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SIZE-DEPENDENT HAEMAGGLUTINATING ACTIVITY IN THE HAEMOLYMPH FROM SUB-ADULT BLUE SHRIMP (*PENAEUS STYLIROSTRIS* STIMPSON)

FRANCISCO VARGAS-ALBORES, ANTONIA GUZMÁN and JOSÉ LUÍS OCHOA
Division of Experimental Biology, Center of Biological Research, P.O. Box 128, La Paz, BCS, 23000, México. Tel.: (682) 5-36-33; Fax: (682) 5-36-25

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Abstract—1. The blue shrimp (*Penaeus stylirostris*) haemolymph is capable of agglutinating the red blood cells of several vertebrates to different titres. However, the haemagglutinin is considered non-specific because it is incapable of differentiating erythrocytes of human blood types A, B and O.

2. Haemagglutinating activity and serum protein content were determined for male and female blue shrimp ranging in size from 8.5 to 16 cm. Haemagglutinating activity decreased significantly with animal size, while protein content was unaffected.

3. The above finding is probably related to maturation of the immune system and could explain the higher susceptibility of young shrimp to parasitic and viral diseases.

INTRODUCTION

The haemolymph of invertebrates contains agglutinins which have been proposed as an important component of its humoral defence system in contrast to vertebrate response which is characterized by immunoglobulins (Rögener and Uhlenbruck, 1984; Vasta and Marchalonis, 1983; Vasta *et al.*, 1984b; Ratcliffe, 1985; Renwratz, 1983, 1986; Sminia and van der Knaap, 1987; Olafsen, 1988). These agglutinins have the ability to precipitate glycoconjugates and to agglutinate cells, bacteria (Bayne, 1980), virus (McCumber *et al.*, 1979) and protozoa (Pereira *et al.*, 1981). In this way, they facilitate phagocytosis by opsonization (Coombe *et al.*, 1984; Renwrantz and Mohr, 1978; Rögener and Uhlenbruck, 1984), or by locating on the haemocyte surface (Vasta *et al.*, 1982, 1984a; Richards and Renwrantz, 1991).

The involvement of agglutinins in the defense system of invertebrates has been supported by studies on the regulation of the synthesis of these proteins after challenging the animal by injury or pathogens (Komano *et al.*, 1980, Kubo *et al.*, 1984) and by studies that show changes in the serum level of haemagglutinating activity during infection (Loker and Hertel, 1987; Couch *et al.*, 1990). However, only a few studies have been published on changes in haemagglutinating activity during the course of development, or when influenced by the age, sex or size of the animal (Komano *et al.*, 1980, 1983; Bellah *et al.*, 1988; Muramoto *et al.*, 1991). Although some serum agglutinins have been studied and characterized from marine crustaceans such as *Homarus americanus* (Hall and Rowlands, 1974), *Macrobrachium rosenbergii* (Vasta *et al.*, 1983) and *Cancer antenarius* (Ravindranath *et al.*, 1985), little information is available about the serum level or activity of these proteins. This study determines the presence of haemagglutinating activity in the blue shrimp (*Penaeus stylirostris*) haemolymph and evaluates its reaction against human and animal erythrocytes, measures the total protein and haemagglutinin in the haemolymph of this decapod, and correlates these values according to shrimp size.

MATERIALS AND METHODS

Shrimp collection and haemolymph extraction

Male and female shrimp (*Penaeus stylirostris*) were collected from San Carlos Bay, B.C.S., México. The water temperature was 21°C with a salinity of 36 ppt. The haemolymph (50–200 μl) was obtained as previously described (Vargas-Albores and Ochoa, 1992) by inserting a sterile 25 gauge needle in the pleopod base of the first abdominal segment. Each sample was put into a 1.5 ml Eppendorf tube and kept at 10°C for transporting. In the laboratory, the cell-free haemolymph, or serum, was recovered by centrifugation for 10 min in an Eppendorf microfuge and subsequent removal of the cell clot. Only the haemolymph from intermoult shrimp was used. For the haemagglutination experiments, we pooled the haemolymphs of 40 shrimp (male and female), obtained as described above.

Experimental design

To determine the influence of animal size on total serum protein concentration and haemagglutinating activity, the whole shrimp population and each sex was separated into three groups of different sizes: group A was comprised of animals ranging in size from 8.5 to 11.0 cm; group B from 11.1 to 13.5 cm, and group C from 13.6 to 16 cm.

Statistical comparison was accomplished using one-way analysis of variance (ANOVA-1W) or Student's *t*-test. Statistical differences between groups were determined using Duncan's multiple range test (Sokal and Rohlf, 1981). Differences were considered to be significant if the *P* value was less than 0.05.

Chemical analysis

The total protein content was measured according to Lowry *et al.* (1951) using bovine serum albumin (BSA) as a standard.

Table 1. Haemagglutinating activity of the blue shrimp hemolymph against human and other animals erythrocytes

Erythrocyte	Titre	RHA
Human A	1:32	1.0
Human B	1:32	1.0
Human O	1:32	1.0
Cow	1:8	0.2
Duck	1:8	0.2
Mouse	1:256	8.0
Rabbit	1:16	0.5
Rat	1:8	0.2
Sheep	1:8	0.2

RHA = Relative haemagglutinating activity (human = 1.0).

Haemagglutination test

The human and animal blood samples (except for mice) were obtained by venous puncture, then collected and stored in sterile Alsever's solution. The mouse blood was obtained by cardiac puncture and stored the same way. Before using, the cells were washed twice by centrifugation (800 *g*, 10°C, 10 min) with saline solution (NaCl 0.15 M) and twice with Tris buffered saline plus calcium (TBS-Ca: 50 mM Tris–HCl, 100 mM NaCl, 10 mM $CaCl_2$, pH 7.5). Finally, the red blood cells (RBC) were suspended to 2% (v/v) in TBS-Ca. The haemagglutination assays were performed on U-plates (Falcon). Two-fold serial dilutions of the shrimp serum were made in 25 μl of TBS-Ca; then, 25 μl of a 2% suspension of erythrocytes were added. The plates were incubated at room temperature (26 ± 2°C) for 1 hr. The control was the substitution of shrimp serum by TBS-Ca. The agglutination titre was recorded as the reciprocal of the last dilution, giving evidence of agglutination at 1 hr of incubation.

RESULTS

Preliminary experiments showed that the blue shrimp (*Penaeus stylirostris*) haemolymph is able to agglutinate human and other animal erythrocytes. The buffer used in the haemagglutination test was supplied with 10 mM Ca^{2+} because in crustaceans, this cation is depleted during clot formation (Durliat and Vranckx, 1981). In addition, a reduction in the haemagglutinating activity was observed when the haemolymph was dialysed against EDTA. To prove the presence of haemagglutinating activity on the blue shrimp haemolymph, the serum from 40 shrimps (male and female) was pooled. A panel of erythrocytes from human and six other species of mammals was used to determine the species/type selectivity of this haemagglutinating activity. Apparently, the haemagglutinating activity was not specific because human RBC of types A, B and O were agglutinated in the same way (Table 1). On the other hand, the blue shrimp haemolymph displayed the highest agglutinability against mouse erythrocytes but did not react with horse RBC. All other erythrocytes from the tested animal species exhibited lower titre than human RBC.

Table 2. Total protein content and haemagglutinating activity (mean ± SD) of the blue shrimp haemolymph

	Male ($N = 14$)	Female ($N = 23$)	P
Size (cm)	12.93 ± 1.49	12.65 ± 1.99	
Weight (g)	15.59 ± 5.06	15.50 ± 5.70	
Total protein (g/l)	53.05 ± 7.92	53.69 ± 7.19	0.8029
Haemagglutinating activity	4.36 ± 1.55	4.65 ± 2.33	0.2163

The titres are expressed as the inverse of maximum dilution which gives positive agglutination. Differences (P) were established by Student's t-test.

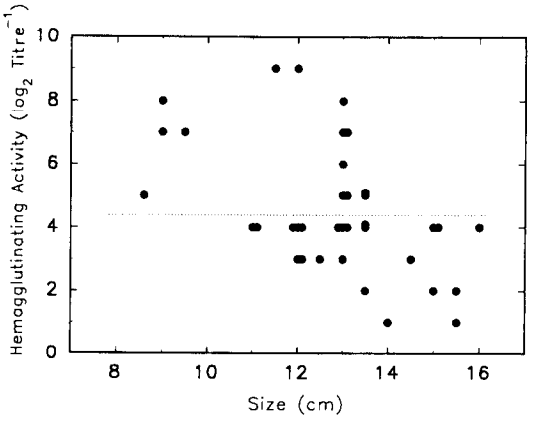

Fig. 1. Haemagglutinating activity of the blue shrimp (*Penaeus stylirostris*) haemolymph. The dotted line represents the mean of all data.

The haemolymph of 23 female and 14 male shrimps were used to determine individual haemagglutinating activity and total protein. Shrimps ranged in size between 8.5 and 16 cm, weighing between 5.4 and 27.2 g. The data obtained are shown in Table 2. The results, analysed by ANOVA test, do not indicate significant sex differences ($P > 0.10$) for either protein content or haemagglutinating activity.

As expected, a good correlation between size and weight was observed in both male ($r = 0.9295$) and female ($r = 0.9652$), as well as in mixed populations ($r = 0.9502$). For this reason, further comparisons refer to size only, assuming that similar results could be obtained with reference to weight. In contrast, a poor correlation between size (or weight) and haemagglutinating activity or protein content was found when the data were analysed separately for female, male, or mixed shrimp populations. Nevertheless, despite the lack of a good linear correlation between size and haemagglutinating activity ($r = -0.5312$), a negative tendency was observed, indicating a size dependency (Fig. 1).

To confirm this, the animals were classified into three groups according to size: A (8.5–11 cm), B (11.1–13.5 cm), and C (13.6–16 cm) and their corresponding data were analysed by ANOVA one-way. The results showed important significant differences ($P = 0.0013$) in haemagglutinating activity levels (Fig. 2), but no differences were observed in protein content. This was also observed when male and female shrimps were analysed separately.

A relationship between protein content and haemagglutinating activity was established. The haemagglutinating unit (HU) could be defined as the minimal protein quantity capable of causing agglutination (protein concentration/titre). Using this quotient, the significant difference was maintained (Fig. 2b), indicating that haemagglutinating activity was independent of protein content.

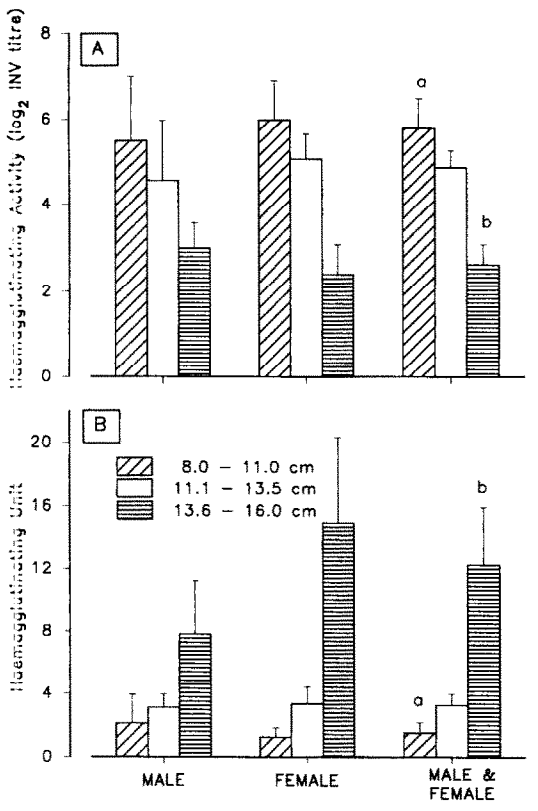

Fig. 2. Size-dependence haemagglutinating activity in the haemolymph from blue shrimp (*Penaeus stylirostris*). The animals were separated into three groups and the titre (A) or haemagglutinating unit (B) were analysed by ANOVA 1W. Differences ($P < 0.05$) are indicated by letter on S.E. bar.

DISCUSSION

Modifications in serum component levels have been observed in invertebrates under different physiological conditions. Example of these include changes in protein composition of American cockroaches haemolymph by immunization with proteins (George *et al.*, 1987) and increases in both ecdysteroids and total protein of haemolymph from *Penaeus vannamei* during proecdysis (Chan *et al.*, 1988). Another good example was the observed decrease in both protein and sugar content of molluscan haemolymph during infection (Cheng *et al.*, 1978, 1983; Muley and Fen, 1981). Some authors support the idea that this phenomenon is produced when parasites consume the serum protein and carbohydrate (Becker and Hirtbath, 1975; Muley and Fen, 1981). However, environmental factors such as temperature and salinity may also influence haemolymph protein and carbohydrate concentration in mollusca (Fisher and Newell, 1986) and in crustacea (Spicer and Taylor, 1987). Thus, it is possible that a resulting reduction in protein and/or carbohydrate concentration can facilitate the establishment of infection. Modifications in serum component levels of shrimps have also been observed under special physiological or environmental conditions. The diet of *Penaeus vannamei* (Cruz-Ricque *et al.*, 1989) and the molt stage in *Penaeus japonicus* (Chan *et al.*, 1988) can alter the protein and/or sugar serum levels. In *P. stylirostris* the influence of animal size on serum osmolality has been observed (Vargas-Albores and Ochoa, 1992). However, in this work, modifications in the total serum protein content was not detected in *P. stylrostris*, indicating that this parameter is unaffected by animal size.

Osmotic pressure in shrimp is maintained by proteins, amino acids and ions in the haemolymph. In the blue shrimp, the ionic components have a good correlation with haemolymph osmolality (Vargas-Albores and Ochoa, 1992) and according to the results shown here, the participation of protein in this phenomenon is apparently low. Like other penaeid species (Castille and Lawrence, 1981a), the blue shrimp is a better osmoregulator during larval stages than when adult. However, they migrate from estuarine to ocean waters where the salinity is higher than isosmotic point. This migration is motivated more by reproductive than by nutritional or osmotic factors (Castille and Lawrence, 1981b). Thus, while the haemolymph ionic component is modified according to animal size and osmotic capacity, alterations in the protein content appear to be independent of these factors.

Similar to agglutinin from other crustaceans (Vasta and Marchalonis 1983), blue shrimp agglutinin is able to agglutinate human and other vertebrate erythrocytes, but lacks serological specificity against human blood types A, B and 0 (Table 1). Since lectins are commonly found in body fluids and, by binding with defined carbohydrate, are able to specifically agglutinate bacteria (Bayne, 1980), viruses (McCumber *et al.*, 1979) and protozoa (Pereira *et al.*, 1981) they were initially considered to function as part of a defense system (Ratcliffe, 1985; Renwrantz, 1986; Olafsen, 1988). Some invertebrate lectins have been shown to function as opsonins (Coombe *et al.*, 1984; Renwrantz and Mohr, 1978; Rögener and Uhlenbruck, 1984; Renwrantz, 1986) and this led some researchers to consider lectin binding as a first step in the recognition of foreign material (Rögener and Uhlenbruck, 1984; Vasta and Marchalonis, 1983; Ratcliffe, 1985; Renwratz, 1983, 1986; Sminia and van der Knaap, 1987; Olafsen, 1988).

When animal size was correlated to haemagglutinating activity, an important difference was observed. The mean haemagglutinating activity for the whole population studied was 4.48, yet animals measuring less than 11.1 cm showed haemagglutinating activity values above the mean (Fig. 1). In contrast, animals 15 cm or larger showed little haemagglutinating activity. Thus, it is possible to establish that shrimp size has a direct influence on haemagglutinating activity: from smaller shrimps (less than 11 cm) having elevated activity (1:32–1:256), to large shrimps (above 14.5 cm) having a lower activity (1:2–1:16). Since size is an indirect parameter of age or maturation, it follows that our results agree with other studies which correlated haemagglutinating activity with age in the erisilkworm (Bellah *et al.*, 1988) and with ovarian development in the acorn barnacle (Muramoto *et al.*, 1991). In the erisilkworm (*Philosamia ricini*), the haemagglutinating titres of the haemolymph were independent of sex (Bellah

et al., 1988), similar to blue shrimp where sex differences were not observed (Table 2). In the acorn barnacle (*Megabalanus rosa*) ovarian maturation was associated with maximum haemagglutinin levels, and decreased lectin levels during the spawning season (Muramoto *et al.*, 1991).

In the absence of information on shrimp agglutinins, and their physiological role, it is difficult to explain their modification in blue shrimp. However, it might be explained by associating our observations with those of other invertebrate studies. Dikkeboom *et al.* (1984), studying the maturation of the immune system of the pond snail (*Lymnaea stagnalis*), found that juveniles had fewer circulating haemocytes that adults. In addition, the cells from juvenile snails showed both mitotic activity and characteristics of less differentiated cells. From these results, they concluded that juvenile snails have a less developed cellular immune system than adults. This is consistent with a previous report on *Biomphalaria glabrata* (Stumpf and Gilbertson, 1978), where a low peroxidase activity is also manifested in the haemocytes from juvenile snails. This reduction in enzymatic activity and immaturity of the immune system is characterized by a higher susceptibility to the parasite *Schistosoma mansoni* (Granath and Yoshino, 1983).

In the blue shrimp, the highest frequency of viral and parasitic disease is found during the early stages of development (Lightner, 1983). If the maturation of the shrimp immune system is similar to mollusca, it is possible that an immature immune system at the cellular level is responsible for the susceptibility found in larval and juvenile blue shrimp. The humoral response is probably more active in the early stages and decreases as the cellular mechanisms (and animal) mature. This hypothesis may explain the decrease in agglutinin levels since these proteins are responsible for humoral responses in invertebrates.

Such immune system behaviour is not exclusive to invertebrate animals. It is also characteristic of immune response development in higher animals where a maturation of the cellular response is followed by maturation of the organism and a replacement of the humoral response (Kay, 1978; Stites and Cadwell, 1978). Therefore, in addition to a functional homology between vertebrate immunoglobulins and invertebrate agglutinins, a similarity in the maturation of both immune systems can be observed. The participation of the serum agglutinins, and the humoral response in early developmental stages, support the idea that, despite the diversity of immune response, the immune system of both invertebrate and vertebrate animals may have a similar development.

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REFERENCES

Bayne C.. (1980) Molluscan immunity: induction of elevated immunity in the land snail (*Helix*) by injection of bacteria (*Pseudomonas aeruginosa*). *Dev. comp. Immun.* **4,** 43–54.

Becker W. and Hirtbach E. (1975) Effect of starvation on total protein and hemoglobin concentration in the hemolymph of *Biomphalaria glabrata*. *Comp Biochem. Physiol.* **51,** 809–814.

Bellah M. E. M., Ridi R. E., Abou-Elela R. and Cooper E. L. (1988) Age related occurrence of natural agglutinins in the erisilkworm, *Philosamia ricini*. *Dev. comp. Immun.* **12,** 707–717.

Castille F. L. Jr and Lawrence A. L. (1981a) The effect of salinity on the osmotic, sodium and chloride concentrations in the hemolymph of euryhaline shrimp of the genus *Penaeus*. *Comp. Biochem. Physiol.* **68A,** 75–80.

Castille F. L. Jr and Lawrence A. L. (1981b) A comparison of the osmotic, sodium and chloride concentrations in urine and hemolymph of *Penaeus setiferus* (L.) and *Penaeus stylirostris* Stimpson. *Comp. Biochem. Physiol.* **70A,** 525–528.

Chan S. M., Rankin S. M. and Keeley L. L. (1988) Characterization of the molt stages in *Penaeus vannamei*: setogenesis and hemolymph levels of total protein, ecdysteroids, and glucose. *Biol. Bull.* **175,** 185–192.

Cheng T. C., Guida V. G. and Gerhart P. L. (1978) Aminopeptidase and lysozyme levels and serum protein concentrations in *Biomphalaria glabrata* (Mollusca) challenged with bacteria. *J. Invertebr. Path.* **32,** 297–302.

Cheng T. C., Howland K. H., Moran H. J. and Sullivan J. T. (1983) Studies on parasitic castration: aminopeptidase activity levels and protein concentration in *Ilyanessa obsoleta* (mollusca) parasitized by larval trematodes. *J. Invertebr. Path.* **42,** 52–50.

Coombe D. R., Ey L. and Jenkin C. R. (1984) Particles recognition by haemocytes from the ascidian *Botrylloides leachii*: evidence that *B. leachii* HA-2 agglutinin is opsonic. *J. comp. Physiol.* **154B,** 509–521.

Couch L., Hertel L. A. and Loker E. S. (1990) Humoral response of the snail *Biomphalaria glabrata* to trematode infection: observations on a circulating hemagglutinin. *J. exp. Zool.* **255,** 340–349.

Cruz-Rique L. E., Guillaume J. and van Wormhoudt A. (1989) Effect of squid extracts of the time course appearance of glucose and free amino acids in haemolymph in *Penaeus japonicus* after feeding: preliminary results. *Aquaculture* **76,** 57–66.

Dikkeboom R., van der Knaap W. P. W., Meuleman E. A. and Sminia T. (1984) Differences between blood cells of juvenile and adult specimens of the pond snail *Lymnaea stagnalis*. *Cell Tissue Res.* **238,** 43–47.

Durliat M. and Vranckx R. (1981) Action of various anticoagulants on hemolymphs of lobsters and spiny lobsters. *Biol. Bull.* **160,** 55–68.

Fisher W. S. and Newell R. I. E. (1986) Seasonal and environmental variation in protein and carbohydrate levels in the hemolymph from american oysters (*Crassostrea virginica* Gmelin). *Comp. Biochem. Physiol.* **85A,** 365–372.

George J. F., Karp R. D., Rellaha B. L. and Lessard J. L. (1987) Alteration of the protein composition in the haemolymph of American cockroaches immunized with soluble proteins. *Immunology* **62,** 505–509.

Granath W. O. and Yoshino T. P. (1983) Characterization of molluscan phagocyte subpopulations based on lysosomal enzyme markers. *J. exp. Zool.* **226,** 205–210.

Hall J. L. and Rowlands D. T. Jr (1974) Heterogeneity of lobster agglutinins. I. Purification and physicochemical characterization. *Biochemistry* **13,** 821–827.

Kay M. M. B. (1978) Aging and the decline of immune responsiveness. In *Basic and Clinical Immunology* (Edited by Fudenberg H. H., Stites D. P., Caldwell J. L. and Wells J. V.), pp. 322–333. Lange Medical Pub, Los Altos, CA.

Komano H., Mizuno D. and Natori S. (1980) Purification of lectin induced in the hemolymph of *Sarcophaga peregrina* larvae on injury. *J. biol. Chem.* **255,** 2919–2924.

Komano H., Nozawa R., Mizuno D. and Natori S. (1983) Measurement of *Sarcophaga peregrina* lectin under various physiological conditions by radioimmunoassay. *J. biol. Chem.* **258,** 2143–2147.

Kubo T., Komano H., Okada M. and Natori S. (1984) Identification of hemagglutinating protein and bactericidal activity in the hemolymph of adult *Sarcophaga peregrina* on injury of body wall. *Dev. comp. Immun.* **8,** 282–291.

Lightner D. V. (1983) Diseases of cultured penaeid shrimp. In *CRC Handbook of Mariculture. Vol. I. Crustacean Aquaculture.* (Edited by McVey J. P.), pp. 289–320. CRC Press, U.S.A.

Loker E. S. and Hertel L. A. (1987) Alterations in *Biomphalaria glabrata* plasma induced by infection with the digenetic trematode *Echinostoma paraensei*. *J. Parasitol.* **73,** 503–513.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with Folin phenol reagent. *J. biol. Chem.* **193,** 265–275.

McCumber L. J., Hoffmann E. M. and Clem L. W. (1979) Recognition of viruses and xenogeneic proteins by the blue crabs, *Callinectes sapidus*: a humoral receptor for T2 bacteriophage. *J. Invertebr. Path.* **33,** 1–9.

Mulvey M. and Fen S. Y. (1981) Hemolymph constituents of normal and *Proctoeces maculatus* infected *Mytilus edulis*. *Comp. Biochem. Physiol.* **70A,** 119–125.

Muramoto K., Kado R., Takei Y. and Kamiya H. (1991) Seasonal changes in the multiple lectin compositions of the acorn barnacle *Megabalanus rosa* as related to ovarian development. *Comp. Biochem. Physiol.* **98B,** 603–607.

Olafsen J. A. (1988) Role of lectin in invertebrate humoral defense. *Am. Fish. Soc. Special Publ.* **18,** 189–205.

Pereira M. E. A., Andrade A. F. B. and Ribero J. M. C. (1981) Lectins of distinct specificity in *Rhodnius prolixus* interact selectively with *Trypanosoma cruzi*. *Science* **211,** 597–600.

Ratcliffe N. A. (1985) Invertebrate immunity—a primer for the non-specialist. *Immunol. Lett.* **10,** 253–270.

Ravindranath M. H., Higa H. H., Cooper E. L. and Paulson J. C. (1985) Purification and characterization of an O-acetylsialic acid-specific lectin from a marine crab *Cancer antenarius*. *J. biol. Chem.* **260,** 8850–8856.

Renwrantz L. (1983) Involvement of agglutinins (lectins) in invertebrate defense reactions: the immuno-biological importance of carbohydrate-specific binding molecules. *Dev. comp. Immun.* **7,** 603–608.

Renwrantz L. (1986) Lectins in molluscs and arthropods: their occurrence, origin and roles in immunity. *Symp. Zool. Soc. Lond.* **56,** 81–93.

Renwrantz L. and Mohr W. (1978) Opsonising effects of serum and albumin gland extracts on the elimination of human erythrocytes from the circulation of *Helix pomatia*. *J. Invertebr. Path.* **31,** 164–170.

Richards E. H. and Renwrantz L. R. (1991) Two lectins on the surface of *Helix pomatia* haemocytes: a Ca^{2+}-dependent, GalNAc-specific lectin and a Ca^{2+}-independent, mannose 6-phosphate-specific lectin which recognizes activated homologous opsonins. *J. comp. Physiol.* **161B,** 43–54.

Rögener W. and Uhlenbruck G. (1984) Invertebrate lectins: the biological role of a biological rule. *Dev. comp. Immun.* **3,** 159–164.

Sminia T. and van der Knaap W. P. W. (1987) Cells and molecules in molluscan immunology. *Dev. comp. Immun.* **11,** 17–28.

Sokal R. R. and Rohlf F. J. (1981) *Biometry*. W. H. Freeman and Co., New York.

Spicer J. J. and Taylor C. (1987) Ionic regulation and salinity related changes in haemolymph protein in the semi-terrestrial beachflea *Orchestia gammarellus* (Pallas) (Crustacea: amphipoda). *Comp. Biochem. Phyiol.* **88A,** 243–246.

Stites D. P. and Cadwell J. L. (1978) Phylogeny and ontogeny of the immune response. In *Basic and Clinical Immunology* (Edited by Fudenberg H. H., Stites D. P. Caldwell J. L. and Wells J. V.), pp. 141–153. Lange Medical Publishers, Los Altos, CA.

Stumpf J. L. and Gilbertson D. E. (1978) Hemocytes of *Biomphalaria glabrata*: factors affecting variability. *J. Invertebr. Path.* **32,** 177–181.

Vargas-Albores F and Ochoa J. L. (1992) Variations of pH, osmolality, sodium and potassium concentration in the haemolymph of sub-adult blue shrimp (*Penaeus stylirostris*) according to size. *Comp. Biochem. Physiol.* **102A,** 1–5.

Vasta G. R., Cheng T. C. and Marchalonis J. J. (1984a) A lectin on the hemocyte membrane of the oyster (*Crassostrea virginica*). *Cell. Immun.* **88,** 475–488.

Vasta G. R. and Marchalonis J. J. (1983) Humoral recognition factors in the Arthropoda. The specificity of Chelicerata serum lectins. *Am. Zool.* **23,** 157–171.

Vasta G. R., Marchalonis J. J. and Kohler H. (1984b) Invertebrate recognition protein cross-reacts with an immunoglobulin idiotype. *J. exp. Med.* **159,** 1270–1276.

Vasta G. R., Sullivan J. T., Cheng T. C., Marchalonis J. J. and Warr G. W. (1982) A cell membrane-associated lectin of the oyster hemocyte. *J. Invertebr. Path.* **40,** 367–377.

Vasta G. R., Warr G. W. and Marchalonis J. J. (1983) Serological characterization of humoral lectins from the freshwater prawn *Macrobrachium rosembergii*. *Dev. comp. Immun.* **7,** 13–20.