On the Question of Hyaluronidase in Sea-Urchin Spermatozoa[1]

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It has recently been reported (*1*, *2*) that a hyaluronidaselike agent can be extracted from sea-urchin sperm and that this is capable of dissolving the jelly coat of the eggs. The possibility does not appear to have been eliminated, however, that apparent dissolution of the egg jelly may have been a consequence of precipitation of the jelly (fertilizin) by antifertilizin in the extracts (*3*) or even merely a consequence of gradual dissolution of the jelly resulting from exposure to sea water.

Evidence for the hyaluronidaselike nature of the factor in sea-urchin sperm extracts appears to be based largely upon the fact that it is reportedly obtained by methods commonly employed for extraction of hyaluronidase from mammalian testes. It has also been reported (*1*) that sea-urchin sperm preparations are capable of reducing to some extent the viscosity of a solution of hyaluronic acid.

At the present time two points appear to require clarification: (1) Is an egg-jelly-dissolving factor extractable from sea-urchin sperm by methods used for hyaluronidase extraction? (2) Does hyaluronidase prepared from bull testis or other sources dissolve or otherwise act upon the material of the gelatinous coat of sea-urchin eggs?

In order to obtain evidence that might bear upon these questions, the author prepared extracts of the sperm of two species of Pacific Coast sea urchins (*Strongylocentrotus purpuratus* and *Lytechinus pictus*) by a number of methods which have been described for extraction of hyaluronidase from mammalian testes. For testing, eggs of the homologous species were treated in the different extracts, in solutions containing purified bull testis hyaluronidase (supplied by the Schering Corporation), and in sea water.

In several tests, jellyless eggs were occasionally found in experimental dishes, but an equal, or in some cases a greater percentage, of jellyless eggs was consistently found in control dishes containing sea water. It was observed that different lots of eggs exhibited considerable variation with respect to the presence of the jelly hull after standing in sea water. This probably depends upon the condition of the jelly in a given lot of eggs, i.e., whether it is initially soft or firm, thick or thin. No significance, therefore, could be attached to those cases in which jellyless eggs were found in treated suspensions.

It is of interest that a weak precipitation membrane was observed about some of the eggs in dishes containing sperm extract prepared by a procedure in which the spermatozoa were frozen and thawed—a treatment known to release antifertilizin from sea-urchin spermatozoa. Antifertilizin was present, therefore, in this preparation, but in low concentration, since in no case did the precipitation membrane become heavy or contract to the egg surface. Precipitation membrane formation was not observed in suspensions of eggs treated with bull testis hyaluronidase.

In other experiments advantage was taken of the fact that fertilizin co-precipitates with added protein in acid solution (*4*). Following a procedure patterned after methods described for turbidimetric assay of hyaluronidase with hyaluronic acid, the effect of sea-urchin sperm extracts, as well as of purified bull testis hyaluronidase fertilizin, was tested by a turbidity reduction method.

Incubation of the various sperm extracts with solutions of purified fertilizin at 37° or at room temperature for periods ranging from 30 min to 2 hr proved to be ineffective in reducing the turbidity upon subsequent acidification and addition of protein. In most cases, as a matter of fact, the turbidity was enhanced. Similar results were obtained when hyaluronic acid (Schering) was incubated with these extracts. On the other hand, incubation of fertilizin with purified bull testis hyaluronidase did not cause enhancement of the turbidity, but neither was there any reduction in turbidity even with relatively high concentrations of the enzyme. Incubation of hyaluronic acid with the same hyaluronidase preparation in low concentration resulted in marked reduction in turbidity.

Considerable data are now available concerning the chemical composition of fertilizin, and these show that it is different in this respect from hyaluronic acid (*3*, *5*). Fertilizin does resemble hyaluronic acid insofar as it co-precipitates with added protein in acid solution, but this indicates only a general resemblance shared with acidic mucopolysaccharides such as chondroitin sulfate.

The evidence reported here shows that treatment of spermatozoa of two species of sea urchin by methods employed for the extraction of hyaluronidase from mammalian testes fails to yield an egg-jelly-dissolving factor. Failure of bull testis hyaluronidase either to dissolve the egg jelly or to reduce the turbidity in a turbidimetric system employing purified fertilizin indicates that this substance does not serve as a substrate for mammalian hyaluronidase. This last point is further strengthened by the difference in chemical composition between hyaluronic acid and fertilizin. In general, the results of numerous experiments with the preparations mentioned here, as well as with other sea-urchin sperm extracts, clearly emphasize the importance of controlling the tests adequately with respect to the action of antifertilizin and gradual dissolution of egg jelly in sea water.

[1] Experiments reported here were performed in the laboratory of Albert Tyler, Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena.

References

1. Monroy, A., and Ruffo, A. *Nature*, **159**, 603 (1947).
2. Chambers, R. *Biol. Revs.*, **24**, 246 (1949).
3. Tyler, A. *Am. Naturalist*, **83**, 195 (1949).
4. Krauss, M. *Biol. Bull.*, **96**, 74 (1949).
5. Vasseur, E. *Acta Chem. Scand.*, **2**, 900 (1948).