THE RICE INSTITUTE

STUDIES ON THE CARBOHYDRATE AND RELATED METABOLISM OF THE CESTODE, HYMENOLEPIS DIMINUTA

by

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Asa C Chandler

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II.. The Problem

This study was initially undertaken to determine whether the role of phosphorus in the carbohydrate metabolism of tapeworms is similar to or different from the role of this element in the chemical economy of other animals. A study was also made of the overall carbohydrate utilization as indicated by some of the end products, and of some aspects of the electron transfer mechanism at the "terminal end" of the carbohydrate metabolism.

III.. Historical

There is abundant evidence that carbohydrates are deposited as polysaccharides in the tissues of cestodes. Brand and Oesterlin (1933) isolated a polysaccharide from Moniezia expansa which had the characteristics of glycogen. Wardle (1937[a]) confirmed this and reported that hydrolysis of the polysaccharide yielded a reducing sugar which was apparently identical with d-glucose. Salisbury and Anderson (1939) isolated glycogen from Cysticercus fasciolaris.

A number of workers have reported the glycogen content to be 20% to 35% of the dry weight in various species (Weinland, 1901; Brand, 1929, 1933; Wardle, 1937 a and b; Smorodinzew and Bebeschin, 1935b). As exceptions, the last authors reported values as high as 60% in Taenia saginata and as low as 5.79% in Taenia plicata; Brand (1929, 1933) reported variations from 8.96 to 48.45% in the glycogen content of Moniezia expansa. The demonstration by Markov (1939) and Reid (1942) that the glycogen content of cestodes falls rapidly during starvation may account for the great variations reported by various workers.

Many workers have studied the carbohydrate content of the cystic fluid of larval cestodes. Bernard and Axenfeld (1856) reported the presence of a carbohydrate in a human hydatid. This was soon corroborated by several other investigators (Lucke, 1860; Naunyn, 1863; Schilling, 1904). Mazzocco (1923) reported that hydatid fluid contained 0.3 to 0.4 grams

of glucose per liter. According to Schopfer (1925[a]) the cystic fluid of Cysticercus tenuicollis contains 1.385 grams of reducing sugar per liter.

Glycogen has been reported in the pericystic and vesicular membranes of larval tapeworms by Brault and Loeper (1904), Déve (1905), and Voss (1940).. Salisbury and Anderson (1939) found that the cysticercus of Taenia pisiformis (= T. taeniaeformis) contained 32.2% glycogen..

Very little experimental study has been devoted to the carbohydrate metabolism of cestodes. Brand (1933) reported a series of experiments on the metabolism of the strobila of Moniezia expansa. The worms were incubated anaerobically for six hours in Ringer's solution or Ringer's solution + 5% glucose.. Glycogen, fat and protein content of the worms were determined before and after incubation. The medium was analyzed for total acidity (minus carbon dioxide), lactic and succinic acids, and ether-soluble substances. Under starvation conditions the glycogen decreased markedly and fats slightly; higher fatty acids, and lactic and succinic acids, were excreted into the medium. In Ringer-glucose the results were somewhat irregular. Glycogen decreased slightly in three out of four experiments, and fat content increased slightly.. Brand concluded that the anaerobic decomposition of glycogen in this cestode yields energy and that fats are waste products; lactic and fatty acids were presumably derived from glycogen degradation, but Brand could not account for the succinic acid.

Brand estimated that 0.4 grams of ether-soluble products and 0.44 grams of carbon dioxide were produced from 1.0 gram of glycogen..

Wardle (1937a) carried out similar experiments with Moniezia. The glycogen content was determined before and after incubation in a variety of media. There was no significant change in glycogen content in a dilute Tyrode's solution, distilled, water, or phosphate saline. In quintuple Tyrode's, 1% glycocholate, or bile saline, there was a glycogen loss. In Tyrode's solution and bile saline containing 1% glucose, a gain in glycogen content was found.

Markov (1939) studied the carbohydrate metabolism of the plerocercoid larvae of Dibothriocephalus latus and Ligula intestinalis and of the strobila of Eubothrium rugosum and Triaenophorus nodulosus. The worms were incubated in Ringer-Locke, Ringer-Locke plus 1% glucose, and fish broth. The glycogen content was determined before and after incubation. Under starvation conditions the glycogen decreased. In D. latus plerocercoids the glycogen fell to 66% of its original level in 38 hours at 15° C. Increasing the temperature to 35° C. caused the glycogen to decrease to 6% of the original content in 72 hours. In glucose-containing media, the glycogen content increased; Ligula plerocercoids showed an 11% increase in glycogen content after 22 days incubation at 15° C.

Brand (1933) reported that "Taenia marginata" from a dog fed a normal diet for eight weeks had a mean glycogen content

of 4.99% (wet weight), while worms from a dog kept on a high carbohydrate diet for a similar period contained 8.83% glycogen.

Reid (1940, 1942) carried out extensive experiments on Raillietina cesticillus, a cestode parasitic in chickens. Glycogen analyses were made of worms from chickens after normal feeding and after 20 hours starvation. Worms from normally fed hosts had a mean glycogen content of 4.60% (wet weight), while worms from starved hosts contained an average of 0.25%. Statistical treatment showed the differences to be highly significant. Reid showed that there was a significant daily fluctuation in the glycogen content of R. cesticillus under normal feeding conditions.

Chandler (1943) investigated the effect of lack of host dietary carbohydrate on the development of Hymenolepis diminuta.. Worms from such hosts showed a severe retardation of growth and the average number of worms which became established was 3.8, as compared with 7.4 in the controls.

Essentially no data are available on the intermediary metabolism of cestodes. Friedheim and Baer (1933a) reported that the oxygen uptake of Dibothriocephalus latus was enhanced by the addition of glucose. On the other hand, Van Grembergen (1944) reported that glucose did not increase the respiration of Moniezia benedeni. According to Bueding (1949), the finding of Chandler (1943) and Addis and Chandler (1945) that some unidentified factor in brewer's yeast is necessary for optimal

growth of Hymenolepis diminuta constitutes circumstantial evidence for the participation of flavine enzymes in the electron transfer mechanism of this helminth. Enzymatic reduction of methylene blue by extracts of Taenia pisiformis and Moniezia benedeni was reported by Pennoit-de-Cooman and Van Grembergen (1942). Dye reduction was accelerated in the presence of succinate. Friedheim and Baer (1933[a]) reported the approximate absorption bands of cytochrome C in Dibothriocephalus latus and Triaenophorus lucii.. The approximate absorption bands of cytochrome B and C were described by Van Grembergen (1944) in extracts of Moniezia benedeni. This worker also reported enhanced respiration upon addition of succinate or para-phenylenediamine to a Moniezia brei.. Similar results were obtained by Ivanov and Burnasheva (1945) with "Thysaniezia ovilla".

These fragmentary data comprise the body of the knowledge of carbohydrate and related metabolism in cestodes.

IV. Materials and Methods

The cestode Hymenolepis diminuta was reared in albino rats by the technique of Chandler (1943). The eggs were fed to semi-starved grain beetles, Tenebrio molitor. After 16 to 20 days the beetles were dissected and the cysticercoids washed out of the body cavity. The cysticercoids were then fed to albino rats. The worms were used, in most cases, three to eight weeks after infecting the host.

A number of chemical methods were used in various experiments. Inorganic ortho-phosphate was determined by the method of Fiske and Subbarow (1925); lactic acid by the method of Barker and Summerson (1941); pyruvic acid by the method of Lu (1939) as modified by Bueding and Wortis (1940); fructose by the method of Roe (1934); pentose by the method of Meijbaum (1939); reducing sugar by the method of Folin and Malmros (1929); nitrogen by the method of LePage (1949[a]); nicotinic acid by the method of Bandier and Hald (1939); glycogen by the method of Good, Kramer and Somogyi (1933); and adenylic acid by the method of LePage (1949[a]). Calibration curves were constructed for each determination and standards determined with each sample.

Tapeworm tissues were prepared for various experiments in several ways. The infected rat was killed by a blow on the head. The small intestine was removed and the worms washed out with normal sodium chloride or Tyrode's solution. For analyses of resting tissue, the worms were washed quickly in ice-cold normal saline and frozen with a dry-ice-acetone

mixture; the tissue was then thoroughly powdered in a mortar chilled with dry-ice-acetone.. Homogenates were prepared in an all-glass homogenizer of the type described by Potter and Elvehjem (1936). Tissue minces were prepared by forcing the tissue through a fine wire screen. In experiments with whole tissues, the middle third of the strobila was used.

All manometric measurements were carried out in small calibrated Warburg vessels at 38° C. The shaking rate in all such experiments was 120/min.

Cytochrome C was prepared by the method of Keilin and Hartree (1945).. Coenzyme I was prepared by the method of LePage (1949) or was purchased from a commercial source; Adenosine triphosphate was prepared by the method of Dounce et al (1948) or was purchased from a commercial source. Glucose-1-phosphate was prepared by the method of Hanes (1940). Fructose-1, 6-diphosphate was obtained from a commercial source.

Other details of methods will be discussed with the appropriate context.

V.. The Partition and Metabolism of Phosphorus

A.. The Acquisition of Isotopically Labeled Inorganic Phosphate *In Vivo*

It seemed desirable to determine the rate at which the worm might acquire inorganic phosphate in its natural environment.. *In vitro* experiments in this laboratory by Read, Forister, Logan and Howard (1948) suggested that the presence of glucose might change the rate of phosphorus uptake.. Therefore, experiments were planned in which phosphate was administered with and without glucose.

The worms were reared as previously described in three- to four-month-old male albino rats. The animals were used for the experiments three to four weeks after feeding each rat ten cysticercoids. Radioactive phosphorus was administered, by stomach tube or intraperitoneal injection, as ortho-phosphoric acid neutralized to pH 7.0 with sodium hydroxide. The trace doses given contained less than one microgram of phosphorus. In experiments where the dose desired was above the trace level, unlabeled sodium dihydrogen phosphate was added as carrier.. To measure the radioactivity in the host gut mucosa, the small intestine was removed, washed out quickly with cold Tyrode's solution, and the mucosa scraped off with a razor blade.. The scrapings were then weighed, wet ashed in nitric and perchloric acids, and the radioactivity determined with a glass-walled dipping counter. The worms were removed from the host gut and rapidly washed in five changes of cold Tyrode's solution. They were then carefully blotted on filter

paper to remove as much adhering fluid as possible, and weighed. This was followed by wet ashing in nitric and perchloric acids, and, after making the digested material to volume, aliquots were removed to counting dishes for determination of radioactivity. Counts of worm samples were made with a thin mica window Geiger-Mueller tube manufactured by Tracerlab, Inc. Sufficient counts were taken on each sample to keep fluctuation error below 2%.

The Uptake of Orally Administered Phosphorus:

Experiment 1a - Two groups of infected rats were starved for twenty-four hours. The rats of one group were given 0.5 ml. of normal saline containing a trace dose of radiophosphorus with an activity of 18×10^4 counts per second. The rats of the second group were given 0.5 ml. of normal saline containing an identical amount of radiophosphate and 0.5% glucose. All materials were administered by stomach tube. At intervals after dosing, the rats were killed by decapitation and the small intestine and worms rapidly removed and prepared as described for the assay of radioactivity. The results of this experiment are summarized in Figure 1. The amount of radioactive phosphorus is expressed in terms of percentage of the dose administered to the rat per gram of tissue assayed. It may be seen that in the animals receiving only phosphate the maximum activity of the host tissues is attained in about seventy minutes, while the maximum activity of the helminths is reached in eighty-five to ninety minutes. In those animals

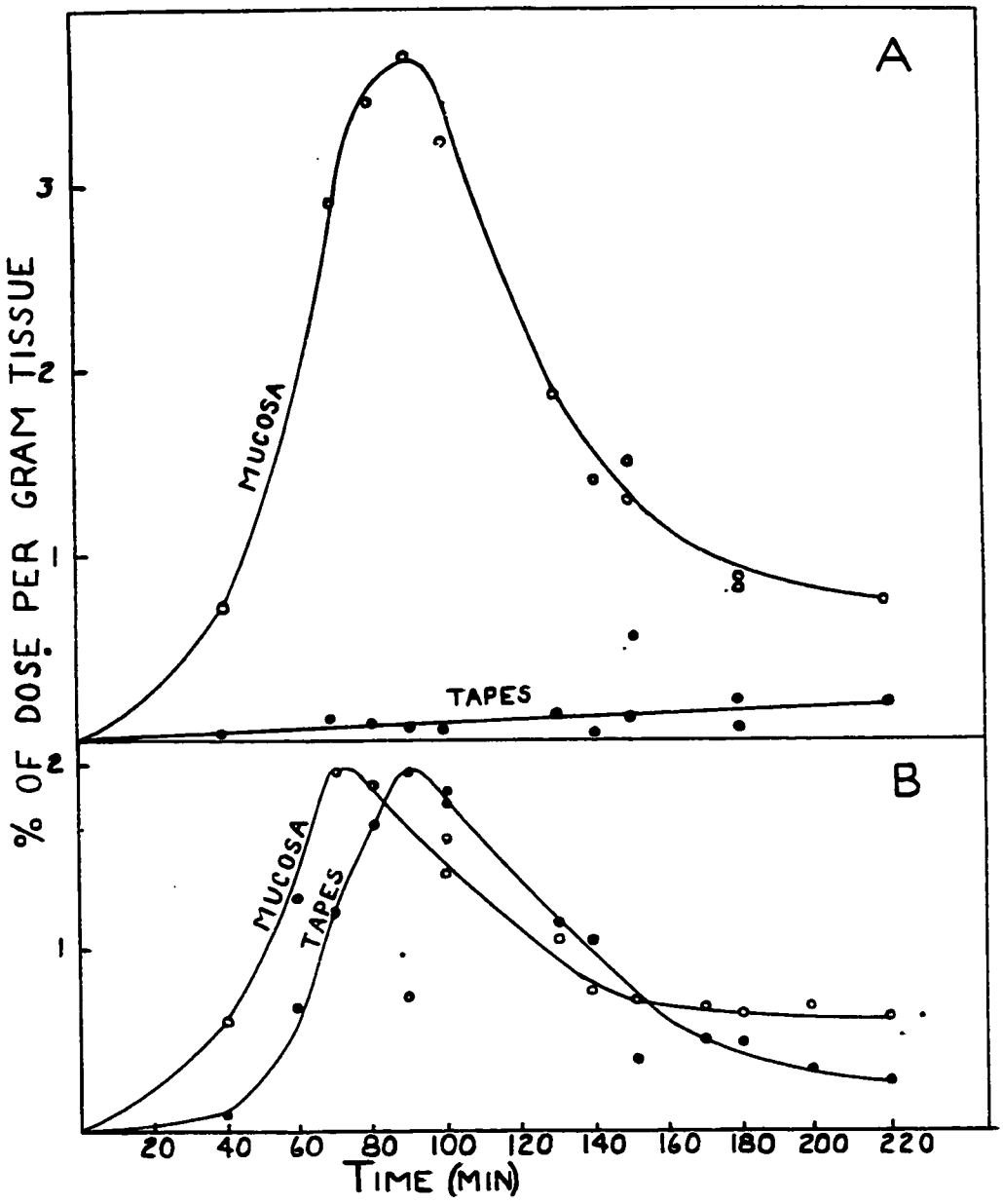


FIG. 1. The uptake of trace amounts of radiophosphate by host tissues and tapeworms when administered to the host *per orum*. A—phosphate plus glucose. B—phosphate without glucose.

receiving phosphate plus glucose the mucosa reached a peak of activity about ninety minutes after administration of the dose. The tapeworms in these latter animals acquired the phosphate in a manner strikingly different from that seen in the animals not receiving glucose.

Two alternative explanations for the above-mentioned difference presented themselves. First, the glucose might exert some inhibitory effect on the worms or cause secretion of some inhibitory substance by the host tissues thus hindering the uptake of phosphate ion. Second, the phosphate might be taken up more rapidly in the anterior part of the small intestine in animals absorbing glucose than in those not absorbing glucose, thus making it unavailable for the worms. It was seen that the second of these explanations could be rather readily tested. Thus, an experiment was planned in which a large enough dose of phosphate was administered to insure its reaching the portion of the small intestine inhabited by the worms.

Experiment 1b - Two groups of infected rats were starved for twenty-four hours. The rats of one group were given 5.0 mg. of labeled sodium phosphate with an activity of 18.5×10^4 counts per second in 1.0 ml. of normal saline, and the animals of the second group were given 5.0 mg. of labeled sodium phosphate and 50.0 mg. of glucose in 1.0 ml. of normal saline, all by stomach tube. At intervals after dosing, the rats were killed and the worms removed for assay as in Experiment I.

This experiment was repeated on two additional groups of rats. The results are summarized in Figure 2..

It will be seen that with larger amounts of phosphate, the uptake of phosphorus by the worms is essentially the same in the presence or absence of glucose.

The Uptake of Parenterally Administered Phosphorus: Experiment 2 - Two groups of infected rats were starved for twenty-four hours. The rats of the first group were given a trace dose of labeled phosphate with an activity of 18.0 X 10^4 counts per second in 1.0 ml. of normal saline intraperitoneally.. The rats of the other group were given 1.0 ml. of saline containing fifty milligrams of glucose by stomach tube, and one hour later an intraperitoneal injection of labeled phosphate in saline identical with that given the rats of the first group.

At intervals after dosing, the rats were killed by decapitation, the intestinal mucosa and worms rapidly removed, and the radioactivity assayed as described above. The results of this experiment are summarized in Figure 3. It may be seen that the worms in these animals acquired phosphorus at a low but steady rate..

Discussion

It was found in the foregoing experiments that when trace amounts of phosphorus were administered orally with glucose the worms acquired labeled phosphorus at a much lower rate than when the phosphorus was given without glucose. It was

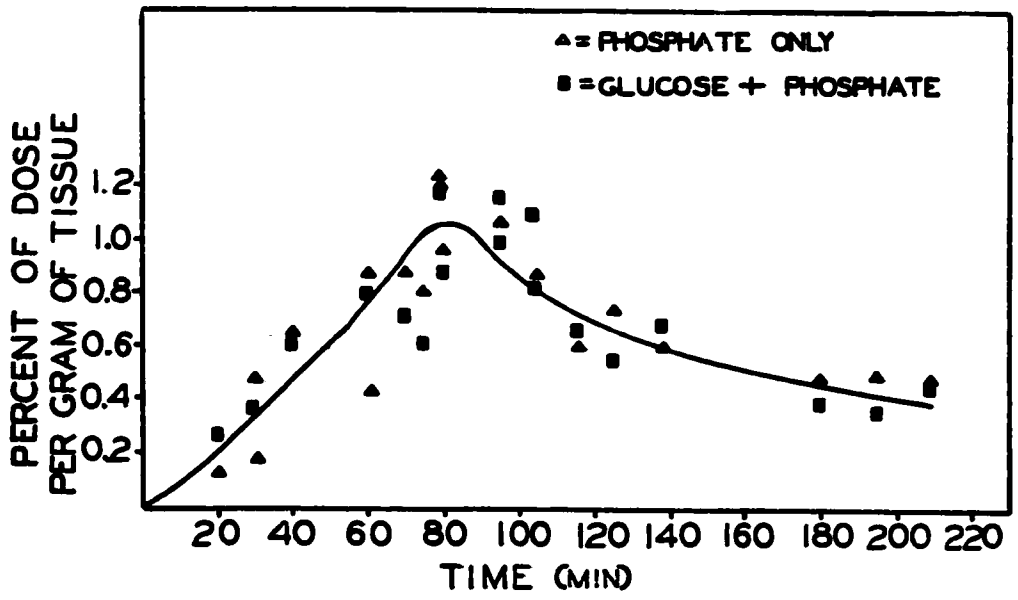


FIG. 2. The uptake of radiophosphate by tapeworm tissues when given, with or without glucose, *per orum* in macro amounts.

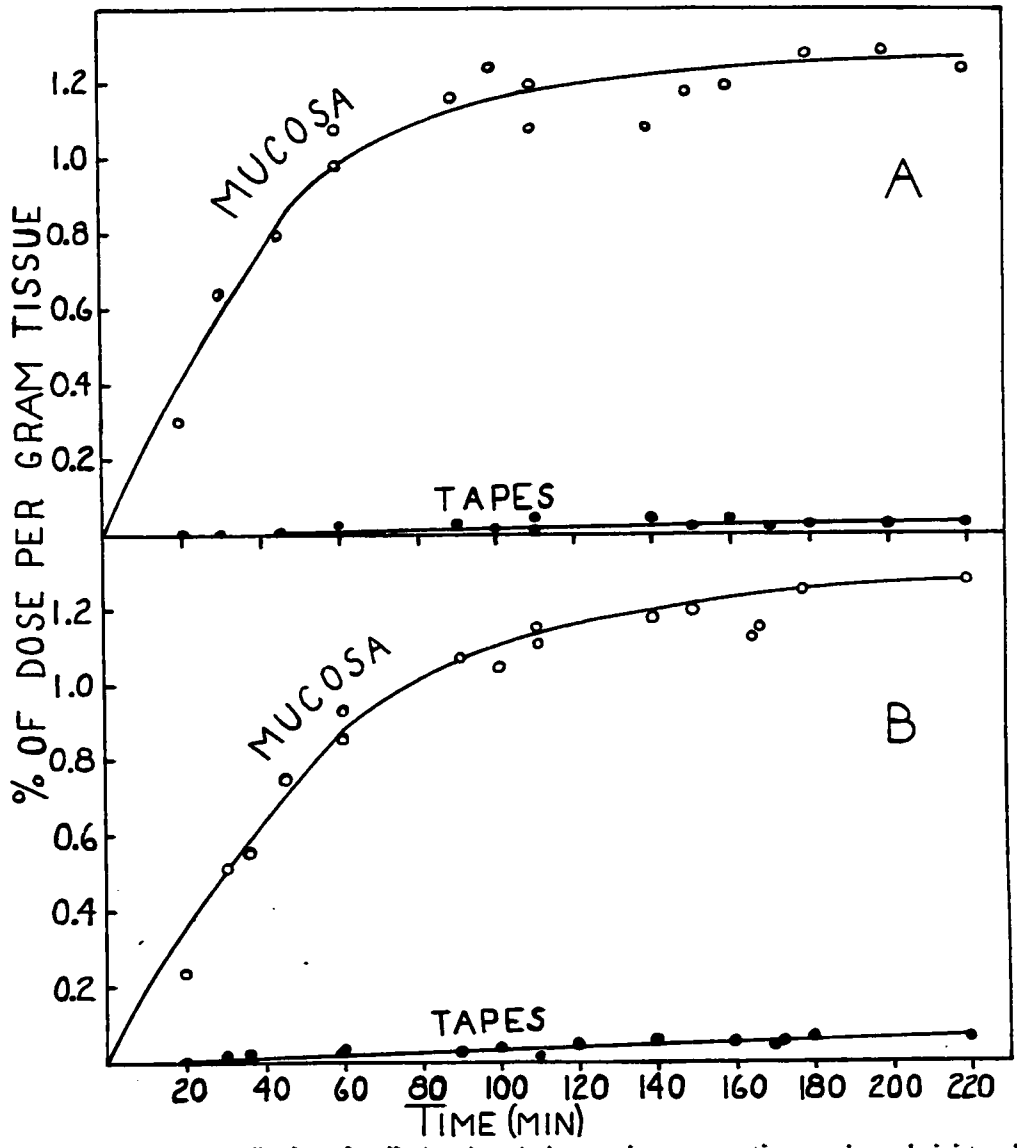


FIG. 3. The distribution of radiophosphate in host and tapeworm tissues when administered to the host by intraperitoneal injection. A—hosts given glucose before administration of phosphate. B—hosts not given glucose before administration of phosphate.

found that when larger amounts of phosphate were given this difference in glucose-fed and non-fed animals disappeared. It seems probable that the results obtained with trace doses of phosphate can be explained as follows: When trace amounts of phosphate are given with glucose the phosphate is absorbed from the lumen of the intestine more rapidly than is the case when no glucose is given. This phenomenon is probably associated with the phosphorylations which are thought to be involved in carbohydrate absorption by the host (Höber, 1949).

The observation that the worms in hosts receiving phosphate by intraperitoneal injection showed a steady increase in P^{32} content with time calls attention to a rather important aspect of the relationship existing between cestode parasites and their hosts: Namely, the fact that the worms obtained phosphorus from endogenous sources. Parasitologists have too infrequently taken into consideration the fact that the gut is a dynamic organ into which materials are constantly being secreted and from which some of these materials are reabsorbed. The writer has discussed this more fully elsewhere (Read, 1950[a & b]).

B. The Acid-soluble Phosphorus Compounds of "Resting" Tissue

The substances in animal tissues which are soluble in dilute trichloracetic acid include the phosphorylated esters which are intermediates in the Meyerhof-Embden cycle of glycolysis. Therefore, analyses were carried out to determine the partition of phosphorus among the compounds extractable from the worm's tissues by cold 10% trichloracetic acid.

The worms were powdered in the frozen state, as described previously, and transferred, still frozen, to a tared tube containing 8 ml. of ice-cold 10% trichloracetic acid. This was stirred vigorously and centrifuged. The supernatant fluid was collected, and the residue was extracted twice with 3 ml. portions of ice-cold 5% trichloracetic acid. These portions were added to the original extract. Total phosphorus was determined on the extract as follows:: A 0.5 ml. aliquot was removed and to it was added 0.5 ml. of 10 N H_2SO_4. This was heated for 4 hours at 110° C, partially cooled, and 1 to 2 drops of 30% H_2O_2 added. The tube was replaced in the oven for 60 minutes, removed, cooled, and 2 ml. of water added. The tube was then heated at 100° C for 10 minutes, cooled, and 2 aliquots removed for determination of inorganic ortho-phosphate.

The acid-soluble compounds were separated into three fractions by a modification of the method of LePage (1945, 1949a). This method takes advantage of the differential

solubilities of the barium salts of known phosphorus esters at pH 8.2. The procedure followed is precisely diagrammed in Figure 4.

Certain phosphorus-containing compounds associated with glycolysis in vertebrate tissues are known to be insoluble in the form of their barium salts. These are adenosine diphosphate (A.D.P.), adenosine triphosphate (A.T.P.), hexose diphosphate (H.D.P.), phosphoarginine, and 3-phosphoglyceric acid.. On the other hand, other known intermediates are soluble in the form of the barium salts, but are insoluble in 50% ethyl alcohol. These are glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, phosphopyruvic acid, triosephosphate, adenylic acid, phosphopyridine nucleotides, and ribose-5-phosphate. LePage (1945, 1949a) has contributed detailed information as to the characterization of these intermediates. The chemical characteristics are summarized in Figure 5.

No attempt was made to study the partition of phosphorus in the barium-soluble, alcohol-soluble fraction. The total phosphorus in this fraction was determined in each case and was invariably a very small portion of the total acid-extractable phosphorus.

In order to minimize the breakdown of the phosphorylated intermediates it is necessary that the analyses be carried out as soon as possible after extraction from the tissues. It was found that working alone the writer could carry out a complete series of analyses in about 18 to 20 hours. With

Unneutralized trichloracetic acid extract treated with equal volume of ice-cold 95% ethyl alcohol and centrifuged

Ppt.: Extractable glycogen. Held for analysis

Supernatant: Neutralized with 25% KOH to faint pink with phenolphthalein (pH 8.2). 3 ml. 25% barium acetate and 3.2 volumes of 95% ice-cold ethyl alcohol added. Chilled and centrifuged

Ppt.: Dissolved in 0.1 N HCl, neutralized to pH 8.2, excess barium acetate added, chilled, and centrifuged

Supernatant: Barium-soluble, alcohol-soluble fraction

Ppt.: Barium insoluble fraction.. Redissolved in 0.1 N HCl

Supernatant: Barium-soluble, alcohol-insoluble fraction

Each fraction was treated immediately with an amount of .05 N H_2SO_4 which was slightly in molar excess of the barium initially added. The precipitate of barium sulfate was removed at once by centrifugation and each fraction neutralized to pH 8.2 with KOH.

Figure 4.. Procedure of Phosphorus Fractionation.

two or more workers this time could be appreciably reduced. Due to the extreme hardship of working alone, only three complete analyses were carried out.. These included all determinations shown in Figure 5, and in addition, determinations of lactic acid, pyruvic acid, arginine (Albanese and Frankston, 1945), and glycerol (Harvey, 1949).. A large number of preliminary determinations were carried out in perfecting the techniques necessary for a complete analyses of single tissue samples.

Thus, on the barium insoluble fraction, the following analyses were carried out: Total phosphorus, inorganic phosphorus, inorganic phosphorus after 7-minute hydrolysis in 1 N HCl, fructose, pentose, reducing sugar, adenine, and inorganic phosphorus after 180-minute hydrolysis in 1 N HCl. On the barium-soluble, alcohol-insoluble fraction the following analyses were carried out: Total phosphorus, reducing sugar, inorganic phosphorus and reducing sugar after 7-minute hydrolysis in 1 N HCl, fructose, phosphorus released by alkaline iodine, phosphorus released by alkaline hydrolysis, adenine, nicotinic acid, pentose. Glycogen was determined on the tissue residue plus the 50% alcohol-precipitable material of the trichloracetic acid extract.

The analyses performed on one sample (sample A) are summarized in Table 1. Using the methods of calculation of LePage (1947, 1949) this data was interpreted in terms of the acid-extractable intermediates known to occur in various other animal tissues. The interpretation of the analyses of

Fraction	Compound	Method of Determination	% Hydrolysis in 1 N HCl at 100° C 7min	180 min	Reducing Value	Supplementary
Barium-insoluble	A.T.P.	Δ7 P,pentose	66	86	0	adenylic
	A.D.P.	Δ7 P,pentose	50	79	0	adenylic
	H.D.P.	fructose (52.5%)	26.5	--	9.5	--
	3-phospho-glyceric acid	resistant to 3 hr. hydrolysis	0	2	0	--
Barium-soluble, alcohol-insoluble	glucose-1-phosphate	Δ7P,reducing sugar after hydrolysis	100	--	0 (66.4 after hydrolysis)	--
	glucose-6-phosphate	reducing sugar	--	10.5	13.2	--
	fructose-6-phosphate	reducing sugar, fructose	--	74	31.6	--
	phospho-pyruvic acid	P released by alkaline iodine	46	100	0	--
	triose phosphate	alkali-labile P	46	100	--	--
	adenylic acid	260 mu absorption	--	58.7	0	pentose
	phospho-pyridine nucleo-tides	nicotinic acid	--	58.7	0	pentose, adenylic
	ribose phosphate	pentose	--	58.7	19.7	--
Glycogen	glycogen	reducing value after acid hydrolysis	--	100	--	yeast treatment

Figure 5. Characteristics of Acid-soluble Phosphorus Compounds

Table 1 are summarized in Table 2. Interpreted findings of the additional series of analyses (samples B and C) are presented in Tables 3 and 4.

Determination	Ba-insoluble	Ba.-sol., alc.-insol.	Ba.-sol., alc.-sol.	Glycogen
Phosphorus	mg.	mg.	mg.	mg..
Total	1.398	0.518	0.274	0.223
Inorganic	0.477	0	--	--
Hydrolyzed in acid(7 min)	0.250	0	--	--
(180 min)	0.841	--	--	--
Alkali-labile	--	0.038	--	--
Released by alkaline I	--	0	--	--
Released by molybdate	0	0	--	--
Fructose	0.427	0.428	--	--
Pentose	0.593	0.646	--	--
Reducing Sugar Without hydrolysis	0.146	0.459	--	0
After 180 min hydrolysis	--	--	--	204.21
Creatine	0	0	--	--
Arginine After 15 hr. hydrol.(.1NHCl)	trace	0	--	--
Nicotinic Acid*	--	0.110	--	--
Adenylic acid	1.249	0.993	--	--

Original extract

Pyruvic Acid	1.007
Lactic Acid	2.681
Glycerol	3.432

* Recovery 91%

Table 1. Analysis A. (Sample = 5.369 g., wet weight).

Compound	Mg.	Mg.%	Fraction
Glucose-1-phosphate	0	0	
Glucose-6-phosphate	0.860	16.0	
Fructose-6-phosphate	1.025	19.1	
Pentose phosphate	0.106	1.8	Barium-soluble, alcohol-insoluble P
Triose phosphate	0.207	3.8	
Adenylic acid	0.649	12.0	
"Coenzyme" (as D.P.N*)	0.674	12.5	
% P of fraction accounted for = 87.1%			
Inorganic P	0.477	8.8	
Hexose diphosphate	1.538	28.6	
Phosphoglyceric acids	1.252	23.3	Barium-insoluble P
A.T.P.	0.852	15.8	
A.D.P.	0.956	17.6	
% P of fraction accounted for = 90.6%			
Lactic acid	2.681	49.7	
Pyruvic acid	1.007	18.7	Other
Glycogen	204.21	3798.3	
Glycerol	3.432	63.8	

* As diphosphopyridine nucleotide

Table 2. Analysis A, Interpretation.

Compound	Mg.	Mg.%	Fraction
Glucose-1-phosphate	0	0	
Glucose-6-phosphate	0.624	12.8	
Fructose-6-phosphate	0.983	20.2	Barium-soluble, alcohol-insoluble P
Pentose phosphate	0.099	2.0	
Triose phosphate	0.187	3.9	
Adenylic acid	0.538	11.1	
"Coenzyme" (as D.P.N.)	0.449	9.2	
% P of fraction accounted for = 78.7			
Inorganic P	0.377	7.8	
Hexose diphosphate	1.261	25.9	
Phosphoglyceric acids	0.995	20.5	Barium-insoluble P
A.T.P..	0.800	16.5	
A.D.P..	0.903	18.6	
% P of fraction accounted for = 87.0			
Lactic acid	1.572	32.4	
Pyruvic acid	0.984	20.3	
Glycogen	177.90	3664.7	Other
Glycerol	1.002	20.6	

Table 3.. Analysis B. (Sample = 4.837 g. tissue).

Interpretation

Compound	Mg.	Mg.%	Fraction
Glucose-1-phosphate	0	0	
Glucose-6-phosphate	0.798	15.9	
Fructose-6-phosphate	1.002	19.9	
Pentose phosphate	0.103	2.0	Barium-soluble, alcohol-insoluble P
Triose phosphate	0.189	3.8	
Adenylic acid	0.607	12.1	
"Coenzyme" (as D.P.N.)	0.597	11.9	
% P of fraction accounted for = 78.8			
Inorganic P	0.432	8.6	
Hexose diphosphate	1.371	27.3	
Phosphoglyceric acid	1.118	22.2	Barium-insoluble P
A.T.P..	0.798	15.9	
A.D.P..	0.941	18.7	
% P of fraction accounted for = 89.4			
Lactic acid	2.432	48.4	
Pyruvic acid	1.011	20.1	Other
Glycogen	220.31	4384.2	
Glycerol	1.116	22.2	

Table 4.. Analysis C. (Sample = 5.003 g. tissue). Interpretation.

C. The Rate of Transformation of Inorganic to Organic Phosphate

It has been shown that inorganic phosphate is readily taken up by the worm and that the tissue phosphorus is distributed in acid-soluble organic compounds resembling those found in the tissues of other animals. Therefore, it seemed desirable to study the rate of organification of inorganic phosphorus.

A group of young infected male rats were fed only 50% glucose solution (three 2.0 ml. doses *per orum*) for 24 hours. At the end of 30 hours the rats were each given a trace dose of radiophosphorus (1×10^4 counts per minute) in 1.0 ml. of normal saline. At intervals the rats were killed, the worms rapidly removed, washed, and frozen in dry-ice-acetone mixture.. The tissue was then extracted and fractionated with barium and alcohol, as described in the preceding experiments. The radioactivity in various fractions was determined by the same procedure as for whole tissues and chemical analyses for the phosphorus in each sample were carried out. The results of this experiment are summarized in Figure 6.

It may be seen that inorganic phosphorus rapidly undergoes organification. This furnishes additional evidence that phosphorylation plays an integral part in the chemical economy of the worm's tissues.

Figure 6. Phosphate Organification.

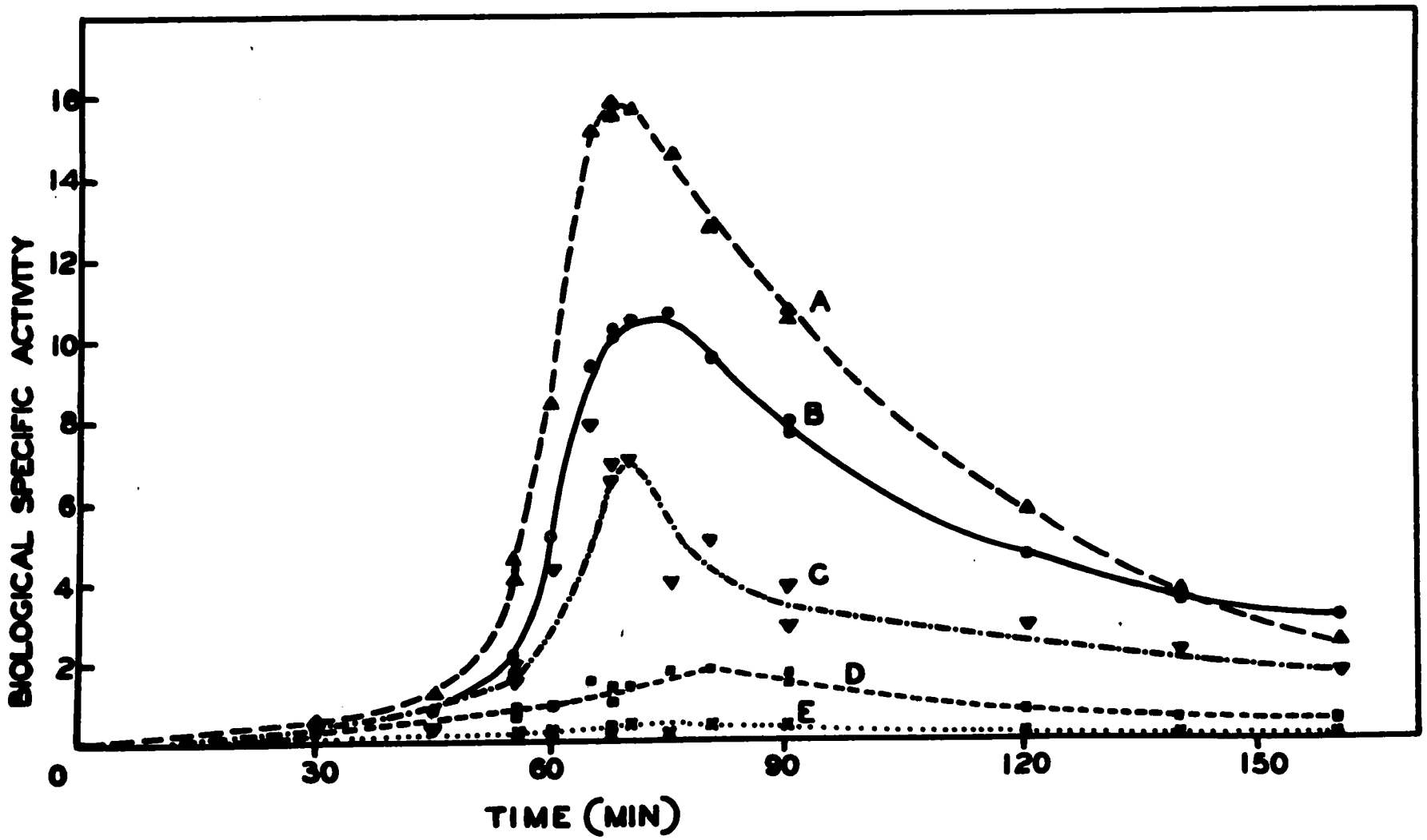
A. = Inorganic phosphate.

B. = Barium soluble-alcohol insoluble.

C. = Barium insoluble (minus inorganic).

D. = Barium soluble-alcohol soluble.

E. = Alkali-labile.


BIOLOGICAL SPECIFIC ACTIVITY
16
14
12
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0
30
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120
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TIME (MIN)
A
B
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E

D. The Enzymes of Phosphorylative Fermentation

The foregoing data suggest very strongly that phosphorylation is involved in the degradation of carbohydrate in the tissues of Hymenolepis. However, unless the enzymes concerned with the formation of phosphorylated compounds can be demonstrated, the evidence must be considered only suggestive. With this in mind, experiments were designed to demonstrate certain enzymes known to be associated with glycolysis in vertebrate tissues.

(1) Hexokinase

In vertebrate tissues and yeast glucose is phosphorylated by a transfer of phosphate from adenosine triphosphate to glucose (Colowick and Kalckar, 1943). This reaction is catalyzed by the enzyme hexokinase and may be formulated thus:

Glucose + A.T.P. ⟶ Glucose-6-phosphate + A.D.P.

Since this is the important initial step in carbohydrate utilization in other animal tissues, its demonstration in the tissues of the tapeworm would be strong evidence for a phosphorylative fermentation..

Partial purification of the enzyme from an extract of the worm's tissues was carried out by a modification of the method of Kunitz and McDonald (1946).. About 70 grams of fresh wet worms were ground thoroughly in a mortar with 30 ml. of toluene warmed to 40°C.. The mixture was warmed in a bath at 40°C. for an hour. It was then removed from the bath and allowed to sit for an hour at room temperature. To the mixture was then added 50 ml. of cold distilled water. This was thoroughly mixed and placed in the refrigerator overnight.. The following day the top layer was removed with a pipette and discarded, and the mixture was rapidly centrifuged. The supernate was then poured off and retained. The residue was washed once with 5 ml. of ice cold distilled water and the washings added to the original supernatant.. The residue was discarded.

The clear fluid was now brought to 0.5 saturation with solid ammonium sulfate (314 grams per liter) and the precipitate which formed was centrifuged off and discarded. The clear filtrate was now brought to 0.7 saturation with ammonium sulfate and the preparation placed in the refrigerator (ca. 5°C.) for eighteen hours.

The precipitate was collected by centrifugation and the supernatant discarded. The precipitate was then dissolved in 3.0 ml. of 1% dextrose and dialyzed in cellophane for eighteen hours against a slowly dripping 1% dextrose solution.

Following dialysis the preparation was used at once to assay for hexokinase activity. The conditions of the assay were as follows: A complete reaction mixture included 0.003 M. A.T.P., 0.005 M. $MgCl_2$, and 0.6 ml. of enzyme preparation which was 1% with respect to dextrose in a total volume of 2.0 ml. The reaction mixture was incubated for 20 minutes at 30° C. Reaction was terminated by the addition of 1.0 ml. of 5% trichloracetic acid. An aliquot was removed at once, and a determination was made of the inorganic phosphorus and of the inorganic phosphorus after 7 minute hydrolysis in 1 N HCl at 100° C. The results of these determinations are summarized in the following figure.

Experiment	Reaction Mixture	Inorganic P (after hydrolysis)	Inorganic P (before hydrolysis)	P transferred
		µg	µg	µg
1HE	Complete	37	10	14
	Without Mg	53	6	4
	Without A.T.P.	0	2*	0
	Without enzyme	62	0	0
	Complete (time=0)	62	1*	--
2HE	Complete	46	8	10
	Without Mg	59	5	5
	Without A.T.P.	1*	0	0
	Without enzyme	64	0	0
	Complete (time=0)	64	0	--

*Accuracy of P determinations below 4 µg is questionable. Optimum range of the method employed is 4 to 40 µg.

These data indicate that hexokinase is present in these preparations and it is concluded that this enzyme is a constituent of the tissues of this tapeworm.

(2) Phosphorylase

In vertebrate tissues glycogen is brought into the chain of phosphorylating reactions of utilization by the action of the enzyme phosphorylase (Cori and Cori, 1936). The reaction may be formulated thus:

glycogen + $H_3PO_4 \rightleftharpoons$ glucose-1-phosphate + H_2O

In the present investigation demonstration of this enzyme was sought by the use of aged, dialyzed extracts prepared by the method of Cori et al (1937). About 100 grams of fresh worms were ground in a mortar with an equal amount of ice-cold distilled water. The mixture was placed in an ice-salt bath and stirred with a motor-driven stirrer for two hours. The mixture was then rapidly centrifuged in the cold and residue re-extracted with the same volume of ice-cold distilled water. The combined extracts were then dialyzed in cellophane against distilled water at about 0° C. for 7 days. Denatured protein which precipitated during this period was removed by centrifugation. This long dialysis removes Mg ion which activates enzymes concerned in the transformation or degradation of glucose-1-phosphate.

The phosphorylase activity of the extract was assayed by the method suggested by Cori et al (1937). In this test 6 ml. of extract are mixed with 3 ml. of M/3 phosphate buffer (pH 7.0) containing 50 mg. of glycogen and 0.2 mg. of adenylic acid. The mixture was incubated for 30 minutes at 25° C. and the reaction was terminated by the addition of 6 ml. of 15%

trichloracetic acid. After thorough mixing, the reaction mixture was brought to pH 8.2 with barium hydroxide and kept at about 5°C. for an hour. The precipitate which formed was separated by centrifugation and discarded.

The supernatant was analyzed for inorganic phosphorus and reducing value before and after 7 minute hydrolysis. The results of these analyses are summarized in the following table:

Expt.	Reaction mixture	Inorganic P (before hydrolysis)	Inorganic P (after hydrolysis)	Reducing value (before hydrolysis)	Reducing value (after hydrolysis)
		µg	µg	µg	µg
1 Ph	Complete	0	190	25	1596
	Without adenylic	0	>20*	28	35
	Complete (time=0)	0	>20	25	>20
2 Ph	Complete	0	201	40	1720
	Without adenylic	0	>20	38	39
	Complete (time=0)	0	>20	0	24

* Due to aliquot size, figures below 20 are outside the accurate range of the method of determination of P.

Glucose-1-phosphate is not precipitated by barium at pH 8.2 and is distinguished from other phosphorus esters by the following properties (Cori et al, 1937). Its phosphate is readily hydrolyzed in 1 N HCl, at 100° C. in 7 minutes. Before hydrolysis the ester has no copper-reducing properties, but after phosphate hydrolysis it gives the reducing value of d-glucose. Adenylic acid is required for phosphorylase

activity. Recognizing these properties, we may see that the data given above indicate that this ester was formed in the reaction mixture. It is concluded that phosphorylase is present in the tissues.

(3) Phosphohexomutase

In mammalian tissues the above-named enzyme catalyzes the following reaction (Cori et al, 1938a):

glucose-1-phosphate ⇌ glucose-6-phosphate

The demonstration of this enzyme in the tapeworm's tissues was attempted using the same extract that was used in the demonstration of phosphorylase. The long dialysis in the preparation of this material removed the inorganic ions. In assaying for phosphorylase this was advantageous since the presence of phosphohexomutase, which is activated by Mg or Mn ions, could have caused the rapid removal of any glucose-1-phosphate formed. The equilibrium for the reaction lies far to the right (Colowick and Sutherland, 1942). It was therefore possible to assay the enzyme preparation for phosphohexomutase activity by using glycogen as the substrate and adding Mg ions to the system. The reaction mixture was identical with that of the phosphorylase assay except that it contained 10 mM $MgCl_2$. The conditions of incubation and barium precipitation were the same as for phosphorylase assay.. The barium-soluble supernatant was analyzed for reducing value, inorganic phosphorus after 7 and 180 minute hydrolysis in 1 N HCl at 100° C., and total phosphorus. The results obtained are presented in the following table:

Experiment	Reaction mixture	P hydrol. 7 min.	P hydrol. 180 min.	Total P
		µg	µg	µg
1 Pg	Complete	35	40	105
	Without Mg	185	191	196
	Complete(time=0)	0	20	30
2 Pg	Complete	39	48	189
	Without Mg	174	186	192
	Complete(time=0)	0	20	20

Glucose-6-phosphate has the following properties: It has a copper-reducing value 13.5% that of an equal weight of d-glucose. Its phosphorus is not hydrolyzed in 7 minutes in 1 N HCl at 100° C., and only 10.5% of its phosphorus is hydrolyzed in 180 minutes under these conditions (LePage, 1947). Examination of the foregoing data indicates that glucose-6-phosphate was formed in the reaction. It is concluded that phosphohexomutase is present in the worm's tissues.

(4) Aldolase

Muscle and yeast contain an enzyme, aldolase, which catalyzes the splitting of fructose-1, 6-diphosphate (Meyerhof and Lohmann, 1934).. This reaction may be formulated thus:

Fructose-1,6-diphosphate $\rightleftharpoons$ 3-phosphoglyceraldehyde + dihydroxyacetone phosphate

Assay for this enzyme was carried out in a relatively simple manner. It has been shown by Meyerhof and Lohmann (1934) that the products of this reaction differ from other phosphorylated esters in that the phosphate is quite readily hydrolyzed by N NaOH at room temperature. As pointed out by Speck and Evans (1945) and Herbert et al (1940) the reaction comes to equilibrium and it is thus necessary to add a fixing agent. Cyanide was used in the present experiments as suggested by Speck and Evans (1945) since it combines with and binds the triose phosphates. This prevents further enzymatic triose degradation.

The assay was carried out as follows: a 5% homogenate of worm tissue was prepared as previously described. The reaction mixture contained 0.5 ml. of homogenate, 1 ml. of 0.30 M H.D.P., and 0.25 M KCN in a volume of 3 ml. The mixture was incubated for 10 minutes at 38° C. and the reaction terminated by addition of 1.0 ml. of 10% trichloracetic acid. Aliquots were then used to determine inorganic phosphorus, and inorganic phosphorus, and inorganic phosphorus after 10 minute hydrolysis at room temperature in N NaOH. The results obtained are

shown in the following table:

Experiment No.	Reaction mixture	Inorganic P (without hydrolysis)	Inorganic P (after hydrolysis)	Alkali-labile P formed
		µg	µg	µg
1 AE	Control (time=0)	0	11	--
	Complete	28	146	107
	Minus cyanide	40	59	8
2 AE	Control (time=0)	2	9	--
	Complete	39	115	67
	Minus cyanide	47	55	0

These results indicate that the tissues contain aldolase.

(5) Glyceraldehyde-3-phosphate dehydrogenase

In vertebrate muscle the above named enzyme catalyzes the first oxidative step in the degradation of carbohydrate in the tissues. This consists of the oxidation of a -CHO to a $COO-PO_3H_2$ group and may be formulated as follows:

Glyceraldehyde-3-phosphate $\rightleftharpoons$ 3-phosphoglyceric acid

The determination of this enzyme was carried out by a method which depends on certain properties of the products. The procedure of Cori, Slein and Cori (1948) for the simultaneous preparation of aldolase and glyceraldehyde-3-phosphate dehydrogenase was applied with some modifications to suit the material under study. It was possible to obtain extracts containing both these enzymes and to use fructose-1,6-diphosphate as a substrate rather than attempting the synthesis of glyceraldehyde-3-phosphate, which is a formidable problem in itself. In vertebrate tissues, the further breakdown of phosphoglycerate is inhibited by fluoride (Lohmann and Meyerhof, 1934) and phosphoglyceric acid can be estimated by analysis of the barium-precipitable, difficultly-hydrolyzed phosphorus (LePage, 1947).

The extract was prepared as follows: about 90 grams of fresh wet worm tissue was ground in a cold mortar with 1 volume of cold .03 N KOH.. This was stirred in the cold for ten minutes and strained through gauze. The residue was re-extracted and the extracts pooled. The material was lightly centrifuged and the residue discarded. To the extract was

added 1 volume of saturated ammonium sulfate solution (pH 7.5 with ammonia) and the mixture left in ice for an hour. The mixture was centrifuged and the residue discarded. Solid ammonium sulfate was added to .80 saturation and the pH adjusted to 8.0 with ammonium hydroxide.. The mixture was left in the icebox overnight. The following morning the mixture was centrifuged and the supernatant discarded. The residue was dissolved in 5 ml. of ice-cold distilled water, and dialyzed for 8 hours against distilled water in the refrigerator.

The glyceraldehyde-3-phosphate dehydrogenase activity was tested in the following system: The complete reaction mixture contained 0.4 ml. of enzyme preparation, 0.005 M $MgCl_2$, 0.1 M KF, and 0.03 M hexose diphosphate in a total volume of 2.0 ml. This was incubated for 15 minutes at 38° C. and .1 ml. of 50% trichloracetic acid added to stop the reaction. This was mixed by shaking, a drop of phenolphthalein added, and the pH adjusted to 8.2. Barium precipitation was carried out as described previously for the tissue analyses. Aliquots of the barium insoluble material were then hydrolyzed in 1 N HCl for periods up to 180 minutes at 100° C. and inorganic phosphorus was determined. Total phosphorus analysis was also carried out on an aliquot of the material precipitated by barium. The phosphorus which was not hydrolyzed in 180 minutes was considered phosphoglyceric acid phosphorus (LePage 1947, 1949a). These data are summarized in the following table:

Experiment No.	Reaction mixture	P hydrolyzed in 180 min.	Total P	Acid-Resistant P
		µg	µg	µg
1 GD	Control (time=0)	3720	3751	--
	Complete	2123	3754	1621
	Minus KF	3094	3750	656
2 GD	Control (time=0)	3703	3742	--
	Complete	2016	3748	1732
	Minus KF	3120	3753	633

The amounts of acid-resistant P formed indicate that the tissues contain a glyceraldehyde phosphate dehydrogenase.

(6) Lactic oxidase

The above named enzyme is perhaps the best known dehydrogenase of animal tissues, having been obtained in crystalline form from vertebrate muscle (Straub, 1940; Kubowitz and Ott, 1943), heart, and liver (Mehler et al, 1948).. Meyerhof (1919) showed that it catalyzed the following reaction:

Lactic acid + D.P.N. $\rightleftharpoons$ Pyruvic acid + $DPNH_2$

Though this enzyme is not an enzyme of phosphorylative fermentation (sens. strict.) it is included at this point because it follows directly from the phosphorylative processes.

Since these compounds had already been demonstrated in the tissues of the worm, it was expected that the enzyme would be present. Therefore, an attempt was made to demonstrate the lactic oxidase manometrically.

A 15% homogenate of worm tissue in isotonic KCl was prepared and tested in the following system:: 0.033 M phosphate buffer (pH 7.4), 0.05 M sodium lactate, 0.5 mg. methylene blue, 0.033 M D.P.N, and 0.3 ml. of homogenate. Total volume of the reaction mixture was 3.0 ml. The results obtained are summarized in the following table.

Reaction mixture	Expt. No. 1 Q_{O_2}*	Expt. No. 2 Q_{O_2}	Expt. No.3 Q_{O_2}
Complete	10.4	9.8	11.3
Minus D.P.N.	4.6	4.9	3.2
Minus lactate	1.0	1.9	1.0
Minus methylene blue	1.4	1.0	1.0

*µl. O_2/mg. tissue (dry wt.)/hour

The data indicate that the tissues of the worm contain a lactic oxidase which is pyridine-nucleotide-linked.

E. The Phosphatases

The phosphatase activity was studied in whole tissue homogenates prepared as 1% homogenates with ice-cold distilled water. The enzyme activity was determined by estimation of the ortho-phosphate formed after incubating the enzyme with an appropriate substrate. Alcohol was used to accelerate color development as suggested by Meyerhof and Oesper (1947). The buffer employed in all reaction mixtures was the veronal-acetate system of Michaelis (1931). The spontaneous formation of orthophosphate from tissue components was determined in each case by incubation of a complete preparation minus substrate. Substrate and trichloracetic acid were added simultaneously at the end of incubation and orthophosphate determined. This blank was used as a correction, but in all cases gave a very low value.

After preliminary experiments, the following procedure was adopted as a standard assay: 0.5 ml. of homogenate was incubated at 38° C. with 1.0 ml. M/10 $MgCl_2$, 0.5 ml. of M/100 substrate, and buffer (pH 7.6) to a total volume of 5.0 ml. After 30 minutes incubation, 2.5 ml. of 25% trichloracetic acid were added to stop the reaction, and the mixture was rapidly centrifuged. Aliquots of 0.5 and 1.0 ml. were removed for analysis. The phosphorus values obtained at these two levels served as an adequate control on the colorimetric procedure.

Of the substrates used in these experiments, muscle adenylic acid, fructose-1,6-diphosphate, sodium beta-glycerophosphate, and sodium pyrophosphate were commercial products of high purity. A.D.P. was prepared according to the method of Lohmann and Shuster (1935). Glucose-1-phosphate and A.T.P. were prepared as previously described.

(1) Effect of Ca and Mg on Phosphatase Activity

The phosphatase activity was markedly increased by the addition of Mg to the system.. This enhancement was not observed when Mg was replaced by Ca, Mn, or Zn.. Activation is maximal after the addition of Mg to a concentration of .02M and does not change if the Mg concentration is increased several fold. Figure 7 illustrates the effect of addition of varying amounts of Mg on the phosphatase activity with sodium pyrophosphate as the substrate. The Mg enhancement of phosphatase activity with several other substrates is shown in the following table.

Substrate	Mg (0.02 M) added (+) or not added (-)	Phosphate liberated μg
Hexose diphosphate	+	78
Hexose diphosphate	-	12
Sodium glycerophosphate	+	36
Sodium glycerophosphate	-	3
Adenylic acid	+	67
Adenylic acid	-	15
Sodium pyrophosphate	+	75
Sodium pyrophosphate	-	49

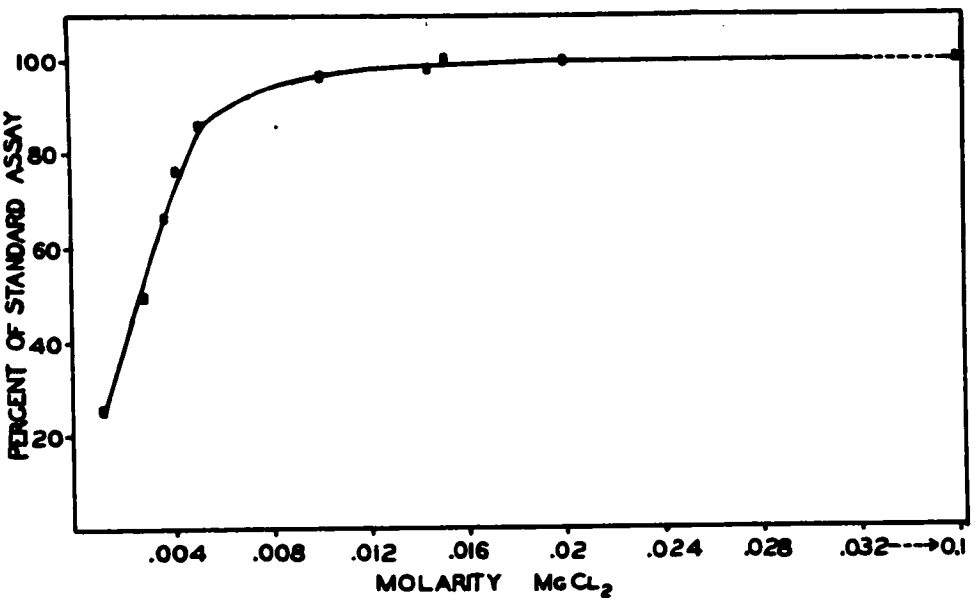


Figure 7. Magnesium Activation of Phosphatase

Calcium ion has an inhibitory effect on the phosphatase activity. The degree of inhibition after the addition of various amounts of Ca to standard assays with sodium pyrophosphate as the substrate is shown in Figure 8. The effect of Ca on phosphatase activity in standard assays of other substrates is shown in the following table.

Substrate	Ca (0.004 M) added (+) or not added (-)	Phosphate liberated γ
Hexose diphosphate	+	45
Hexose diphosphate	-	76
Sodium glycerophosphate	+	22
Sodium glycerophosphate	-	35
Adenylic acid	+	40
Adenylic acid	-	68
A.T.P..	+	46
A.T.P..	-	72

(2) Effect of Substrate Concentration

It was found that increasing the amount of substrate resulted in a decrease in the percent substrate hydrolyzed in the standard period. This is illustrated in Figure 9. This is the result to be expected if the enzyme concentration in the reaction mixture is a limiting factor.

(3) Effect of pH

The results of experiments designed to determine the optimum pH for phosphatase activity on several substrates are graphically summarized in Figure 10. It may be seen that the

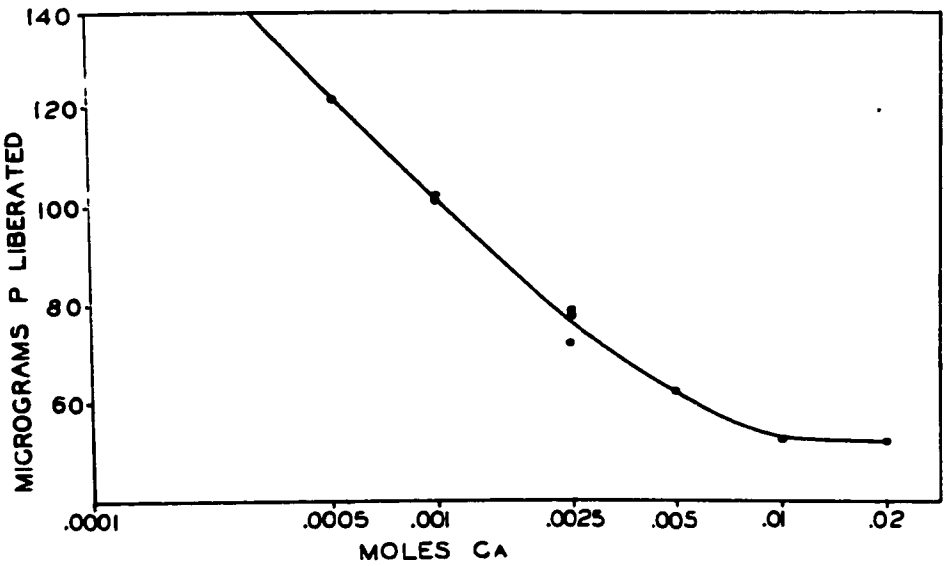


Figure 8. Calcium Inhibition of Phosphatase.

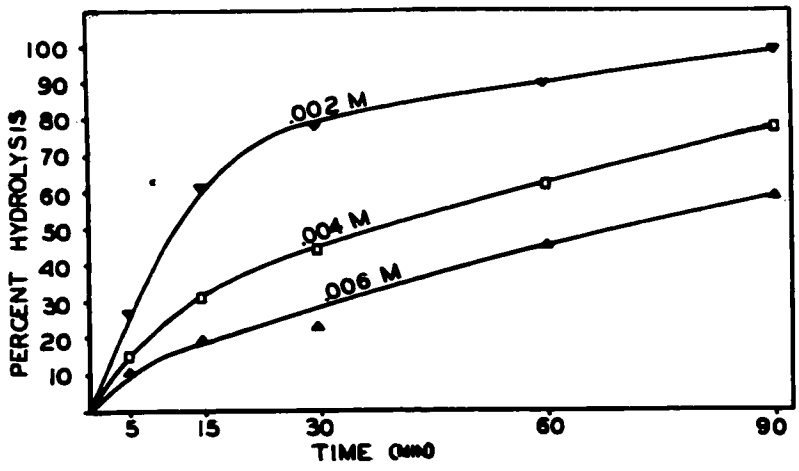


Figure 9. Phosphatase Activity with Varying Concentration of Substrate.

Figure 10. Effect of pH on Phosphatase Activity with Various Substrates.

A = Sodium pyrophosphate

B = A.T.P.

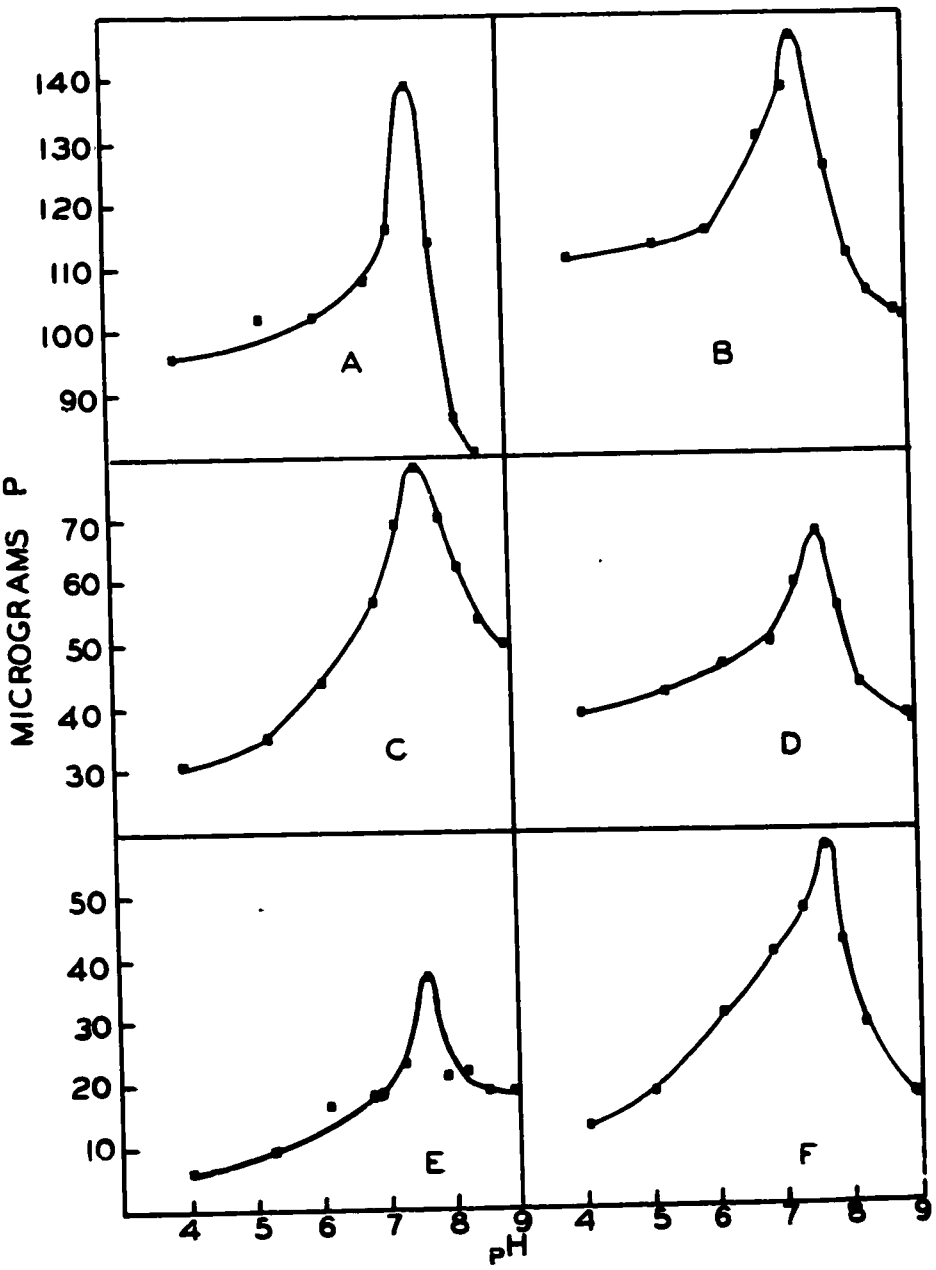
C = H.D.P.

D = Adenylic acid

E = Sodium beta-glycerophosphate

F = Glucose-1-phosphate

(Each Point Is Average of Three Determinations.)


MICROGRAMS P
140
130
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110
100
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A
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4
5
6
7
8
9
pH

optimum hydrogen ion concentration is rather sharply delineated at pH 7.6 in the case of all substrates tested. It may also be observed that the degree of activity with various substrates is quite different.

(4) The Phosphatase Gradient

It might be expected that there would be differences in the enzyme activity along the length of a tapeworm, since the various parts of the strobila are in varying states of physiological activity.

In order to test this hypothesis the following experiment was carried out: Individual worms were stretched on a glass plate and linearly divided into three equal portions. Corresponding thirds of ten worms were pooled into tissue samples representing anterior, middle, and posterior thirds. Each sample was carefully weighed and made up as a 1% homogenate. Each homogenate was assayed by the previously described standard procedure. The results are summarized in Table 5.

It may be seen that the anterior and middle thirds gave quite similar results, but the posterior third seemed somewhat irregular. Therefore, another experiment was carried out. The worms were treated as in the foregoing experiment except that the animals were divided into fourths. The results are summarized in Table 5..

It may be seen that this represents good evidence for a phosphatase gradient. The relative phosphatase activity is greatest in the anterior portion and decreases posteriorly in the second and third quarters. In the fourth quarter

Experiment	Anterior Third	Middle third	Posterior third
1	75	24	46
2	68	39	20
3	84	50	64

Experiment	Anterior fourth	Second fourth	Third fourth	Posterior fourth
4	94	72	43	98
5	99	83	56	91
6	84	41	32	100
7	72	36	12	88

All values = µg orthophosphate liberated

Substrate - sodium pyrophosphate

Table 5.. Phosphatase Activity in Different Parts of the Strobila

phosphatase activity increases. This can probably be ascribed to the eggs developing in the segments of this region.

(5) Discussion

This investigation has shown that the tissues of this cestode contain powerful phosphatase systems. Rogers (1947) reported phosphatase activity in the cuticle of mature segments of Moniezia, but found no activity in the anterior portion where the cuticle was undeveloped. Rogers' failure to demonstrate phosphatase activity more generally distributed in the tissues in his experimental material may be explained by consideration of the method which he used in his study. The histochemical method for the demonstration of "alkaline" phosphatase, described by Gomori (1939) and used by Rogers, entails the use of a relatively high concentration of calcium in the reaction mixture which is at a pH above 9. The present study indicates that Ca inhibits the phosphatase activity and that 9.0 is not an optimal pH for phosphatase activity in the tissues of at least one species of cestode. The fact that Mg is not added to the reaction in the Gomori assay would also result in a lower degree of enzyme activity. Further, it will be seen from the data of this study that beta-glycerophosphate is hydrolyzed to a markedly lower degree than other substrates. It would seem from these considerations that the Gomori method, if applied to H. diminuta, would yield minimal values in determining the phosphatase activity.

Roger's suggestion that the phosphatase in the cuticle may be associated with carbohydrate metabolism may well be true. However, it should be pointed out that these enzymes may serve the animal in another respect by preventing the passage into the animal of phosphorylated compounds. Rothstain and Meier (1948) have presented good evidence that the "surface" phosphatases of yeasts function in this way.

It is of interest to compare the results of this study with those of Novikoff (1949) who assayed the phosphatase activity of homogenates of various organs of the rat. Novikoff found that of the tissues which he assayed, intestinal mucosa was the only one in which the pH activity curves were similar with all substrates tested. The pH optimum for intestinal mucosa phosphatases was 7.4.

F. Discussion of Significance of Findings

The experimental data thus far presented furnishes strong evidence that the primary steps of carbohydrate utilization in this tapeworm are essentially the same as in vertebrate tissues and yeast. The reactions involved, as known in vertebrate tissues, are thoroughly reviewed by Barron (1943), Meyerhof (1943), Lippman (1941), and Lardy (1949), and are summarized in Figure 11. In the present study, evidence has been obtained for the presence of all intermediates shown in this reaction scheme, with the exception of phosphopyruvic acid. It may be mentioned that this compound is extremely labile and is normally found in very low concentration in vertebrate tissues (LePage, 1947). Furthermore, by the use of radioactive phosphorus, it has been shown that, *in vivo*, inorganic phosphate is rapidly incorporated in the organic, acid-soluble compounds.

Of the enzymes shown to be concerned in the reactions diagrammed in Figure 11, several have been demonstrated in the present study. These are phosphorylase (Reaction 1), phosphohexomutase (Reaction 2), hexokinase (Reaction 3), aldolase (Reaction 7), phosphoglyceraldehyde dehydrogenase (Reaction 9), and lactic oxidase (Reaction 14).

The powerful phosphatase system which has been shown to be present in the worms' tissues indicates an order of metabolic activity in the handling of phosphorus compounds comparable to the phosphatases of vertebrate tissues (Novikoff, 1949).

REACT.	ENZYME
1	PHOSPHORYLASE
2	PHOSPHOHEXO-MUTASE
3	HEXOKINASE
4	PHOSPHATASE
5	PHOSPHOHEXO-ISOMERASE
6	PHOSPHOHEXO-KINASE
7	ALDOLASE
8	TRIOSE ISOMERASE
9	PHOSPHOGLYCERALDE-HYDE DEHYDROGENASE
10	----------
11	TRIOSE MUTASE
12	ENOLASE
13	PHOSPHATASE
14	LACTIC OXIDASE

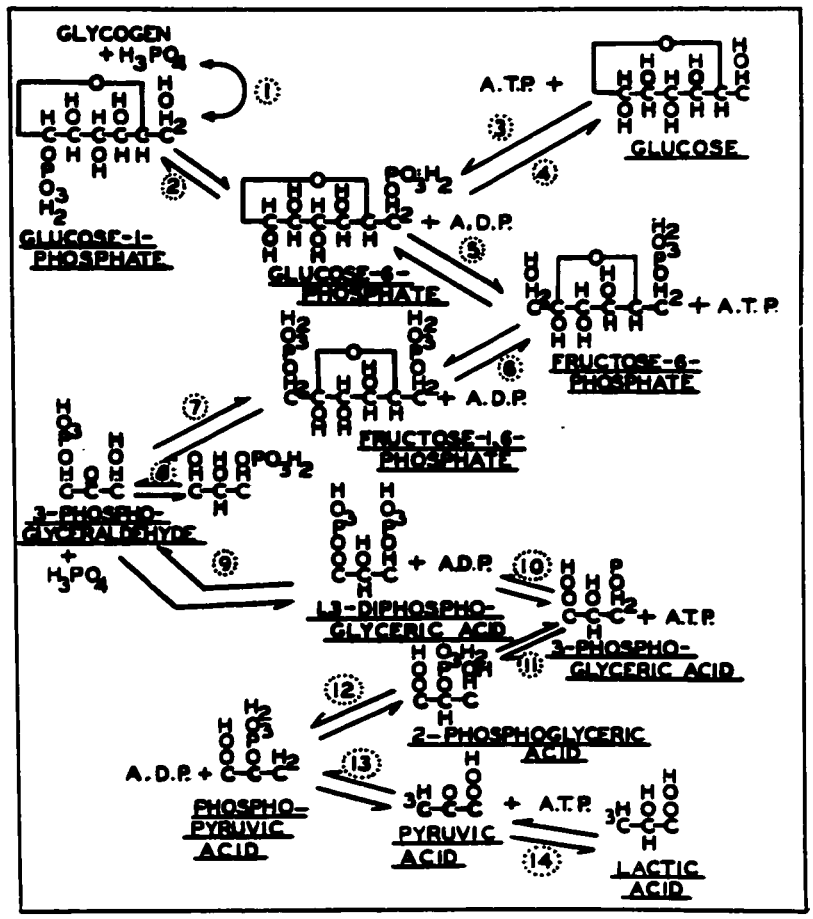


Figure 11. The Meyerhof-Embden Reactions of Glycolysis.

The demonstration of an enzyme (hexokinase) in which A.T.P. transphosphorylation is involved implies that energy is transferred via high energy phosphorus (Lippman, 1941).

The failure to demonstrate phosphagens such as occur in all free-living animals thus far investigated (Baldwin, 1945) has some interesting implications. It is now recognized that creatine phosphate or arginine phosphate function in animal tissues as "storage" substances for high energy phosphorus. When the demand arises, this high energy phosphorus is transferred to A.D.P., forming A.T.P.. This latter substance is evidently the direct source of energy for all metabolic activity (Baldwin, l.c.). Since arginine phosphate is found in free-living Plathelminthes and this substance apparently is not present in Hymenolepis, it is suggested that concomitant with the adoption of the parasitic way of life the tapeworm has lost the phosphagen energy-storage mechanism. This is perhaps not surprising when we consider that this animal is not forced to expend energy in rapid bursts in the process of obtaining its food or in escaping its enemies.. We may also perceive that this animal is feeding almost continuously.. In contrast, free-living flatworms may go for fairly long periods without feeding and for this reason "need" an energy-storage mechanism. By adapting an endoparasitic way of life the animal lives a "sheltered existence."

VI. Carbohydrate Utilization and End Products *In Vitro*

In order to obtain further information on the metabolism of this animal, it seemed desirable to study the utilization of carbohydrate and to identify some of the end products of carbohydrate metabolism under aerobic and anaerobic conditions. Experiments were carried out with unbroken tissues and cell-free homogenates.

A. Whole Tissues

Worms were removed from an infected host and washed in several changes of Krebs-Ringer phosphate buffer (pH 7.4) during a 20 to 25 minute period. A section of the strobila (60 to 90 mg.) from the middle of each worm was used in an experimental vessel. The remainder of the worm was placed in 30% KOH and held for analysis of the glycogen content. The experimental vessels containing tissue and Krebs-Ringer phosphate or bicarbonate buffer with .015 M glucose were placed in a water bath at 38° C. and incubated in an atmosphere of air or nitrogen for a period of 90 minutes. At the end of the incubation period the tissue was removed and placed in 30% KOH for glycogen analysis. The suspending medium was analyzed for lactate, succinate, or acetate in various experiments.

In the first experiments only lactate was determined in the suspending medium. The suspending medium was Krebs-Ringer bicarbonate buffer plus .015 M glucose. The results obtained are summarized in the following table.

Experiment	Carbohydrate utilized (µM glucose per gm. tissue)	Lactate produced (µM per gm. tissue)	% carbohydrate accounted for
Anaerobic			
1	42.8	33.0	38
2	51.0	37.1	36
Aerobic			
1	36.2	16.5	22
2	31.0	16.4	26

Obviously, formation of lactate failed to account for the carbohydrate utilized in the experimental period. Other products were sought in the following manner: About 500 mg. of worm tissue was incubated anaerobically in 300 ml. of Krebs-Ringer bicarbonate (pH 7.4) plus .05 M glucose at 38° C. for 3 hours. The worms were removed and one-fifth volume of 1 M phosphate buffer (pH 1.8) was added to the medium. The mixture was then steam-distilled, the distillate being collected in an iced flask. After 200 ml. of distillate had been collected, the material was titrated with .005 N NaOH (bromthymol blue). Nitrogen was bubbled through the distillate during titration. After it was found that a steam-volatile acid was being formed by the worms, the acid was identified as acetic acid in the following manner: Elsden (1946) showed that volatile fatty acids are eluted at different rates from a silica gel column by chloroform-butanol mixtures. These acids are eluted at rates inversely proportional to the length

of the carbon chain. In the present study silica gel columns were prepared according to Elsden with bromcresol green as the indicator. A portion of the neutralized steam distillate containing 15 microequivalents of the steam-volatile acid was evaporated to dryness. One gram of $KHSO_4$ was added to the residue and the material was extracted with four 2 ml. portions of 5% butanol in chloroform. The extracts were pooled and made up to 10 ml. Five ml. of the extract were placed on the silica gel column. A single yellow band was observed on the column during the elution. This indicated that the distillate contained only one acid. The rate of elution was equal to or slightly higher than the rate of elution of known solutions of acetic acid of the same concentration. The acid in the steam distillate was quantitatively eluted by 5% butanol in chloroform; this behavior is also characteristic of acetic acid. This identification of the unknown acid as acetic was confirmed by a strongly positive lanthanum nitrate test (Feigl, 1946).

Further experiments were carried out to determine whether acetic acid formation would account for a significant portion of the unaccounted for carbohydrate utilized by the worms. Acetic acid was determined by titration with .005 N NaOH (with bubbling nitrogen) of a steam-distillate of the medium. The experimental conditions were identical with those of the preceding experiments. The results are summarized in the following table.

Experiment	Carbohydrate utilized (µM/gm. tissue)	Lactate produced (µM/gm. tissue)	Acetate produced (µM/gm. tissue)	% Carbohydrate accounted for
Anaerobic				
1	47.0	34.1	3.1	39.5
2	41.3	33.2	5.4	46.6
Aerobic				
1	38.4	16.4	0.1	21.3
2	32.6	17.1	0.1	26.2

It is clear that acetate formation does not account for a very large part of the carbohydrate utilized by the worm under anaerobiasis. Even less acetate is formed under aerobic conditions.

Attempts were made to prepare derivatives of the non-volatile acids. In all cases the melting point determinations indicated that the derivatives obtained were mixtures of more than one substance. The amounts of material obtained for study did not allow the application of some of the methods of organic analyses which require relatively large amounts of material.

Since Brand (1933) found succinic acid to be an end product of the metabolism of Moniezia it was suspected that this acid might be a product of the metabolism of Hymenolepis. After manometric equipment became available, it was possible to determine qualitatively and quantitatively whether succinic acid was being excreted into the medium by Hymenolepis. The

procedure used was that of Cohen (1949). This method utilizes a heart muscle succinoxidase preparation to which is added the water-soluble residue of the benzine-extractable material. The enzyme preparation was standardized by the addition of known amounts of succinate. These experiments were highly successful in that succinic acid was found to be present and reproducible quantitative data were obtained. The experimental conditions were the same as those described above. The results obtained are summarized in the following table.

Experiment	Carbohydrate utilized (µM/gm. tissue)	Lactate produced (µM/gm. tissue)	Succinate produced (µM/gm. tissue)	% utilized Carbohydrate accounted for
Anaerobic				
1	43.0	33.6	6.0	46.0
2	44.6	39.4	5.7	50.6
3	50.2	40.8	8.3	48.9
Aerobic				
1	32.2	15.2	0	23.6
2	31.0	17.1	0	27.5
3	37.3	16.7	0	22.4

These experiments were repeated using Krebs-Ringer phosphate buffer in place of the bicarbonate system. Under these conditions lactate production was essentially the same as in the above experiments, but succinate was produced only in trace amounts anaerobically. The implications of this observation will be discussed in Section X.

It may be seen that the identified end products account for about half of the utilized carbohydrate. However, the unidentified end products still constitute a significant fraction and merit further investigation. The finding that succinate is not formed aerobically is of some interest. The possible implications of this finding will be treated in the general discussion (Section X).

B. Homogenates

It seemed desirable to study the processes of fermentation in cell-free, isotonic KCl homogenates. LePage (1948) described a system in which he was able to maintain glycolysis and phosphorus esterification by tumor tissue homogenates. In the present study LePage's system was used as a basis and the ingredients varied in different experiments. The following procedure was used in all experiments: Reaction vessels were prepared before the tissue was homogenized. All reactants were added except the homogenate, and the vessels were kept in chopped ice until the addition of the homogenate. The homogenates were prepared with ice-cold isotonic KCl (made alkaline by adding 8.0 ml. of .02 M $KHCO_3$ per liter). The basic system contained 0.0024 M K_2HPO_4, 0.025 M $KHCO_3$, 0.040 M nicotinamide, 0.00033 M A.T.P., 0.00020 M D.P.N., 0.002 M hexose diphosphate, 0.005 M pyruvate, 0.0066 $MgCl_2$, 0.010 M KF, .010 M glucose, and 0.2 ml. of 25% homogenate. Total volume of the reaction mixture was 3.0 ml. After addition of the homogenate the vessels were placed in the water bath at 38°

and pure nitrogen was bubbled into the vessels through a capillary tube during the incubation period. This served the double purpose of providing anaerobic conditions and keeping the material agitated during incubation. After 30 minutes incubation 0.2 ml. of 100% trichloracetic acid was added to each reaction vessel and thoroughly mixed with the contents to terminate the reactions. After neutralization with strong (30%) KOH and centrifugation, aliquots of the medium were analyzed for lactic acid, inorganic phosphorus, and glycerol. In all experiments unincubated reaction mixtures were analyzed. From these data it was possible to calculate lactate and glycerol formed and phosphate esterified in the incubated mixture.. The results obtained in these experiments are summarized in tabular form as follows:

Experiment	Component varied	P esterified µM	Lactate formed µM	Glycerol formed µM
1	Pyruvate:			
	.005 M	+ 2.33	+ 1.52	4.43
	0	-1.74	+ 0.34	3.33
2	.005 M	+ 2.06	+ 1.61	4.56
	0	- 1.48	+ 0.24	3.20

Experiment	Component varied	P esterified µM	Lactate formed µM	Glycerol formed µM
3	Hexose diphosphate:			
	.002 M	+ 1.81	+ 1.95	4.23
	.001 M	+ 1.52	+ 1.55	3.46
	0	+ 1.20	+ 0.88	1.06
4	.002 M	+ 2.01	+ 1.69	3.95
	.001 M	+ 1.65	+ 1.12	2.64
	0	+ 1.08	+ 0.64	1.21
5	$MgCl_2$			
	.0033M	+ 2.39	+ 1.24	3.30
	.0066M	+ 1.94	+ 1.73	4.21
	.0099M	+ 1.84	+ 0.95	5.42
6	.0033M	+ 2.09	+ 1.19	2.69
	.0066M	+ 1.89	+ 1.80	4.14
	.0099M	+ 1.41	+ 0.71	4.98
7	A.T.P.			
	2×10^{-4}M	+ 3.75	+ 1.24	3.24
	4×10^{-4}M	+ 4.48	+ 2.03	4.30
	8×10^{-4}M	+ 2.52	+ 1.55	4.42
8	2×10^{-4}M	+ 3.53	+ 1.49	3.71
	4×10^{-4}M	+ 4.63	+ 2.14	4.41
	8×10^{-4}M	+ 2.07	+ 1.78	4.62

Experiment	Component varied	P esterified µM	Lactate formed µM	Glycerol formed µM
9	D.P.N.			
	2×10^{-4} M	- 0.38	+ 0.81	0.64
	4×10^{-4} M	+ 1.62	+ 1.67	5.01
	6×10^{-4} M	+ 3.81	+ 1.98	4.71
10	2×10^{-4} M	- 0.68	+ 0.24	0.79
	4×10^{-4} M	+ 1.87	+ 1.79	4.84
	6×10^{-4} M	+ 3.90	+ 2.13	3.96
11	Nicotinamide			
	.02 M	+ 1.42	+ 1.36	4.30
	.04 M	+ 2.16	+ 2.27	4.69
	.08 M	+ 3.58	+ 2.61	5.02
12	.02 M	+ 1.31	+ 1.14	4.20
	.04 M	+ 2.02	+ 1.88	4.48
	.08 M	+ 3.44	+ 2.89	4.99
13	Inorganic P			
	.0010 M	+ 0.11	0	0.28
	.0015 M	+ 1.41	+ 0.65	2.58
	.0025 M	+ 2.94	+ 1.78	4.01
14	.0010 M	+ 0.10	+ 0.09	0.08
	.0015 M	+ 1.07	+ 1.12	1.99
	.0025 M	+ 2.61	+ 1.94	3.87

Experiment	Component varied	P esterified µM	Lactate formed µM	Glycerol formed µM
15	KF			
	.0066 M	+ 2.97	+ 0.95	3.01
	.0100 M	+ 1.62	+ 2.02	4.50
	.0200 M	0	+-1.61	3.96
16	.0066 M	+ 2.48	+ 0.65	2.86
	.0100 M	+ 1.95	+ 1.87	4.07
	.0200 M	0	+ 1.30	3.22

These experiments show that anaerobic fermentation can be maintained in a cell-free system, with a homogenate of the tissues of this tapeworm as the source of the enzymatic catalysts. LePage's system which is optimum for mammalian tumor tissues is not optimum for fermentation by worm tissue homogenates. The data indicate that a more nearly optimum system for worm tissues contains the following: 0.005 M pyruvate, 0.002 M H.D.P., 0.0033 M $MgCl_2$, 0.0004 M A.T.P., 0.0006 M D.P.N., .08 M nicotinamide, .0025 M phosphate, 0.0066 M KF, 0.025 M $KHCO_3$, 0.010 M glucose, and homogenate. Further study of the system will be necessary to establish that these conditions are actually optimum in all respects.

As far as the writer is aware these are the first experiments of this type with invertebrate tissues and the writer feels that it may furnish a very valuable tool for the practical testing of anthelmintic drugs.. Bueding (1949) has recently pointed out that we must develop techniques for

attacking parasites at susceptible points in their metabolism. The greater the difference of such points from the corresponding points in the host's metabolism, the more susceptible these points will be to chemotherapeutic attack. In a system such as has been studied here, a long series of enzymatic mechanisms may be exposed to the effects of a drug and the search may thus be narrowed. Studies of this type may help workers to abandon the search-at-random, empirical drug testing procedures..

VII. Glycogen Fluctuation *In Vivo*

Reid (1942) showed that the glycogen content of the cestode, *Raillietina cesticillus*, parasitic in chickens, was markedly reduced by starving the host for as little as 20 hours.. Further, Reid showed that there is a diurnal fluctuation in the glycogen content of the worm which may be correlated with the feeding of the host. Since Chandler (1943) showed that *Hymenolepis diminuta* is also very sensitive to deletion of carbohydrate from the host diet, it seemed desirable to study the glycogen fluctuations in this species under conditions of host starvation and normal feeding.

A. Carbohydrate Depletion

In the first experiment 10 infected rats were divided into two groups of 5 animals each. One group was given only water for 20 hours, while the other group was fed *ad libitum* on the ordinary animal house fare. The host animals were killed and the worms from each animal washed in Tyrode's solution, blotted on filter paper and weighed. Each worm was then placed in a tube containing 1.0 ml. of 30% KOH and held for glycogen analysis. The glycogen was determined as previously described. The results of these determinations are summarized in Table 6.

The data indicate that starving the host animals for the short period of 20 hours reduces the glycogen content of the worms to a value about one-tenth that of worms from hosts under normal feeding conditions. This is further evidence that the worms are dependent on the host diet for the satisfaction of their carbohydrate requirements.

Hour killed and treatment	Rat No.	No. worms	Worms in sample	% Glycogen	Weighted mean % glycogen
8-9 A.M.	1C	6	1	5.71	
			1	5.34	
On feed until killed			1	5.59	
			1	5.02	
			2	5.82	5.55
	2C	4	1	5.28	
			1	5.38	
			1	4.92	
			1	4.99	5.14
	3C	6	1	6.51	
			1	6.02	
			2	6.32	
			2	5.90	6.16
	4C	2	1	5.59	
			1	3.98	4.78
	5C	3	1	6.51	
			2	6.15	6.27
			Mean -	5.59	5.58
10-11 A.M.	1E	5	1	0.51	
			2	0.55	
No food for 20 hours			2	0.30	0.44
	2E	6	1	0.47	
			1	0.37	
			1	0.49	
			1	0.54	
			2	0.42	0.45
	3E	5	1	0.69	
			2	0.60	
			2	0.74	0.67
	4E	3	1	0.47	
			1	0.48	
			1	0.71	0.55
	5E	4	1	0.55	
			1	0.42	
			2	0.69	0.59
			Mean -	0.54	0.54
			Difference in Means-	5.04*	5.03*
			Standard error of difference-	0.641	0.567

*Highly significant

Table 6.

B. Normal Fluctuation

It now seemed desirable to ascertain whether this tapeworm showed a normal fluctuation in glycogen content such as Reid (loc. cit.) reported for Raillietina. Accordingly, ten infected rats were divided into two groups of five animals each. These were kept in the experimental cages for six days and fed the ordinary animal house diet. This was done to be certain that feeding was not upset by the psychological trauma of handling and of being placed in new cages. On the day of the experiment the two groups of rats were killed at 4 A.M. and 4 P.M. respectively, and the glycogen content of the worms determined. The results of this experiment are summarized in Table 7.

It may be seen that the glycogen content of the tapeworm fluctuates under normal conditions of host feeding and, in view of the results obtained by starving the host, it seems probable that this is dependent on the feeding habits of the host.

C. Effect of Lactate

In vertebrate tissues the resynthesis of polysaccharide from lactic acid is a well recognized phenomenon. It seems probable that small invertebrates may be limited in carrying out such a resynthesis through loss of products by excretion into the medium (Brand, 1945). It should also be pointed out that so far as is known, resynthesis of glycogen from lactic acid only occurs aerobically (Barron, 1943).

Hour killed	Rat no.	No. worms	Worms in Sample	% Glycogen	Weighted mean % glycogen
4 P.M..	16A	8	5	2.46	
			3	3.01	2.66
	17A	10	5	3.42	
			5	3.27	3.34
	18A	8	5	2.90	
			3	3.14	2.99
	19A	6	3	4.02	
			3	2.98	3.50
	20A	3	3	3.08	3.08
				Mean - 3.14	3.11
4 A.M.	21A	6	3	6.87	
			3	5.94	6.41
	22A	4	4	7.03	7.03
	23A	8	5	5.86	
			3	6.21	5.99
	24A	9	5	6.01	
			4	6.31	6.14
	25A	6	3	5.45	
			3	6.10	5.77
				Mean - 6.20	6.26
			Difference in Means-	3.06*	3.15*
			Standard error of difference-	0.656	0.523

*Highly significant

Table 7.. Normal Glycogen Fluctuation.

With these facts in mind, an experiment was planned in which host animals would be given lactate only and the effect, if any, on the glycogen stores of the worm would be determined. Two groups of five animals each were starved for a period of 24 hours. During this period the animals of one group were given doses of 180 mg. of sodium lactate at 6, 12, and 18 hours after the beginning of the experiment. This was administered by stomach tube. At the end of 24 hours all animals were sacrificed and the worms analyzed for glycogen. The results are summarized in Table 8.

The data indicate that feeding lactate resulted in a lessened lowering of the glycogen content of the worms. The standard error of the difference between the animals receiving lactate and those not given this substance indicates that the difference is definitely significant. The writer believes that this experiment constitutes strong evidence for some aerobic metabolism <u>in vivo</u>. It is not known whether this represents an actual resynthesis of glycogen from lactate or an aerobic utilization of pyruvate produced by the action of the lactic dehydrogenase which is present in the tissues. One or both of these may be involved. Studies with C^{14}-labeled lactate might furnish an answer to this problem.

Treatment	Rat No.	No. worms	Worms in sample	% Glycogen	Weighted mean % glycogen
Starved 24 hours	26A	6	2	0.43	
			2	0.35	
			2	0.21	0.33
	27A	8	2	0.41	
			2	0.18	
			4	0.37	0.33
	28A	10	2	0.19	
			2	0.49	
			2	0.20	
			4	0.38	0.32
	29A	3	3	0.42	0.42
	30A	8	2	0.31	
			2	0.74	
			4	0.21	0.37
			Mean -	0.35	0.35
Fed Sodium lactate only	31A	5	1	1.65	
			2	1.34	
			2	1.05	1.28
	32A	8	2	1.97	
			2	1.48	
			4	1.59	1.66
	33A	7	1	2.01	
			2	1.37	
			2	1.54	
			2	1.84	1.64
	34A	7	2	1.32	
			2	1.88	
			3	0.99	1.34
	35A	6	2	1.90	
			2	1.72	
			2	1.82	1.81
			Mean -	1.54	1.55
			Difference in Means -	1.19*	1.20*
			Standard error of difference -	0.343	0.204

*Significant

Table 8. Glycogen-sparing Effect of Lactate

VIII.. Respiratory Metabolism

As pointed out by Bueding (1949), essentially nothing is known about the respiratory metabolism of parasitic helminths. Arguments have raged back and forth for many years as to whether intestinal helminths live aerobically or anaerobically.. Brand (1945) and Bueding (1949) have surveyed what evidence is available and have concluded that dependence on aerobic metabolism varies greatly from one parasite to another, and that this feature of the metabolism must be investigated for each individual species. With these points in mind some attempt was made to determine the extent to which H. diminuta might depend on aerobic metabolism.

A.. The Oxygen Debt

No report of the development of an oxygen debt by cestodes under anaerobiasis has been found in an extensive search of the literature. It seemed of interest, therefore, to determine whether or not this cestode would develop an oxygen debt under anaerobic conditions.. Sections from the middle of the strobila (40 to 70 mg.) were used in the determinations. Krebs-Ringer phosphate solution containing 1% glucose was used as the suspending medium. After an initial control period of 60 minutes, the worms were gassed with pure nitrogen and kept under anaerobiasis for the following 60 minutes. At the end of this period the flasks were flushed with air, and the oxygen uptake determined for a period of 60 minutes. The results of this experiment are shown in the following table.

	Expt. No. 1	Expt. No. 2	Expt. No. 3	Expt. No. 4	Expt. No. 5	Expt. No. 6
	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}
Control period	1.9	3.8	2.7	3.4	2.9	2.0
First hour after anaerob.	2.8	5.6	3.9	4.1	3.8	3.1

The data indicate that under anaerobiasis the animal contracts an oxygen debt. Though the substances involved were not actually determined in this study, it seems probable that lactic and/or succinic acids formed under anaerobic conditions are probably oxidized in the recovery period. Both of these substances have been identified in the course of this investigation as products of the anaerobic metabolism of this worm. Furthermore, the tissues have been shown to be capable of oxidizing lactic acid and, as will be shown presently, are capable of oxidizing succinic acid. To what extent this recovery from anaerobiasis may be extrapolated to the conditions *in vitro* is somewhat difficult to assay. The gut is a larger container than the flasks used in these experiments and more of the materials formed under anaerobiasis would probably be lost to the worm than is the case in the closed system of this investigation. Furthermore, the biochemical activities of microorganisms and absorption of substances by the host might result in greater loss to the worm.

It may be mentioned that Dr. K. B. Kerr (personal communication) has recently found that *Raillietina cesticillus*, a chicken tapeworm, also contracts an oxygen debt under anaerobiasis.

B. Respiratory Enzymes

To attain real understanding of the respiratory metabolism of an animal, investigation of the catalytic mechanisms involved is a necessity. For this reason some experiments were performed to determine the presence or absence of certain respiratory enzymes.

(1) Cytochrome oxidase

This enzyme is known to play a prime role in the disposition of electrons during the respiratory process of many animal tissues (Lardy et al, 1948). Keilin and Hartree (1938) named the enzyme and showed that it was essentially the same as the indophenol oxidase of earlier workers.

It has not been conclusively demonstrated that cytochrome oxidase actually exists in the tissues of any parasitic helminth. Some studies on the effect on worms of substances known to be inhibitors of cytochrome oxidase have been reported. Stannard, McCoy, and Latchford (1938) reported that low concentrations of cyanide strongly inhibited the respiration of Trichinella larvae. On the other hand, Brand (1945) reported that low concentrations of cyanide or azide had little effect on the respiration of Eustrongyloides larvae, and with concentrations of 2.5×10^{-3} to 1×10^{-2} M cyanide or azide the respiration was only inhibited 72 and 85%, respectively. According to Friedheim and Baer (1933) and Friedheim (1934) the oxygen uptake of Dibothriocephalus (= Diphyllobothrium) was reduced to 15% with the very high cyanide concentrations of 5×10^{-3} to 1×10^{-2} M. Partial

respiratory inhibition in the presence of high concentrations of cyanide has been reported by Wilmoth (1945) in Taenia sp., by Van Grembergen (1944) in Moniezia benedeni, and by Rogers (1948) in Ascaridia galli, Nematodirus sp., and Nippostrongylus muris. Complete inhibition of the respiration of Neoaplectana glaseri with low cyanide concentrations was reported by Rogers (1948). Bueding (1949 a and b) reported similar findings with Litomosoides carinii and Schistosoma mansoni. Laser (1944) showed that cyanide concentrations up to 10^{-2} M did not inhibit the respiration of Ascaris. However, as Bueding (1949) pointed out, this apparent lack of cyanide inhibition in Ascaris might be due to removal of oxaloacetic acid by cyanohydrin formation, oxaloacetic having been shown by Laser (loc. cit.) to inhibit markedly the respiration of Ascaris.

It may be pointed out that cyanide inhibition is only suggestive evidence that the cytochrome system is involved in respiratory activity. As a case in point, Bueding (1949) reported that although the respiration of Litomosoides was more sensitive to cyanide than that of most other parasitic helminths, he was unable to demonstrate cytochrome oxidase activity in homogenates of the worm's tissues.

The increase in respiration in the presence of paraphenylenediamine observed with Eustrongyloides by Brand (1945) and with Moniezia by Van Grembergen (1944) does not prove the presence of cytochrome oxidase since there is a definite

possibility that this substrate may be oxidized by other enzymes. The data of Stotz et al (1938) indicate that indophenol oxidase is not an entity identical with cytochrome oxidase. Laser's demonstration (1944) of the formation of hydrogen peroxide in the aerobic metabolism of Ascaris indicates that cytochrome oxidase is probably not involved to any significant extent in hydrogen transfer; cytochrome oxidase reacts with oxygen to form water rather than hydrogen peroxide (Potter, 1949).. This is further supported by the report of Herrick and Thede (1945) that cytochrome oxidase could not be demonstrated in homogenates of Ascaris. These workers used cytochrome C as the substrate and ascorbate as the reducing agent.

Since the previous evidence for the presence of cytochrome oxidase in cestode tissues was considered inconclusive, it seemed that an examination of the tissues of Hymenolepis for this enzyme would be highly desirable. The biological substrate of the enzyme, cytochrome C, was employed in these experiments since this would remove all doubt as to substrate specificity, and the cytochrome oxidase was studied manometrically in the tissue homogenate system described by Schneider and Potter (1943).. Homogenates were prepared as previously described and components of the reaction system varied.. The complete system contained 1.0×10^{-4} M cytochrome C, 4×10^{-4} $AlCl_3$, .0114 M sodium ascorbate, 3.3×10^{-2} M phosphate buffer (pH 7.4), 0.3 ml. of 20% homogenate, and

distilled water to a total volume of 3.0 ml. The data are summarized in the following table.

Reaction mixture	Experiment 1	Experiment 2	Experiment 3
	Q_{O_2}*	Q_{O_2}	Q_{O_2}
Complete	13.5	14.0	12.8
Without Cytochrome C	1.6	1.9	1.6
Without Ascorbate	0.0	0.0	1.0
Without Al Cl_3	12.4	12.5	12.5

*Q_{O_2} = µl. O_2/ mg. tissue (dry wt.)/hour

The data indicate that cytochrome oxidase is present in the tissues of the worm.

(2) Succinic dehydrogenase

This enzyme is known to catalyze an important step in the so-called "citric acid cycle" of Krebs (1943). The reaction may be formulated:

$$\text{Succinic acid} \underset{+2H}{\overset{-2H}{\rightleftharpoons}} \text{Fumaric acid}$$

Since succinic acid has been shown in the present paper to be at least one of the end products of the metabolism of this worm, the demonstration of this enzyme would seem to be important. In vertebrate tissues this reaction normally passes electrons to the cytochrome system and thence to molecular oxygen. It has already been shown that cytochrome oxidase is present in the worm's tissues. Therefore, it seemed desirable to determine whether the succinic dehydrogenase, if present, could transfer electrons to oxygen via the cytochrome

system. For this purpose the manometric assay of Schneider and Potter (1943) was used as a basis for studying the enzyme.

The reaction system consisted of the following: 0.033 M phosphate buffer (pH 7.4), 0.05 M sodium succinate, 1 X 10^{-4} M cytochrome C, 4 X 10^{-4} M $AlCl_3$, 4 X 10^{-4} M $CaCl_2$, and 0.3 ml. of 25% tissue homogenate. The mixture was made to a volume of 3.0 ml. with distilled water. The results obtained with this system are summarized in the following table.

Reaction mixture	Expt. 1	Expt. 2	Expt. 3
	Q_{O_2}	Q_{O_2}	Q_{O_2}
Complete	4.1	2.4	3.4
Minus cytochrome C	0.5	0.5	--
Minus succinate	0.5	1.0	0.5
Minus Al Cl_3	3.8	1.7	--
Minus Ca Cl_2	3.6	1.8	--
Complete + .001 M KCN	--	0	0

The tissues of the animal definitely possess cytochrome-linked succinic dehydrogenase activity. However, compared to vertebrate tissues, it is of a very low order. It was at first thought that cytochrome C concentration might be a limiting factor. An experiment was performed using the same system described above except that 15% homogenate was tested and the cytochrome C concentration was varied.. The results of this experiment are shown in Figure 12.. The data indicate that cytochrome C was not the limiting factor in the concentration used in the first experiment.

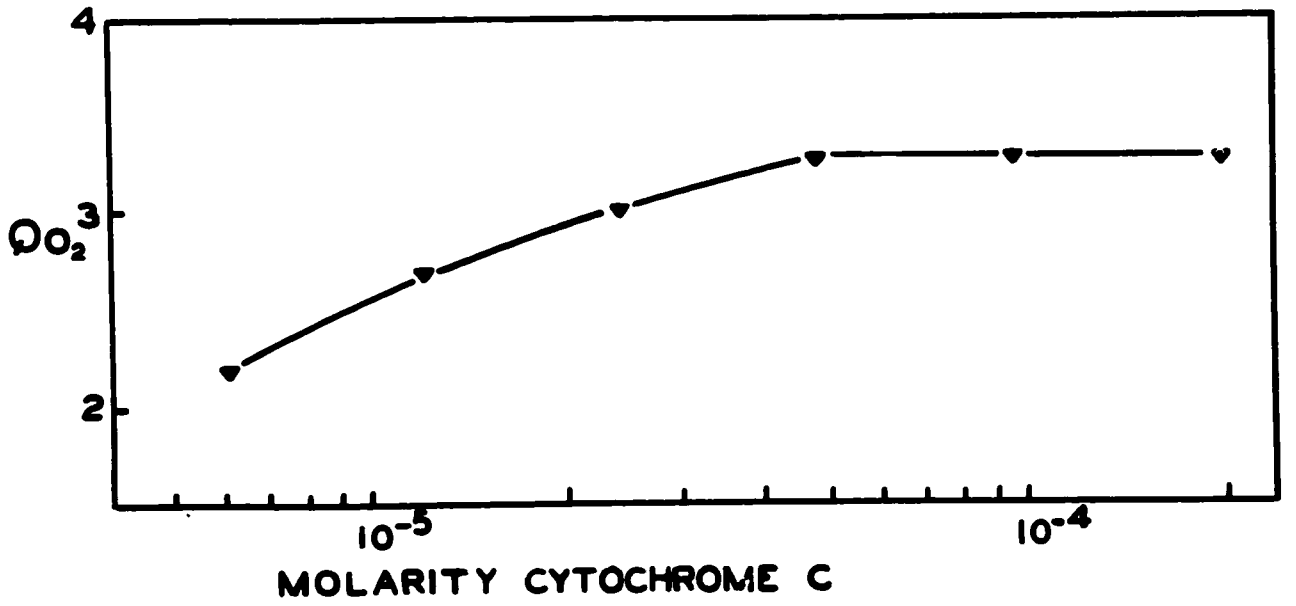


Figure 12. Succinic Dehydrogenase Activity with Varying Concentrations of Cytochrome C.

Various workers have shown that the succinic dehydrogenase of vertebrate tissues may also transfer electrons to redox dyes such as cresyl blue or methylene blue (Lardy et al, 1949). The degree of activity is lower with these dyes than with the cytochrome system (Lardy et al, 1949). It seemed profitable to test one of these dyes as an electron acceptor for the succinic dehydrogenase of Hymenolepis. The auto-oxidizable dye, methylene blue, was chosen and the reaction followed manometrically. The system tested had the following components: 0.033 M phosphate buffer (pH 7.4), 0.05 M sodium succinate, 14 X 10^{-4} M $CaCl_2$, 4 X 10^{-4} $AlCl_3$, 2.0 mg. of methylene blue, and 0.3 ml. of 25% tissue homogenate. The total volume of the mixture was 3.0 ml. This was compared with the activity with the cytochrome system using the cytochrome assay previously described. The results are summarized in the following table:

Reaction mixture	Expt. 1	Expt. 2	Expt. 3
	Q_{O_2}	Q_{O_2}	Q_{O_2}
Methylene blue system			
Complete	12.5	11.0	14.1
Minus methylene blue	1.1	0.7	1.0
Minus succinate	0.9	0.8	1.2
Complete + .001 M KCN	13.0	11.2	14.1
Cytochrome system			
Complete	3.3	2.8	--
Complete + .001 M KCN	0	0	--

The results obtained were somewhat surprising. The Q_{O_2} obtained with methylene blue as the electron acceptor is more

than four times as great as the Q_{O_2} obtained with the cytochrome system.

The succinic dehydrogenase potential of the worm's tissues was obviously not satisfactorily determined in the experiments using cytochrome C as the electron acceptor. Restudy of the Q_{O_2} values obtained in the cytochrome oxidase assay (page 72) seemed to indicate that the oxidase was probably not the limiting factor in the transfer of electrons from succinic dehydrogenase to oxygen.

Quite by accident, it was found that if a water tissue homogenate was left in the refrigerator (ca. 5° C) for 24 hours it lost the ability to transfer electrons from succinic dehydrogenase to oxygen via the cytochrome system. When such a homogenate was tested for succinic dehydrogenase activity with methylene blue, the activity was only slightly lower than that obtained with the fresh homogenate. In addition, cytochrome oxidase was still quite active in the aged homogenate. The data supporting the foregoing statements are summarized in the following table.

Reaction mixture	Expt. 1 Q_{O_2}	Expt. 2 Q_{O_2}	Expt. 3 Q_{O_2}
Fresh 15% homogenate			
Methylene blue system	11.0	12.2	12.6
Cytochrome system	3.3	3.9	3.6
Cytochrome oxidase	13.5	12.9	11.0
Same homogenate aged 24 hours			
Methylene blue system	10.9	12.0	12.1
Cytochrome system	0.5	0.5	0
Cytochrome oxidase	13.1	12.1	10.8

This data is interpreted to indicate that the succinic dehydrogenase is not linked to oxygen by the simple linkage:

Dehydrogenase ⟶ Cyto. C ⟶ Cyto. oxidase ⟶ O_2

Instead, some other factor is involved between the dehydrogenase and cytochrome C. The relationship can be re-formulated thus:

Dehydrogenase ⟶ X ⟶ Cyto. C ⟶ Cyto. oxidase ⟶ O_2

This is comparable with some of the known properties of succinic dehydrogenase from mammalian tissues. Using purified succinic dehydrogenase from mammalian heart it has been shown that some unknown factor from pig heart muscle (Straub, 1942) and ox or rabbit liver (Stoppani, 1947) is necessary for the coupling between succinic dehydrogenase and cytochrome C. The nature of the substance is not known.

From the great difference in cytochrome-linked and redox-dye-linked dehydrogenase activity and from the demonstration of a labile intermediate step between the dehydrogenase and cytochrome C, it seems probable that this unknown component is probably the limiting factor in the activity of the dehydrogenase <u>via</u> the cytochrome system. Isolation and addition of this linking substance to an homogenate system would perhaps have furnished evidence of this. However, time was not available for further study of the system.

(3) Malic dehydrogenase

In vertebrate tissues this enzyme catalyzes the following reaction:

Malic acid + DPN $\rightleftharpoons$ oxaloacetic acid + DPN·H

The reaction was studied in detail by Green (1936), Straub (1942 b), and Green et al (1948).. In order to demonstrate the enzyme in the worm's tissues, a modification of the manometric method of Straub (l.c.) was used. This method utilizes methylene blue as the mediator between reduced diphosphopyridine nucleotide (D.P.N.·H) and molecular oxygen. Since the equilibrium for the reaction is far to the left, cyanide is added to bind the oxaloacetate which is formed. The reaction system employed contained the following: 0.033 M phosphate buffer (pH 7.4), 0.01 M nicotinamide, .05 M sodium malate, 1.5 mg. methylene blue, 0.08 M KCN, 0.3 ml. of an isotonic KCl 20% homogenate, 1.0 mg. of D.P.N., and distilled water to a total volume of 3.0 ml. The D.P.N. was added from the side arm after a 7-minute equilibration period. The results obtained are summarized in the following table.

Reaction mixture	Expt. 1 Q_{O_2}	Expt. 2 Q_{O_2}	Expt. 3 Q_{O_2}
Complete	8.7	11.1	9.4
Minus nicotinamide	3.1	4.2	3.9
Minus malate	0	0	0
Minus methylene blue	0	0	0
Minus KCN	0.3	0.6	0.4
Minus DPN	2.6	1.9	1.6

The data clearly indicate that the tissues contain a pyridine-nucleotide-linked malic dehydrogenase. This was substantiated by a second series of experiments for which a new method of assay was developed.

Kun and Abood (1949) described a technique for succinic dehydrogenase assay which depended on the reduction by dehydrogenase activity of a soluble colorless substance, triphenyltetrazolium (TPT), to an insoluble, colored substance, formozan. This colored product is readily dissolved in an organic solvent and estimated colorimetrically. Kun and Abood (l.c.) suggested acetone as the solvent. However, it was found by the writer that acetone, being miscible with water, tended to produce cloudiness in some cases. This rendered reproducibility of experiments difficult. It was found that the use of an immiscible solvent, xylene, gave much better results. The application of this TPT reduction to a malic dehydrogenase assay was then attempted. The complete system contained the following: 0.04 M sodium malate, 0.033 M phosphate buffer, 0.1 M KCN, 3 mg. D.P.N., 0.017 M nicotinamide, 1.0 mg. T.P.T., .5 ml. of 20% KCl homogenate and distilled water to a total volume of 3.5 ml. The mixture was incubated for 30 minutes at 38° C and the reaction stopped by the addition of 0.2 ml. of 100% trichloracetic acid with shaking. The mixture was now extracted with three 2 ml. portions of redistilled xylene. The extracts from each sample were pooled and the formozan estimated colorimetrically.

Addition of known amounts of formozan to acidified homogenates and extraction with xylene showed that over 95% of the colored compound was recovered by this procedure. The results obtained are summarized in the following table.

Reaction mixture	Expt. 1	Expt. 2	Expt. 3
	µg formozan	µg formozan	µg formozan
Complete	315	294	271
Minus KCN	10*	26	29
Minus DPN	115	100	116
Minus nicotinamide	206	161	148
Minus malate	164	92	90
Minus homogenate	0	0	0

*Lowest limit of accurate estimation.

These data support the conclusion that the worm contains the malic dehydrogenase. Furthermore, the use of TPT for assay of this enzyme may be of some value to other workers.

(4) Fumarase

This enzyme is important in the "citric acid cycle" in vertebrate tissues (Green, 1949). It catalyzes the following reaction:

$$\text{Fumaric acid} + H_2O \rightleftharpoons \text{Malic acid}$$

Evidence for this reaction in a tissue preparation may be obtained by actually analyzing the material for malic and fumaric acids, or by using a preparation contining malic dehydrogenase and measuring the oxidation of the malic acid formed by the action of fumarase. In the present investigation

the latter method was used to obtain evidence for fumarase in the tissues of the worm. The T.P.T.. assay was set up exactly as described for the assay of malic dehydrogenase except that 0.05 sodium fumarate was used as the substrate. The results of the experiments are summarized in the following table.

Reaction mixture	Experiment 1 µg formozan	Experiment 2 µg formozan	Experiment 3 µg formozan
Complete	111	143	97
Minus nicotinamide	69	74	51
Minus fumarate	40	46	30
Minus KCN	20	20	20
Minus D.P.N.	52	68	31
Minus homogenate	0	0	0

The data indicate that fumarase is probably present in the tissues of the worm.

IX. The "Crowding Effect"

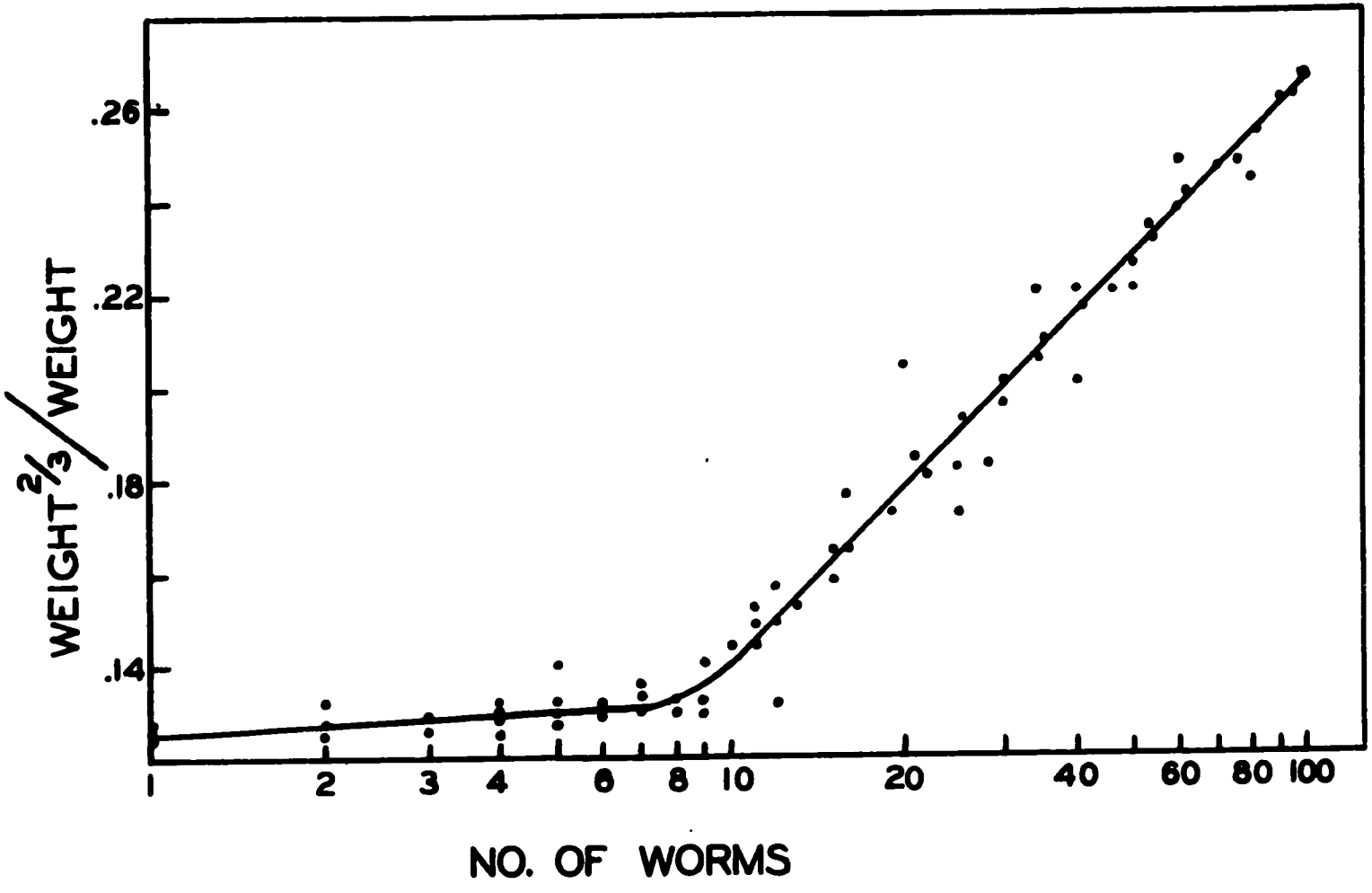
Several workers have observed that in a cestode infection the size of the worms is, roughly speaking, inversely proportional to the number of worms in the given infection. This has been called the "crowding effect". Woodland (1924), Shorb (1933), and Hunninen (1935) demonstrated this phenomenon in rats and mice infected with Hymenolepis nana. Chandler (1939) and Hager (1941) reported this effect in studies on Hymenolepis diminuta. Reid (1942) obtained similar results with Raillietina cesticillus. Reid's data may be somewhat more accurate from a quantitative standpoint since he used weight as a measure of size, whereas previous workers relied on linear measurements as criteria. Wardle and Green (1941), studying the rate of growth of Diphyllobothrium latum, used the weight-length ratio as an index of average cross sectional area. This seems to be a valid approach to problems of this type.

In connection with other studies on the metabolism of Hymenolepis diminuta the writer has gathered considerable data on the mean wet weights of worms from infections varying in size from one to one hundred worms. In selecting data for study, particular care was taken to be certain that the worms were of similar age (38 to 44 days after infection) and that the host rats were males of similar size (190 to 208 grams). The wet weights of 1694 worms were available for consideration after imposing the restrictions indicated above.

In studying the data it was obvious not only that the worms become smaller in infections with increasing numbers of worms, but with decreasing size the area per unit of weight of the individual worm rapidly becomes larger. Since the relationship Area $\propto$ Weight$^{2/3}$ or Area = (k)(Weight)$^{2/3}$ has been demonstrated in the case of other animals (Brody, 1945), it seems reasonable that for practical purposes when comparing worms of the same species we may arbitrarily assume k = 1 and obtain the relation, Area = Weight $^{2/3}$. Applying this to the data at hand we may obtain the relative area in each case, and on dividing the "area" by the original weight we derive a comparative index of the area per unit weight of worm. If the indices so obtained are graphically plotted against the logarithm of the number of worms in the respective infections, it is found that with infections with about eight or more worms, the ratio is proportional to the logarithm of the number of worms. This plot is shown in Figure 13.

Reconsideration of the data of Reid (1942) seemed desirable. Reid's mean wet weights of worms in infections with up to 150 worms were presented in graphical form. Therefore, the writer estimated the mean wet weights from Reid's graph and, applying the same calculations as were applied above, obtained the plot shown in Figure 14. It may be seen that the relationship in the Raillietina cesticillus infections studied by Reid is very similar to that found in H. diminuta infections.

Figure 13. Plot of "Relative Surface Area" of *Hymenolepis diminuta* in Infections of Varying Intensity.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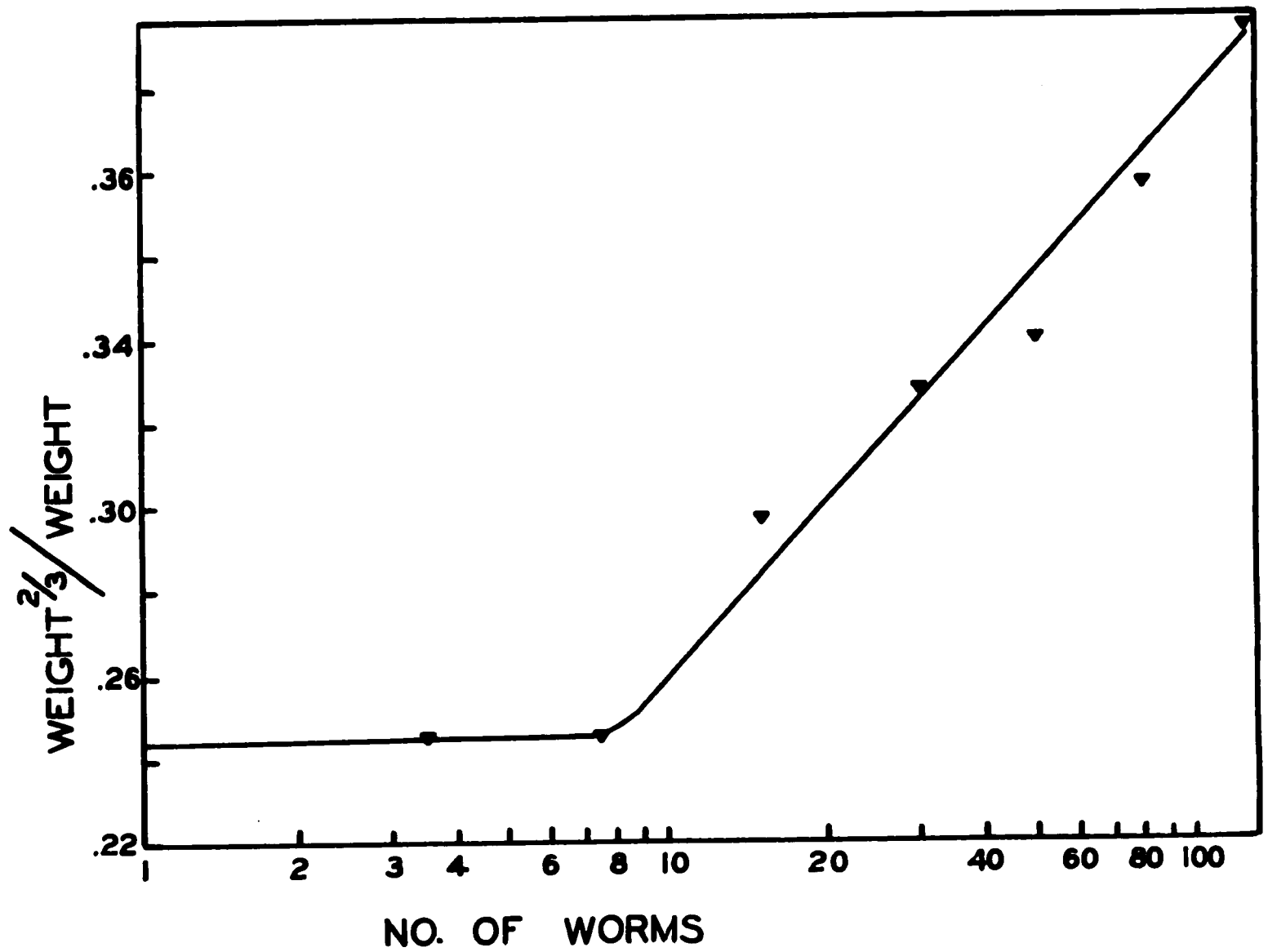
10
20
40
60
80
100
NO. OF WORMS

Figure 14. Plot of "Relative Surface Area" of Raillietina cesticillus in Infections of Varying Intensity. (Points calculated from data of Reid, 1942)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WEIGHT 2/3 / WEIGHT
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10
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40
60
80
100
NO. OF WORMS

Study of the work of Shorb (1933) with H. nana and of Chandler (1939) with H. diminuta suggested the possibility of re-evaluation of these workers' data. According to Brody (1945) the area of an animal is, in general, proportional to the square, and the weight to the cube of linear measurement. Thus, using the linear data of Shorb to calculate the relation $\frac{\text{Length}^2}{\text{Length}^3}$ and plotting each ratio against the logarithm of the number of worms in the respective infection, we obtain the plot shown in Figure 15, Curve A. While the plot so obtained is highly suggestive there is, unfortunately, a considerable gap in the data between 11 and 62 worms. Hence, it is not feasible to attempt estimation of the critical number of worms at which the Area/Weight ratio begins to increase sharply with an increase in worm burden. Applying the same method to Chandler's linear data we obtain the plot seen in Figure 15, Curve B. Again the data is valuable in showing changes in the Area/Weight ratio, but the greatest intensity of infection in Chandler's animals was 36 worms, which does not allow sufficient data for significant application in the present study.

These evaluations support the view that the crowding effect is related to the Area/Weight ratio, and suggest that some factor, required by the worms in a minimal amount per unit weight of tissue and obtained by the worms through the body surface, is present in the external environment in limited amount. As the number of worms increases, the satisfaction

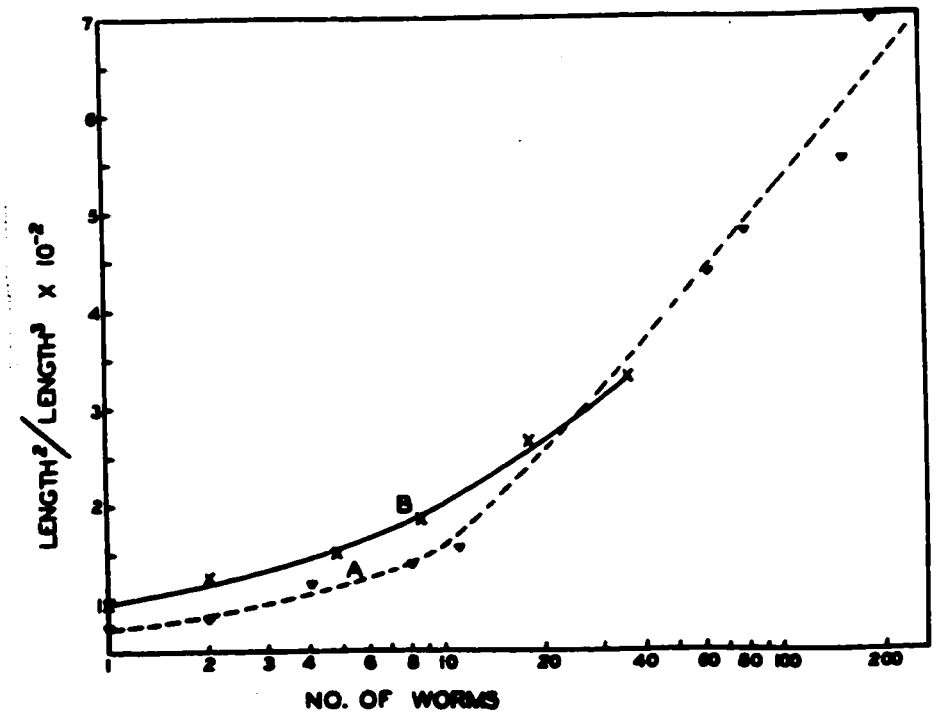

Figure 15. Plot of "Relative Area" of Hymenolepis nana (Curve A) and H. diminuta (Curve B) in Infections of Varying Intensity. Data of Curve A from Shorb (1933) and of Curve B from Chandler (1939).

of the requirement by the individual worm becomes increasingly difficult and is only possible by maintaining a relatively larger surface area per unit of weight. In other words, growth beyond a certain size may be inhibited because the reduced efficiency of a lower Area/Weight ratio may well serve as a brake on further growth. Let us consider what this necessary factor may be.

As Reid (1942) pointed out, there is little reason to believe that the stunting of the worms is due to insufficient carbohydrate in lumen of the gut. The amount of carbohydrate ingested by the host is hundreds of times the greatest requirement of an excessive number of large worms. Chandler (1943) has reported that H. diminuta is apparently independent of protein in the host diet. Smyth (1947) in his excellent review of the knowledge of cestode physiology points out that present evidence at least suggests that cestodes may have no fat requirements. Chandler and Addis (1944, 1946) showed that some heat-stable factor present in brewer's yeast and liver extracts is necessary for normal growth of H. diminuta in female or castrated male rats. The identity of the substance and the amount required by the worms has not been determined. However, Hager (1941) showed that limiting the food of her experimental rats to one-half or one-third the normal intake had no apparent effect on the egg production of H. diminuta, whereas crowding caused an inhibition of reproduction. Hager's observations take on added

significance in the present study and make it possible to state that the hypothetical limiting factor in the crowding effect is probably not a food substance obtained from the foodstuffs ingested by the host.

A substance which may fulfill the criteria suggested for the limiting factor is oxygen. A little evidence for some aerobic metabolism in vivo by H. diminuta has recently been obtained by the writer (1949), and it seems probable that a limited amount of aerobic metabolism is necessary. It should be pointed out that with an increasing number of worms there may be less contact of the individual worm with the mucosal surface of the gut, if only because of more contact with other worms. Presumably, oxygen normally present in the gut enters by diffusion from the surrounding tissues, and it seems logical to assume that it may be present in the largest amount in the region of greatest proximity to the mucosal surface. Such an hypothesis may well explain the lessened effect with 1 to 8 worms. The recent report of Rogers (1949) that oxygen tensions in the rat small intestine, near the mucosa, averaged 24 mm. of Hg furnishes definite evidence that oxygen may be present in sufficient amount in the rat gut to support some aerobic metabolism of an intestinal parasite.

X. General Discussion

The great limiting factor, time, required that this investigation be terminated at this point. Obviously, the facts are not all in. However, it seemed to the writer that enough data are available to formulate a hypothesis as to a general scheme of carbohydrate metabolism. Admittedly, some of the ideas to be presented in the following discussion are of a somewhat speculative character, but they may be capable or experimental verification and may orfer an explanation of some complicated and, at first sight, unrelated observations. In this regard, the words of G. N. Lewis (1922) have been kept in mind: "...while the sort of vague surmise which is not based upon experimental evidence nor capable of experimental test has no place in our scientific method, rational speculation must always be regarded as the advance guard of experimental science."

The main portion of a hypothetical scheme of carbohydrate metabolism in Hymenolepis diminuta has been formulated and is presented in Figure 16. The evidence to support this concept may be summarized as follows:

1. Data have been presented to show that the tissues of this worm are capable of carrying out every step, with the exception of 7, shown in Figure 16.
2. Acetic acid, lactic acid, and succinic acid have been isolated and identified as products of the anaerobic metabolism of this worm.

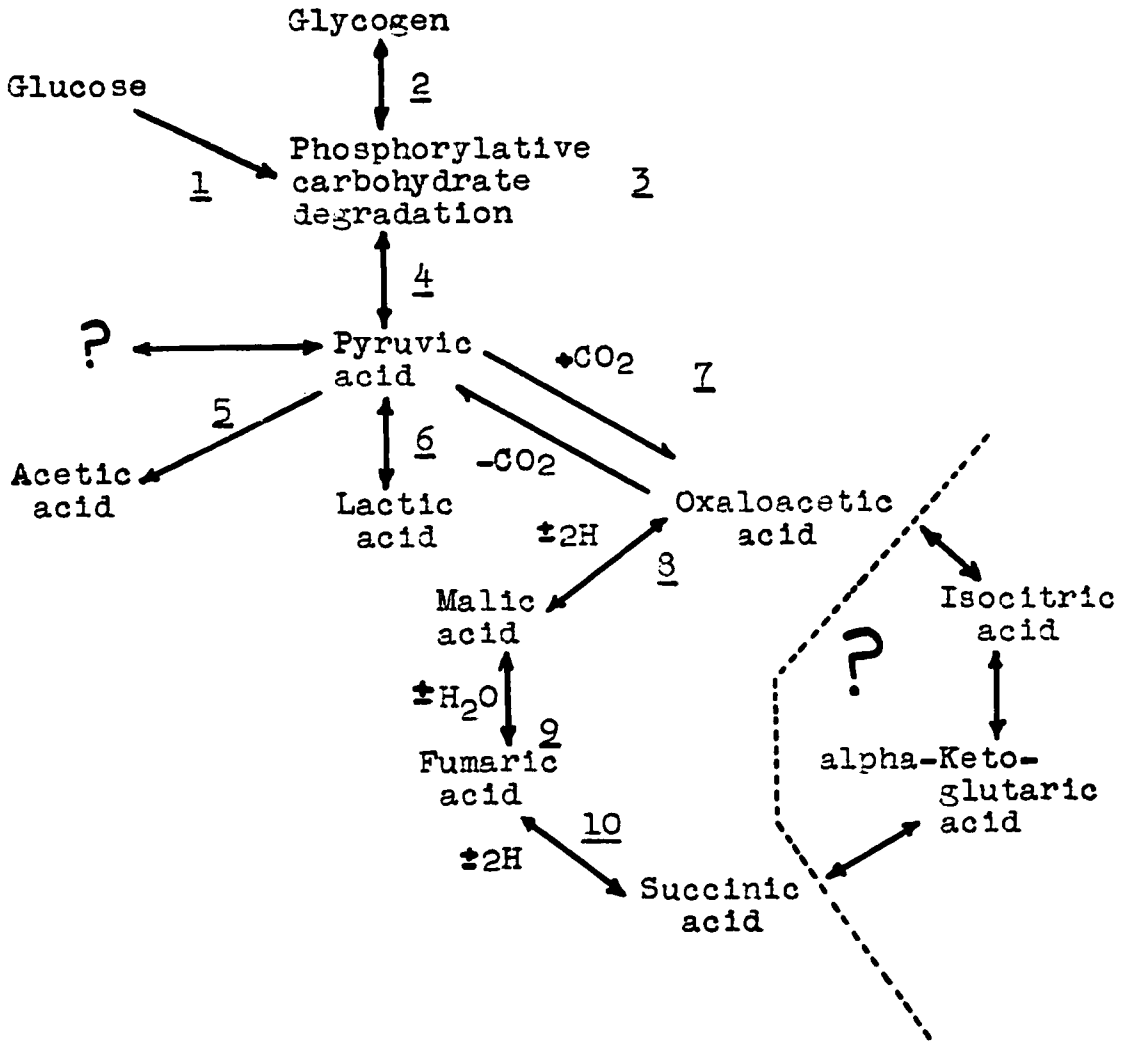

Figure 16. A Postulated Scheme of Metabolism for Hymenolepis diminuta

3. Smaller amounts of succinate are produced in phosphate buffer than in bicarbonate buffer. The writer feels that this observation furnishes strong circumstantial evidence for the occurrence of reaction 7 in the sequence of reactions leading to the formation of succinate.

The fixation of carbon dioxide postulated in reaction 7 is not an original concept. It was recognized several years ago that by fixation of carbon dioxide bacteria are able to form a number of end products, including succinate. This work has been reviewed by Werkman and Wood (1942). There is evidence that the carboxylation of pyruvic acid occurs in animal tissues. Searle and Reiner (1941) postulated a somewhat similar series of reactions to explain the production of succinate by Trypanosoma lewisi. Thomas (1942) reported that the production of succinate by the protozoan, Tetrahymena gelei, occurred through this reaction. This was corroborated by Van Niel et al (1942) who showed that the inclusion in the medium of bicarbonate labeled with radioactive carbon, C^{11}, resulted in the formation of succinate containing a high percentage of the radioactivity. The labeled carbon was in the terminal position on the succinate molecule as would be expected in carbon dioxide fixation. Utter and Wood (1946) demonstrated that the reaction occurs in mammalian liver. The anaerobic formation of succinate by the cestode, Moniezia expansa, (Brand, 1933) probably takes place by a similar mechanism. The closely allied species, M. benedeni, is known to have a well developed succinic dehydrogenase system (Van Grembergen, 1944).

It is of interest to contemplate the possible advantages to be derived by this cestode from this type of carbohydrate metabolism. As a background it is well to consider the special environmental conditions in which it lives. The pH, osmotic pressure, and oxidation-reduction potential are relatively constant. The gas phase includes a carbon dioxide tension of about 100 mm. Hg and an oxygen tension of 16 to 20 mm. (Read, 1950b).

With these gas tensions in the external environment, what would be the oxygen and carbon dioxide tensions in the tissues of the worm? In a recent study of the respiratory metabolism of small nematodes, Rogers (1949b) calculated that an oxygen tension of 16 mm. would result in a zero tension in the central tissues of Nippostrongylus muris, a parasite of the small intestine of the rat. Since this nematode is not as thick an animal as H. diminuta, it seems highly probable that the oxygen tensions in the central tissue of this cestode are probably zero under the conditions prevailing in the intestine of the rat. This is further supported by the fact that the tapeworm can only obtain oxygen through the external surface. Small nemas, on the other hand, may obtain a portion of their oxygen through the gut and may possess an oxygen transport system of sorts (Rogers, 1949b). Cestodes may thus be considered unique in that there is a regularly decreasing oxygen tension, progressing toward the central tissues.

Are there any morphological peculiarities of cestodes

which may be correlated with this oxygen gradient? Yes! It is striking to note that the central tissues of tapeworms consist of the reproductive organs and supporting parenchymatous tissue; the nervous system, musculature, and the absorptive surface are located in the external regions. The structures in the central tissues are primarily associated with the physiological functions of growth and differentiation while those structures in the outer part of the animal are associated not only with these functions, but also with work processes, such as muscle action and active transfer (absorption). These latter activities call for a higher level of generation of phosphate-bond energy than is required for growth and differentiation (Brody, 1945).

The generation of phosphate-bond energy for the most part accompanies the end oxidations of carbohydrate metabolism. In the breakdown of a molecule of glucose to lactic acid there is a net gain of two high-energy phosphate groups; on the other hand, in the oxidative reactions from succinate to oxaloacetate (Figure 16) five high-energy phosphate bonds are generated. In the operation of the complete "citric acid cycle" twenty-eight high-energy bonds are generated from the oxidation of the products of a single glucose molecule (Lipmann, 1946; Green, 1948).

With the foregoing discussion in mind, an attempt may be made to derive a hypothetical metabolic picture. It may be visualized that the anaerobic metabolism predominating in

the internal tissues of the worm results in the production of succinate. This readily diffusible substance passes into the outer tissues where it may be oxidized to furnish the energy for the work functions. In short, it is postulated that the reaction, succinate ⟶ oxaloacetate, is effectively operating in one part of the animal and that the reverse reaction, oxaloacetate ⟶ succinate, is the net reaction operating in the inner tissues of the animal. If such a metabolic mechanism is operating in the tissues of this and perhaps other tapeworms, it presents an interesting biochemical adaptation in an animal of singular morphology to an environment possessing peculiar physical and chemical characteristics.

The need for further study of the physiology and metabolism of this cestode is indicated. The mechanism of reactions resulting in the production of acetic acid requires elucidation. The data suggestthat the worm may utilize acetate under aerobic conditions. The ability of the worm to oxidize this and other fatty acids invites investigation. The carbon dioxide fixation resulting in succinate production may be rather easily verified by the use of isotopically-labeled bicarbonate. The nature and operation of the respiratory enzymes needs further attention. Evidence that the cytochrome system may operate in the worm has been adduced in the present study, but no data are available as yet on the actual amounts of biologically active cytochrome C to be found in the tissues of the animal. The possibility of flavin electron transport

systems linked directly with molecular oxygen or other environmental electron acceptors should bear investigation.

As with most other animals, nothing is known of the protein metabolism of this animal. The deaminase and transaminase activities of the tissues are unknown. Possible linkage of amino acids to carbohydrate metabolism should be investigated.

Of all cestodes this one probably is the most convenient for laboratory study. It is readily maintained in albino rats, and is relatively long-lived. The writer is convinced that a great wealth of fundamental knowledge may be attained by a comprehensive study of the physiology of this tapeworm.

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