Journal of Evolutionary Biochemistry and Physiology, Vol. 41, No. 4, 2005, pp. 428—436. Translated from Zhurnal Evolyutsionnoi Biokhimii i Fiziologii, Vol. 41, No. 4, 2005, pp. 343—349.

COMPARATIVE AND ONTOGENIC PHYSIOLOGY

Encystment *in vitro* of the Cercariae *Himasthla elongata* (Trematoda: Echinostomatidae)

A. M. Gorbushin* and I. A. Levakin**

** St. Petersburg State University, Russia*

*** Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia*

Received June 10, 2003

Abstract—Trigger and toxic effects of *Mytilus edulis* (Bivalvia) hemolymph on encystment of cercariae *Himasthla elongata* obtained from infestated *Littorina littorea* (Prosobranchia) was evaluated as a result of 24-h experiments *in vitro*. The contact of *H. elongata* larvae with the whole hemolymph or mussel acellular plasma led to an intensive transformation of cercariae into metacercariae. In both tested media, the cercariae had to complete the encystment phase as fast as for 2 h, otherwise the risk of the larvae injury by humoral and cellular components of the mussel hemolymph would increase dramatically. The cercaria mortality after 24 h in the whole hemolymph was twice higher than in plasma (40% and 20%, respectively) and much higher than in the control medium (sea water). Both toxic and trigger effects of plasma was revealed to depend on its concentration, with the maximal larva mortality in the undiluted medium and with the highest number of successful transformations in the medium diluted more than 4 times. There is shown both the strong individual variability of toxicity of the individual mussel hemolymph for cercariae and the variability of the resistance to the toxic factors of the cercariae obtained from various *L. littorea* individuals. These experiments not only offer a method of the massive encystment of *H. elongata* cercariae, but also propose a perspective model for the study of the systemic defensive response of Bivalvia to invasion of multicellular parasite.

INTRODUCTION

The parasite infectivity and the host resistance are the most mysterious phenomena characterizing each mollusc—trematode system. The host specificity varies in very wide limits: some trematodes develop successfully in representatives of several mollusc families, while others, only in one or the limited number of species [1]. Analysis of compatibility of molluscs and trematodes is performed from several points of view: morphological [2], behavioral [3, 4], and physiological. The latter implies that after invasion into the mollusc organism the parasite has to resist to attacks of the host defense system that has rather effective mechanisms of elimination of the potential pathogen [5, 6].

The importance of the cellular and humoral components of the host defense in determining the compatibility level in the mollusc—trematode system has been confirmed in studies both *in vitro* and *in vivo* [6, 7]. The success in cultivation of some trematodes in xenic and axenic media [8, 9] allows performing analysis of fine mechanisms of interaction between a parasite and the host tissues. Provided that the main technical problems are overcome, the *in vitro* systems are very useful due to an evident possibility of the complete control of experimental conditions. However, in spite of the potential di-

0022-0930/05/4104-0428

versity of experimental models, studies of interactions of molluscs and trematodes have been so far focused on a small number of the mollusc—trematode combinations. Only three digenean families, Schistosomatidae, Echinostomatidae and Fasciolidae, selected because of their medical and veterinary significance, have received *in vitro* investigations. The diversity of mollusc hosts studied is even more limited; only basommathophoran snails (Pulmonata) have been used in experiments.

To gain a better insight into mechanisms that govern mollusc-trematode compatibility, it would be helpful to use a comparative approach and include in the analysis additional models, in which the host is phylogenetically distant from pulmonates. Since even those models already studied seem to differ from one to another [10]) and the level of host specificity has changed in the Diginea evolution [11], investigation of host parasite systems less specialized than pulmonate-based ones is very reasonable. In terms of this paradigm the most interesting are marine prosobranchia and lamellibranchia molluscs, first, because the main peculiarities of their internal defense system differ from those of pulmonate snails [12] and, second, in some cases their trematode parasites have well studied relatives infecting pulmonate hosts. The latter circumstance is especially essential for performing the adequate comparative analysis.

The trematode *Himasthla elongata* (Echinostomatidae) develops successively in three hosts: the prosobranch snail *Littorina littorea*, the lamellibranch mollusc *Mytilus edulis*, and lastly, in gulls [13]. Parthenogenetic reproduction takes place in periwinkle and is completed by the mass production of freely swimming larvae (cercariae). Mussels are infestated by the cercariae in the process of water filtration. Assuming the epithelial barrier is overcome, the cercaria must then contend with the host internal defense system that causes encapsulation and death of the invader in a nonsusceptible mussel. One of the specific mechanisms allowing cercariae to adequately resist the mollusc defence response is formation of the cyst in *M. edulis* muscle tissues, mostly in the foot and contractible parts of the mantle. However, details of the process of the cercaria encystment have been poorly studied. There is also little information on the toxicity of humoral and cellular components of *M. edulis* hemolymph to metazoan pathogens. In the present study, we used experiments *in vitro* to solve these problems. Our aims are not only to clarify the method for *in vitro* encystment of *H. elongata* cercariae, but also suggest promising model for the study of systemic defense response in Bivalves on invasion with metazoan parasite.

MATERIALS AND METHODS

Experimental animals. Snails *Littorina littorea* were collected at littoral near the White Sea Biological Station "Kartesh" of Zoological Institute of the Russian Academy of Sciences (the Chupa Bay, the Kandalaksha Gulf of the White Sea). Non-infected 5–7-year old *Mytilus edulis* individuals were collected in the same region from the commercial substrates (the Nikolskaya Bay). Periwinkles from the 5–19-year old group were divided into the infected (emitting cercariae) and non-infected, the former being used in the experiments as a source of trematode larvae. Both *L. littorea* (supplied with food *ad libitum*—fucoid seagrass) and *M. edulis* were kept until used in experiments in separate cages located 50 m away from each other.

Derivation of cercariae. To gain mass-emission of cercariae, infected periwinkles were removed from cages and kept overnight in humid cameras. Then snails were placed individually in 60 ml bowls filled with filtered sea water (FSW) of natural salinity (24‰). Two hours later the water with emitted cercariae was poured from the bowl into several conical tubes and cercariae were concentrated by placing tubes vertically in ice. Under 1–4°C larvae lost their motility and aggregate on the bottom of the tube. After warming up to normal temperature cercariae entirely recovered and showed normal reactions and swimming behavior. Two qualitatively different groups of larvae were tested in experiments: cercarial "clones"—larvae emitted by one host individual, and cercarial pool—ones obtained from several snail individuals and pooled together.

Effects of mussel hemolymph on cercariae encystment. Mytilus edulis hemolymph was collected from the posterior adductor using a sterile 1ml syringe with 0.8 mm diameter needle. A minimum 400 μl was sampled from each individual. To remove hemocytes, half of the hemolymph volume was centrifuged at 110 g for 10 min. The resulting su-

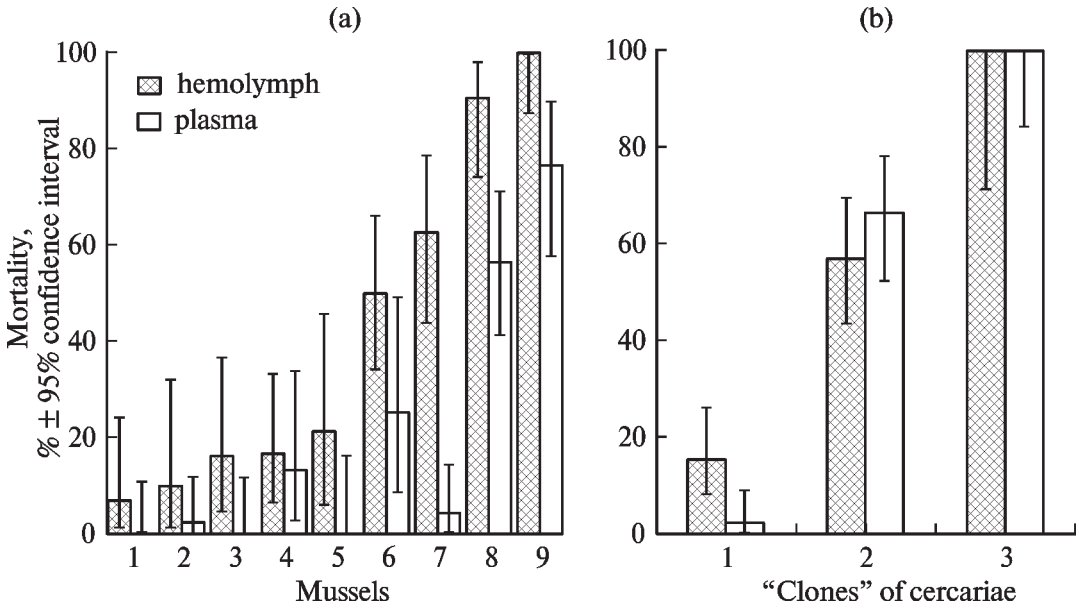

Fig. 1. Differences in survival of one cercaria "clone" in hemolymph and plasma of 9 individuals of mussels (a) and of three cercaria "clones" in hemolymph and plasma of one mussel individual (b). For simplicity, mussels and "clones" are arranged by their mortality.

pernatant was termed plasma. Larvae were exposed to whole hemolymph, plasma and diluted plasma to perform the test of the effects of mussel hemolymph on cercariae behavior and survival. About 30 cercariae of one "clone" in 10 μl of sea water were placed into each of 3 wells in a 96-wells flat-bottom plate. Within each set, first well received 100 μl of whole hemolymph, second—100μl of plasma of the same individual and the last well received 100μl of FSW, which served as a control. The remaining hemolymph and plasma was used for testing of other larva "clones." At 45 min, 2 and 24 h, cercarial condition was examined using a dissection and inverted microscopes. Surviving, body movement and encystment stage were assessed for each larva. In total, 15 cercarial "clones" (6859 larvae) and 40 mussel individuals were used in the experiment.

Estimation of plasma concentration-dependent effect on cercaria encystment was performed as follows. Cercarial pool (1754 larvae) was obtained from 4 infected periwinkles and plasma pool from 5 mussel individuals. The first well of 96-wells flat-bottom plate received 100 μl of undiluted plasma, in following wells the plasma was diluted by serial two-fold dilutions of the 100-μl sample with equal volume of FSW. After dilution of the pooled sample, 30–40 "pooled" cercariae in 10 μl of sea water were added in each well. FSW served as control medium. Cercariae condition was examined as above at 0.5, 1, 2, 4 and 24 h intervals. All experiments were carried out in thermostabilized chamber (12°C) under 24 h illumination with cold light sources.

Data analysis. The arcsin-square root transformed data from encystment experiments were analyzed with help of the statistical analysis software Statistica for Windows v5.5. Confidence intervals (95%) of means were retransformed after computation. Means were compared using two-tailed *t*-test for depended samples, significantly different pairs indicated by "*" (Fig. 3). In the case of plasma concentration-dependent effect determination, percentages of cercarial encystment and mortality were compared with control estimates using two-tailed Fisher exact test. The same approach was used in individual comparisons (see Figs. 1a, 1b). In the last case, confidence intervals (95%) for percentages were computed using Fisher exact formula [14].

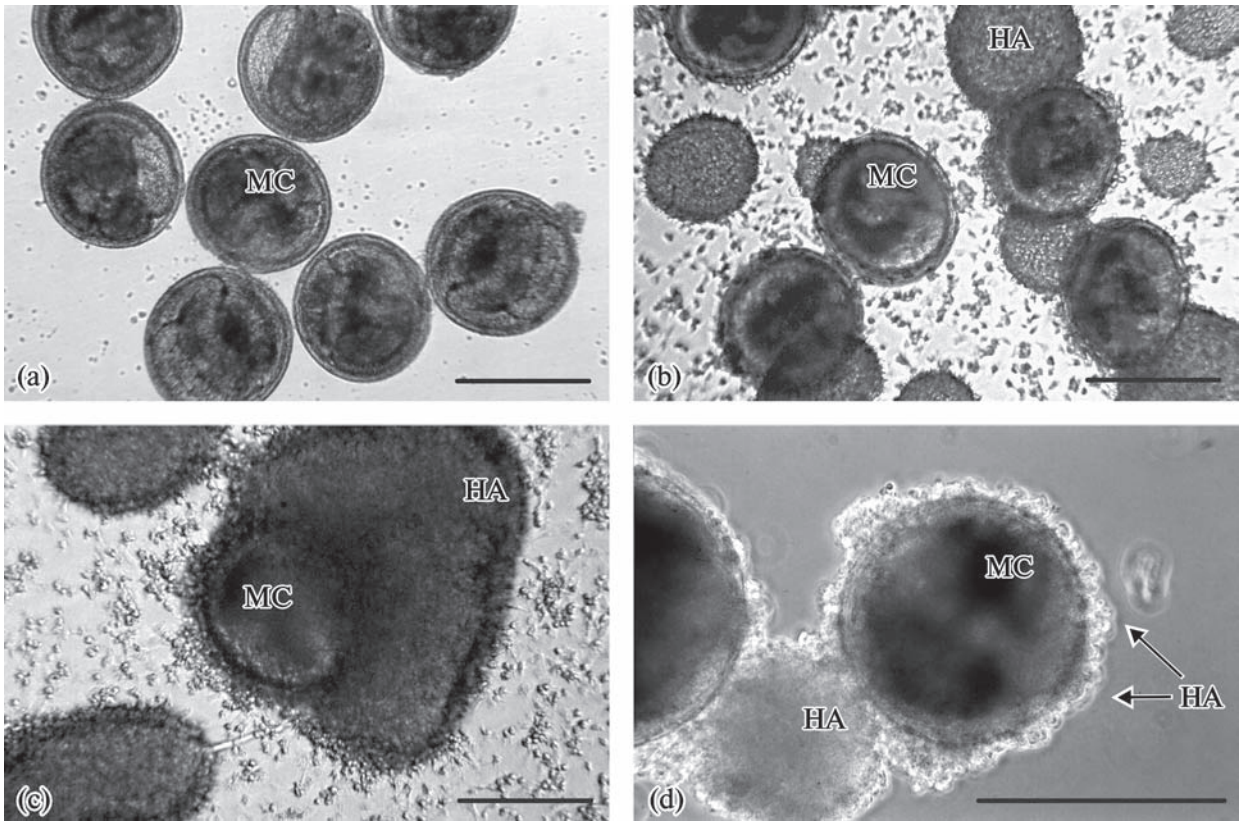

Fig. 2. The external view of *H. elongata* transformed *in vitro* in plasma (a), the whole hemolymph (b), and encapsulated by mussel hemocytes ((c) inverted microscope, (d) phase contrast). MC—Metacercariae, HA—hemocytes accumulation. Scale bar—200 μm.

RESULTS

Encystment triggering effect of hemolymph. Cercariae of *Himasthla elongata* in FSW (control medium) were observed actively swimming and sometimes attaching to a plastic surface or an air–water interface. Cercariae were rather motile in the beginning of the experiment but they progressively slowed down over 24 h. Towards the end of this period most of the larvae sunk to the well bottom and remained there, contracting slightly and failing to swim up. For FSW condition, the mean numbers of cercariae that had spontaneously encysted and that were dead after 24 h were 0.4% and 0.8% respectively.

Exposure of *H. elongata* cercariae to either whole hemolymph or plasma of blue mussel resulted in a vigorous increase in motility and in rate of change of swimming direction. In the case of contact with bottom or wall of a well, they attached to the surface, showed several crawling movements scanning plastic with anterior sack, detached and continued dynamic swimming. Such active searching for substrate suited to an encystment was attended with gradual reducing of the swimming period and ascending time of the scanning. Thereupon larvae stopped their swimming, rounded off and began the formation of a cyst sealing it off from an aggressive environment. During the last stage cercariae released the secretions of their cystogenic glands onto their body surface and slowly twisted in all directions forming a round shape envelopes. After the process had finished successfully, larvae might be termed metacercariae (Figs. 2a, 2b, 2c, 2d) and began metamorphosis into immature maritae.

Figure 3a shows dynamic of the successful encystment of cercariae in hemolymph and plasma. Within initial 45 minutes, rate of encystment in

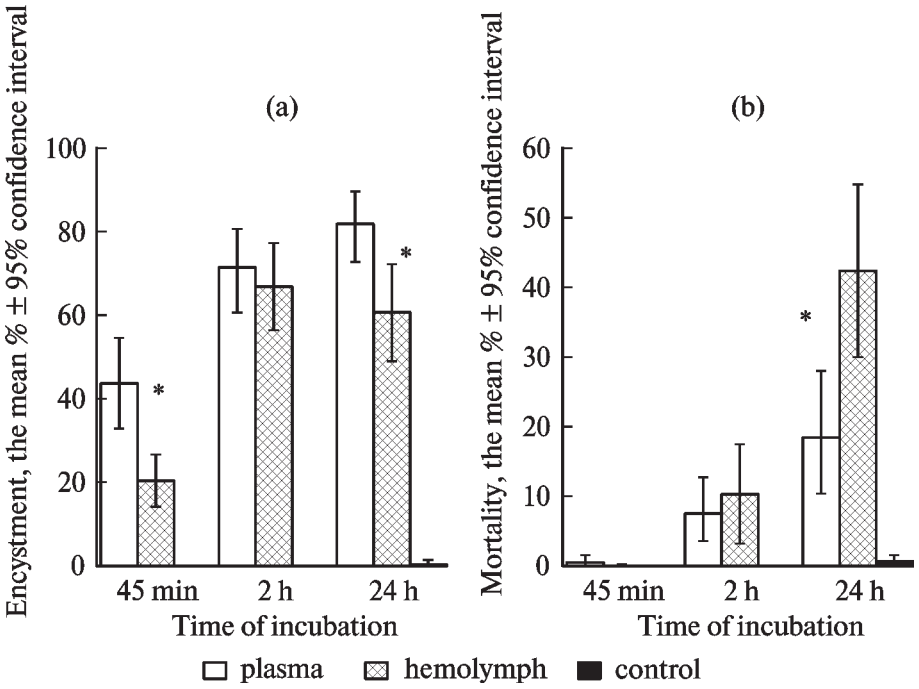

Fig. 3. Effect of the whole hemolymph and its plasma on success of encystment (a) and mortality (b) of *H. elongata* cercariae after the 45-min, 2-h, and 24-h exposition. FSW serves a control.

plasma was about 40% and twice higher ($p < 0.05$) than in whole hemolymph. In 2 h mean numbers of encysted cercariae increased to up to about 70% and were similar in plasma and hemolymph ($p > 0.05$). After 24 h the rate of encystment has progressed ($p < 0.05$) up to about 80% in plasma and kept constant ($p < 0.05$) in hemolymph. With respect to control estimates (in FSW), the extent of encystment in both test mediums was significantly higher ($p \ll 0.001$).

The plasma of *M. edulis* showed a dose-dependent triggering effect on cercarial encystment. In experiment with cercarial pool treated with pooled hemolymph, encystment rate decreased with plasma dilution (Fig. 4; transparent surface). In this, triggering effect was significant ($p < 0.05$) by 2 h in 16-fold diluted plasma (Fig. 4; point 1). Moreover, 60% of cercariae encysted by 24 h even though the plasma was 32-fold diluted (Fig. 4; point 2). In this experiment maximum estimates of successful encystment were found in 4–8—fold diluted plasma. Low success in undiluted plasma was evidently related with toxic effect of this medium.

Toxic effect of hemolymph. In whole hemolymph small clumps of hemocytes, or individual hemocytes, or both, were observed loosely attached to the surface of cercaria bodies and tails. Typically most of them were flushed away while larvae retained highly motile. However, the situation changed drastically once cercaria reduced motility and began the encystment process—hemocytes extensively bound to the tegument of transforming larvae. The extent of binding by hemocytes increased with time and led to encapsulation culminating in the metacercarial stage (Fig. 2c, 2d). It is during this crucial encystment phase that relatively high mortality of cercariae was observed. Both whole hemolymph and cell-free plasma mediums were toxic; after only 2 h about 10% of larvae were dead in either ones (Fig. 3b). The majority of dead parasites were unable to end cyst formation and were simply ejected from an abnormal cyst envelope. Without any covering, re-exposure to hemolymph killed them within a hour. Note that at the close of the experiment the mean percentage of live metacercariae in plasma was significantly higher ($p < 0.05$) than in hemolymph (Fig. 3b). With respect to control estimates, the extent of

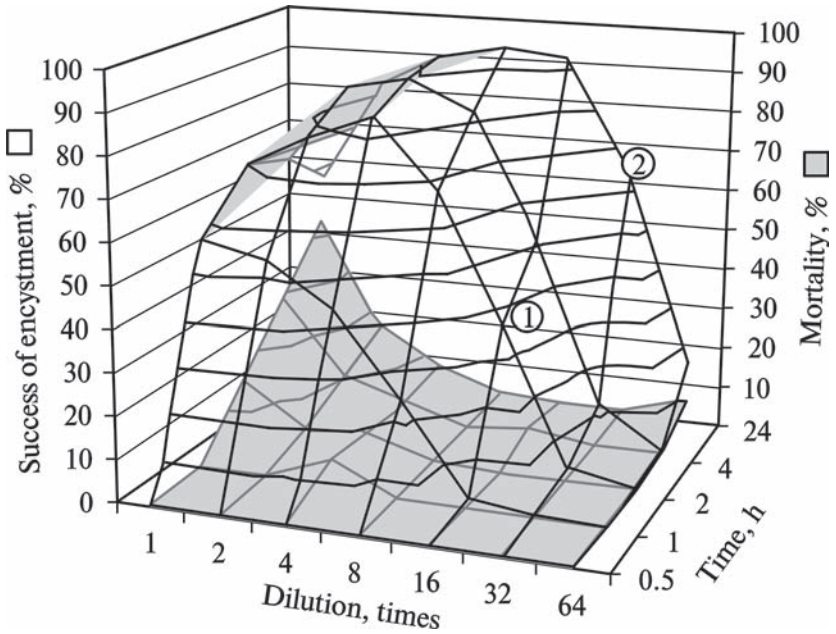

Fig. 4. Effect of the mussel plasma depending on concentration on success of encystment (the light surface) and mortality (the gray surface) of *H. elongata* cercariae after the 45-min, 2-h, and 24-h exposition.

mortality in both test mediums was significantly higher ($p \ll 0.001$).

Dose-dependent toxic effect of plasma had pattern like those of triggering effect—mortality rate decreased with plasma dilution (Fig. 4; gray surface). As this take place, the past harm concentration of plasma can result from 8 and more fold dilution.

It is important to note that the toxic effect described in the "dose-dependent" experiment, that dealt with hemolymph pool against pool of cercariae, seems to contradict the results found in the previous experiment that involved examining the plasma of individual mussels against separate "clones" of cercariae. Specifically, in the former experiment the total mortality of larvae after 24 h was twice higher than the mean mortality in the later one; 43 and 20% respectively. The reason for this conflict is that being in a pool, the highly toxic plasma of only one individual can significantly influence the total toxicity. Indeed, "killing" features of blue mussel hemolymph varied greatly between individuals. Figure 1a shows example of survival of one cercarial "clone" after 24 h exposure to whole hemolymph and plasma of 9 mussel individuals. In this, "low toxic" (mussels 1–5), "middle toxic" (mussels 6, 7) and "highly toxic" (mussels 8, 9) groups induced significantly different ($p < 0.05$) mortality. On the other hand, 3 different cercarial "clones" showed significantly distinct ($p \ll 0.01$) resistance to a toxic effect of hemolymph of one and the same mussel individual (Fig. 1b).

DISCUSSION

Before encystment, larvae must rely on special signals to identify the specific host. Other echinostome cercariae studied up to now in respect to host-finding behavior responded to small molecular weight components of snail-conditioned water, such as amino acids, peptides, sugars and lipophilic compounds [15–18]. Our observations have shown soluble factors of the mussel hemolymph to produce a pronounced trigger effect on the larval encystment. Identification of these factors will allow potentially determining whether they are specific trigger agents or represent only unspecific "ag-

gressive" molecules that force cercariae to look for protection under the cyst cover.

The rate of the *M. edulis* invasion by *H. elongata* metacercariae in populations of the White Sea varies from 8% to 100% [19]. The latter evaluation on the whole confirms the idea that encystment is a very effective tactics of resistance to attacks of the host defensive system. However, as the present study shows, this very critical phase is relatively time consuming and requires realization of a precise program with respect to all properties of the host defense. For both mediums examined, as judged by *in vitro* experiments, cercariae must end the transformation into metacercaria within 2 hours. Otherwise, the risk of being harmed by mussel defense reactions increases dramatically. In this, mussel hemocytes clearly have an important role to play in the host defence against trematode larvae. They can be divided into basophilic and eosinophilic groups, the former including hyaline (agranular) and granular cells and the latter only granular cells. Mussel granular hemocytes contain two defferent types of granules, distinguishable by size, lectin staining characteristics, and enzyme content [20, 21]. The presence of numerous hydrolytic enzymes including glucosidases, proteinases, and sulfatases in large granules indicates their relationship with lysosomes [22]. Besides, an increased production of the active oxygenic metabolites is revealed in the *M. edulis* hemocytes [23]. In our study the cytotoxic properties of the mussel hemocytes [24, 28] seem very essential to determine the metacercaria mortality 2 h after incubation in the host hemolymph.

On the other hand, the plasma soluble components can play a great role in the injury of the *H. elongata* larvae and on the whole in determination of compatibility of cercariae and mussels. First of all, lectins are involved in recognition of the "alien" in molluscs [26] and they are established to play an essential role in this process in *M. edulis* [27, 28]. Further, we have found in the mussel plasma a significant cytolytical activity to human (groups A and B), mouse, dog, rabbit, rat, and pig erythrocytes (prepared for publication). Moreover, a cytotoxic protein complex has been detected in plasma of the closely related species *M. galloprovincialis* [29]. This complex consisting of several different proteins (320 kDa) is able to destroy selectively the eukaryote cells, mouse tumor cells, and parasitic protozoa. Similar complexes might also exist in *M. edulis*; they lead to the omission of the encystment phase in *H. elongata* by damaging the cercaria tegument or disturbing the larval physiological processes.

The mussel hemolymph toxicity revealed in this study is characterized under *in vitro* conditions by significant individual variability. Such high variability can reflect either the different degree of the preactivity of the defense system of some mussels or individual genetic differences in both mussel susceptibility and the larvae "clone" infectivity. In either case, the method of the *in vitro* encystment of the *H. elongata* larvae is a very perspective method of analysis of results of interaction between the defense system of the mollusc individuals and single "clones" of digenetic larvae. Indeed, a large hemolymph volume (0.4–1.5 ml) not contaminated with mucus or extravisceral fluid of an individual allows testing several cercaria "clones." In the case of the careful hemolymph sampling, mussels survive very well, and this procedure can be repeated after the recovery period. In studies of the individual variability of the host defensive response, by using different methods up to the populational one, these distinct peculiarities of the *M. edulis/H. elongata* model are evident advantages as compared with the traditional "pulmonate" systems (*Biomphalaria, Bulinus, Lymnaea*).

ACKNOWLEDGMENTS

We are grateful to V.Ya. Berger, Biol. Sci. D. (head of the White Sea Biological Station) who arranged for us excellent conditions for work in the Station "Kartesh". We are grateful to N.V. Yakovleva and T.G. Shaposhnikova for critical comments and to D. Kanaikin participating in this study at its preliminary stage.

The work is supported by the Russian Foundation for Basic Research (projects nos. 02-04-63026, 03-04-49392, 03-04-63166).

REFERENCES

1. Write, C.A., The Pathogenesis of Helminthes in the Mollusca, *Helminthol. Abstr.,* 1971, vol. 35, pp. 207–224.

2. Gorbushin, A.M., Comparative Morpho-Functional Analysis of the Gastropod–Trematode Interactions, *Parazitologiya,* 2000, vol. 34, pp. 502–514.
3. Haas, W., Gui, B., Haberl, M., and Strobel, M., Miracidia of *Schistosoma japonicum*: Approach and Attachment to the Snail Host, *J. Parasitol.*, 1991, vol. 77, pp. 509–513.
4. Kalbe, M., Haberl, B., and Haas, W., Snail Host Finding by *Fasciola hepatica* and *Trichobilharzia ocellata: C*ompound Analysis of "Miracidia-Attractmg Glycoproteins", *J. Parasitol.,* 2000, vol. 96, pp. 231–242.
5. Bayne, C., Humoral Factors in Molluscan Parasite Immunity, *Aspects of Developmental and Comparative Immunology*, Solomon, J., Ed., Oxford: Pergamon, 1980, vol. 1, pp. 113–124.
6. Adema, C.M. and Loker, E.S., Specificity and Immunobiology of Larval Digenean–Snail Associations, *Advances in Trematode Biology*, Fried, B. and Graczyk, T.K., Eds., Roca Raton: CRC, 1997, pp. 230–253.
7. Yoshino, T.P. and Vasta, G.R., Parasite–Invertebrate Host Immune Interactions, *Advances in Comparative and Environmental Physiology*, Cooper, T., Ed., Berlin: Springer, 1996, pp. 125–167.
8. Laursen, J.R. and Yoshino, T.P., *Biomphalaria glabrata* Embryonic (Bge) Cell Line Supports *in vitro* Miracidial Transformation and Early Larval Development of the Deer Liver Fluke, *Fascioloides magna*, *Parasitol.,* 1999, vol. 118, pp. 187–194.
9. Coustau, C. and Yoshino, T.P., Flukes without Snails: Advances in the *in vitro* Cultivation of Intramolluscan Stages of Trematodes, *Exper. Parasitol.,* 2000, vol. 94, pp. 62–66.
10. Sapp, K.K. and Loker, E.S., Mechanisms Underlying Digenean–Snail Specificity: Role of Miracidial Attachment and Host Plasma Factors, *J. Parasitol.,* 2000, vol. 86, pp. 1012–1019.
11. Gibson, D.I. and Bray, R.A., The Evolutionary Expansion and the Host–Parasite Relationships of the Digenea, *Intern. J. Parasitol.,* 1994, vol. 77, pp. 798–800.
12. Yakovleva, N.V., Samoilovich, M.P., and Gorbushin, A.M., The Diversity of Strategies of Defense from Pathogens in Molluscs, *J. Evol. Biochem. Physiol.,* 2001, vol. 37, pp. 358–367.
13. Werding, B., Morphologie, Entwicklung und Okologie Digener Trematogen-larven der Strandschnecke *Littorina littorea*, *Marine Biol.,* 1969, vol. 3, pp. 306–333.
14. Zhivotovskii, L.A., *Populyatsionnaya biometriya* (Populational Biometry), Moscow, 1991.
15. Freied, B., Frazer, B.A., and Reddy, A., Chemoattraction and Penetration of *Echinostoma trivolvis* and *E. caproni cercariae* in the Presence of *Biomphalaria glabrata, Helisoma trivolvis* and *Lymnaea elodes* Dialysate, *Parasitol. Res.,* 1997, vol. 83, pp. 193–197.
16. Haas, W., Korner, M., Hutterer, E., Wegner, M., and Haberl, B., Finding and Recognition of the Snail Intermediate Hosts by 3 Species of Echinostome Cercariae, *Parasitol.,* 1995, vol. 110 (Pt 2), pp. 133–142.
17. Korner, M. and Haas, W., Chemo-Orientation of Echinostome Cercariae towards Their Snail Hosts: Amino Acids Signal a Low Host-Specificity, *Int. J. Parasitol.,* 1998, vol. 28, pp. 511–516.
18. Korner, M. and Haas, W., Chemo-Orientation of Echinostome Cercariae towards Their Snail Hosts: Amino Acids Signal a Low Host-Specificity, *Int. J. Parasitol.,* 1998, vol. 28, pp. 517–525.
19. Fateev, A.E., Granovitch, A.I., and Slusarev, G.S., Comparative Analysis of Symbiotic Fauna in *Mytilus edulis* from Natural and Industrial Populations in Kandalaksha Bay, *Experience of Iindustrial Farming of Blue Mussel in the White Sea* (Trudy Biologicheskogo Nauchno-Issledovatelskogo Instituta SPbGU), Minichev, Yu.S. and Maksimovitch, N.V., Eds., St. Petersburg: St. Petersburg State University, 2000, vol. 46, pp. 155–172.
20. Dyrynda, E.A., Pipe, R.K., and Ratcliffe, N.A., Sub-Populations of Haemocytes in the Adult and Developing Marine Mussel, *Mytilus edulis,* Identified by Use of Monoclonal Antibodies, *Cell Tissue Res.,* 1997, vol. 289, pp. 527–536.
21. Pipe, R.K., Farley, S.R., and Coles, J.A., The Separation and Characterization of Haemocytes from the Mussel *Mytilus edulis, Cell Tissue Res.,* 1997, vol. 289, pp. 537–545.
22. Pipe, R.K., Hydrolytic Enzymes Associated with the Granular Haemocytes of the Marine Mussel *Mytilus edulis, Histochem. J.,* 1990, vol. 22, pp. 595–603.
23. Winston, G.W., Moore, M.N., Kirchin, M.A., and Soverchia, C., Production of Reactive Oxygen Species by Hemocytes from the Marine Mussel, *Mytilus edulis:* Lysosomal Localization and Effect of Xenobiotics, *Comp. Biochem. Physiol.,* 1996, vol. 113, pp. 221–229.
24. Wittke, M. and Renwrantz, L., Quantification of Cytotoxic Hemocytes of *Mytilus edulis* Using a Cytotoxicity Assay in Agar, *J. Invertebr. Pathol.,* 1984, vol. 43, pp. 248–253.
25. Leippe, M. and Renwrantz, L., Release of Cytotoxic and Agglutinating Molecules by Mytilus Hemocytes, *Dev. Comp. Immunol.,* 1988, vol. 12, pp. 297–308.
26. Horak, P. and van der Knaap, W.P.W., Lectins in Snail–Trematode Interactions: A Review, *Fol. Parasitol.*, 1997, vol. 44, pp. 161–172.
27. Mullainadhan, P. and Renwrantz, L., Lectin-Dependent Recognition of Foreign Cells by Hemocytes

of the Mussel, *Mytilus edulis, Immunobiol.,* 1986, vol. 171, pp. 263–273.
28. Renwrantz, L., Daniels, J., and Hansen, P.D., Lectin-Binding to Hemocytes of *Mytilus edulis, Dev. Comp. Immunol.,* 1985, vol. 9, pp. 203–210.
29. Hubert, F., Cooper, E.L., and Roch, P., Structure and Differential Target Sensitivity of the Stimulable Cytotoxic Complex from Hemolymph of the Mediterranean Mussel *Mytilus galloprovincialis, Biochim. Biophys. Acta,* 1997, vol. 1361, pp. 29–41.