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GEOGRAPHICAL DIFFERENCES IN LECTINIC ACTIVITY OF ALBUMEN GLAND EXTRACTS OF THE PLANORBID SNAILS BIOMPHALARIA GLABRATA AND B. TENAGOPHILA

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SUMMARY

Extracts of albumen gland of the planorbid mollusc **Biomphalaria glabrata**, intermediate host of **Schistosoma mansoni**, showed lectinic activity on human red blood cells of certain blood groups of the ABO system, according to the geographical origin of the snails, which were collected in Brazil (States of Paraná, Goiás, Bahia, Minas Gerais and Maranhão) and Venezuela (Valencia Lake at Maracay). Cells of the B group were agglutinated by Paraná, Bahia and Venezuela extracts, and were not agglutinated by Goiás, Minas Gerais and Maranhão extracts. Paraná extracts agglutinated cells of all A, B and AB groups and subgroups. Those of Maranhão agglutinated only A_1 cells. Goiás, Minas Gerais and Maranhão extracts did not agglutinate A_2 cells. All extracts agglutinated A_1 cells and were inactive against O cells. Extracts of digestive gland (hepatopancreas) and ovotestis of **B. glabrata** and another planorbid, **Helisoma duryi** were inactive, as well as those of the albumen gland of the latter species. A single examined sample of **Biomphalaria tenagophila** from Rio de Janeiro showed similar activity to that of **B. glabrata** from Goiás. Hemolymph of both **Biomphalaria** species was inespecifically hemolytic.

INTRODUCTION

Extracts from several species of molluscs have lectinic activity on human red blood cells (rbc). This has been repeatedly demonstrated in many families of Gastropoda, including Planorbidae [7]. In 1899, CAMUS [2] reported agglutinating activity of albumen gland extracts of **Helix pomatia** on rbc of different animal species, including man. That paper, which has remained apparently unnoticed since then, was published 10 years after the first report of the agglutinating effect of a plant extract by STILLMARK [11], and two years before the classical paper of LANDSTEINER [4] on blood groups. GILBERTSON & ETGES [3] reported differences in the activity of hemolymph of **Biomphalaria glabrata** from Brazil; in some cases the hemolymph was inactive and in some others it agglutinated human rbc inespecifically.

We now report lectinic activity of **B. glabrata** and **B. tenagophila**, limited to albumen gland extracts, with "virtually specific" [1] differences of activity according to the geographical origin of the snails. A preliminary account of our results was rendered in a short communication [5].

MATERIALS AND METHODS

Snails were kept at 24°-26°C in aquaria containing **Elodea canadensis**, and fed with

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lettuce leaves. In most experiments they were used within one week from arrival at the laboratory. Snails' shells were lightly crushed to expose the internal organs; under a dissecting microscope the hemolymph was aspirated from the venous sinuses with 27 gauge needle and tuberculin syringe. Albumen glands, digestive glands and ovotestes were removed and washed in cold saline (0.85% NaCl). The hemolymph and the different organs of each lot were pooled separately and frozen at -20°C. Sonic extracts of each lot of organs in the proportion of 1.35 ml of saline to 200 mg of tissue were prepared in tubes kept in crushed ice; after centrifugation at 1500 rpm for 15 minutes each supernatant (crude extract) was stored at -20°C or diluted 1:30 with saline, and also stored at -20°C.

Sonic extracts of albumen glands (AG), digestive glands (DG), and ovotestes (OT) were obtained from specimens of **B. glabrata** from different areas of Brazil and Venezuela, and of **B. tenagophila** from Rio de Janeiro. Typed human rbc from randomly selected donors were obtained from two blood banks in Brasília, capital of Brazil. No effort was made to track the racial or geographical origin of donors. Since Brasília is only 16 years old, all the individuals came from many places of the country, thus constituting a sample of Brazilian population. Blood from adult male donors was collected in equal parts of Alsever solution, kept at 4°C, and used within two weeks, in general. Suspensions of three times washed rbc (1.5% in saline) were used for the tests.

Lectinic activity was measured with microdilution technique using microtiter equipment (Cook Laboratory Products, Alexandria, Virginia, USA). Crude extracts and hemolymph were used for panels of rbc representative of blood groups and subgroups of the ABO system; diluted extracts were used with all available blood samples. Extracts of hemolymph were dropped (0.05 ml) in the first line of wells of disposable U-plates; serial two-fold dilutions with saline were made using microdiluters, beginning at the second line of wells; then, the 1.5% suspension of rbc was dropped (0.05 ml) in all wells. The final dilutions, in the case of crude extracts, ranged from 1:2 to 1:4096. The plates were incubated at room temperature in a moist chamber for 2 hours. Readings of the pattern of agglutinated or non-agglutinated cells were recorded from 0 to 4-plus. Only 3- and 4-plus were considered positive.

RESULTS

The results are shown in Table I, II and III. Differences in lectinic activity with AG extracts were observed according to the area where the snails were collected. No activity was observed with DG and OT extracts. AG, DG and OT extracts obtained from **Helisoma duryi**, an originally Nearctic planorbid introduced into Lagoa da Pedra, State of Goiás, were also inactive.

DISCUSSION

From our results (Tables I, II and III) it appears that differences in lectinic activity of AG extracts are related to the geographical origin of the snails (Fig. 1). There was a sharp difference in the activity of extracts of **B. glabrata** from Moreira Sales (State of Paraná, near the border with Paraguay) and from São Luís (State of Maranhão, in the northern part of Brazil). Except for O type, Moreira Sales extract agglutinated all rbc types in relatively high titers, while São Luís extracts were active only on A_1 cells. This may explain why other Authors [9], working in Paraná, reported that "agglutinin acting strongly on blood groups A and B was present in crude extract of the albumin gland of **B. glabrata**", while that of **B. tenagophila** showed a "powerful anti-A activity and weak anti-B activity".

Salvador (State of Bahia) and Venezuela diluted extracts, while showing activity on B rbc, had variable activity on other rbc types (Table I). In general, A_1 rbc were agglutinated by all **B. glabrata** and **B. tenagophila** AG extracts, while A_2 rbc were not agglutinated, except by Moreira Sales extract. More striking was the fact that A_2B rbc were agglutinated only by Moreira Sales, Salvador and Venezuela **B. glabrata** extracts, while A_2 rbc were not agglutinated, in most cases, by Salvador and Venezuela extracts.

TABLE 1 - Lectinic activity of diluted albumen gland extracts of planorbid snails. Differences of activity according to geographical areas.

Origin of snails	Blood samples tested	Blood groups (positive/total)											
		A*	A_1	A_2	$A_{Int.}$	B	AB*	A_1B	A_2B	O*	O_{MM}	O_{MN}	O_{NN}
BRAZIL													
B. glabrata													
Moreira Sales, PR	235	69/70	21/21	7/7	1/1	27/27	9/9	4/4	2/2	1/81	0/4	0/6	0/4
Rio Preto, GO	774	41/63	159/175	4/48	2/7	0/97	4/4	11/17	1/11	1/280	0/16	0/40	0/15
Salvador, BA	585	48/61	88/97	9/30	0/1	51/55	2/2	8/10	12/12	0/235	0/18	0/47	0/17
Belo Horizonte, MG	114	3/5	22/25	0/9	-	1/12	-	3/3	0/2	0/41	0/4	0/10	0/3
Santa Luzia (albino), MG	216	24/25	34/35	3/9	0/1	0/27	2/2	2/2	0/1	0/75	0/4	0/12	0/5
São Luiz, MA	199	-	54/60	0/16	0/2	0/14	-	0/1	0/2	0/58	-	-	-
B. tenagophila													
Rio de Janeiro, RJ													
Normal	187	-	57/57	5/12	2/2	0/16	-	2/2	0/2	0/96	-	-	-
Infected	171	3/4	51/52	1/15	1/3	0/18	-	1/2	0/2	0/75	-	-	-
H. duryi													
Formosa, GO	39	0/12	-	-	-	0/3	0/2	-	-	0/22	-	-	-
VENEZUELA													
B. glabrata													
Maracay and Valencia	118	3/6	20/22	2/8	-	8/8	-	4/4	2/2	0/46	0/4	0/16	-
Samples tested	2281												

Abbreviations for the Brazilian states: PR - Paraná, GO - Goiás, BA - Bahia, MG - Minas Gerais, MA - Maranhão
*Subgroups not typed

TABLE II

Lectinic activity of albumen gland crude extracts from snails collected in different areas of Brazil

Blood group panel	**Biomphalaria glabrata**						**B. tenagophila**		
	Moreira Sales, PR (*)		Lagoa Feia and Rio Preto, GO		São Luís, MA		Rio de Janeiro, RJ		
	I	II	I	II(**)	I	II	I	II	III
A_1	256(***)	64	64	64	256	256	128	32	256
A_2	—	32	2	8	16	8	8	16	8
A_{Int}	64	32	8	32	64	128	—	4	16
B	64	32	0	4	0	0	4	0	4
A_1B	128	—	16	128	64	64	128	32	256
A_2B	—	64	—	4	8	0	2	0	4
O_{MM}	—	—	0	—	—	—	—	—	—
O_{MN}	—	—	0	—	—	—	—	—	—
O_{NN}	—	—	0	—	—	—	—	—	—
O	0	0	—	2	0	0	0	0	0

(*) Abbreviations for the Brazilian States: PR — Paraná, GO — Goiás, MA — Maranhão, RJ — Rio de Janeiro

I, II, III, Samples of snails

(**) Infected snails

(***) Reciprocal of highest agglutination titer

Since crude AG extracts of both snail species were slightly active on A_2 rbc and some of them showed very weak activity on B rbc, while Moreira Sales extracts were quite active on A_2 and B rbc (Table II), the comments of BOYD [1] about the relative "specificity" of plant extracts apply to our results.

It is interesting to note that GILBERTSON & ETGES [3] reported lack of activity of hemolymph of **B. glabrata** from Puerto Rico, Belo

TABLE 3 - General trend of lectinic activity of diluted albumen gland extracts from snails of different geographical areas.

Origin of snails	Blood groups					
	A		B	AB		O*
	A_1	A_2		A_1B	A_2B	
BRAZIL						
B. glabrata						
Moreira Sales, PR**	+	+	+	+	+	–
Rio Preto and Lagoa Feia, GO	+	>–	–	>+	>–	–
Salvador, BA	>+	>–	+	>+	+	–
Belo Horizonte, MG	>+	–	–			–
Santa Luzia, MG (albino)	+	>–	–			–
São Luiz, MA	+	–	–	–	–	–
B. tenagophila						
Rio de Janeiro, RJ	+	>–	–	>+	–	–
VENEZUELA						
B. glabrata						
Maracay and Valencia	+	>–	+	+	+	–

* Includes O_{MM}, O_{MN} and O_{NN}
** Abbreviations for the Brazilian states: PR - Paraná, GO - Goiás, BA - Bahia, MG - Minas Gerais, MA - Maranhão
>+, mostly positive >–, mostly negative

Horizonte (Minas Gerais) and Venezuela, and activity in those from Surinam and Salvador (Bahia). In our experiments, hemolymph of **B. glabrata** from Catolândia (Bahia) and Lagoa Feia (Goiás), and of **B. tenagophila** from Rio de Janeiro (infected and non infected snails) was inespecifically hemolytic in low titers (1:2 and 1:4). Low-titer inespecific agglutination (including O rbc) was observed when the hemolymph of **B. tenagophila** was further diluted (1:8 and 1:16).

The present experiments seem to corroborate GILBERTSON & ETGES' [3] hypothesis that lectinic activity could be used to differentiate between populations of planorbid snails. Concerning the role of lectins in Planorbidae, it is apparently unknown. For other invertebrates, it has been suggested that they are important in the defense mechanisms against potential pathogens [6], and the name of protectin has been given to them [8]. While a more decisive explanation in awaited, we may agree with the opinion of SHARON & LIS [10] in their review on lectins: The role of lectins in nature — whether in plants or in other organisms — remains a mystery.

RESUMO

Diferenças geográficas na atividade lectínica de extratos da glândula do albúmen dos planorbídeos Biomphalaria glabrata **e** B. tenagophila

Extratos da glândula do albúmen do planorbídeo **Biomphalaria glabrata,** hospedeiro intermediário do **Schistosoma mansoni,** apresentaram atividade lectínica sobre hemácias humanas de certos grupos sanguíneos do sistema ABO, segundo a origem geográfica dos moluscos, que foram coletados no Brasil (Paraná, Goiás, Bahia, Minas Gerais e Maranhão) e na Venezuela (lago de Valencia, em Maracay). As hemácias do grupo B foram aglutinadas por extratos do Paraná, Bahia e Venezuela, e não foram aglutinadas por extratos de Goiás, Minas Gerais e Maranhão. Os

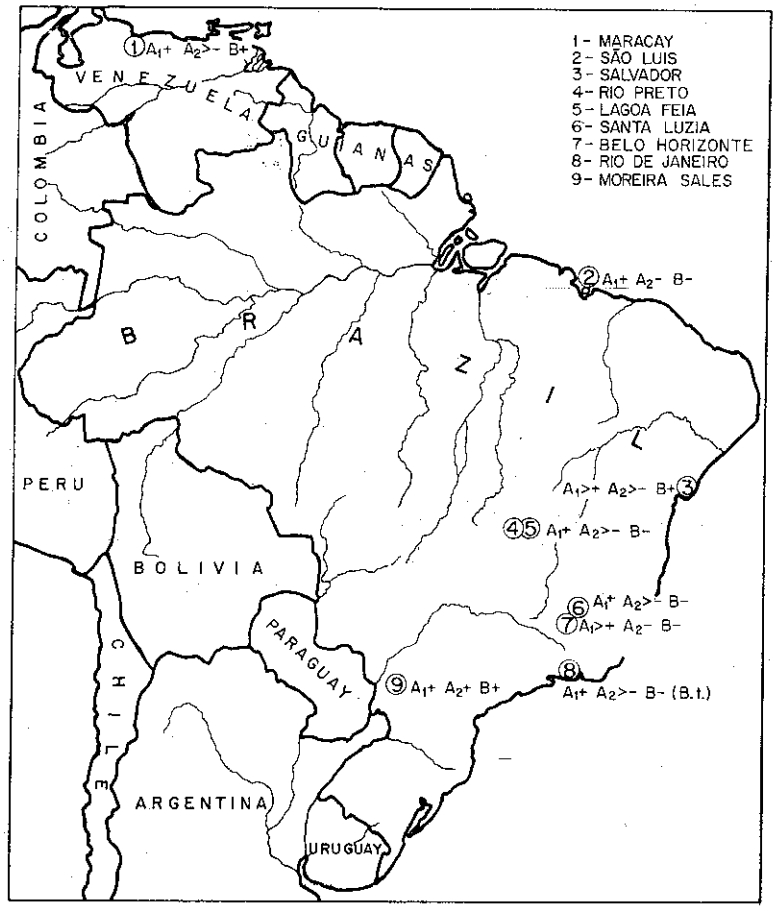

Fig. 1 — Lectinic activity of albumen gland extracts of **Biomphalaria glabrata** and **B. tenagophila** according to geographical origin of snails. B. t. = **B. tenagophila** (See Table III for details).

extratos do Paraná aglutinaram as hemácias de todos os grupos e subgrupos A, B e AB. Os do Maranhão aglutinaram apenas hemácias A_1. Os extratos de Goiás, Minas Gerais e Maranhão não aglutinaram as hemácias A_2. Todos os extratos aglutinaram as hemácias A_1 e foram inativos contra as do grupo O. Os extratos da glândula digestiva (hepatopâncreas) e do ovoteste de **B. glabrata** e de outro planorbídeo, **Helisoma duryi**, foram inativos, assim como os da glândula do albúmen desta última espécie. Uma única amostra examinada de **Biomphalaria tenagophila** do Rio de Janeiro apresentou atividade semelhante à de **B. glabrata** de Goiás.

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