

Agglutination of Spermatozoa of Clams and of Human A, B, and O Blood Groups by Phytagglutinins (33621)

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Numerous reports have appeared in recent years that phytagglutinins are specific for human (1-9) and animal red cells (10-19). The presence of antigens on sperm has also been investigated (20-25). Kashiwabara's studies (24) using phytagglutinins prepared from soy bean extracts on bull spermatozoa led us to investigate the possibility that the presence of antigens on spermatozoa of invertebrates might be detected by similar methods. We chose to study the reactivity of 50 seed extracts against the spermatozoa of the mussels, *Venus mercenaria* and *Mytilus mytilis*, and to test the reactivity of these same extracts against human blood group antigens.

Materials and Methods. Saline extracts of 50 seeds were prepared as previously described by Brilliantine and Allen (5). Testes were obtained from freshly killed clams and stored in 20 vol of sterile buffer solution, pH 6.8, at room temperature, 22°. The sperm suspensions were washed twice with gentle centrifugation at 800g for 15 min and placed in a plastic vial. The spermatozoal suspensions were used in the tests between 1-24 hr after removal from the live clams. Microscopic observations of the pattern of agglutination were recorded 30-45 min after mixing 1 drop of phytagglutinin with 1 drop of sperm suspension to which had been added 1 drop of methylene blue. Direct saline agglutination tests against human erythrocytes were carried out at room (20-22°) temperature. Human erythrocytes were washed 3 times with physiological saline and a 2% suspension of blood cells, groups A, B, and O were used as the test panel. Equal parts of seed extract and cell suspension were mixed using the test tube technique. The mixture was incubated at room temperature for 30 min and centrifuged in the Sero-Fuge for 30 sec. The sedimented cells were gently dislodged and ob-

served macroscopically. One drop of a 5% solution of polyvinylpyrrolidone was added to all negative or weakly positive tests to detect any changes in agglutination reaction. Titrations were performed to determine the agglutination titer.

Direct inhibition tests were carried out at room (20-22°) temperature. Solutions of glucose, alpha lactose, *N*-acetyl glucosamine, and raffinose were prepared and diluted to a final concentration of 80 mmoles/ml. One drop of each sugar solution was added to 1 drop of lectin that had been mixed with 1 drop of spermatozoal suspension to note any change or inhibition in the pattern of agglomeration.

Seeds selected for this study were obtained from the United States, Mexico, Ecuador, Chile, and Uruguay, and represent 18 different botanical families. Of the 50, 27 extracts were prepared from legumes or belong to the family Leguminosae. The seeds include: *Acacia baileyana*, *Acacia brachybotrya*, *Acacia cyclops* nos. 823 and 822, *Acacia decora*, *Acacia discolor*, *Acacia* sp., *Agave Victoriae Reginae*, *Andromeda glaucophylla*, *Annona cherimola*, *Avena amarilla*, *Basiloxylon braziliensis*, *Bauhinia candicans*, *Beta* sp., *Brassica oleracea* var. *capitata*, *Caesalpinia guardinerlana*, *Caesalpinia peltophoroides*, *Cassia appendiculata*, *Cassia sylvestris*, *Cebeda bordenave*, *Cichorium intybus*, *Citrus aurantifolia*, *Criolla* sp., *Curpessus lamerlina*, *Cybistax aritisyphilitica*, *Delphinium hesperium*, *Dolichos lablab*, *Ephedra aspera*, *Eriosema* sp., *Lupinus succulentes*, *Melilotus alba*, *Mucuna pruriens*, *Penstemon Bridgesii*, *Phaseolus angularis*, *Phaseolus limensis* nos. 805 and 777, *Phaseolus onungo*, *Puerro porrum*, *Pyrus* sp., *Ricinus* sp., *Rynchosia minima*, *Spondias lutea*, *Tephrosia brevipes*, *Tephrosia cardida*, *Tilandsia* sp., *Tretium* sp.,

TABLE I. Reactivity of Phytagglutinins against Clam Spermatozoa and Human Blood Group Antigens.

	Total no.
Agglomeration (tail to tail)	23/50*
No agglomeration	27/50
Nonspecific for human blood groups	13/50
Specific for human blood groups	6/50
Nonreactive against human red blood cells	29/50
Hemolytic for human red blood cells	2/50

* Total number (50) of lectins tested, 23/50 indicates 23 of 50 phytagglutinins were specific for clam spermatozoa and showed a pattern of tail to tail agglomeration.

Tretium sp., *Ulex europaeus*, Legume nos. 1123 and Legume 1142.

Results and Discussion. The results showed that no spontaneous head to head nor tail to tail agglutination of spermatozoa was noted when microscopic observations were performed 1–24 hr after the sperm had been collected from either species, *Venus* or *Mytilus*. When 50 seed extracts were tested for reactivity against the sperm of either species, 23 showed a head to head type of agglutination pattern, whereas 27 had no visible effect. Of the 50 extracts used to detect probable antigens on the spermatozoa, 13 agglutinated the erythrocytes of all human blood groups and were classified as nonspecific (Table I); 5 contained anti-H + anti-B + anti-A agglutinins in titers ranging from 1:16 to 1:256 (Table II); one was specific for the B antigen; and 29 showed no reactivity against human blood groups. Although 2 of the 50 extracts tested for spermatozoan agglutination were hemolytic for human erythrocytes, they did not lyse the sperm. In those seed extracts that exhibited between 80–100% degree of agglutination, raffinose, in a concentration of 80 mmoles/ml, was found to be the most inhibitory of the 4 saccharides tested. It inhibited the largest percentage of aggregates formed between sperm and lectin. Galactose exhibited the least inhibitory effect. Curiously, all the saccharides appeared to inhibit the agglutination between the clam sperm and the seed extract prepared from *Acadia Baileyana* (Table III).

Since *Dolichos lablob* was the only seed that remained in sufficient quantity, this extract was absorbed with A, B, and O human erythrocytes and then retested for agglutinating properties against the clam spermatozoa of *Mytilus mytilis*. After absorption techniques were used, there was a 20% agglutination pattern noted between the lectin and sperm. We were not successful in removing every trace of the A₁ agglutinating ability of the lectin and the results are not conclusive. With this evidence, it cannot be stated that this phytagglutinin detected antigens present on the sperm spermatozoa different from those present on human erythrocytes. We intend to investigate this aspect of the problem further.

The specific mechanisms of agglutination between phytagglutinin and erythrocytic antigen or phytagglutinin and spermatozoic antigens is not clearly understood at this time. Why the patterns of agglutination vary between certain seed extracts and tissue antigens warrants further investigation.

Kashiwabara detected a tail to tail type of agglomeration of bull spermatozoa by testing phytagglutinins from soy bean. Our results showed a head to head type of agglutination using both seed extracts from legumes and other sources on clam spermatozoa. Until the agglutinating substances of both the head and tail parts of various species are characterized by immunochemical means, this phenomenon of agglutination cannot be adequately explained.

Summary. Sperm suspensions of *Venus mercenaria* and *Mytilus mytilis* were agglutinated by 23 of 50 different phytagglutinins exhibiting a head to head type of agglutina-

TABLE II. Titers of Phytagglutinins against Human Blood Groups.

Genus and species	Reactivity in saline (22°)
1. <i>Andromeda glaucophylla</i>	Anti-H (1:64) >B>A
2. <i>Beta</i> sp.	Anti-H (1:16) >B>A
3. <i>Brassica oleraceae</i>	Anti-H (1:4) >B>A
4. <i>Eriosema</i> so.	Anti-A (1:8) >H>B
5. <i>Ephedra aspera</i>	Anti-B (1:8)
6. <i>Ricinus</i> sp.	Anti-H (1:256) >A>B

TABLE III. Comparison of the Inhibitory Effects of Four Saccharides on the Agglutinating Activity of Phytagglutinins and Clam Spermatozoa.

Genus	Before saccharide	Agglutination (%) after addition of saccharide ^a			
		Lactose	Galactose	Glucosamine	Raffinose
<i>Andromeda glaucophylla</i>	90-100	50	90	60	10
<i>Dolichose lablob</i>	90-100	90-100	90-100	60	5
<i>Acacia brachybotyra</i>	80-90	10-20	50	10	10
<i>Acacia baileyana</i>	80	50	50	50	50

^a Concentration of saccharide = 80 mmoles/ml.

ting pattern. Of the 4 saccharides tested, raffinose proved most inhibitory to the agglutination reaction. Five of the seed extracts contained human anti-H, anti-B, and anti-A agglutinins and one was weakly specific for the human B antigen.

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