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BIOCHEMICAL CHARACTERIZATION OF HUMAN SCHISTOSOMES AND THEIR MOLLUSCAN HOSTS BY ELECTROPHORETIC TECHNIQUES

BY

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التمييز البيوكيميائي للشستوسوما الآدمية وعائلها من الرخويات باستخدام تقنيات الهجرة الكهربية

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أدى التطبيق الحديث للطرق البيوكيميائية في علم التصنيف إلى تحسُّن واضح في تحديد الأنواع وتمييز السلالات في مختلف الكائنات الحية ، وبدأت هذه الاساليب تحل تدريجيا محل المعايير التقليدية لخصائص التشريح والبنيان والمستخدمة عادة لتعريف الطفيليات الحيوانية وبخاصة تلك ذات الاهمية الطبية أو البيطرية . ويندرج ضمن هذه الطرق تقنيات الهجرة الكهربية والتركز عند نقطة التعادل الأيوني ، والتي أثبتت كفايتها الفائقة في هذا المجال .

ويتضمن البحث دراسات تفصيلية لأنماط الهجرة الكهربية أو التركز عند نقطة التعادل الايوني للبروتينات الذائبة وإنزيمات محللة للاسترات والانزيم الحمضي المحلل للفوسفات وانزيم ناقل للاسبارتات ونازع الهيدروجين من فوسفات الجلسرين ، وذلك في بعض عشائر القواقع الناقلة للطفيلي وكذلك في بعض سلالات طفيلي الشستوسوما .

وقد لوحظت بعض الاختلافات البينية ذات الدلالة لبعض عشائر القواقع وكذلك بعض سلالات الطفيلي ، مؤكدة أهمية التعرف البيوكيميائي على عشائر القواقع وسلالات الطفيلي وقد تمت مناقشة العلاقة بين تلك الاختلافات البيوكيميائية والفروق المشاهدة في أنماط قابلية العشائر المختلفة من القواقع للعدوى بسلالات الشستوسوما الآدمية التي تضمنتها الدراسة .

Key Words: Human Schistosomes, Molluscan hosts, Electrophoresis, Iso-Electric focusing, Esterases, Acid phosphatase, Soluble proteins.

ABSTRACT

The recent application of biochemical techniques in taxonomy markedly improved the specific identification and strain characterization of a wide range of living organisms. These techniques are gradually replacing classical anatomical and morphological indices commonly used for characterization of animal parasites particularly those of medical and veterinary importance. Amongest these biochemical techniques, both electrophoresis and iso-electric focusing proved to be most useful and reliable tools in this respect. The present investigation includes a detailed study of the electrophoretic and/or the iso-electric focusing patterns of soluble proteins, non specific esterases, acid phosphatases, aspartate amino transferase (AST), glutamate - oxaloacetate transaminase (GOT) and α-glycerophosphate dehydrogenase in some snail populations as well as in some strains of schistosomes. Certain inter-population and inter-strain variations are observed in the patterns of these proteins and enzymes proving the importance of the biochemical characterization of populations of snails and strains of schistosomes. The significant correlation between these observed biochemical variations and the differences observed in the susceptibility patterns of different populations of snails to infection with human schistosomes has been also attempted.

INTRODUCTION

The past three decades have witnessed great interest in the identification and characterization of strains of parasites and their vectors or intermediate hosts. This is particularly evident in the case of human schistosomes and their intermediate hosts where these infraspecific variations directly affect the epidemiology of the disease and thus influencing effective control [1]. Distinct infraspecific variations in several biological and pathological characteristics (infectivity, prepatent period, egg distribution into tissues, pathogenicity and virulence) of human schistosomes in the definitive host have been documented for two main species of human schistosomes: *S. japonicum* and *S. mansoni* [2-4]. Substantial evidence also accumulated on the infraspecific differences between strains of schistosomes in their intermediate snail hosts [5, 6].

In view of the great difficulties encountered in determining the morphological, anatomical and other biological characteristics of parasite strains and populations of snail intermediate hosts, scientists appealed for more research, to develop reliable methods for identifying these strains and populations [7].

The intrinsic infraspecific identification and characterization of schistosomes received widespread attention. The pioneering electrophoretic studies of Wright *et al.* [8. 9] and Fletcher *et al.* [10] have contributed greatly to our understanding of infraspecific variations in schistosomes and their intermediate hosts. These studies are also complemented by the recent application of DNA analytical procedures which extend our knowledge of the genetic diversity of schistosomes [11, 12].

Recently, the proplem of genetic variability became more complicated by demonstrating that isolates of parasites and intermediate hosts from the same geographical region might differ in their response to chemotherapy [12] and their infectivity to snail intermediate hosts [14, 15]. Accordingly, the present work was initiated in an attempt to complement these observations by extending modern electrophoretic studies to identify the range of variability of both schistosomes and their intermediate hosts in Egypt. In the present work the range of variability in the electrophoretic and isoelectric focusing patterns of proteins and certain enzymes in some populations of both *Biomphalaria* and *Bulinus* (the intemediate hosts of *S. mansoni* and *S. haematobium* respectively) as well as certain strains of human Egyptian schistosomes are studied. In the meantime, possible correlation between these patterns with the observed differences in the infectivity of the parasite strains and the snail intermediate hosts is attempted.

MATERIAL AND METHODS

The snail populations of *Bulinus truncatus* used in this study were collected from some areas in Egypt and from one locality in Sudan. The populations of wild snails used were collected from Alexandria, Zagazig, Qanater, Abo Rawwash, Giza, Menia, Quena, Luxor, Aswan in Egypt and Gezira in Sudan.

Two strains of *Schistosoma haematobium* are used for the susceptibility studies of the snails. These are Abo-Rawash and Qena strains. The strains were maintained in the laboratory in the local strain of *B. truncatus* and golden hamsters as intermediate and definitive hosts respectively.

Three species of *Biomphalaria* were used in this work: *Biomphalaria alexandrina, Biomphalaria glabrota* and *Biomphalaria pfeifferi*. Wild species of *Biomphalaria alexandrina* were collected from the following sites in Egypt; Alexandria, Zagazig, Al Marg, Suez, Aswan and Abo Rawwash. A laboratory colony of *Biomphalaria glabrota* were raised from a stock originally obtained from the Center of Tropical Diseases, University of Lowell, Lowell, Mass, U.S.A. A laboratory colony of *Biomphalaria pfeifferi* was raised from a stock originally obtained from an irrigation canal at Al Gezira in Sudan.

Two strains of *Schistosoma mansoni* were used one from Egypt and the other one from Puerto Rico. The Egyptian Strain was isolated from stool samples of young untreated parasitologically positive cases, living in a small village, Abbis, near Alexandria. Eggs of the Puerto Rico Strain were obtained from a hamster infected with cercariae shed from infected snails mailed to Cairo from the Center of Tropical Diseases, University of Lowell, Lowell, Mass, U.S.A. Both strains were subsequently maintained at Ain Shams Laboratory for Snail and Parasite Biology using the respective snails and golden hamsters as intermediate and defenitive hosts respectively.

Two weeks old snails grown on the blue green algae, *Nostoc muscorum* [16] were used for the susceptibility studies or maintenance of the parapite strains. The snails were examined after 5 weeks post - exposure for the presence of infections. For this purpose snails were covered with black cloth sixteen hours before being tested for shedding. The snails were put in 20 ml conditioned water then placed under a fluorescent light for about two hours. The water was then checked for cercariae.

Starch Gel electrophoresis was performed on the snails according to the method described by Jelnes [17]. Cellogel electrophoresis was performed according to the method of Chemetron [18]. The staining of the protein bands and the iso-enzymes was performed according to Jelnes [17]. The technique of isoelectric focusing used was that according to Righetti [19].

RESULTS AND DISCUSSION

The results of the present work (Tables 1-4) indicate that although, mostly, there is a characteristic electrophoretic pattern for proteins and enzymes common to all individual snails from various species and populations, there are always differences sufficiently marked and consistent to distinguish one species and one population from others. The results of the isoelectric focusing studies confirm this in snails as well as in certain strains of *Schistosoma mansoni* (Tables 5-8). Accordingly these interspecies and population differences may be thus of great importance in characterizing various species and populations.

The results are also highly suggestive of the presence of a correlation between the number and distribution of the anodic migrating electrophoretic bands of total soluble proteins of *Biomphalaria* snails and their respective susceptibility to infection with *S. mansoni*. *Biomphalaria globrata* (P1) and *Biomphalaria alexandrina* from Alexandria (P3) which showed the least susceptibility to infection with *S. mansoni* showed

Table 1

Comparison of Survival and Susceptibility of Snail Population of *Bulinus truncatus* Infected with Abo Rawwash and Quena Strains of *S. haematobium*

Parasite Strains	Abo Rawwash Strain					Quena Strain				
Snail Populations	No. Exposed	Survivals No.	%	Infected No.	%	No. Exposeed	Survivlas No.	%	Infected No.	%
1- Alexandria	150	110	73.3	42	38.18	75	58	77.33	35	60.35
2- Zagazig	200	158	79.00	57	36.08	100	77	77.00	46	59.74
3- Qanater	85	43	50.59	22	51.16	85	39	45.88	24	61.54
4- Abo Rawwash	200	138	69	55	39.88	93	63	67.74	29	46.03
5- Giza	103	73	70.87	32	43.84	75	58	77.33	24	41.38
6- Menia	73	40	54.79	21	52.5	100	78	78.00	38	48.72
7- Quena	113	91	80.53	48	52.74	75	38	50.67	26	68.42
8- Luxor	100	64	64.00	23	35.94	47	25	53.19	9	36.00
9- Aswan	150	113	75.33	33	29.20	100	72	72.00	35	48.61
10- Sudan (Gezira)	100	70	70.00	25	35.71	100	62	62.00	35	56.65
Total	1274	900	70.64	358	39.78	850	570	67.06	301	52.81

Table 2

Densitometry % of Anodic Migrating Total Soluble Body Proteins and Enzymes in *Bulinus* Snail Populations

Snail Population	Protein Fractions %						Non Specific Esterase Fractions %			Glutamic Oxaloacetic Transminase Fractions (GOT) %		α-Glycerophosphate Dehydrogenase Fractions %		
	I	II	III	IV	V	VI	I	II	III	I	II	I	II	III
P1	72.90	22.91	1.82	2.34	-	-	18.01	71.5	10.46	50.00	49.82	-	-	-
P2	76.91	9.56	3.37	2.94	2.98	3.05	28.74	71.27	-	-	-	-	77.82	22.17
P3	72.05	23.34	2.25	2.34	-	-	15.02	49.13	34.21	39.95	6.04	75.37	10.00	14.03
P5	74.67	10.97	2.95	3.59	1.98	5.8	36.04	60.88	2.85	37.88	62.00	-	89.03	10.47
P6	70.02	13.83	3.77	5.86	6.49	-	21.91	65.00	13.07	31.25	68.55	-	77.37	22.04
P7	68.59	25.00	0.84	1.44	4.11	-	73.35	20.31	5.28	-	-	-	-	-
P8	-	-	-	-	-	-	-	-	-	-	-	49.14	31.60	18.9
P9	73.44	20.05	6.49	-	-	-	59.40	23.42	17.15	40.56	59.04	77.36	13.25	9.39
P10	59.43	3.72	8.28	8.40	20.06	-	-	-	-	-	-	-	-	-

P1 : *B. truncatus* from Alexandria
P2 : *B. truncatus* from Zagazig
P3 : *B. truncatus* from Abo Rawwash
P5 : *B. truncatus* from Luxor
P6 : *B. truncatus* from Aswan
P7 : *B. truncatus* from The Sudan
P8 : *B. truncatus* from Quena
P9 : *B. tropicus* from Zimbabwe
P10 : Control - Human Serum

Table 3

Comparison of Survival and Susceptibility of Snail Populations of *Boiomphalaria alexandrina*, Biomphalaria glabratus and *Biomphalaria pfeifferi* infected with an Egyptian Strain from Alexandria and a Puerto Rican Strain of *Schistosoma mansoni*

Snails		Parasite Strains									
		Alexandria Strain					Puerto Rico Strain				
Species	Populations	No. Exposed	Survivals		Infected		No. Exposed	Survivals		Infected	
			No.	%	No.	%		No.	%	No.	%
Biomphalaria alexandrina	Alexandria	55	28	51.1%	8	27.86%	50	35	70%	0	0
	Zagazig	110	83	73.96%	47	55.86%	50	43	86%	0	0
	Al Marg	40	29	72.5%	16	55.04%	0	0	0	0	0
	Suez	125	105	84.6%	52	50.79%	50	44	88%	0	0
	Aswan	45	35	77%	18	52.37%	0	0	0	0	0
Total		375	280	71.83%	141	48.38%	150	122	81.33%	0	0
Biomphalaria glabrata	Puerto Rico	150	140	93.33%	21	14.99%	135	124	92.35%	109	88.08%
Biomphalaria pfeifferi	Sudan	95	47	47.66%	14	31.87%	0	0	0	0	0

Table 4

Densitometry of Soluble Body Proteins and Enzymes in *Biomphalaria* Snail Populations

Snail Populations	Anodic Migrating Protein Fraction %						Cathodic Migrating Protein Fraction %			Non Specific Esterase % Anodic Migrating				Cathodic Migrating	a-Glycerophosphate Dehydrogenase			Enzyme Fractions % (GOT) Glutamic Oxoloacetic Transaminase			
																		Anodic Migrating		Cathodic Migrating	
	I	II	III	IV	V	VI	I	II	III	I	II	III	IV	I	I	II	III	I	II	I	II
P1	7.76	88.48	3.72	-	-	-	0.6	99.39	-	1.78	13.82	17.49	58.6	8.26	29.92	51.43	18.63	23.00	34.83	33.44	8.67
P2	1.88	18.07	68.25	3.91	7.84	-	13.49	86.50	-	10.23	2.23	10.98	57.2	10.19	1.14	89.79	9.02	47.01	18.66	33.50	-
P3	5.01	80.28	14.70	-	-	-	21.37	32.38	46.23	-	1.54	31.04	60.47	6.92	3.93	84.25	11.71	46.92	24.37	28.61	-
P4	5.31	7.77	7.42	18.7	31.34	29.07	10.63	55.67	33.64	-	-	29.54	64.19	6.26	27.67	70.28	2.03	47.97	18.44	33.55	-

P1 = *Biomphalaria glabrata* from Puertrico
P2 = *Biomphalaria alexandrina* from Zagazig
P3 = *Biomphalaria alexandrina* from Alexandria
P4 = *Biomphalaria alexandrina* from Suez

Table 5
Comparison Between The Survival and Susceptibility of Snail Populations of *Biomphalaria alexandrina* Infected with Qualuobiya, Zagazig and Warrak Strains of *Schistosoma mansoni*

Snail Populations	Strains of *S. mansoni*														
	Qualuobiya Strain					Zagazig Strain					Warrak Strain				
	No. exposed	Survivals		Infected		No. exposed	Survivals		Infected		No. exposed	Survivals		Infected	
		No.	%	No.	%		No.	%	No.	%		No.	%	No.	%
Alexandria	250	244	97.60	32	13.11	125	119	95.20	11	9.24	125	112	89.60	6	5.35
Zagazig	250	229	91.60	119	51.97	125	90	72.00	24	26.66	125	98	78.40	52	53.06
Abo-Rawwash	250	196	78.40	76	38.77	125	98	78.40	46	46.93	125	84	67.20	43	51.19
Suez	250	244	97.60	132	54.10	125	89	71.20	51	57.30	125	98	78.40	72	73.46
Menia	250	247	98.80	216	87.45	125	78	62.40	51	65.38	125	85	68.00	63	73.11
Total	1250	1160	92.80	575	49.56	625	474	75.84	183	38.60	625	477	76.32	236	49.47

Table 6a
Number of Bands Obtained by Iso-electric Focusing of Esterases Extracted from Various Populations of Snails at pH 3.5 - 9.5

pH	(I) Whole Snail					(II) Digestive Gland					(III) Head Foot Organ				
	P1	P2	P3	P4	P5	P1	P2	P3	P4	P5	P1	P2	P3	P4	P5
4.5-5.5	2	3	3	3	3	3	3	3	3	3	1	1	1	1	-
3.5-5.5															
5.5-6.5	6	6	6	6	6	6	7	8	6	6	6	6	3	7	6
5.5-7.5															
6.5-7.5	2	2	4	5	3	5	3	4	4	3	1	1	-	2	2
7.5-8.5	-	3	5	-	5	-	2	6	2	5	-	-	-	-	-
7.5-9.5															
8.5-9.5	1	3	4	2	3	3	3	4	3	4	1	3	2	2	2

Snail Population: P1 - Alexandria, P2 - Zagazig, P3 - Abo Rawwash, P4 - Sum, P5 - Menie.

Table 6 b

Number of Bands Obtained by Iso-electric Focusing of Acid Phosphatases Extracted from Various Populations of Snails at pH 3.5 - 9.5

pH	(I) Whole Snail					(II) Digestive Gland					(III) Head Foot Organ				
	P1	P2	P3	P4	P5	P1	P2	P3	P4	P5	P1	P2	P3	P4	P5
4.5-5.5	2	2	3	2	3	2	2	3	2	2	1	2	2	2	3
3.5-5.5															
5.5-6.5															
5.5-7.5															
6.5-7.5															
7.5-8.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7.5-9.5															
8.5-9.5															

Snail Population: P1 - Alexandria, P2 - Zagazig, P3 - Abo Rawwash, P4 - Sum, P5 - Menie.

Table 6c

Number of Bands Obtained by Iso-electric Focusing of Soluble Proteins Extracted from Various Populations of Snails at pH 3.5 - 9.5

pH	(I) Whole Snail					(II) Digestive Gland					(III) Head Foot Organ				
	P1	P2	P3	P4	P5	P1	P2	P3	P4	P5	P1	P2	P3	P4	P5
4.5-5.5															
3.5-5.5	17	17	21	21	20	18	17	19	18	18	13	19	20	18	23
5.5-6.5															
5.5-7.5	7	9	13	11	15	7	10	11	9	7	3	8	8	8	10
6.5-7.5															
7.5-8.5															
7.5-9.5	4	4	5	4	6	3	4	4	5	5	2	2	4	2	4
8.5-9.5															

Snail Population: P1 - Alexandria, P2 - Zagazig, P3 - Abo Rawwash, P4 - Sum, P5 - Menie.

Table 7

Number of Bands Obtained by Iso-electric focusing of Esterases and Acid Phosphatases Extracted from Worms of Various Strains of *Schistosoma mansoni* at pH 3.5 - 9.5

pH	Esterases								Acid Phosphatases						
	Female Worms				Male Worms				Male Worms				Female Worms		
	Strains				Strains				Strains				Strains		
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
4.5-5.5	-	-	-	-	-	-	-	-	-	2	2	-	-	-	-
5.0-6.5	4	4	7	5	5	4	6	6	1	2	2	-	-	-	-
6.5-7.5	-	-	-	-	-	-	-	-	2	2	3	1	1	1	1
6.5-8.0	1	1	8	-	3	4	8	5	-	-	-	-	-	-	-
7.5-8.5	-	-	-	-	-	-	-	-	2	2	3	2	2	2	2
8.0-9.5	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
8.5-9.5	-	-	-	-	-	-	-	-	4	1	4	3	2	1	2

Numbering of strains of *S. mansoni*

1: Qualuobiya 2: Warrak 3: Zagazig 4: Puerto Rican

Table 8
Number of Bands Obtained by Iso-Electric Focusing of Soluble Proteins Extracted from Worms of Various Strains of *Schistosoma mansoni* at pH 3.5 - 9.5 and 5.5 - 8.5

pH	Female Worms				Male Worms				pH	Female Worms				Male Worms			
	Strains				Strains					Strains				Strains			
	1	2	3	4	1	2	3	4		1	2	3	4	1	2	3	4
3.5-5.5	9	13	18	15	22	18	20	17	5.5-6.5	3	2	2	2	6	5	6	6
5.5-5.7	4	4	6	5	16	13	18	15	6.5-7.5	3	1	2	5	5	3	3	3
7.5-9.5	1	1	4	1	5	3	5	4	7.5-8.5	1	1	1	1	3	3	3	4

Numbering of strains of *S. mansoni*
1: Qualuobiya
2: Warrak
3: Zagazig
4: Puerto Rican

smaller number of electrophoretic protein bands than *B. alexandrina* from Zagazig (P2) and that from Suez (P4) which are much more highly susceptible to infection (Tables 3 and 4).

In case of cathodic migrating electrophoretic proteins, *Biomphalaria glabrata* (P1) showed a pattern distinctive from the three populations of Biomphalaria alexandrina from Zagazig, Alexandria and Suez (P2 , P3 and P4) that showed a characteristic pattern of its own that could not be related to the susceptibility to each infection with *S. mansoni*. This is also true for α-glycerophosphate dehydrogenase and glutamic oxaloacetic transaminase (GOT). In case of the esterases each species and population also showed a different zymogen picture. Esterases in *Biomphalaria globrata* exhibited pronounced intra- and inter-population variations that were correlated with snail susceptibility to infection with schistosomes. However, species identification was not possible on the basis of esterase patterns, whereas, reliable species identification was possible from the study of four enzymes but often the esterase pattern alone could provide tentative identification. Genetic polymorphism has been described in esterases, glucose 6-phosphate dehydrogenases and alcohol dehydrogenases in natural populations of *Biomphalaria globrata* and *Biomphalaria tenagophila* [20].

In the present study, the electrophoretic protein pattern of *Bulinus tropicus*, a diploid snail from Zimbabwe which completely resists infection with *Schistosoma haematobium* [21] was compared with that of some populations of *Bulinus truncatus*, a polyploid snail susceptible to infection with schistosoma haematobium. The diploid snail showed a different pattern of proteins than those of all the populations of the polyploid snails. Burch and Lindsay [22] studied, using disc electrophoresis, the esterases of foot muscle extracts of *Bulinus tropicus* and *Bulinus truncatus*. They observed a stable difference between the diploid and polyploid samples.

However, more than one protein pattern was also found between polyploids. In the present study three different protein patterns were found in different populations of *Bulinus truncatus* from Egypt and Sudan (Tables 1-2). The presence of more than one protein pattern among polyploids has been observed before in egg protein [23].

When esterase isoenzymes of *Bulinus truncatus* were separated using cellogel and starch gel electrophoresis, similar results were obtained (Tables 1-2). This proves that the interpopulation differences in esterase zymogen is a constant finding which does not depend on the different experimental conditions. This difference in the zymogen pattern might be relevant to the interpopulation differences in susceptibility of snail populations of *Bulinus truncatus* to Abo-Rawash strain of *Schistosoma haematobium* (Tables 1-2). Enzymes, being protein in nature, have rates of migration that are relative to the rate of migration of total body proteins. This has been examined by some authers [22, 23] in case of total body proteins and esterases. This relation was found to be also true according to the results of the present work (Tables 1-2).

The differences in the zymogens of GOT and 3-hydroxybutyrate dehydrogenase in *Bulinus tropicus* and *Bulinus truncatus* snails in the present study was found to be related to the susceptibility of the snails to infection with Abo-Rawwash strain of *Schistosoma haematobium*. This result however, was not completely in consistence with that obtained for the susceptibility to infection with Quena strain of *Schistosoma haematobium*. Similarly, Mohamed [24] reported certain correlations between the mobility of GOT bands and the mobility and the distribution of α-glycerophosphate dehydrogenase and non specific esterases in *Bulinus* snails and their susceptibility to infection with *Schistosoma haematobium*.

The behaviour of *Schistosoma* species in the defenitive (final) host varies between strains and affects diagnosis and treatment [1, 25]. Biochemical features has been considered by helminth taxonomists as valid characteristics for classification [10, 26].

In the present work, isoelectric focusing has been used to characterize populations of *Biomphalaria alexandrina* as well as certain strains of *Schistosoma mansoni*. Various systems have been attempted in order to determine those techniques which may be conventionally used for that purpose. The differential characteristics of the salient bands for populations of snails and strains of *S. mansoni* are shown in tables [6-8].

It is clear that isoelectric focusing of soluble proteins extracted from whole snails gives better results than those obtained from the digestive gland or the head foot organ. Similarly strains of *S. mansoni* are differentiated by isoelectric focusing of proteins extracted from male and female worms. Isoelectric focusing of esterases extracted from tissues of the digestive gland of snails, provides another important tool for the characterization of populations of *Biomphalaria alexandrina* from Egypt. The same is also true for acid phosphatases extracted from the digestive gland.

Various studies have been made about the variation in the level of resistance induced by different pools of cercariae [27]. In each of two experiments separate groups of C57 BI mice were exposed to 20 to 30 *S. mansoni* cercariae from two different pools and challenged 12 weeks later with a common pool of cercariae. In both experiments the two initial infections induced quite different levels of resistance (27-78% and 10-60%).

Dean *et al.* [28] stated that cercarial heterogenity may be unusual in laboratory strains, since other workers had obtained more consistent levels of resistance when the same strain of mouse had been examined repeatedly under similar conditions [29]. The level of resistance is directly related to the magnitude of the granulomatous response. Dean *et al.*, [28] suggested an important role for the granuloma in the development of resistance. It is possible that the mechanisms of this resistance are independent T-cell mediated interactions with schistosomes since similar relationships between resistance and the magnitude of the granulomatous relation have been demonstrated for S. japonicum in mice [30]. Harrison *et al.* [31] showed that unisexual cercarial infection, which does not lead to granulomatous liver disease, fails to induce significant resistance to re-infection even with the addition of eggs.

The major problem of bilharziasis in endemic areas, up till now, is the repetition of infection. Bogliola [32] found that the granulomas in repeatedly infected patients were more numerous, but natural evolution is still to fibrous scars. In addition, the diameter of the granulomata in sensitized animals, as suggested

by Cheever [33] was greater than unsensitized animals, while Habib [34] stated that on each infection the pathological lesions were detected with formation of cellular granulomata in relation to the freshly deposited ova. He added that the development of pipe-stem fibrosis, in cases of repetition of infection, was faster than in single infection, and the first was undoubtedly responsible for the final picture of Symmer's fibrosis.

In spite of all these inter- and intraspecific variations in the proteins and enzymes in the snails as well as in the schistosomes, fortunately, from work on *S. mansoni* there appears to be little intraspecific diversity of the major surface antigens of adult schistosomes. This obviously augurs well not only from the point of view of serological diagnosis but also with regard to vaccine production. Thus, in schistosomes, once effective vaccination is achieved it may be universally applicable [12].

A number of live vaccines have been used against schistosomasis in animal experiments. These included freeze, thawed [35] or irradiated parasites [36] and vaccines based on extracted or purified antigens [37]. Despite all this activity satisfactory sterile immunity has never been obtained; this is at least partly due to an evasion mechanism which allows the parasite to persist in the host making schistosomiasis a chronic disease. The adult forms of the worm can survive in the blood vessels of the lower intestine or bladder for years, by disguising themselves with a coat of host like surface proteins. These proteins include the major histocompatibility antigens of the host [38], which are mainly blood group antigens. Immature forms of the parasite do not have this disguise. The expression of *Schistosoma mansoni* genes which encodes for surface antigens has been described [39].

Wakelin [40] stated that, although there is some agreement on the components which function as effectors in vaccine induced resistance, it has not been possible to associate variations in specific components with variations in resistance. However, Kelly and Colley [41] suggested that perhaps differences between good and poor responders may depend less upon differences in capacity to respond than upon differences in immunoregulating mechanisms which allow the expression of response capacity.

Strain variations in immuno-pathological responses to *S. mansoni* are also evident in mice. Dean *et al.* [28] found that the varying degrees of resistance seen in a panel of 10 strains of mice after re-infection with S. mansoni were linked primarily with the degree of portal hypertension and the number of lung granulomata, both reflections of pathological changes in the liver. Fanning and Kazura [42] showed that the modulation of granulomata size which ameliorates the severity of pathological reactions late in infection was also strain variable.

Immunity to schistosomiasis in mice is more strongly expressed against re-infection than against primary infections. Neverthless there is substantial strain dependent variation in the degree to which primary infections develop. Such variations are apparent in the number of adult worms, egg output, pathological response and inflammatory changes [43]. Butherworth *et al.* [44] demonstrated that there is some correlation with resistance to re-infection and recognition of particular antigens.Accordingly, the importance of establishing the basis of the differences and the similarities in the biochemical pictures especially proteins and enzymes in the snails as well as in schistosomes is highly important not only from the point of view of classification but also for the studies of resistance to infection and re-infection as well as for the success of producing a successful vaccine against schistosomiasis.

This is highly important in view of the fact that although, there is a powerfull and reliable chemotherapeutic drug, Praziquantel which can be used to treat schistosomiasis, the drug is relatively expensive and the disease is soo widespread so that research into a vaccine has been going on for several years, since reports came from Zaire [45]. The latter investigators showed that serious side effects can develop with the drug and the treatment does not prevent re-infection, thus the effort put into development of a vaccine has been considerably increased. To overcome the problem of drug resistance, Katz *et al.* [46] reported that treatment with alternative drugs (oxaminiquine and praziquantel) in children not cured with the first treatment resulted in negative stool in 11 out of 12 cases, examined one month after the second round of therapy. In order to minimize the risk of the development of drug resistance, they suggested that infected patients should be treated with one drug and in therapeutic failures with another.

REFERENCES

[1] **Nelson, G.S., 1970.** The epidemiological significance of infraspecific variations in helminths of medical importance, with particular reference to *Trichinella* and *Schistosoma* In: Single KSY, Tandan BC(eds) HD Srivasta Commemoration volume. Indian Veterinary and Research Institute, Izatnagar, pp. 19-25.

[2] **Saoud, M.F.A., 1965.** Morphological and experimental studies on some Schistosomes of man and animals, Ph.D. Thesis, University of London.

[3] **Saoud, M.F.A., 1966.** The infectivity and pathogenecity of geographical strains of *Schistosoma mansoni*, Trans. Roy. Soc. Trop. Med. Hyg. 60-585-600.

[4] **Nelson, G.S.** and **M.F.A. Saoud, 1968.** A comparison of the pathogenicity of two geographical strains of *Schistosoma mansoni* in rhisus monkeys, Helminth., 42, 339-362.

[5] **Saoud, M.F.A., 1965.** Susceptibility of various snail intermediate hosts of *Schistosoma mansoni* to different strains of the parasite, J. Helminth 39, 363-375.

[6] **Theron, A., 1984.** Early and late shedding patterns of *Schistosoma mansoni* carcariae: ecological significance in transmission to human and murine hosts, J. Parasitol. 70: 652-655.

[7] **Thompson, R.C.A., 1988.** Infraspecific variation and Epidemiology. In: Parasitology in Focus. Heing Mehlhorn (Ed) Springer verlag.

[8] **Wright, C.A.** and **D. Rollinson, 1979.** Analysis of enzymes in the *Bulinus africanus* group (Mollusca, Planorbidae) and their detection. Parasitology: 79, 95-105.

[9] **Wright, C.A.; D. Rollinson,** and **P.H. Goll, 1979.** Parasites in *Bulinus senegalensis* (Mollusca, Planorbidae) and their detection, Parasitology, 79: 95-105.

[10] **Fletcher, M., D.S. Woodruff, P.T. Loverde** and **H.L. Arch 1980.** Genetic differentiation between *Schistosoma mekongi* and *S. japonicum,* an electrophoretic study. Molacological Review (Supplement 2) 113-122.

[11] **Carter, C.E.** and **D.G. Colley, 1986.** The molecular biology of Schistosomes, Parasitol. Today 2: 84.

[12] **Simpson, A.J.G., 1986.** The influence of molecular heterogencity in helminth identification, protective immunity and immunodiagnosis In: Parasitology quo vadit, Howell M.J. (ed). Australian Academy of Science, Canberra, pp. 69-77.

[13] **Araujo, N., N. Katz, E.P. Dias** and **C.P. Desouza, 1980.** Susceptibility to chemotherapeutic agents of strains of *Schistosoma mansoni* isolated from treated and untreated patients, Am. J. Trop. Med. Hyg. 29: 890-94.

[14] **Fawzi, S.M., 1983.** Biological studies on *Schistosoma mansoni* and its relationships with snails of the genus *Biomphalaria,* M.Sc. Thesis, University of Ain Shams, Cairo.

[15] **Saoud, M.F.A., J.L. Bruce, S.H. Mohamed** and **S.M. Fawzi, 1993.** Infectivity of some Egyptian strains of *Schistosoma mansoni* in certain local populations of *Biomphalaria alexandrina* with observations on isoenzyme characterization of snail populations and parasite strains, International Conference on Schistosomiasis (FEB. 14-18, 1993 Cairo, [Abstracts. 29].

[16] **Liang, J.S., 1973.** Cultivation of *Bulinus (Physopsis) globosus* (Morelet) and *Biomphalaria pfeifferi* (Krauss), snail hosts of Schistosomiasis. Sterkiana, 53: 7-23.

[17] **Jelnes, J.E., 1977.** Experimental taxonomy of Bulinus. II. Recipes for horizontal starch gel electrophoresis of ten enzymes in *Bulinus* and description of internal standard systems and of two new species of the *Bulinus forskalii* complex, J. Chromatogr., 170: 405-411.

[18] **Chemetron 1963.** Cellogel electrophoresis: International chemical exhibition in Milan.

[19] **Righetti, P.G., 1984.** Laboratory techniques in biochemistry and molecular biology. Isoelectric focusing: Theory, methodology and applications, Work T.S. and Burdon, R.H. (Eds) Elsevier, Biochemical Press, Amsterdam, New York, Oxford.

[20] **Henriksen, U.B.** and **J.E. Jelnes, 1980.** Experimental taxonomy of *Biomphalaria* (Gastropoda, Planorbidae). I. Methods for experimental taxonomic studies on Biomphalaria done by horizontal starch gel electrophoresis and staining of 12 enzymes, J. Chromotogr., 188(1): 169-179.

[21] **Wright, W.H., 1973.** Geographical distribution of schistosomes and their intermediate hosts In: Epidemiology and control of schistosomiasis, Ed.: N. Ansari, University Park Press, pp. 32-249.

[22] **Burch, J.B.** and **G.K. Lindsay, 1967.** Electrophoretic analysis of esterases in *Bulinus,* Ann. Rep. Am. Molac. Union, 34, 39-40.

[23] **Brown, D.S.** and **C.A. Wright, 1972.** On a polyploid complex of fresh water snails (Planorbidae: Bulinus) in Ethiopia, J. Zool. London, 167: 97-132.

[24] **Mohamed, S.H., 1982.** On certain aspects of the biology and host-parasite relationships of *Schistosoma haematobium* in Egypt, M.Sc. Thesis. Ain Shams University.

[25] **Cheever, A.W., 1985.** *Schistosoma japonicum:* The pathology of experimental infection, Exp. Parasitol. 59: 1-11.

[26] **Viyant, V., E. S. Upatham,** and **S. Siriteramongkol, 1985.** Enzyme analysis of *Bithma* (Mollusca, Bithyniidae) by isoelectric focusing, Malacological Review, 18: 15-20.

[27] **Smith, M.A.** and **J.A. Clegg, 1979.** Different levels of immunity to *S. mansoni* in the mouse. The role of varient cercariae, Parasitology 78: 311-321.

[28] **Dean, D.A., M.A. Bukowsiu** and **A.U. Cheever, 1981.** Relationship between acquired resistance portal hypertension and lung granulomas in ten strains of mice infected with Schistosoma mansoni, Am. J. Trop. Med. Hyg. 30: 806-814.

[29] **Long, J.; R.D. Tanaka,** and **A.J. Macinnis, 1980.** Development of a cell free protein. synthesizing system from S. mansoni, J. Parasitology, 66(3): 424-427.

[30] **Moloney, N.A. G. Webbe,** and **A. Luty, 1984.** Factors affecting the Acquisition of resistance against S. japonicum in the mouse. I. The correlation between egg deposition and worm elimination, Parasitology. 89: 345-60.

[31] **Harrison, R.A., Q. Bickle,** and **M. Doenkoff, 1982.** Factors affecting the acquisition of resistance against S. mansoni in the mouse. Evidence that the mechanisms which mediate resistance during early patient infections may lack immunological specificity, Parasitology, 84: 93-110.

[32] **Bogliolo, L. 1957.** The anatomic picture of the liver in hepatosplenic *Schistosoma mansoni,* Ann. Trop. Parasitology, 51: 1-14.

[33] **Cheever, A.W., 1961.** Hepatovascular lesions in mice infected with *Schistosoma mansoni,* Arch. Pathol. 72: 648-657.

[34] **Habib, A.K., 1970.** Studies on the role of repeated infection with *S. mansoni* and repeated treatment of animals on the liver pathology, M.Sc. Thesis, Cairo University.

[35] **James, S.L., 1985.** Induction of protective immunity against *Schistosoma mansoni* by a non living vaccine is dependent on the method of antigen presentation, J. Immunol. 134: 1956-1960.

[36] **Dalton, B.I.P. M. Strand, B.L. Mangold** and **D.A. Dean, 1986.** Identification of *Schistosoma mansoni* glycoproteins recognized by protective antibodies from mice immunized with irradiated cercariae, J. Immunol. 136: 4689-4694.

[37] **Sher, A., E. Pearce, S. Hieny** and **S. James, 1988.** Induction of protective immunity against *Schistosoma mansoni* by a non living vaccine IV. Fractionation and antigenic properties of a soluble adult worm immunoprophylactic activity, J. Immunol. 136: 3878-3883.

[38] **Sher, A., B.F. Hall** and **M.A. Vadas, 1978.** Acquisition of murini major histocompatibility complex gene products by Schistosomula of *Schistosoma mansoni*, J. Exp. Med. 148: 46-57.

[39] **Taylor, D.W., I.S. Cordingley, D.W. Dunne, K.S. Johnson, W.I. Massov; C.E. Hormaeche, V. Nene** and **A.E. Butterworth 1986.** Malecular cloning of Schistosoma genes, Parasitology, 92: 73-81.

[40] **Wakelin, D., 1988.** In: Parasitology in Focus. Facts and trends, Heinz Mehlhorn (Ed) Springer - Verlag, p. 665.

[41] **Kelly, E.A.** and **D.G. Colley, 1986.** Effects of immunomanipulations on resistance induced by irradiated *Schistosoma mansoni* cercarial sensitization of C57 BL/6 and CB A/J mice, Am. Trop. Med. Hyg. 35: 803-811.

[42] **Fanning, M.M** and **J.W. Kazura 1984.** Genetic-linked variation in susceptibility of mice to *Schistosoma mansoni*, Parasite immunol 6: 95-103.

[43] **Kee, K.C.; D.W. Taylor, J.S. Cordingley, A.E. Butterworth,** and **A.J. Munro, 1986.** Genetic influence on the antibody response to antigens of *Schistosoma mansoni* in chronically infected mice, Parasite Immunol. 8: 565-574.

[44] **Butherworth, A.E., M. Capron, J.S. Cordingley, P.R. Dalton, D.,W. Dunne, H.C. Kariuki, G. Kimani, D. Koech, M. Dugambi, J.H. Ouma, M.A. Prentice, B.A. Richardson, T.K. Arap Siongok, R.F. Sturrock,** and **Taylor D.W. 1985.** Immunity after treatment of human Schistosomiasis mansoni. II. Identification of resistant individuals and analysis of their immune responses, Trans. R. Soc. Trop. Med. Hyg. 79: 393-408.

[45] **Polderman, A.M., B.Gryseels, J.L. Gerold, K. Mpamila** and **J.P. Manshand, 1985.** Side effects of Praziquantel in the treatment of *Schistosoma mansoni* in Maniema, Zaire. Trans. R. Soc. Trop. Med. Hyg. 78: 752-754.

[46] **Katz, N.; R.S.; Rocha, C.P. De-Souza, F.P. Coura, J.I. Bruce, Coles,** and **G.K. Kinoti, 1991.** Efficacy of alternating therapy with oxamniquine and praziquantel to treat *S. mansoni* in children following failure of first treatment, Am. J. Trop. Med. Hyg. vol. 44(5): 509-12.