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Studies on metabolism of lung flukes genus *Paragonimus*

II. Paper chromatography of sugars and hexose phosphates in uterine eggs, larvae and adults

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Sugars and hexose phosphates are substances associated with the Embden-Meyerhof pathway. Detection of the sugars and hexose phosphates in lung flukes of the genus *Paragonimus* is important in biochemical studies on carbohydrate metabolism of these organisms.

Detection of sugars and hexose phosphates in some helminths has been studied by von Brand and Otto (1938), Odlaug (1955), Vernberg and Hunter (1956), von Brand (1957), Agosin et al. (1957), Fairbairn and Passey (1957), Goil (1957), Fairbairn (1958 a, b), Laurie (1959), Mansour (1959), Ueno (1960), etc. However, studies on the detection of sugars and hexose phosphates in the lung flukes are scanty. Therefore, the present study was carried out to detect sugars and hexose phosphates in uterine eggs, larvae and adults of these parasites.

Materials and Methods

Preparation of Samples:

Uterine eggs, mature rediae and cercariae of *Paragonimus westermani* (Kerbert, 1878), metacercariae of *Paragonimus miyazakii* Kamo, Nishida, Hatsushika et Tomimura, 1961 and adults of *P. westermani* were harvested and their homogenates were prepared as previously described (Hamajima, 1966).

Analyses of Sugar and Hexose Phosphate:

For these studies, extraction of sugar and hexose phosphate was carried out by the same procedure that was used for the amino acids and aminosugar (Hamajima, 1966). Using a standardized micropipet, a 10 to 50 μl-portion was spotted on Toyo Roshi No. 50 filter paper for one-dimensional chromatographic separation. The filter paper was washed with 2 N acetic acid in the case of analysis of hexose phosphates. Solvents used for analysis of sugars were a mixture of acetone, n-butanol and water (7 : 5 : 2, v/v); phenol saturated with water : and collidine saturated with water. Chromatography was carried out at room temperature. The presence of sugars was detected by using iodine; 0.01 M potassium periodate and 3.5 % sodium borate, the latter solution included 0.8 % potassium iodide, 0.9 % boric acid and 3 % soluble starch; anilinhydrogenphthalate; resorcinol; and ammoniacal silver nitrate as indicators. In addition to the above analyses, glycogen was detected by the method of Carroll et al. (1956). Solvents used for analysis of hexose phosphates were a mixture of acetone and 35 % formic acid (3 : 2, v/v); acetone and 35 % trichloroacetic acid (TCA) (3 : 2, v/v); and methanol and 2 N ammonia (7 : 3, v/v). The presence of hexose phosphates was detected by using the method of Hanes and Isherwood (1949).

Results

Table 1 shows sugars found in the uterine eggs, larvae and adults. By paper chromatography of sugar in the uterine eggs, one

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positive spot was yielded by iodic reaction and nonreducing sugar reaction. Four positive spots were produced by aniline phthalic acid reaction ; two positive spots by ketose reaction ; and two positive and two faint spots by silver reaction. Thus, five spots corresponded in Rf value to glycogen, two polysaccharides or oligosaccharides, glucosamine and galactose were detected with the authentic samples (Table 1). In the case of the adults, one positive spot was yielded by iodic reaction and nonreducing sugar reaction. Nine positive spots were yielded by aniline phthalic acid reaction ; three positive spots by ketose reaction ; and six positive and four faint spots by silver reaction. The eleven spots corresponded in Rf value to glycogen, two polysaccharides or oligosaccharides, lactose, maltose, glucosamine, galactose, glucose, fructose, an unknown sugar and fucose were detected with the authentic samples (Table 1). In the rediae and cercariae, and metacercariae, iodic reaction and nonreducing sugar reaction showed one positive spot in both the cases of the rediae and cercariae, and metacercariae, respectively. The aniline phthalic acid reaction produced five positive spots in the case of the rediae and cercariae and six in the case of the metacercariae. The ketose reaction yielded three positive spots in both the cases of the rediae and cercariae, and metacercariae, respectively. And the silver reaction produced four or five positive and two faint spots in the rediae and cercariae, and in the metacercariae. The seven spots corresponded in Rf value to glycogen, two polysaccharides or oligosaccharides, glucosamine, galactose, glucose and fructose were detected in both the rediae and cercariae, and metacercariae with the authentic samples. There also was one spot of an unknown sugar in the case of the metacercariae. On the other hand, reaction for glycogen by Anthrone reagent was positive in the samples of the uterine eggs, metacercariae and adults.

Hexose phosphates and an unknown phosphoric compound found in the uterine eggs, larvae and adults are given in Table 2. By paper chromatography five spots corresponded in Rf value to glucose-1-phosphates, glucose-6-phosphates, fructose-6-phosphates, fructose-1, 6-diphosphates and to an unknown phosphoric compound (spot 5) were detected in the uterine eggs, rediae and cercariae, metacercariae and adults.

Table 1. Sugars in *Paragonimus westermani* uterine eggs, *P. westermani* rediae and cercariae, *Paragonimus miyazakii* metacercariae and *P. westermani* adults

Spot	Sugar	Acetone 7 n-Butanol 5 Water 2					Phenol saturated with water				Collidine saturated with water			
		Rf value	UE	RC	MC	A	Rf value	UE	MC	A	Rf value	UE	MC	A
1	Glycogen	0.00	+	+	+	+	0.00	+	+	+	0.00	+	+	+
2	Unknown	0.01	+	+	+	+	0.09	+	+	+	0.08	+	+	+
3	Unknown	0.03	+	+	+	+	0.25	+	+	+	0.34	+	+	+
4	Lactose	0.06	−	−	−	+	0.37	−	−	+	0.39	−	−	+
5	Maltose	0.09	−	−	−	+	0.34	−	−	+	0.49	−	−	+
6	Glucosamine	0.16	+	+	+	+	0.13	+	+	+	0.46	+	+	+
7	Galactose	0.20	+	+	+	+	0.40	+	+	+	0.48	+	+	+
8	Glucose	0.23	−	+	+	+	0.35	−	+	+	0.52	−	+	+
9	Fructose	0.30	−	+	+	+	0.47	−	+	+	0.54	−	+	+
10	Unknown	0.35	−	−	+	+	0.45	−	+	+	0.59	−	+	+
11	Fucose	0.40	−	−	−	+	0.61	−	−	+	0.55	−	−	+

UE=Uterine eggs. RC=Rediae and Cercariae. MC=Metacercariae.
A=Adults. +=Positive. −=Negative.

Table 2. Hexose phosphates in *Paragonimus westermani* uterine eggs, *P. westermani* rediae and cercariae, *Paragonimus miyazakii* metacercariae and *P. westermani* adults

Spot	Hexose phosphate	Acetone 3 / 35 % TCA 2				Acetone 3 / 35 % Formic acid 2			Methanol 7 / 2 *N* Ammonia 3				
		Rf value	UE	MC	A	Rf value	UE	A	Rf value	UE	RC	MC	A
1	Glucose-1-phosphate	0.60	+	+	+	0.51	+	+	0.70	+	+	+	+
2	Glucose-6-phosphate	0.65	+	+	+	0.53	+	+	0.53	+	+	+	+
3	Fructose-6-phosphate	0.70	+	+	+	0.57	+	+	0.64	+	+	+	+
4	Fructose-1, 6-diphosphate	0.71	+	+	+	0.55	+	+	0.33	+	+	+	+
5	Unknown	0.77	+	+	+	0.68	+	+	0.40	+	+	+	+

Abbreviations are the same as in Table 1.

Discussion

It is well known that parasitic helminths are characterized by glycogen content (Bueding, 1949 ; von Brand, 1950, 1960). Similarly, in the lung flukes, the author has reported that glycogen was detected in the uterine eggs, larvae and adults as shown in Table 1 (Hamajima, 1964, 1965). In histochemical studies on the adult flukes *Paragonimus ohirai* Miyazaki, 1939 and *P. westermani*, glycogen has been demonstrated (Yokogawa and Yoshimura, 1957 ; Yoshimura and Yokogawa, 1958 ; Ro et al., 1963 ; Kang et al., 1963). In addition, periodic acid-Schiff reaction has been observed histochemically in cyst walls of the metacercariae of *P. miyazakii* (Kamo et al., 1963). Furthermore, glycogen has been found in various species as shown in Table 3. Polysaccharides except glycogen have been demonstrated histochemically in the adults of *P. westermani* by Yoshimura and Yokogawa (1958). In the present study, two spots (spots 2 and 3) did not correspond to monosaccharide or disaccharide sites. Moreover, Agosin et al. (1957) have reported that scolices of *Echinococcus granulosus* were shown to contain polysaccharide containing galactose and glucosamine. In the present study, traces of lactose and maltose were found in the adult of the lung flukes. However, trehalose as found in unembryonated eggs of *Ascaris lumbricoides* by Fairbairn and Passey (1957), larvae of *Porrocaecum decipiens* by Fairbairn (1958 a, b), adults of *A. lumbricoides* by Fairbairn and Passey (1957), and *Ascaris suum* by Feist et al. (1965) and *Moniliformis dubius* by Laurie (1959) was not found in the uterine eggs, larvae and adults of the lung flukes. Relatively little trehalose has been found in *Fasciola hepatica* (von Brand, 1960). In the present study, aminosugar has been demonstrated in the uterine eggs, larvae and adults of the lung flukes as previously described (Hamajima, 1966). Kreuzer (1953) has demonstrated the presence of glucosamine and N-acetylglucosamine in acid hydrolysates of the chitinous membrane of *A. lumbricoides* eggs. Ogimoto (1956) has reported that a spot corresponding to spot yielded by glucose was detected paper-chromatographically in the adult fluke of *P. ohirai*. Fairbairn (1958 a, b) found glucose as a constituent of *P. decipiens* larvae and Fairbairn and Passey (1957) found a spot corresponding to Rf of glucose in tissues of *A. lumbricoides*, but not in unembryonated eggs. These facts are very similar to those revealed in studies on the uterine eggs and adults of the lung flukes in the author's data. Furthermore, Ando (1957) found glucose in larval and adult worms of *Gnathostoma spinigerum*.

Ueno (1960) found glucose-6-phosphate, fructose-6-phosphate and other acid-soluble phosphoric compounds in muscles of *A. lumbricoides* var. *suum*. In the present study of the lung flukes, glucose-1-phosphate, glucose-6-phosphate, fructose- 6-phosphate and fructose-1,6-diphos-

Table 3. Parasitic helminths that possess glycogen

Species	Material	Glycogen	Author
Trematoda			
Schistosoma japonicum	uterine eggs & miracidia	+	Axmann ('47)
Schistosoma mansoni		+	
Gorgodera amplicava	sporocysts	+	Cheng ('63a)
Echinoparyphium sp.	ceacariae	+	Cheng ('63b)
Glypthelmins pennsylvaniensis		+	Snyder & Cheng ('61) ; Cheng & Snyder ('62)
Gorgodera amplicava		+	Cheng ('63a)
Schistosoma japonicum		+	Axmann ('47)
Schistosoma mansoni		+	
Clinostomum attenuatum	metacercariae	+	Axmann ('47) ; Odlaug ('55)
Gynaecotyla adunca		+	Vernberg & Hunter ('56)
Allassostoma magnum	adults	+	
Fasciola hepatica		+	
Fascioloides magna		+	
Gorgodera amplicava		+	
Gorgoderina attenuata		+	Axmann ('47) ; Odlaug ('55) ; Mansour ('59)
Haematoloechus complexus		+	
Haematoloechus medioplexus		+	
Polystomoidella oblongum		+	
Schistosoma japonicum		+	
Schistosoma mansoni		+	
Haplometra cylindracea		+	Dawes & Muller ('57)
Gastrothylax crumenifer		+	Goil ('57)
Paramphistomum explanatum		+	
Cestoda			
Echinococcus granulosus	scolices	+	Agosin et al. ('57)
Diphyllobothrium sp.	plerocercoids	+	Archer & Hopkins ('58)
Hymenolepis diminuta	adults	+	Read ('56)
Nematoda			
Ascaris lumbricoides	unembryonated eggs	+	Fairbairn & Passey ('57)
Ancylostoma caninum	larvae	+	Sato ('58)
Porrocaecum decipiens		+	Fairbairn ('58a b)
Ancylostoma caninum	adults	+	von Brand & Otto ('38) ; Fernando & Wong ('64)
Ascaris lumbricoides		+	Fairbairn & Passey ('57)
Dioctophyme renale		+	von Brand ('57)
Heterakis gallinae		+	Glocklin & Fairbairn ('52)
Nippostrongylus brasiliensis		+	Roberts & Fairbairn ('65)
Acanthocephala			
Macracanthorhynchus hirudinaceus	adult females	+	Ward ('52)
Moniliformis dubius	adult males & females	+	Read & Rothman ('58) ; Laurie ('59) ; Graff ('64)

phate were detected in the uterine eggs, larvae and adults. Glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate and fructose-1, 6-diphosphate are intermediate metabolites. Consequently, it was suggested that such hexose phosphates have demonstrated the presence of the Embden-Meyerhof pathway in the uterine eggs, larvae and adults.

Summary

The sugars and hexose phosphates in the uterine eggs, larvae and adults of the lung flukes of the genus *Paragonimus* were detected by one-dimensional paper chromatography. Glycogen, two oligosaccharides or polysaccharides, glucosamine and galactose were detected in the uterine eggs, larvae and adults. In addition glucose, fructose and an unknown sugar were found in the larvae and adults. Additionally, lactose, maltose and fucose were isolated in the adults. Glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1, 6-diphosphate and an unknown phosphoric compound were detected in the uterine eggs, rediae and cercariae, metacercariae, and adults.

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肺吸虫の代謝に関する研究
II. 子宮卵，幼虫，成虫における糖およびヘキソース燐酸のペーパークロマトグラフ

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一次元ペーパークロマトグラフによつて，子宮卵，幼虫，成虫における糖およびヘキソース燐酸の検出をおこなつた．その結果，グリコーゲン，2 種のオリゴ糖またはグリコーゲン以外の多糖類，グルコースアミンおよびガラクトースが検出された．其他，グルコース，フラクトースおよび1種の不明糖が幼虫および成虫より検出され，さらにラクトース，マルトースおよびフコースが成虫より検出された．一方，ヘキソース燐酸としてグルコース-1-燐酸，グルコース-6-燐酸，フラクトース-6-燐酸，フラクトース-1，6-ジ燐酸および不明燐化合物がそれぞれの試料から検出された．