat was applied to the distilling flask to keep the volume of the sample constant. The steam distillate was saturated with nitrogen (5 minutes) and then titrated with 0.005 N NaOH (indicator bromothymol blue). Nitrogen was passed through the distillate throughout the whole period of titration in order to keep contamination by CO_2 at a minimum.

The total carbohydrate content of the worms was determined by the following procedure. The organisms (20 to 60 mg. wet weight) were ground in an all glass homogenizer with 5 N H_2SO_4 (2 ml.). The mixture was transferred to a calibrated centrifuge tube and was heated for 20 minutes in a boiling water bath (during this period the mouth of the tube was covered with a glass marble). As demonstrated by Sahyun (12), complete hydrolysis of glycogen occurs under these conditions; no further increase in reducing sugar was observed when heating of the ground worms in 5 N H_2SO_4 was continued up to 180 minutes. After cooling, neutralization, and precipitation with Somogyi's (9) $Ba(OH)_2$ and $ZnSO_4$ reagents, aliquots of the filtrate were taken for the determination of fermentable sugar (9), both before and after treatment with yeast (13). Glucose added to such filtrates was fermented completely by this treatment.

For the analysis of filarial polysaccharide, the worms (20 to 60 mg.) were ground in 30 per cent KOH and the mixture was heated for 20 minutes in a centrifuge tube covered with a glass bulb. The polysaccharaide was then precipitated according to the method described by Good, Kramer, and Somogyi for glycogen (14). The residue was suspended in 5 N H_2SO_4 (2 ml.) and hydrolysis and determination of fermentable sugar were carried out in the same manner as described above for the analysis of total filarial carbohydrate.

The determination of cyanide in the filarial medium was based on the reaction of cyanide with iodine to form cyanogen iodide (15). This reaction can be measured by the decrease in the optical density in the presence of starch. The starch-iodine solution was prepared in the following manner: 1 ml. of a KIO_3 solution (0.01 N) was placed in a 250 ml. volumetric flask containing about 150 ml. of distilled water. After addition of 2 ml. of KI (5 per cent), 1 ml. of H_3PO_4 (0.17 M), and 2 ml. of freshly prepared starch solution (1 per cent) the mixture was diluted to 250 ml. with distilled water. To 5 ml. of this starch-iodine solution in a colorimeter tube was added 0.8 ml. of the cyanide solution (concentration range, 1×10^{-4} to 2×10^{-5} M; solutions with a higher concentration of cyanide should be diluted appropriately). To a control tube containing 5 ml. of the starch-iodine solution was added 0.8 ml. of basic filarial medium. The contents of the tubes were mixed well, allowed to stand for 15 minutes in the dark at room temperature, and the optical densities of the solutions were read in a colorimeter using a red filter (filter No. 66 of the Klett-Summerson instrument). The decrease in optical density is proportional to the cyanide concentration. The accuracy of this prodecure is ± 5 per cent.

RESULTS

Oxygen Uptake of L. carinii.—When filariae were incubated in basic filarial medium they remained motile for a much longer period of time in an atmosphere of air than in an atmosphere of nitrogen. Thus, it became evident that these organisms take up oxygen and that at least part of the energy required by them for motility is supplied by oxidative reactions. When the medium contained no substrate the oxygen uptake of the filariae gradually decreased over a period of 7 hours (Table I). In the presence of glucose the oxygen uptake of the organisms was about twice as high as in the absence of this substrate and it remained

constant for at least 7 hours (Table I). The motility of the organisms was preserved in the presence of glucose; otherwise a gradual decrease in the motility of the worms occurred. Fructose and mannose were as effective as glucose in increasing the oxygen uptake and in preserving the motility of the filariae. On the other hand, addition of the following substances to the medium produced no change in the rate of respiration and in the motility of the worms: galactose, ribose, sodium lactate, pyruvate, succinate, fumarate, glutamate, formate, acetate, propionate, butyrate, or valerate. The respiratory quotient of the filariae in the absence of glucose varied in 12 experiments between 0.35 and 0.57 (average, 0.44; standard deviation, ±0.07); in the presence of glucose the R.Q. varied between 0.87 and 1.03 (14 experiments; average, 0.94; standard deviation, ±0.07).

TABLE I

Oxygen Uptake of Filariae in the Absence and in the Presence of Glucose

Time after start of experiment	Q_{O_2}*									
	No glucose					Glucose (0.04 M)				
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 1	No. 2	No. 3	No. 4	No. 5
min.										
0– 60	0.92	0.80	0.74	0.77	0.78	1.80	2.07	1.81	1.92	1.66
60–120	0.78	0.72	0.67	0.74	0.65	1.87	2.22	1.86	2.02	1.74
120–180	0.82	0.72	0.67	0.77	0.65	1.87	2.16	1.96	2.06	1.78
180–240	0.78	0.72	0.65	0.63	0.54	1.82	2.36	1.86	1.98	1.78
240–300	0.68	0.70	0.57	0.66	0.52	1.89	2.15	1.92	2.06	1.74
300–360	0.63	0.58	0.48	0.55	0.46	1.82	2.22	1.92	2.02	1.81
360–420	0.58	0.57	0.45	0.55	0.46	1.87	2.18	1.84	2.00	1.78

* Q_{O_2}, milliliters of oxygen taken up by 1 gm. filariae (wet weight) in 1 hour.

It has been observed with many invertebrates that exposure of the organisms to anaerobiosis resulted in a subsequent increase in respiration (16–19). This temporary rise in the oxygen uptake occurring immediately after anaerobiosis did not occur with the filarial worm, *L. carinii*. The oxygen uptake of this organism was the same before and immediately after a period of anaerobiosis (Table II).

Products of Glucose Metabolism of Filariae.—Aerobic or anaerobic incubation of the filariae with glucose resulted in a marked lowering of the pH of the medium. Under these conditions one of the acids formed was *d*-lactic acid. This compound was identified in the following manner.

About 500 mg. of filariae were incubated aseptically at 37°C. for 24 hours in basic filarial medium (100 ml.) containing glucose (0.05 M). The medium was acidified (pH 1.0) and extracted for 24 hours with peroxide-free ether in a Soxhlet apparatus. The dried ether extract was then treated with *o*-phenylenediamine and the reaction product isolated according to a

procedure described by Moore, Dimler, and Link (20). *o*-Phenylenediamine reacts with hydroxycarboxylic acids to form benzimidazole derivatives (21). The isolated compound had the characteristics of *d*-lactic benzimidazole described by Dimler and Link (22) and had a melting point of 175–176°C. (melting point of authentic *d*-lactic benzimidazole; 175–177°C.): mixed melting point with *d*-lactic benzimidazole, 175–176°C.; optical rotation (C_4, ethyl alcohol), −33.6° (*d*-lactic benzimidazole, −33.4°); elementary analysis,[1] C, 66.15 per cent; H, 6.43 per cent; N, 17.23 per cent (calculated for lactic benzimidazole ($C_9H_{10}ON_2$): C, 66.6 per cent; H, 6.23 per cent; N, 17.28 per cent). Thus, *d*-lactic acid is a product of the metabolism of glucose by filariae.

TABLE II

Oxygen Uptake of Filariae before and after Anaerobiosis

The filariae were incubated for 30 minutes in air at 37.5°C. Then their oxygen uptake was recorded for the following 90 minutes; whereupon vessels and manometers were saturated with nitrogen (previously passed through alkaline pyrogallol and red hot copper wire) for 10 minutes. The manometers were replaced in the water bath (37.5°C.) and shaken for 3 hours. Following anaerobiosis the vessels were removed from the manometers and their contents saturated with air by rotating them gently for 3 minutes. The oxygen uptake of the worms was recorded for the following 2 hours. Glucose concentration of the medium, 0.05 M.

Period	Q_{O_2}		
	No. 1	No. 2	No. 3
Control period	2.07	1.82	1.93
1st hr. after anaerobiosis	2.04	1.82	1.96
2nd hr. after anaerobiosis	2.09	1.87	1.91

In addition to *d*-lactic acid, a steam-volatile acid was formed during aerobic and anaerobic incubation of the nematodies with glucose. The acid in the steam distillate was identified as acetic acid by two different methods:

1. Elsden (23) has demonstrated that volatile fatty acids are eluted at different characteristic rates from silica gel columns by chloroform-butanol mixtures. The rate of elution of these acids is inversely proportional to the length of the carbon chain. In Elsden's series valeric acid was eluted most rapidly and formic acid least rapidly.

The steam distillate to be analyzed (containing 5 to 15 micromoles of acid) was neutralized and was evaporated to dryness. After addition of 1 gm. of $KHSO_4$ the residue was extracted with successive portions (2 ml.) of butanol (5 per cent) in chloroform, and the volume was made up to exactly 10 ml. Five ml. of the extract was placed on the silica gel column which had been prepared according to Elsden (23). Bromcresol green served as the indicator.

A single yellow band was observed on the column during the elution, indicating that the distillate contained only one acid. The acid was eluted quantitatively with the 5 per cent butanol-chloroform mixture, and the rate of elution was equal to or slightly lower than that obtained with known solutions of acetic acid of similar concentration. This excluded the

[1] The elementary analysis of this compound was performed by William Saschek.

possibility that the steam-volatile acid in question was propionic, butyric, or valeric acid. Furthermore, formic acid remains fixed at the top of the column, and can be eluted only by increasing the butanol concentration to 20 per cent (23).

The absence of formic acid was confirmed by a negative chromotropic acid test (15)[2] observed in concentrated steam distillates containing 2 microequivalents of the organic acid, whereas, this test was positive with 0.1 microequivalent of formic acid added to this distillate.

2. Carboxylic acids react with *S*-benzylthiuronium chloride to form crystalline derivatives having well defined melting points (24, 25). Accordingly, a derivative of the steam-volatile acid formed by the filariae was prepared: 450 mg. of filariae were incubated aerobically for 4 hours at 37.5°C. in 20 ml. of basic filarial medium containing glucose (concentration 0.05 M). After the incubation the acid steam distillate (250 ml.) obtained from the medium was neutralized to pH 8.0 and was concentrated *in vacuo* to dryness. The residue was dissolved in water and the resulting solution was transferred to a centrifuge tube. A small insoluble residue was centrifuged off and the supernatant (2 ml.) was transferred to an evaporating dish. Of this supernatant, 0.05 ml. gave a strongly positive lanthanum nitrate test (26, 15).[3] This test is positive in the presence of acetate or propionate, but negative with formate. The supernatant was then evaporated to dryness and was dissolved in 0.3 ml. of water following which the solution was transferred to a 2 ml. centrifuge tube. The evaporating dish was then washed twice with 0.2 ml. of water and the washings were combined with the original solution. The pooled solutions were then made slightly acid (pH about 6.0) and 20 mg. of benzylisothiourea hydrochloride was added. After cooling the solution in an ice bath a crystalline precipitate formed which was centrifuged down after 1 hour. After four recrystallizations from 50 per cent alcohol, the cystals melted at 134°C. This melting point did not change on further recrystallization. When mixed with an authentic specimen of the *S*-benzylthiuronium derivative of acetic acid (melting point, 134–135°C.) (24, 25), a melting point of 135°C. was observed. The mixed melting point of the propionate derivative (melting point, 151–152°C.) (25) with the isolated material was 129°C. Similarly, a mixture of the authentic acetate and propionate derivatives had a melting point of 129°C. The identity of the *S*-benzylthiuronium derivative of the isolated acid with that of acetic acid was confirmed by the spectroscopic data reproduced in Fig. 1 (curves A and B). No differences in the absorption of infrared radiation in the region from 7.43 to 15.08 μ could be detected that are not to be ascribed to differences in the concentration of crystalline material in the optical path. The propionate derivative on the other hand was markedly different (curve C).[4]

Aerobic incubation of the worms in glucose containing basic filarial medium resulted in an increase in the total carbohydrate content of the filariae. The carbohydrate stored by these organisms is a polysaccharide which was completely hydrolyzed to a fermentable sugar after heating the homogenized worms in 5 N H_2SO_4 at 100°C. for 20 minutes. Isolation of the polysaccharide according to Bell and Young (27) yielded a white powder which with iodine gave a red-brown color characteristic of glycogen. The amount of total carbohydrate synthesized by the filariae during aerobic incubation with glucose was equal to the increase in intrafilarial glycogen as determined by the method of Good,

[2] Feigl (15), page 397.

[3] Feigl (15), page 397.

[4] The infrared spectra were kindly determined by Dr. Hans Hirschmann, to whom the author wishes to express his great appreciation.

Kramer, and Somogyi (14) (Table III). On the other hand, filariae contain a polysaccharide which is not identical with glycogen, because the total carbohydrate content of the worms before aerobic incubation with glucose (Table III) was found to be significantly higher than the initial filarial glycogen.

Anaerobic and Aerobic Carbohydrate Balances of Filariae.—When the filariae were incubated anaerobically the polysaccharide content of the organisms de-

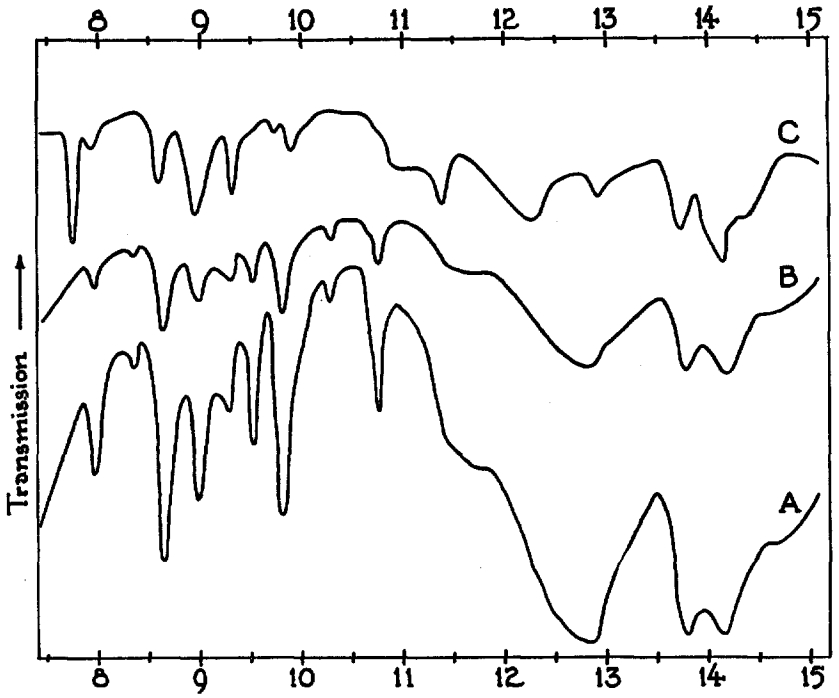


FIG. 1. Infrared transmission curves of the *S*-benzylthiuronium derivatives of acetic acid (A); of the steam-volatile acid isolated from filarial medium (B); and of propionic acid (C). Measurements were made on nujol mulls with a Perkin-Elmer infrared spectrometer (Model A-B-12). Abscissa, wavelength in microns.

creased, even when glucose was present in the medium. Under these conditions, over 80 per cent of the total carbohydrate was converted to lactic acid, the remainder to acetic acid (Table IV). Thus, under anaerobic conditions, lactic and acetic acid production accounted completely for the disappearance of polysaccharide from the worms and the removal of glucose from the medium.

Aerobically a much smaller proportion of the removed glucose was converted to lactic acid (30 to 40 per cent). On the other hand, the filariae formed more acetic acid from glucose under aerobic than under anaerobic conditions. The formation of acetic acid accounted for 25 to 35 per cent of the glucose removed by the filariae in an atmosphere of air. The organisms produced approximately

3 times more acetic acid under aerobic than under anaerobic conditions. Synthesis of polysaccharide accounted for 10 to 20 per cent of the glucose removed

TABLE III

Aerobic Polysaccharide Synthesis by Filariae

The filariae were shaken in a water bath (37.5°C.), for a period of 3 hours, in 3 ml. basic filarial medium containing 0.02 M glucose. Atmosphere, air.

At the end of the incubation period, total carbohydrate was determined in one aliquot of worms and glycogen (9) in another ("final" samples). The "initial" total carbohydrate and glycogen contents of the filariae were determined before incubation of the worms in the glucose-containing medium; 21 to 28 mg. of filariae were used for each determination.

Experiment No.	Sample	Total fermentable carbohydrate		Glycogen	
		Per cent	Increase per gm. worms (wet weight)*	Per cent	Increase per gm. worms (wet weight)*
			mg.		*mg.*
1	Initial	1.74	—	0.73	—
	Final	3.51	17.7	2.56	18.3
2	Initial	0.99	—	0.81	—
	Final	2.62	16.3	2.48	16.7
3	Initial	1.23	—	0.79	—
	Final	1.95	7.2	1.50	7.1

* Expressed as glucose.

TABLE IV

Anaerobic Glucose Balance of Filariae

Filariae (70 to 130 mg. wet weight) incubated in atmosphere of nitrogen for 90 minutes in basic filarial medium (2 ml.). Temperature, 37.5°C. All results expressed in micromoles per hour per gram of filariae.

Experiment No.	Substrate	Glucose removed	Decrease in polysaccharide*	Total carbohydrate removed*	Lactic acid	Acetic acid	Removed carbohydrate accounted for	
		μM	*μM*	*μM*	*μM*	*μM*	*μM*	*per cent*
Control	—	—	18	18	33	0	16.5	92
1	Glucose 0.015 M	78	16	94	159	26	92.5	98.5
2	Glucose 0.015 M	69	10	79	130	23	76.5	97

* Expressed as glucose.

from the medium. Of the glucose utilized by the worms, 20 to 25 per cent could not be accounted for by the formation of lactic acid, acetic acid, or polysaccharide. In Table V two representative aerobic carbohydrate balances of filariae (see control samples) are reproduced.

TABLE V

Aerobic Glucose Balance of Filariae

Filariae (22 to 32 mg. wet weight) incubated for 240 minutes in 2 ml. basic filarial medium containing glucose (0.015 M). Temperature, 37.5°C. Atmosphere, air. All results expressed in micromoles per hour per gram of filariae.

Experiment No.	Na fluoroacetate	Cyanine dye No. 348	O_2 uptake	Glucose removed	Lactic acid	Percent of glucose removed accounted for*	Acetic acid	Pyruvic acid	Polysaccharide synthesis‡	Glucose removed accounted for§	
	μM		μM	μM	μM		μM	μM	μM	μM	*per cent*
1	—	—	99	118	77	32	81	0.8	13.2	92.6	79
	4×10^{-3}	—	40	88	122	70	42	7.0	3.7	87.4	99
	1×10^{-3}	—	76	111	93	41	81	4.3	5.3	94.4	85
2	—	—	100	127	90	35.5	66	0.6	20.0	98	77
	—	2.6×10^{-7}	30	131	231	88.5	18.7	0.6	4.2	129	98.5

* By lactic acid formation.

‡ Expressed as glucose.

§ By lactic acid, pyruvic acid, acetic acid, and polysaccharide formation.

TABLE VI

Effect of Ionic Environment on Metabolic Activity and Motility of Litomosides carinii

Incubation period, 2 hours. Basic filarial medium containing 0.015 M glucose.

Experiment No.	NaCl	KCl	$CaCl_2$	$MgCl_2$	Sodium phosphate buffer (pH 7.56)	Potassium phosphate buffer (pH 7.56)	Ionic strength	Q_{O_2}	Q_G*	Q_L‡	Motility§
	M	*M*	*M*	*M*	*M*	*M*	μ				
1	0.155	0.0027	0.0003	0.0022	0.06	—	0.34	1.88	28.4	11.1	+++
	—	0.0027	0.0003	0.0022	0.06	—	0.185	1.45	19.8	8.2	+
	—	0.0027	0.0003	0.0022	0.11	—	0.34	1.50	21.2	8.8	++
	—	0.0027	0.0003	0.0022	0.22	—	0.665	1.29	19.6	7.6	+
	—	0.0027	0.0003	0.0022	0.38	—	1.145	0.96	18.3	6.0	0
	—	0.0027	0.0003	0.0022	—	0.11	0.335	1.16	16.1	7.3	++
	0.06*	0.0027	0.0003	0.0022	0.06	—	0.245	1.73	25.6	10.7	++
	0.32	0.0027	0.0003	0.0022	0.06	—	0.495	1.61	27.6	11.6	±
	0.64	0.0027	0.0003	0.0022	0.06	—	0.825	0.38	9.3	4.8	0
2	0.155	0.0027	0.0003	0.0022	0.06	—	0.34	1.63	25.0	9.9	+++
	0.0027	0.155	0.0003	0.0022	—	0.06	0.34	1.34	9.6	4.2	±
	0.155	—	—	—	0.06	—	0.335	1.17	14.3	4.8	+
	0.155	0.0027	—	—	0.06	—	0.335	1.46	20.2	7.8	+
	0.155	—	0.0003	—	0.06	—	0.335	1.49	18.5	6.3	+
	0.155	—	—	0.0022	0.06	—	0.335	1.43	20.7	8.1	±
	0.155	0.0027	0.0003	—	0.06	—	0.34	1.58	22.6	8.9	++
	0.155	0.0027	—	0.0022	0.06	—	0.34	1.62	23.3	9.1	++
	0.155	—	0.0003	0.0022	0.06	—	0.34	1.52	21.3	7.8	+
	0.155	0.0027	0.0009	0.0022	0.06	—	0.34	1.26	14.3	6.4	+

* Milligrams of glucose removed by 1 gm. filariae (wet weight) in 1 hour.

‡ Milligrams of lactic acid formed by 1 gm. filariae (wet weight) in 1 hour.

§ Motility is expressed by the following arbitrary symbols: +++, very high motility; ++, high motility; +, fair motility; ±, sluggishness; 0, no motility.

Aerobically the filariae removed 1.4 to 2.2 mg. (15.5 to 24.5 micromoles) of lactic acid per gram per hour (15 experiments). The rate of aerobic utilization of lactic acid was much smaller (approximately 10 times) than that of glucose. This was also borne out by the fact that in contrast to glucose, lactate failed to produce an increase in the oxygen uptake of the filariae.

The rate of aerobic acetate utilization was extremely small. The highest observed value (8 experiments) amounted to 0.07 mg. (1.2 micromoles) acetic acid per gram per hour.

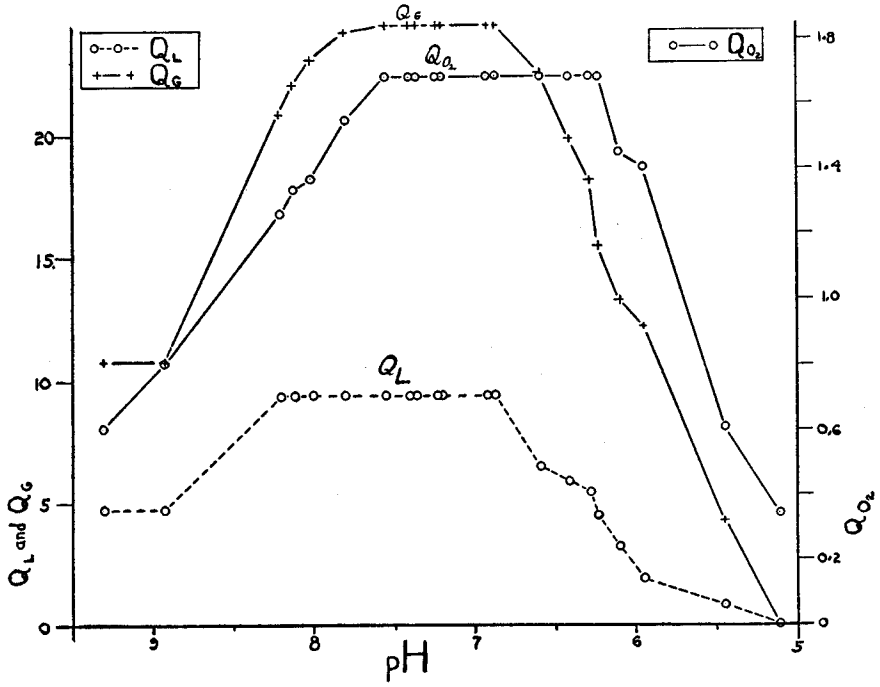


FIG. 2. Effect of pH on oxygen uptake (Q_{O_2}), glucose utilization (Q_G), and lactic acid production (Q_L) of *L. carinii*.

Effect of Ionic Environment (Table VI) and of the pH of the Medium on Filarial Metabolism.—Exclusion of sodium chloride produced a marked decrease in filarial metabolism and motility even if the ionic strength of the medium without NaCl was equal to that containing this salt. The concentration of NaCl optimal for metabolic activity and motility appeared to be close to 0.14 M. When the medium contained high concentrations of potassium instead of sodium ions metabolic activity and motility of the filariae were markedly decreased. When sodium was the only cation of the medium carbohydrate metabolism and respiration of the worms were lower than with a medium containing low concentra-

tions of KCl, $CaCl_2$, and $MgCl_2$. Usually the metabolic activity of the filariae was higher when all three of the latter salts were present in the medium instead of a combination of two of them. A decrease in the metabolic activity of the

TABLE VII

Anaerobic Metabolism of Pyruvate by Filariae

Filariae (70 to 100 mg.) incubated in an atmosphere of nitrogen in 2 ml. basic filarial medium containing sodium pyruvate (0.03 M). Temperature, 37.5°C. All results expressed in micromoles per hour per gram of filariae (wet weight).

Experiment No.	Sodium fluoroacetate	Pyruvic acid removed	Lactic acid formed*	Acetic acid formed	CO_2 production	Removed pyruvic acid accounted for	
	M	*μM*	*μM*	*μM*	*μM*	*μM*	*per cent*
1	—	158	80	75	—	155	98
	4×10^{-3}	161	79	73	—	152	94
2	—	163	78	80	82	158	97
3	—	78	38	42	39	80	103

* Corrected for lactic acid formed in the absence of pyruvate.

TABLE VIII

Aerobic Metabolism of Pyruvate by Filariae

Filariae (130 to 180 mg. wet weight) incubated for 180 minutes in 3 ml. of basic filarial medium containing sodium pyruvate (0.02 M). Atmosphere, air. Temperature, 37.5°C. All results expressed in micromoles per hour per gram of filariae (wet weight).

Experiment No.	Sodium fluoroacetate	O_2 uptake	Pyruvic acid removed	Lactic acid formed*	Acetic acid formed*	Pyruvic acid unaccounted for
	M	*μM*	*μM*	*μM*	*μM*	*μM*
1	—	30	47.5	15.8	25.6	5.7
	4×10^{-3}	16	29.6	15.8	13.3	0.2
	8×10^{-4}	23	40.3	16.7	21.8	1.8
2	—	33	51.8	12.6	19.0	20.2
	1×10^{-3}	23	38.8	11.6	18.3	8.9
	2.5×10^{-4}	32	51.3	11.8	19.2	20.3
3	—	39	46.3	19.2	21.2	5.9
	3×10^{-3}	19	30.5	18.2	13.0	−0.5
	1×10^{-3}	37	45.0	20.0	20.0	5.0

* Corrected for lactic acid and acetic acid formation in the absence of pyruvate.

worms was observed if the concentration of $CaCl_2$ in the medium was increased from 0.0003 M to 0.0009 M. Two typical experiments listed in Table VI illustrate the above mentioned effects of ions on the metabolism of the worms.

The presence of low concentrations of sulfate or manganese ions in the basic

filarial medium had no effect on the metabolic activity of the organisms. Because of the production of large amounts of lactic and acetic acids by *L. carinii*, a high buffer concentration of the medium was required to prevent a sharp drop in pH. Since variations in the concentration of the phosphate buffer between 0.006 M and 0.06 M had no effect on the metabolic activity of the worms it was decided to keep the concentration of the buffer in the basic filarial medium at 0.06 M.

In Fig. 2 the effects of the pH of the medium on oxygen uptake, utilization of glucose, and production of lactic acid of the filariae are represented.

TABLE IX

Effect of Fluoroacetate on the Aerobic Metabolism of L. carinii

Basic filarial medium containing 0.015 M glucose. Incubation period, 120 minutes.

Sodium fluoroacetate	Oxygen uptake		Glucose removal		Lactic acid production		Per cent of glucose removed accounted for by lactic acid production	Pyruvic acid formed*	Motility at end of experiment
	Q_{O_2}	Change	Q_G	Change	Q_L	Change			
M		*per cent*		*per cent*		*per cent*			
—	1.81	—	24.6	—	8.6	—	35	0.05	+++
1×10^{-2}	0.69	−62	19.3	−22	15.6	+80	80	0.94	+
1×10^{-3}	1.36	−25	24.3	−1	10.3	+24	48	0.36	++

* Milligrams per gram of filariae (wet weight) per hour.

Pyruvate Metabolism of Filariae.—The filarial worms removed pyruvate present in the medium under aerobic as well as under anaerobic conditions. Anaerobically pyruvate was removed at a faster rate than aerobically (Tables VII and VIII). It should be noted that in an atmosphere of air pyruvate was metabolized at a considerably slower rate than glucose. Anaerobically, all the pyruvate removed by the organisms could be accounted for by conversion to equimolar amounts of lactate and acetate. Acetic acid was identified by partition chromotography (23) of the steam distillates. One mole of CO_2 was produced for each mole of acetic acid formed (Table VII). Consequently in the filariae the following reaction occurred anaerobically:

$$2\ CH_3 \cdot CO \cdot COOH + H_2O \rightarrow CH_3 \cdot CHOH \cdot COOH + CH_3 \cdot COOH + CO_2$$

This dismutation of pyruvate to lactate, acetate, and CO_2 has also been demonstrated to occur in brain (28) and in certain bacteria (29, 30).

Under aerobic conditions, acetate production from pyruvate exceeded the formation of lactate (Table VIII, controls). Furthermore, a significant proportion of the metabolized pyruvate (10 to 40 per cent) could not be accounted for by either acetate or lactate formation.

Effect of Sodium Fluoroacetate on the Metabolism of Filariae.—It has been postulated recently that fluoroacetate inhibits the respiration of mammalian tissues, bacteria, and yeast because of a competitive inhibition of acetate oxidation (31–33). This compound was also found to inhibit the respiration and the motility of *L. carinii* (Table IX). The decrease in oxidative metabolism was associated with an increase in aerobic glycolysis because a larger proportion of the removed glucose was converted to lactic acid (Table IX). Aerobic incubation of filariae with fluoroacetate resulted in the accumulation of a keto acid, as determined by the dinitrophenylhydrazone method (11). Since worms in an amount adequate for isolation of the hydrazone could not be obtained, attempts were made to establish the identity of the keto acid in the following manner:

1. Oxalated human blood removes pyruvic acid (34) but does not remove α-ketoglutaric and other keto acids (35). Media in which filariae had been incubated with fluoroacetate were added to oxalated human blood; this mixture was allowed to stand at room temperature for various intervals of time. The rate of disappearance of the keto acid was identical with that of sodium pyruvate added to oxalated human blood.

2. Friedemann and Haugen (11) have shown that with their method only 19 per cent of the hydrazone of oxalacetic acid is extracted with benzene, while 79 per cent of this compound is extracted with ethylacetate. On the other hand, 93 per cent of the hydrazone of pyruvic acid is extracted with benzene and 81 per cent with ethylacetate (11). We were able to confirm these observations and to use them for the characterization of the keto acid formed by filariae in the presence of fluoroacetate and glucose. The hydrazone of this keto acid had the same solubilities in benzene and ethylacetate as pyruvic acid dinitrophenylhydrazone and its extinction (filter 54 of the Klett-Summerson colorimeter) after the addition of NaOH was 11 per cent higher when benzene instead of ethylacetate was used as the solvent. If the keto acid formed by the filariae were oxalacetic or α-ketoglutaric acid the extinction after extraction with benzene would have been considerably lower (76 per cent and 61 per cent respectively) than with ethylacetate. It was concluded, therefore, that the aerobic incubation of filariae with glucose and fluroacetate resulted in an accumulation of pyruvic acid. On the other hand, this was not the case under anaerobic conditions.

These observations suggested that fluoroacetate inhibits the aerobic utilization of pyruvate. As shown in Table VIII, fluoroacetate decreased the aerobic utilization of pyruvate by filariae. Low concentrations (1×10^{-3} M) of this inhibitor decreased markedly the amount of pyruvate removed which could not be accounted for by acetate or lactate production. Thus, the mechanism by which pyruvate oxidation is inhibited is one that is not concerned with the conversion of pyruvate to acetate. With higher concentrations (4×10^{-3} M) of fluoroacetate, oxidation of pyruvate to acetate was inhibited also, as well as the production of acetate from glucose (Table V). Low concentrations (1×10^{-3} M) of fluoroacetate did not affect acetate production from glucose although a significant accumulation of pyruvate and a decrease in the respiration of the organisms were observed (Table V). Fluoroacetate did not affect the anaerobic dismutation of pyruvate to acetate and lactate (Table VII) nor the reduction of pyruvate to lactate under aerobic conditions (Table VIII).

Effect of p-Chloromercuric Benzoate on Filarial Metabolism.—Respiration and carbohydrate utilization of the filariae were decreased by sulfhydryl inhibitors, such as iodoacetate or *p*-chloromercuric benzoate (36). In a concentration of 1×10^{-5} M, iodoacetate inhibited the oxygen uptake of the filariae to an extent of 30 per cent while an 80 per cent inhibition of respiration occurred with a concentration of 1×10^{-4} M. As illustrated in Table X, it appears that glycolysis of the worms was slightly more sensitive to inhibition by *p*-chloromercuric benzoate than respiration, because the production of lactic acid was inhibited to a significantly greater extent than the oxygen uptake of the organisms. Inhibition of filarial metabolism by this compound was associated with a reduction in or a disappearance of the motility of the worms.

TABLE X

Effect of p-Chloromercuric Benzoate on the Metabolism of Filariae

The filariae were incubated for 1 hour in basic medium containing 0.013 M glucose, with and without *p*-chloromercuric benzoate, then washed in a similar medium (containing no *p*-chloromercuric benzoate), and finally incubated for another hour in basic filarial medium of a similar glucose content, with and without SH compounds.

1st hour							2nd hour						
p-Chloromercuric benzoate	Oxygen uptake		Glucose removal		Lactic acid production		Added SH compound 1×10^{-2} M	Oxygen uptake		Glucose removal		Lactic acid production	
	Q_{O_2}	Inhibition	Q_G	Inhibition	Q_L	Inhibition		Q_{O_2}	Inhibition	Q_G	Inhibition	Q_L	Inhibition
M		per cent		per cent		per cent			per cent		per cent		per cent
—	1.80	—	22.3	—	7.8	—	—	1.87	—	21.6	—	8.2	—
1×10^{-4}	1.24	31	11.9	47	2.6	66	—	1.17	37	8.9	58	2.3	72
1×10^{-4}	1.11	38	10.8	52	2.3	70	Sodium thioglycolate	1.22	35	8.3	61	2.1	74
1×10^{-4}	1.19	34	9.8	56	1.8	77	Cysteine	1.10	41	8.9	58	2.2	73
1×10^{-4}	1.17	35	10.5	53	2.1	73	Glutathione	1.14	39	8.5	60	2.0	76

When the filariae were first incubated in a medium containing *p*-chloromercuric benzoate and were then washed in and subsequently transferred to basic filarial medium containing no *p*-chloromercuric benzoate, inhibition of respiration, glucose utilization, and lactic acid production persisted for many hours. This inhibition was not reversed by the addition of an excess of thioglycollate, cysteine, or glutathione (Table X). Furthermore, no reversal of this inhibition was observed with hydrogen sulfide.[5]

Effect of Cyanide on the Oxidative Metabolism of Filariae.—Respiration of the

[5] Although the possibility exists that some H_2S was absorbed by the alkali in the center cup, the presence of this gas in the medium at the end of the experiment was verified by a positive iodine-azide reaction (31).

filariae was strongly inhibited by cyanide. Riggs (37) has demonstrated that the usual methods (38, 39) employed to prevent the loss of cyanide from the medium because of absorption by alkali in the inset of the Warburg vessel are inadequate. Consequently, the concentration of cyanide and of KOH in the center cup necessary to keep the cyanide concentration of the solution in the main compartment constant were predetermined empirically. Furthermore, the cyanide concentration of each medium was measured at the end of the experiment. As illustrated in Table XI, cyanide inhibited completely the respiration and the motility of the worms in a concentration of 2×10^{-4} M. A five-times lower concentration (4×10^{-5} M) inhibited the oxygen uptake of the filariae to an extent of 63 per cent. The inhibition of filarial respiration produced by cyanide was associated with a compensatory increase in glycolysis

TABLE XI

Effect of Cyanide on the Metabolism of L. carinii

25 to 35 mg. filariae incubated for 1 hour in basic filarial medium containing 0.01 M glucose. Concentration of KOH in center cup, 0.5 per cent.

Initial concentration of KCN in medium	Final concentration of KCN in medium	Concentration of KCN in inset	Oxygen uptake		Q_G	Q_L	Per cent of glucose removed accounted for by lactic acid production	Motility
			Q_{O_2}	Inhibition				
M	M	M		*per cent*				
—	—	—	1.93	—	32.1	9.7	30	+++
1×10^{-3}	1.06×10^{-3}	1	0.04	98	22.7	22.3	98	0
2×10^{-4}	1.93×10^{-4}	0.7	0.06	97	24.0	24.1	101	0
4×10^{-5}	4.10×10^{-4}	0.11	0.68	65	30.3	20.9	69	±

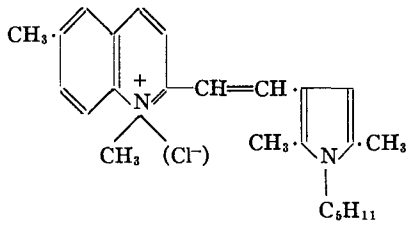
because a greater percentage of the glucose utilized was converted to lactic acid in the presence of this inhibitor than in its absence.

Effect of Cyanine Dyes on the Metabolism of L. carinii.—It has been reported recently from this and another laboratory that various cyanine dyes exert marked chemotherapeutic activity in filariasis of the cotton rat (5, 6). Members of this group of compounds inhibited in very low concentrations the respiration of the filarial worm, *L. carinii*. The cyanine dyes (40) contain the resonating amidinium ion system in which a quarternary nitrogen is linked to a tertiary nitrogen by a conjugated chain of an uneven number of carbon atoms:

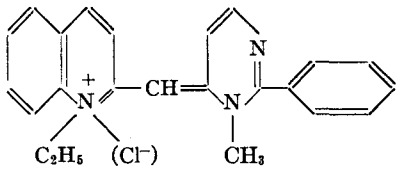
$$>\overset{+}{N}=\overset{\prime}{C}-\overset{\prime}{(C=C)_n}-N< \longleftrightarrow >N-\overset{\prime}{C}=\overset{\prime}{(C=C)_n}=\overset{+}{N}<$$

The relationship between the chemical structure of cyanine dyes and their ability to inhibit the respiration of filariae will be discussed elsewhere. However, it should be pointed out that high antifilarial activity *in vitro* was not re-

stricted to any particular ring, but that any structural modification which destroyed the possibility of amidinium ion resonance caused a disappearance of high antifilarial activity *in vitro* and *in vivo*. The cyanine dyes used in the present investigation were 1-amyl-2,5-dimethylpyryol-(3)-1,6-dimethylquinoline-(2)-dimethincyanine chloride (Chemotherapy Center No. 348),



and 1-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2-cyanine chloride (Chemotherapy Center No. 863).



For the sake of brevity these two compounds will be referred to subsequently by their respective Chemotherapy Center numbers.

The effect of a cyanine dye on filarial respiration was measured by recording the oxygen uptake of the worms before and after addition of a solution of the cyanine dye from the side arm of a Warburg flask. In a concentration of 6.5×10^{-8} M (1:40,000,000) cyanine dye 348 produced a significant inhibition of the oxygen uptake of filariae (Table XII). This inhbition became gradually more pronounced for a period of 60 to 90 minutes and then remained constant for several hours. Filarial respiration was almost completely inhibited (80 to 90 per cent) by No. 348 in a concentration of 5.2×10^{-7} M (1:5 million). Even when the concentration of the dye was increased another ten-fold (1:500,000), respiration of the worms was not completely inhibited and was maintained at about one-tenth of the level which occurred in the absence of the dye. It should also be noted that concentrations of No. 348 as high as 5.2×10^{-6} M decreased the motility of the worms only to a slight degree.

The decrease in the oxidative metabolism of the filariae was associated with an increase in aerobic glycolysis: a much greater proportion of the utilized glucose was converted to lactic acid in the presence of a cyanine dye than in its absence when respiration was not inhibited. As shown in Table XIII, the greater the inhibition of respiration the more pronounced was the increase in aerobic glycolysis. Another result of the inhibitory effect of the cyanine dye

on filarial respiration consisted in the decreased rate of acetic acid production and of polysaccharide synthesis (Table V).

The action of the cyanine dyes on filarial metabolism is not restricted to conditions *in vitro* but it can be observed also after the administration of these compounds to cotton rats infected with filariasis. Chemotherapeutic studies in

TABLE XII

Effect of Cyanine Dye No. 348 on the Oxygen Uptake of Filariae

Basic medium containing 0.02 M glucose. Immediately following the control period (90 minutes), the cyanine dye was tipped from the side arm in the main compartment of the Warburg vessel.

1st period (Control) (No cyanine dye)	2nd period (Experimental)						
		1st 30 min.		2nd 30 min.		3rd 30 min.	
Q_{O_2}	No. 348	Q_{O_2}	Change	Q_{O_2}	Change	Q_{O_2}	Change
	M		*per cent*		*per cent*		*per cent*
1.80	—	1.86	+3	1.78	−1	1.83	+2
1.94	6.5×10^{-8}	1.80	−7	1.58	−17	1.49	−21
1.88	1.3×10^{-7}	1.60	−15	1.30	−31	1.18	−37
1.77	2.6×10^{-7}	1.24	−31	0.80	−55	0.68	−62
1.96	5.2×10^{-7}	1.13	−42	0.41	−79	0.31	−86

TABLE XIII

Effect of Cyanine Dyes on Carbohydrate Metabolism of Filariae

Incubation period, 120 minutes. Basic medium containing 0.015 M glucose.

Cyanine dye No.	Concentration of cyanine dye	Oxygen uptake		Q_G	Q_L	Per cent of glucose removed accounted for by lactic acid production
		Q_{O_2}	Change			
	M		*per cent*			
—	—	1.72	—	34.5	13.1	38
348	1.3×10^{-7}	1.10	−36	27.3	18.5	68
348	5.2×10^{-7}	0.27	−84	23.4	23.1	99
863	6.5×10^{-8}	1.13	−34	26.0	19.7	76
863	2.6×10^{-7}	0.31	−88	25.6	24.8	97

this laboratory have demonstrated that after the intraperitoneal injection into these animals of 0.4 mg. of No. 863 per kg. during 5 successive days all worms found in the pleural cavity are dead.[6] When one-quarter of this dose was used the worms remained alive and motile, but their oxygen uptake was decreased and the rate of their aerobic glycolysis was increased (Table XIV). On the other

[6] Peters, L., Welch, A. D., and Higashi, A., unpublished observations.

hand, the cyanines had no significant effect on anaerobic glycolysis of the filariae, because lactic acid production in an atmosphere of nitrogen was practically the same, regardless of whether cyanine dyes had been administered or not.

Effect of Cyanine Dyes on the Activities of Cytochrome Oxidase and of Cytochrome C.—Since the cyanines in very low concentrations inhibited the respiratory metabolism of filarial worms their effect was tested on the activities of two

TABLE XIV

Metabolism of Filariae Removed from Cotton Rats Treated with Subcurative Doses of Cyanine Dye No. 863

Dosage schedule, 0.1 mg. No. 863 per kg. injected intraperitoneally on each of 5 successive days. Basic filarial medium containing 0.015 M glucose. Duration of experiment, 120 minutes.

Filariae from treated cotton rat No.	Atmosphere	Oxygen uptake		Q_G	Q_L	Per cent of glucose removed accounted for by lactic acid production
		Q_{O_2}	Change			
			per cent			
Controls (average from 20 untreated cotton rats)	Air	1.81	—	32.2	11.4	35
F2389		0.91	−50	23.4	20.1	86
F2390		1.21	−32	17.8	15.2	86
F2386		0.95	−48	23.3	20.8	89
F2387		0.43	−76	13.2	10.8	82
Controls (average from 20 untreated cotton rats)	Nitrogen	—	—	—	13.2	—
F2389		—	—	—	12.1	—
F2386		—	—	—	13.4	—
F2387		—	—	—	11.4	—

respiratory enzymes of mammalian tissues: cytochrome oxidase and cytochrome C. Cytochrome oxidase was prepared according to Haas (41) and its activity was assayed in the presence of an excess of hydroquinone and of cytochrome C (prepared according to Keilin and Hartree (42)) at 25°C. (37). Under these conditions the activity of the system was proportional to the concentration of cytochrome oxidase. An inhibitory effect of the cyanine dyes on the activity of cytochrome oxidase was observed only in concentrations of 2.6×10^{-4} M (Table XV). This was a concentration 4,000 times higher than was required to inhibit the respiration of the filariae.

TABLE XV

Effect of Cyanine Dyes on the Activity of Cytochrome oxidase

Phosphate buffer (pH 7.1), 0.05 M. Substrate, 3 mg. of hydroquinone. Total volume, 1 ml. Temperature, 25°C. Gas phase, air. The solution of cytochrome oxidase was incubated in Warburg flasks (volume, 4.0 to 5.5 ml.) with the cyanine dye and phosphate buffer at 37°C. for 30 minutes previous to assay (total volume, 0.7 ml.). After the incubation, the vessels were allowed to cool to room temperature, cytochrome C was added to the main compartment, the hydroquinone solution was placed in one side arm, and, in the other, enough of the cyanine solution to keep the initial concentration of the dye constant after tipping. The contents of the side arms were tipped in the main compartment after temperature equilibration and closing of the taps.

Cytochrome oxidase	Cytochrome C	Cyanine dye No.	Concentration of cyanine dye	Oxygen (25 min.)	Inhibition
ml.	*M*		*M*	*c. mm.*	*per cent*
0.035	3.9×10^{-8}	—	—	23	—
0.07	3.9×10^{-8}	—	—	48	—
0.07	1×10^{-7}	—	—	48	—
0.07	3.9×10^{-8}	863	5.2×10^{-4}	17	65
0.07	3.9×10^{-8}	863	2.6×10^{-4}	38	21
0.07	3.9×10^{-8}	863	1.3×10^{-4}	49	0
0.07	3.9×10^{-8}	348	5.2×10^{-4}	33	31
0.07	3.9×10^{-8}	348	2.6×10^{-4}	44	9
0.07	3.9×10^{-8}	348	1.3×10^{-4}	48	0

TABLE XVI

Effect of Cyanine Dyes on the Activity of Cytochrome C

Phosphate buffer (pH 7.1), 0.05 M. Substrate, 4 mg. of sodium ascorbate. Total volume, 1 ml. Temperature, 37.5°C. Gas phase, air.

Cytochrome C solution was incubated in Warburg vessels (4.0 to 5.5 ml.) for 30 minutes in phosphate buffer at 37°C. This was followed by cooling to room temperature and addition of cytochrome oxidase. Hydroquinone solution was then placed in one side arm and, in the other, enough of the cyanine solution to keep the concentration of the dye in the reaction mixture constant after tipping. The contents of the side arms were tipped in the main compartment after temperature equilibration and closing of the taps.

Cytochrome C	Cytochrome oxidase	Cyanine dye No.	Concentration of cyanine dye	Oxygen (30 min.)
moles	*ml.*		*M*	*c. mm.*
3.9×10^{-9}	0.1	—	—	17.5
1.56×10^{-8}	0.1	—	—	68
1.56×10^{-8}	0.2	—	—	68
7.8×10^{-9}	0.1	—	—	34
7.8×10^{-9}	0.1	863	5.2×10^{-4}	35
7.8×10^{-9}	0.1	348	5.2×10^{-4}	34

The activity of cytochrome C was tested in the presence of an excess of cytochrome oxidase. Under these conditions cytochrome C was the rate-limiting factor. As shown in Table XVI, the activity of cytochrome C was not inhibited even when the concentrations of the cyanine dyes were as high as 5.2×10^{-4} M.

DISCUSSION

It is evident that oxidative metabolism is essential for the survival of the filarial worm, *Litomosoides carinii*. The intraperitoneal injection of cyanine dyes into cotton rats infected with filariae caused an inhibition of the respiration of the worms. Although this effect resulted in a compensatory increase in glycolysis, anaerobic reactions appeared to be inadequate to maintain the organism alive because the administration of only three to four times higher doses of cyanines produced the death of the filariae (5, 6). This behavior contrasted with that of another parasitic helminth, *Schistosoma mansoni*. Although the cyanines were as effective in inhibiting the oxygen uptake of the latter organism, the parasite survived even when its respiration was inhibited almost completely by the administration of cyanine dye 863 to rabbits infected with schistosomiasis (43). Moreover, indications are available that other parasites can survive under anaerobic conditions (44[7]).

The absence of a postanaerobic increase in the oxygen uptake differentiates the filarial worm, *L. carinii*, from all other invertebrates whose respiration has been measured before and after anaerobiosis (44[8]). It is assumed generally that this repayment of the oxygen debt incurred during anaerobiosis is due to the accumulation products of anaerobic metabolism which are oxidized as soon as aerobic conditions are reestablished (44[8]). This interpretation is consistent with the observed absence of a postanaerobic increase in the respiration of filariae. Anaerobically these organisms converted carbohydrate to lactic acid and acetic acid. The latter was not oxidized significantly. While lactic acid was removed aerobically to a small extent by the filariae the addition of lactate to the medium did not produce an increase in the oxygen uptake of these organisms. Thus, the accumulation of lactate and acetate in the medium during anaerobiosis did not result in a higher rate of postanaerobic respiration.

In contrast to tissues from vertebrate animals and to many bacteria only a few invertebrates convert the major portion of the carbohydrate utilized anaerobically to lactic acid (44[9]). Among helminths the filarial worm, *L. carinii*, appears to be the only one whose main end-product of anaerobic carbohydrate metabolism is lactic acid. In other worms the anaerobic utilization of glycogen and of glucose results predominantly in the production of valeric, formic, acetic, butyric and non-volatile higher fatty acids (44[9]).

[7] Von Brand (44), pages 74 to 84.
[8] Von Brand (44), pages 236 to 247.
[9] Von Brand (44), pages 199 to 216.

The rate of polysaccharide synthesis from glucose by filariae was considerable. To our knowledge a similarly high rate of polysaccharide synthesis has not been reported for any other invertebrate organism. The synthesis of polysaccharide by the filariae appeared to require oxidative energy because this process did not occur under anaerobic conditions. Furthermore, inhibition of the oxidative metabolism of the parasite produced by the cyanines or by fluoroacetate resulted in a decreased rate of polysaccharide synthesis (Table V).

The filariae are well adapted to their natural habitat, the pleural cavity, because their respiration has been found to remain optimal even when the pH of the medium was much lower than the physiological pH of the body fluids. Since the worms produce a large amount of lactic acid, the pH of the pleural fluid in cotton rats heavily infected with filariae was found to be lower than 7.4, varying between 7.0 and 6.5. The more the pH of the medium fell below 6.9, the more pronounced was the decrease in lactic acid production (Fig. 1) while the oxygen uptake of the organism remained optimal until a pH of 6.1 was reached. Since oxidative metabolism was essential for survival of *Litomosoides carinii*, the lowered acid production at a pH below 6.9 prevented a lowering of the pH of the medium below 6.1 at which the respiration of the worms began to decrease.

It is likely that the rate of diffusion of glucose into the filarial cells is more rapid than that of pyruvate and of lactate. This is indicated by several observations: (1) glucose increased the oxygen uptake of filariae, while this was not the case with pyruvate or lactate; (2) the rate of aerobic utilization of glucose was much greater than that of pyruvate or of lactate; (3) the rate of anaerobic lactate production was higher with glucose as the substrate than with pyruvate. However, these results do not exclude the possibility that glucose may be partially metabolized by filariae through pathways which do not involve the formation of pyruvate. Definite information about this problem must await the preparation of metabolically active cell-free filarial extracts and a detailed study of the enzymes of filariae concerned with the metabolism of carbohydrate.

It appears that fluoroacetate does not inhibit the respiration of filariae through a competitive inhibition of acetate oxidation. Fluoroacetate did not produce an accumulation of acetate when the worms were incubated aerobically with glucose or pyruvate. On the contrary, high concentrations of this inhibitor (4×10^{-3} M or more) reduced the amount of acetate present in the medium. On the other hand, our experiments indicate that fluoroacetate can inhibit at least two mechanisms concerned with the oxidation of pyruvate by filariae. Low concentrations of fluoroacetate produced a decrease in the aerobic utilization of pyruvate through a reaction which did not result in its conversion to acetate. Possibly this mechanism involves the tricarboxylic cycle (45). Higher concentrations of fluoroacetate inhibited also the oxidation of pyruvate

to acetate. Therefore, the hypothesis that fluoroacetate is a specific competitive inhibitor of acetate oxidation (31–33) could not be verified with filariae. On the other hand, our results are consistent with the conclusion of Hutchens and MacMahon (46) that in yeast cells fluoroacetate inhibits primarily the oxidation of pyruvate rather than that of acetate.

Oxidative metabolism appears to be required for maintaining the motility of the filariae. Their motility was lost (within 60 to 90 minutes) in an atmosphere of nitrogen while reestablishment of aerobic conditions rapidly restored their motility. Furthermore, inhibition of filarial respiration produced by cyanide or fluoroacetate resulted in a decrease or a loss of motility. However, concentrations of the cyanines which produced a very marked decrease of their oxygen uptake had no apparent effect on the motility of the worms. It should be noted that the cyanines, even in relatively high concentrations inhibited the respiration of the worms to an extent of no more than 85 to 90 per cent. Possibly the residual respiration was catalyzed by enzyme systems which are not affected by the cyanines and whose actions supply the energy for the motility of filariae.

The observed inhibitory effect of the cyanines on filarial respiration is probably explained by an interference with a respiratory enzyme or coenzyme concerned with the transfer of electrons, rather than by an inhibition of a specific dehydrogenase. This is indicated by the fact that the cyanines inhibited the oxygen uptake of filariae in the absence of glucose, even when the polysaccharide stores of the worms had been depleted by previous anaerobic incubation. Therefore, this group of compounds decreased the respiration of the parasite regardless of whether carbohydrate or other substrates were oxidized. In contrast to filariae, concentrations of cyanine dyes up to 1×10^{-5} M did not affect the oxygen uptake of slices or homogenates of mammalian tissues[10] nor the activity of cytochrome C or cytochrome oxidase. This indicates that the cyanines inhibit in these filarial worms an enzyme system which plays no rôle, or only a minor one, in mammalian tissues.

The high cyanide sensitivity of the respiration of filariae indicates that these organisms contain one or several heavy metal-containing enzymes. However, the latter are not identical with cytochrome C or with cytochrome oxidase because neither of these two enzymes could be detected in suspensions of filariae. Further work is necessary to establish the mechanism of hydrogen transfer in this organism and to determine its rôle in other invertebrates.

SUMMARY

The filarial worm, *Litomosoides carinii*, has a high rate of aerobic and anaerobic glucose metabolism. Aerobically 30 to 45 per cent of the glucose utilized

[10] Unpublished observations.

was converted to lactic acid, 25 to 35 per cent to acetic acid, and 10 to 20 per cent to a polysaccharide. Anaerobically over 80 per cent of the total carbohydrate removed by the filariae was metabolized to lactic acid, the remainder was accounted for by the production of acetic acid.

The high rates of aerobic and anaerobic lactic acid production and of aerobic polysaccharide synthesis, as well as the absence of a postanaerobic increase of the oxygen uptake, differentiate the filarial worm, *L. carinii*, from the known metabolic characteristics of all other helminths and of most other invertebrates.

The rate of aerobic lactate and pyruvate utilization by the filariae appears to be much slower than that of glucose. Anaerobically, dismutation of two moles of pyruvate to one mole of lactate, one mole of acetate, and one mole of CO_2, occurred. Aerobically, acetate production from pyruvate exceeded that of lactate. A significant proportion of the pyruvate metabolized aerobically by the filariae was not oxidized to acetate.

In the presence of fluoroacetate, aerobic incubation of the filariae in a glucose-containing medium produced a marked decrease in the respiration of the organisms, an accumulation of pyruvate, a decreased formation of acetate, and an increase in aerobic glycolysis. Low concentrations of fluoroacetate (1×10^{-3} M) inhibited the oxidative metabolism of pyruvate which did not result in the conversion of pyruvate to acetate; higher concentrations of this inhibitor produced also a decreased oxidation of pyruvate to acetate. No evidence has been obtained that fluoroacetate inhibits the respiration of the filariae because of a competitive inhibition of acetate oxidation.

Respiration and glycolysis of filariae were markedly decreased by low concentrations of *p*-chloromercuric benzoate. This inhibition could not be reversed by a large excess of thioglycollate, cystein, glutathione, or H_2S.

Respiration of the filariae was completely inhibited by cyanide (2×10^{-4} M).

The cyanine dyes, a group of compounds possessing high chemotherapeutic activity in filariasis of the cotton rat, inhibited in low concentrations (6.5×10^{-8} M) the oxygen uptake of the filarial worms. This decrease in oxidative metabolism was associated with a compensatory increase in aerobic glycolysis of the worms and with decreased rates of acetate production and of polysaccharide synthesis. The same metabolic changes were observed in filariae removed from cotton rats to which subcurative doses of a cyanine dye had been administered.

Concentrations of cyanine dyes which produced an almost complete inhibition of filarial respiration had no effect on the rate of anaerobic glycolysis of the worms nor on the activity of cytochrome C or of cytochrome oxidase.

It is concluded that, in contrast to many other parasitic invertebrates, oxidative metabolism is essential for the survival of the filarial worm, *L. carinii*, and that the chemotherapeutic activity of the cyanine dyes in filariasis of the cotton rat is due to the inhibitory effect of this group of compounds on the respiratory metabolism of the parasite.

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