

presence of the thalassemic gene. Evidence in favour of such a mechanism could be gathered by studying appropriate erythropoietic parameters in subjects carrying only thalassemia and in carriers of both traits.

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ABO agglutinins from *Biomphalaria straminea* snails

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Summary. Extracts from *B. straminea* spawn are active against A and B normal red cells. A₁ and A₂ subgroups may be differentiated with trypsin-, papain- and pronase-treated cells. O cells treated with papain, pronase and neuraminidase react weakly to the extracts.

Analysis of agglutinins in the Basommatophora (water snails) have revealed differences between this order and the Stylommatophora (land snails), since few members of the Basommatophora (4 out of 16 species) contain agglutinins whereas these are frequent in the Stylommatophora². The Basommatophora snails are significant in public health considerations in Brazil, since 3 species of *Biomphalaria*, *B. glabrata*, *B. straminea* and *B. tenagophila* are the principal intermediary hosts of *Schistosoma mansoni*. This communication describes the ABO agglutinins for 1 of those species, *Biomphalaria (Australorbis) straminea*.

Material and methods. The samples consisted of 2 pools of 275 and 296 individual specimens of fresh spawn from pigmented animals reared in the laboratory over 4 years. The spawn were washed and suspended in saline solution (1 g/1 ml) and after 15 min of sonication were centrifuged for 10 min at 800 × g. Methods for papain- and trypsin-treatment of red cells are described elsewhere³. Pronase: 1 part of 2% red cell suspension in saline and 2 parts of the working solution of pronase (protease type VI Sigma, 6.5 units/mg) were incubated for 15 min at 37°C and afterwards centrifuged for 5 min at 925 × g. The cells were washed again, and resuspended at a 2% final concentration. The working solution of the enzyme was made by mixing 1 part of 1% pronase concentration in saline and 9 parts of buffered saline (pH = 7.3). Neuraminidase: 1 part of the enzyme (from *Virus influenzae*, A₂ Hong Kong/68 strain, 400 units/ml, kindly supplied by Prof. Raimundo D. Machado, Institute of Microbiology, UFRJ) was diluted with 11 parts of saline. 1 vol. of cell suspension and 2 vol. of the working solution of the enzyme were incubated for

30 min at 37°C and afterwards centrifuged for 5 min at 925 × g. The packed cells were resuspended at a 2% final concentration after 3 washings with saline. A control of this enzyme included normal, papain- and neuraminidase-treated O cells which yielded 0, 0 and 256 end-point titers, respectively, against an *Arachis hypogaea* extract. Titrations were made incubating normal, trypsin-, papain- and pronase-treated cells with the spawn extract for 30 min at 37°C; neuraminidase-treated cells, for 15 min at room temperature. The cells were centrifuged for 15 sec at 1000 rpm (Adams Sero-Fuge) and the results were read macroscopically.

Results and discussion. The results in the table show an anti-A, B agglutinin against normal cells. The degree of reactivity with the A antigens was higher than with B group cells. Differences between A₁ and A₂ were not significant. A low titer with O cells when they are treated with papain, neuraminidase and pronase, was also found. The last enzyme yielded the highest titrations with all the ABO groups. Neuraminidase-treatment does not allow a distinc-

Average end-point titers of *B. straminea* spawn

Treatment	A ₁	A ₂	B	O
Normal	109	40	5	0
Papain	7332	272	137	4
Trypsin	1067	130	21	0
Pronase	20171	4096	193	4
Neuraminidase	4360	2048	49	2

tion between the 2 A subgroups. In addition, its reaction with O cells may not be related to the T antigen because of the positive agglutination with papainized and pronased O cells. A high anti-A,B activity was also obtained with trypsin, papain and pronase and the differences between the A subgroups were significant.

The hemolymph of *B. straminea* does not agglutinate human O, rabbit, hamster and duck red cells⁴; our extracts did not react with the cells of a New World monkey, an interspecific hybrid of the genus *Callitrix*. We have not found hemolysins against human erythrocytes in this snail.

Agglutinins and hemolysins from *Biomphalaria tenagophila* snails

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Summary. A saline anti-A and incomplete anti-A,B agglutinin were found in spawn extracts of *Biomphalaria tenagophila* snails. Significant differences after papain and pronase treatments were also found for A₁ and A₂ subgroups. The hemolysin titers are high, and unspecific.

The snail *Biomphalaria tenagophila*, one of the most important intermediary hosts of *Schistosoma mansoni* in Brazil, has been shown to contain agglutinins and hemolysins. A strong anti-A and a weak anti-B activity in the albumen gland have been described previously². In 2 specimens from Rio de Janeiro an anti-A,B agglutinin more active with A₁ than with A₂ was reported; and in a 3rd, an anti-A agglutinin against normal red cells, which does not differentiate between the A subgroups³. In addition, the hemolymph from infected and non-infected snails was found to be unspecifically hemolytic in low titers (1:4)³.

This communication a) identifies the agglutinins and hemolysins found in fresh spawn, b) describes their reaction with red cells treated with neuraminidase and proteolytic enzymes and c) their hemolytic patterns.

Materials and methods. 3 pools of 70,54 and 33 individual samples of fresh spawn from pigmented animals reared in the laboratory were extracted and titrated following techniques already described elsewhere^{4,5}.

Results and discussion. An anti-A agglutinin against normal cells and an anti-A, B, weak anti-B and high anti-A, against trypsin treated cells, are the principal features of the spawn extracts of this snail. It is possible to differentiate

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between the A subgroups using papain and pronase (table 1).

The hemolysin titers are high and unspecific (table 2). All the enzymes have practically identical patterns of hemolytic activity with all the ABO antigens. The hemolysins appear to be highly characteristic of this species, since they have never been found in the spawn of *B. glabrata*⁶ or in *B. straminea*⁵.

Our extracts agglutinate and hemolyze normal and neuraminidase-treated red cells from a New World monkey, a hybrid of *Callitrix penicillata* × *Callitrix jacchus*. Variations have been shown for the lectins of *B. glabrata*³. This variation may be attributed in part to geographic differences and possible consequent genetic differences^{6,7}. However, it may also be explained by 1. differences in serological methods, for instance, tube test or slide test technique; 2. manual or sonicated extracts; 3. diverse concentrations of red cell suspensions; 4. variability of the lectinic content among the different tissues of the animal and 5. 'complete' or 'incomplete' nature of the agglutinin. A weak activity can be easily overlooked when a whole body extract is used instead of the most active organ or tissue.

Table 1. Agglutinin average end-point titers from spawn of *B. tenagophila*

Treatment	A ₁	A ₂	B	O
Normal	65	33	0	0
Papain	10735	1458	259	90
Trypsin	272	97	7	0
Pronase	57052	258	—	—

Table 2. Hemolysin average end-point titers from spawn of *B. tenagophila*

Treatment	A ₁	A ₂	B	O
Normal	129	258	82	82
Papain	515	515	258	129
Trypsin	65	129	65	65
Pronase	515	515	182	92
Neuraminidase	65	65	65	33

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