Fertilization is at least partly due to the specific interaction between the chemical constituents of the sperm and egg surface. There are several methods for studying the chemical characteristics of the gametes, especially those which could play a certain role in fertilization. One of the best methods is the immunochemical analysis introduced by Tyler 2. This method, which with regard to specificity is far beyond the classical chemical analyses, uses the very sensitive and highly specific antigen-antibody reaction to discover the chemical composition of biological material.

Using the immunochemical analyses Perlmann 3 found that in gamete extracts of Paracentrotus lividus, a common Mediterranean sea urchin, a great number of antigenically strongly different components are present. Furthermore, he showed that some of these components have a definite and specific effect when combined with the corresponding antisera. Köhler and Metz 4 found a great number of different antigens in sperm extracts of various sea urchins and some of them proved to be associated to the sperm surface 5.

It seemed worthwhile to continue these investigations and compare the results obtained with those of the investigations on homologous and heterologous fertilization 6-8.

Material and Methods

Gametes of four different sea urchin species, common along the west Istrian coast of the Adriatic sea (Paracentrotus lividus Lam., Arbacia lixula L., Sphaerichinus granularis Lam. and Psammechinus microtuberculatus Biv.) were used.

Freshly caught animals were opened by a circular cut with scissors and the ripe gonads were removed, separated according to sex and species, cut in smaller pieces and placed over dense gauze in a plastic funnel. The extruded gametes were collected in bottles, cooled in ice and used freshly or freeze-dried.

Antisera were obtained by hyperimmunization of rabbits with fresh or freeze-dried gametes mixed with adjuvant 9. Ten days after the last injection of antigen the blood of rabbits was collected, sera were separated by centrifugation and stored at —20 °C until use.

The following symbols were used for the antigens and the corresponding antisera:

2 B. E. Hagström and S. Lönnig, Sarsia 4, 5 [1961].
4 B. E. Hagström, Sarsia 17, 33 [1964].
Jₚ, Jₛ, Jₚₛ and Jₐ — extracts of *Paracentrotus*, *Sphaerechinus*, *Psammechinus* and *Arbacia* eggs;
Sp, Sₛ, Sₚₛ and Sₐ — extracts of *Paracentrotus*, *Sphaerechinus*, *Psammechinus* and *Arbacia* sperm;
Anti Jₚ and Anti Sp — antisera to *Paracentrotus* eggs and sperm;
Anti Jₛ and Anti Sₛ — antisera to *Sphaerechinus* eggs and sperm.

Extracts of gametes were prepared by homogenization in a tissue grinder. Freeze-dried gametes were homogenized as 1% suspensions. Fresh gametes were homogenized and diluted with sea water or water of the same osmotic value. Both homogenates were left standing 24—48 hours, filtered through rough filter paper, and the filtrates used as gamete extracts.

In absorption experiments, to each ml of antiserum 0.5 ml of undiluted fresh gametes was added. After 12 hours of standing at +4 °C the mixture was centrifuged and the clear supernatant used for experiments.

For diffusion in gel a slight modification of OUCHTERLONY’s diffusion method in Difco bacto agar was used. Agar plates of 6 mm thickness were prepared in Petri dishes. Holes (Φ = 12 mm) in the agar were punched at distances of 15 mm. The wells were usually filled with antisera or gamete extracts only once to give a flat meniscus and the plates were then stored in humid atmosphere at room temperature or at +4 °C until precipitation lines were developed.

**Results and Discussion**

In the first series of experiments the reaction between the extracts of *Paracentrotus* and *Sphaerechinus* gametes and the corresponding antisera were studied. Using various antisera and extract combinations in a great number of analyses the maximally obtainable number of precipitation lines was established.

The results of these investigations are schematically represented in Fig. 1 where the position, shape and relative thickness of the precipitation lines correspond to the actual situation. The lines are designated for easier orientation.

By this method it has been shown that eggs contain at least eight and sperm six different antigenic determinants (Table 1), because one should keep in mind that each precipitation line may represent more than one antigen-antibody combination.

![Fig. 1. Scheme of precipitation lines between the gamete extracts and the corresponding antisera.](image)

**Table 1.** Antigenic determinants of *Paracentrotus* and *Sphaerechinus* sperm and eggs. The symbols correspond to those used in Fig. 1.

<table>
<thead>
<tr>
<th>obtained in combination</th>
<th>antigenic determinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jₚ/Anti Jₚ</td>
<td>Aₚ Bₚ Cₚ Dₚ Eₚ Fₚ Gₚ Hₚ</td>
</tr>
<tr>
<td>Jₛ/Anti Jₛ</td>
<td>Aₛ Bₛ Cₛ Dₛ Eₛ Fₛ Gₛ Hₛ</td>
</tr>
<tr>
<td>Sp/Anti Sp</td>
<td>aₚ bₚ cₚ dₚ eₚ fₚ sₚ</td>
</tr>
<tr>
<td>Sₛ/Anti Sₛ</td>
<td>aₛ bₛ cₛ dₛ eₛ fₛ sₛ</td>
</tr>
</tbody>
</table>

**Table 2.** Common antigenic determinants of *Paracentrotus*, *Sphaerechinus*, *Psammechinus* and *Arbacia* gametes. The identical determinants are in the same vertical column. The identified determinants of *Psammechinus* and *Arbacia* gametes are marked only with +.

<table>
<thead>
<tr>
<th>antigen</th>
<th>antigenic determinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jₚ</td>
<td>Aₚ (?) Bₚ Cₚ</td>
</tr>
<tr>
<td>Jₛ</td>
<td>Aₛ (?) Bₛ Cₛ</td>
</tr>
<tr>
<td>Jₚₛ</td>
<td>+ +</td>
</tr>
<tr>
<td>Jₐ</td>
<td>+ +</td>
</tr>
<tr>
<td>Sₚ</td>
<td>aₚ (?) bₚ cₚ</td>
</tr>
<tr>
<td>Sₛ</td>
<td>bₛ cₛ</td>
</tr>
<tr>
<td>Sₚₛ</td>
<td>+ +</td>
</tr>
<tr>
<td>Sₐ</td>
<td>+ +</td>
</tr>
</tbody>
</table>

**Table 2.** Common antigenic determinants of *Paracentrotus*, *Sphaerechinus*, *Psammechinus* and *Arbacia* gametes. The identical determinants are in the same vertical column. The identified determinants of *Psammechinus* and *Arbacia* gametes are marked only with +.

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[O. Ouchterlony, Ark. Kemi, B 26, 1 (1949).]
with the extracts of *Arbacia* and *Psammechinus* gametes. All comparisons were made only with anti Jₚ and Anti Sₚ sera. It was found that egg extracts of these species (Jₚ and Jₙ) also have antigenic determinants corresponding to those denoted as Bₚ, Cₚ and Bₙ, Cₙ in *Paracentrotus* and *Sphaerechinus* eggs. With sperm extracts (Sₚ and Sₙ) only one precipitation line, corresponding to bₚ and bₙ in *Paracentrotus* and *Sphaerechinus* sperm, was obtained.

Thus it was established that all extracts had one common “group specific” antigenic determinant. Furthermore, all egg extracts as well as all sperm extracts had a common “sex specific” determinant. The absence of clear “species specific” antigenic determinants common to sperm and eggs of one species is surprising, but it can be partly attributed to generally weak reaction obtained with sperm extracts.

The results of these analyses were confirmed in experiments in which antisera were absorbed with gametes in various combinations. Anti Jₚ, for instance, absorbed with any sperm loses its capacity to precipitate Cₚ, but other precipitation lines remained, although somewhat weakened.

The relative concentration of “group specific” antigenic determinants was measured using serial dilutions of gamete extracts and antisera to *Paracentrotus* gametes (Anti Jₚ and Anti Sₚ). On the basis of the highest dilutions which still gave the precipitation line, the relative concentration of the “group specific” antigenic determinants could be estimated as 100 : 80 : 25 : 1 between the gametes of *Paracentrotus, Psammechinus, Sphaerechinus* and *Arbacia* respectively.

A good correlation was found by comparing the relative concentration of “group specific” antigenic determinant with the cross fertilization rate in experiments where one of the gametes was from *Paracentrotus*. This coincidence should not, however, reflect a causal connection but shows that species with antigenically more different gametes are less compatible for successful cross-fertilization.