Interspecific relationships of oviducal materials as related to fertilization in Amphibia

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SUMMARY

Experiments are described in which eggs of *Rana* species were treated prior to insemination with antisera prepared against oviducal materials of *Rana, Bufo*, and *Ambystoma* species. Three types of treatment with antisera were employed.

Eggs of *Rana pipiens* and *R. clamitans* were treated with antisera against materials prepared from whole oviducts, or from upper and lower segments of oviducts, of *R. pipiens, R. clamitans, R. sylvatica, R. catesbeiana, Bufo americanus, B. marinus* and *Ambystoma mexicanum*.

Eggs of *R. pipiens* were treated with antisera against materials from whole oviducts, or from upper, middle or lower oviducal regions, of *R. pipiens*, which had been absorbed with materials from whole oviducts of the species named above.

Eggs of *R. pipiens* were treated with antisera against materials from whole oviducts, or from upper, middle or lower oviducal segments of *R. pipiens*, which antisera had each been absorbed with material from whole, upper, middle or lower oviducts of *Rana* species.

The results indicated that treatment with antisera against oviducal materials of *Rana* origin was inhibitory to fertilization of *Rana* eggs, while treatment with antisera against oviducal materials from other amphibian genera was not.

The experiments with absorbed antisera indicated that the secretion by the oviducts of *R. pipiens* of components also occurring in other *Rana* species is regionally localized in the oviduct.

The relationships of secretions of amphibian oviducts to fertilization is discussed in connexion with the histochemically demonstrable complexity of the jelly envelopes around eggs.

INTRODUCTION

A number of investigators (see Shaver, 1966, for references; McLaughlin, 1967) have demonstrated that the jelly coats surrounding amphibian eggs are necessary for fertilization. Experiments with ranid species have shown that antisera produced against egg-jelly materials can inhibit the fertilizability of eggs treated with the antisera prior to insemination (Shaver & Barch, 1960; Shivers, 1965). Further analysis of the role of anuran jelly-coat material in fertilization indicated that there may be regional differences in the jelly material secreted by the oviduct (Barch & Shaver, 1963; Shivers, 1965).

For example, Glick & Shaver (1963) observed that eggs taken from the

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middle and lower portions of the oviducts of ovulating *Rana pipiens* fertilized in much higher numbers than eggs from the upper third of the oviduct. The authors interpreted these results to mean that secretions of the middle and lower portions of the oviduct were necessary for successful sperm–egg interaction(s). The secretions of the middle and lower thirds of the oviduct were presumed to correspond to the middle and outer of the three jelly layers which are visible in *R. pipiens* after immersion of eggs in fluid. Cells in roughly the upper third of the oviduct were supposed to have secreted the inner jelly layer.

Other evidence that secretions from different levels of the oviduct of *R. pipiens* may differ with respect to their interactions with spermatozoa in fertilization has come from experiments reported by Barch & Shaver (1963) and Shaver (1966). In these experiments, mature eggs of *R. pipiens* were treated prior to insemination with antisera prepared against homogenized whole oviducts of *R. pipiens* and homogenized upper, middle, and lower thirds of the oviduct (excluding the ovisac). The results indicated that antibodies against the material from whole oviducts inhibited fertilization more than antibodies against the lower third, while antibodies against the latter were more inhibitory than those against middle and upper portions.

Shivers (1965, and cited in Shaver, 1966) demonstrated by immunofluorescent studies that antigens present in the inner and middle jelly layers of *R. pipiens* eggs were also present in the egg jellies of *R. clamitans*. Antigens specific to *R. pipiens* egg jelly were located in the middle and outer layers. Shaver (1966) has proposed that fertilization in *R. pipiens* may involve interactions between *R. pipiens* spermatozoa and the species-specific components of the egg jelly. Successful fertilization in crosses between *R. pipiens* eggs and spermatozoa of other ranid species may involve interactions between the spermatozoa and the egg-jelly components common to *R. pipiens* and the other species.

In an attempt to extend the analysis of the role of oviducal secretions (and hence of the different jelly layers around eggs) in fertilization, further experiments have been performed, as follows:

1. Eggs of *R. pipiens* and *R. clamitans* were treated prior to insemination with antisera against oviducal material from *R. pipiens*, *R. clamitans*, and other ranid species, as well as from other anuran and urodele genera. On the basis of the hypothesis referred to above (Shaver, 1966), it would be predicted that antibodies against homologous oviduct material would inhibit fertilization to a greater degree than those against heterologous ranid jellies, and these, in turn, would be more inhibitory than antibodies against oviduct material from the other genera.

2. Eggs of *R. pipiens* were treated prior to insemination with antisera against oviducal materials of *R. pipiens* which had been absorbed with oviducal extracts from a variety of amphibian species. The absorptions were performed to remove from the *R. pipiens* antisera any antibodies representing oviduct components common to *R. pipiens* and the species from which the absorbing materials were
derived. If such common components are involved in the fertilization process of *R. pipiens*, then antibodies against them should cause inhibition of fertilization; and removal of such antibodies by absorption might remove the inhibition roughly in proportion to the quantity of such components in the *R. pipiens* oviduct material.

3. Eggs of *R. pipiens* were also treated with antisera against *R. pipiens* oviducal segments; these antisera had been absorbed with materials from different levels of ranid oviducts. The purpose of these experiments was to determine if components common to *R. pipiens* and the other species could be localized in a particular segment of any of the ranid oviducts.

**MATERIALS AND METHODS**

Animals were purchased from commercial dealers, except for *Ambystoma mexicanum* (kindly supplied by Dr R. R. Humphry, Department of Zoology, Indiana University, Bloomington, Indiana, U.S.A.). All the females used had fully developed ovaries containing mature oocytes. The methods used for the preparation of antigens and antisera were those described in previous publications (Barch & Shaver, 1963; Shaver, Barch & Shivers 1962), with some modifications. Oviducts were dissected from pithed animals, and completely straightened on glass plates; they were cut into thirds when segments were desired. The whole oviducts, or segments, were cut into small pieces and stirred in large volumes of distilled water at 4 °C for 24 h. The gelatinous material extruded from the oviducal cells resulted in a viscous fluid which was filtered several times through glass wool. When the filtrate was devoid of visible tissue debris it was lyophilized; 5 mg of the lyophilate were mixed with 1 ml of one-tenth full-strength Holtfreter's solution, and emulsified with an equal volume of complete Freund's adjuvant. The antigen-adjuvant mixture was injected into the subscapular muscles of rabbits. One week later an equivalent dose of antigen and incomplete adjuvant was administered. Booster shots containing 5 mg of antigens with incomplete adjuvant were given every 4–6 weeks during the period when serum was being obtained from the animal, usually about 6 months.

Blood for control serum was withdrawn from animals prior to immunization. Fifty ml of blood were taken from a marginal ear vein 5 weeks after the first immunizing injection; bleeding was repeated every other week during the period of serum collection. Sera were dialysed against 0.65% NaCl. Each sample was tested individually on double diffusion plates, according to the method of Ouchterlony (1949), as modified by Shaver (1961). At least two rabbits were immunized against the same antigen, and their sera were pooled after testing had indicated the presence of antibodies. Sera were used full-strength except in experiments in which unabsorbed sera were diluted in the same proportion as absorbed sera. The latter were absorbed by allowing mixtures of equal volumes
of serum and absorbing antigen to stand at 4 °C for 24 h. The absorbed sera were clarified by centrifugation before use.

Mature eggs were obtained by hormone treatment (2.5–5 mg progesterone in corn oil plus one adult frog pituitary per female; Wright & Flathers, 1961). Eggs were extruded on to glass microscope slides and covered with antiserum, control serum or one-tenth full-strength Holtfreter’s solution for 2 min. The solutions were washed off the eggs by thorough pipetting with one-tenth full-strength Holtfreter’s solution. The eggs were then inseminated with sperm suspension (one testis macerated in 10 ml of one-tenth full-strength Holtfreter’s solution) and, after 10 min exposure to spermatozoa, were placed in bowls of aerated tap water. Four to five hours later the numbers of cleaving eggs and the total number of eggs were counted and recorded. Previous observations had established that treatment with effective antisera actually inhibits fertilization, as judged by failure of the eggs to undergo the rotation of orientation or to emit the second polar body. Cleavage is considered an accurate end-point of these experiments, since it can be observed that almost all uncleaved eggs which had been treated with effective antisera prior to insemination actually had not been fertilized. Simply to count the number of animal hemispheres seen by the observer looking down on the eggs, as a measure of fertilization, would not be accurate, since, by chance, eggs which may not have been fertilized might have been extruded on to the slide with their animal hemispheres ‘up’ (to the observer).

Samples of eggs from one female frog were always treated with all the antibodies and control solutions in any series of treatments. A number of females were used to replicate the treatments. The percentage cleavage was calculated, transformed into arc sin equivalents, and significant differences were established by an analysis of variance for each experiment. Significant differences between means were calculated by the Q test (Snedecor, 1956).

RESULTS

1. Treatment of eggs of Rana pipiens and R. clamitans with antisera against whole, upper and lower oviducal materials from species of Rana, Bufo, and Ambystoma

Antibodies were prepared against materials from the whole oviducts, as well as from the upper and lower thirds of the oviducts, of *R. pipiens*, *R. clamitans*, *R. catesbeiana*, *Bufo americanus*, *B. marinus*, and *Ambystoma mexicanum* (Aholotl). Antibodies were prepared against whole oviducts only of *R. sylvatica*, there not being enough material available to prepare antigens from the separate segments.
(a) Analysis of antisera

Double diffusion plates were prepared with the antisera listed above and *R. pipiens* and *R. clamitans* oviducal antigens. Drawings of representative interactions on such plates are shown in Fig. 1. It can be seen that the materials from both species interact with the antibodies to the oviducal materials from all of the ranid species, but that there are no interactions with the Axolotl material, with the exception of that between *R. pipiens* oviduct material and the antibodies against the lower third of the Axolotl oviduct. In the ranid species, differences

![Fig. 1. Drawings of double diffusion plates showing reactions of whole oviducal material of *R. pipiens* (p, center wells) and *R. clamitans* (cl, center wells) with antisera against whole (O), upper (U) and lower (L) oviducal materials of *R. pipiens* (Op, Up, Lp), *R. clamitans* (Ocl, Ucl, Lcl), *R. catesbeiana* (Oca, Uca, Lca) and *A. mexicanum* (OA, UA, LA). 1 = precipitation zones representing components found in upper thirds of oviducts but not in lower thirds. 2 = precipitation zones representing components found in lower thirds of oviducts but not in upper thirds.](image-url)
Fig. 2. Fertilization of eggs, in arc sin equivalents of percentages, of *R. pipiens* (unshaded bars) and *R. clamitans* (shaded bars) after 2 min treatment with antisera against whole (*W*), upper (*U*), and lower (*L*) oviducal material of *R. pipiens* (*pip.*), *R. clamitans* (*clam.*), *R. catesbeiana* (*cat.*), *Bufo americanus* (*amer.*), *B. marinus* (*mar.*), *Ambystoma mexicanum* (*mex.*); and whole oviducal material of *R. sylvatica* (*syl.*). *C* = control serum, *N* = one-tenth full-strength Holtfreter's solution. * = value significantly lower than control value.
can be seen between the interactions of the upper and lower oviduct materials of the same species. Antibodies against the materials from the bufonid species did not interact with the materials from *R. pipiens* or *R. clamitans* oviducts.

(b) Treatment of eggs with antisera

The eggs of twenty-one *R. pipiens* and eighteen *R. clamitans* females were treated with the antisera listed above. Fig. 2 illustrates the results, in arc sin equivalents of percentages of cleaved eggs. The results are essentially the same for both species. Treatment of the eggs with antisera against ranid materials from either level of the oviducts, as well as from the whole oviducts, was inhibitory to fertilization. Since there was no significant difference in the inhibition of fertilization caused by antisera against the upper and lower segments of oviducts of the ranid species, and since antigenic differences between these two regions can be demonstrated on double diffusion plates, it can be concluded that the different components represented on the plates are either not involved in the fertilization reactions, or that they affect fertilization in the same way, or that components visualized on plates were not represented in sufficient quantities in the sera to affect fertilization.

Only *R. pipiens* eggs are inhibited by treatment with antisera against materials from the whole oviducts of other genera. These data, however, were only of border-line statistical significance. Antisera against the upper oviducal materials of *Bufo americanus* inhibited the fertilization of *R. pipiens* eggs, while *R. clamitans* eggs had their fertilization rate depressed by antisera against the lower oviducal antigens of *B. americanus*.

2. The effects on the fertilizability of *Rana pipiens* eggs treated with antisera against *R. pipiens* whole or regional oviducal materials; antisera absorbed with materials from whole oviducts of a variety of amphibian species.

Antibodies were prepared against the whole, upper, middle, and lower thirds of oviducts of *R. pipiens*. Each of the four antisera was absorbed with whole oviducal material from *R. pipiens*, *R. clamitans*, *R. sylvatica*, *R. catesbeiana*, *B. americanus*, *B. marinus* and *A. mexicanum*.

(a) Analysis of antisera

Double diffusion plates were prepared using unabsorbed antisera and the whole oviducal antigens used for the absorptions. Plates were also prepared using the absorbed antisera and *R. pipiens* whole oviducal antigens. Drawings of representative interactions of the unabsorbed and absorbed antisera against the lower one-third of *R. pipiens* oviduct can be seen in Fig. 3. In Fig. 3A, unabsorbed antiserum against the lower one-third of *R. pipiens* oviduct was reacted with materials from whole oviducts of various species. Only the ranid materials interact with the unabsorbed antiserum. Fig. 3B shows the reactions
between materials from the lower one-third of *R. pipiens* oviduct and antisera against this material which had been absorbed with materials from the whole oviducts of the different species. Here it can be seen that only the ranid absorbing materials reduced the number of zones of precipitation, indicating the presence of common components; i.e. components in the absorbing material, which are complementary to antibodies in the *R. pipiens* antiserum, neutralized the antibodies, thus preventing the formation of precipitation complexes in the gel.

(b) Treatment of eggs with antisera

It had been described in Section 1 of these results that treatment of eggs with unabsorbed antisera against *R. pipiens* oviducal material inhibited fertilization. It would be predicted that absorption of these antisera with materials containing antigens complementary to the inhibitory antibodies would reduce the inhibition of fertilization of the eggs treated with them. Thus, absorption with material from a species having a larger number of components in common with *R. pipiens* oviduct should remove the inhibition to a greater degree than absorption with material from species having fewer common components.

The eggs of 20 females were used for these experiments. Fig. 4 illustrates the results in arc sin equivalents of cleavage percentages. The data can be summarized as follows:

1. It can be seen that absorption of the antisera against *R. pipiens* whole oviducal material with materials from oviducts of all ranid species removes inhibitory antibodies. Absorption with the homologous material removes all the inhibition,
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Fig. 4. Fertilization values in arc sin equivalents of percentages of cleaving *R. pipiens* eggs after 2 min pre-insemination treatment with antisera against whole (W), upper (U), middle (M), and lower (L) oviducal material of *R. pipiens* absorbed with whole oviducal material of several different species. *pip.* = *R. pipiens*; *clam.* = *R. clamitans*; *syl.* = *R. sylvatica*; *cat.* = *R. catesbeiana*; *amer.* = *B. americanus*; *mar.* = *B. marinus*; *mex.* = *A. mexicanum*. C = control serum; N = one-tenth full-strength Holtfreter's solution. * = values significantly different from control serum value. ** = values significantly different from both the control serum values and those after treatment with unabsorbed antiserum.

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*pip.* = *R. pipiens*; *clam.* = *R. clamitans*; *syl.* = *R. sylvatica*; *cat.* = *R. catesbeiana*; *amer.* = *B. americanus*; *mar.* = *B. marinus*; *mex.* = *A. mexicanum*. C = control serum; N = one-tenth full-strength Holtfreter's solution. * = values significantly different from control serum value. ** = values significantly different from both the control serum values and those after treatment with unabsorbed antiserum.
and absorption with *R. sylvatica* oviduct also significantly neutralizes inhibiting antibodies. The values obtained after treating eggs with antisera against *R. pipiens* whole oviducal antiserum which had been absorbed with materials from oviducts of *R. clamitans* and *R. catesbeiana*, are significantly different both from the data obtained after treatment with control serum or with unabsorbed serum. There was no effect on the inhibition of fertilization by absorption of the *R. pipiens* antiserum with oviducal materials from the other genera.

2. Absorption of antisera against material from the *upper thirds* of oviducts of *R. pipiens* with materials from whole oviducts of other species resulted in the neutralization of inhibitory antibodies only in the case of *R. clamitans* and *R. pipiens*. Absorption with materials from the other species had no effect on the inhibition.

3. When antisera against material from *middle thirds* of *R. pipiens* oviducts were absorbed, only material from *R. pipiens* significantly removed inhibitory antibodies. Absorption with materials from *R. sylvatica* and *R. catesbeiana*, and subsequent treatment of eggs with the absorbed antisera, produced results which were significantly different both from the results obtained after treatment with control sera and with unabsorbed antisera. Absorption with material from *R. clamitans*, or from the other genera, had no effects on the inhibition by the antisera against the *middle thirds* of oviducts of *R. pipiens*.

4. Absorption of antisera prepared against the *lower thirds* of *R. pipiens* oviducts resulted in the neutralization of inhibitory antibodies in the cases of *R.
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pipiens, R. sylvatica and R. catesbeiana, the latter being especially effective. Absorption with material from R. clamitans had no significant effects on the inhibitory properties of the antisera, nor did absorption with materials from the other genera.

The data obtained from the experiments described in this section are presented in terms of the commonality of oviducal components in Table 1. It can be seen that the major portion, if not all, of the components in the R. pipiens oviduct which are shared with R. clamitans are located in the upper third of the R. pipiens oviduct. Components of R. pipiens oviducts common also to R. sylvatica and R. catesbeiana appear to be localized in the middle and lower segments of the R. pipiens oviduct. The antigenic materials of R. pipiens responsible for the inhibitory activity appear to be equally distributed along the length of the oviduct. No components common to the oviduct materials of R. pipiens are found in the oviducts of the other genera tested.

3. The effects on the fertilizability of Rana pipiens eggs of treatment with antisera against R. pipiens whole oviducts, or segments thereof; antisera absorbed with materials from different levels of ranid oviducts

The experiments described in Section 2 of these results showed that there are components in oviducal materials common to R. pipiens and the other ranid species tested, and gave some indication of their localization in the R. pipiens oviduct. The experiments described in this section were designed to ascertain whether these common components could also be localized in different regions of the oviducts of the other ranid species.

(a) Preparation of antisera

Antibodies were prepared against material from the whole oviducts and from the upper, middle, and lower thirds of oviducts of R. pipiens. Each of these antisera was absorbed with material from whole oviducts, or from upper, middle and lower thirds of oviducts of R. pipiens and R. catesbeiana; absorptions with material from R. clamitans were made only with whole oviducts, or with the upper or lower thirds of the oviducts.

(b) Treatment of eggs

Due to the large number of treatments involved, it was necessary to perform the experiments in two parts: (1) treatments with antisera against material from whole and upper thirds of oviducts, absorbed with the materials noted above; (2) treatments with antisera against the middle and lower thirds of oviducts, absorbed with the same materials as in (1). Eggs from separate sets of twenty females each were used in the experiments. Statistical analyses were made, independently, of the two parts of the experiment, but these could not be directly compared, owing to the nature of the statistical methods employed. Table 2 presents the results in arc sin equivalents of percentages of eggs which cleaved.
With the exception of one case, absorption of any of the antisera with any of the whole or fractional oviducal materials removed the inhibition of fertilization in eggs treated with the sera. The exceptional case was that of an antiserum against material from the upper third of *R. pipiens* oviduct absorbed with material from the lower third of *R. clamitans* oviduct. After treatment with this absorbed antiserum, the percentage of fertilization was decreased significantly from that of the control.

Table 2. Results, in arc sin equivalents of percentages of cleaving eggs of *Rana pipiens*, of treatment with antisera against whole and regional oviducal materials of *R. pipiens* which were absorbed with whole or regional oviducal materials of species of *Rana*.

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<td><em>Le</em></td>
<td>45-2</td>
<td>39-6***</td>
<td>43-6</td>
<td>41-6</td>
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<td><em>Wca</em></td>
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<td><em>Uca</em></td>
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<td><em>Mca</em></td>
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<td><em>Lea</em></td>
<td>46-8</td>
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* *W* = whole oviducal material; *U* = upper oviducal material; *M* = middle oviducal material; *L* = lower oviducal material; *p* = *R. pipiens*; *c* = *R. clamitans*; *ca* = *R. catesbeiana*.

** Antigen not available.

*** Significantly different from control serum value.

Control values: eggs treated with one-tenth full-strength Holtfreter’s solution prior to insemination = 69-7; 61-8. Eggs treated with control serum prior to insemination = 57-6; 52-5.

Although there were no statistically significant differences between the results of the different treatments, several consistent trends can be noted.

1. Absorption of any of the *R. pipiens* antisera with *R. pipiens* materials neutralized more of the inhibiting antibodies than did absorption with heterologous ranid material, from any level of the oviduct, as reflected in the higher fertilization values.

2. Among the *R. pipiens* materials used as absorbants, those from the upper third of the oviduct consistently removed less of the inhibition than materials from other levels. This may indicate that there are fewer components in this oviducal segment which were involved in the fertilization reaction.
3. Furthermore, these components seem to consist largely of a type located also in the upper third of the oviduct of *R. clamitans*, since absorption of antiserum against the upper third of *R. pipiens* oviduct with material from the upper third of *R. clamitans* oviduct neutralizes more inhibiting antibodies than does absorption with material from other regions of the *R. clamitans* oviduct; e.g. the case noted above where absorption of antiserum against *R. pipiens* materials from the upper oviduct with lower oviduct of *R. clamitans* removed almost none of the inhibiting antibodies.

4. In general, absorption of any of the antisera against *R. pipiens* oviducal material with any oviducal material from *R. clamitans* resulted in the removal of less inhibition to fertilization than did absorption with any other of the ranid materials.

5. When *R. catesbeiana* materials were used as absorbants, the results were intermediate between those obtained when the homologous (*R. pipiens*) absorbants were employed and those when *R. clamitans* absorbants were used.

6. There is a small but consistent difference in the results obtained using *R. pipiens* antiserum which had been absorbed with materials from the upper or from the lower levels of *R. catesbeiana* oviducts, the latter having absorbed more of the inhibiting antibodies than the former. Thus it would appear that components shared with *R. pipiens* are located in the lower third of the *R. catesbeiana* oviduct.

**DISCUSSION**

The experiments in which there were no significant differences in the effects on fertilizability of the antisera to materials from upper and lower thirds of oviducts raise questions concerning the roles of these components in fertilization. It had been suggested by Shaver (1966) that two types of components of the egg-jelly coats of *R. pipiens* might be involved in fertilization reactions: those which are species-specific, and those which are shared with other ranid species. Shivers (as cited in Shaver, 1966) had observed, by means of fluorescent antibody staining reactions, that species-specific components in *R. pipiens* appeared to be localized mainly in the outermost and middle of the jelly layers, whereas the components shared with *R. clamitans* were mainly in the innermost and middle layers. Shivers (1961) also demonstrated that fertilization was inhibited most strongly by antiserum against species-specific components in *R. pipiens*. These antiserum were produced against whole *R. pipiens* egg jelly and were absorbed with egg jelly of *R. clamitans*, thus retaining the species-specific components.

One question raised by the results of the present experiments involves the identification of the components of the upper and lower regions of oviducts, which are clearly distinguishable on double-diffusion plates (cf. Fig. 1) with common components and species-specific components, respectively. Presumably, the egg-jelly components identified by Shivers as being species-specific, would have been secreted around the egg in the lower regions of the oviduct, since they were mainly localized in the outer and middle layers. Likewise, the jelly com-
ponents shared with *R. clamitans*, which were seen by Shivers to be mainly in the inner and middle layers, were supposed to have been secreted by cells in the upper regions of the oviduct.

One difficulty in equating the species-specific components observed by Shivers in egg jellies with materials from precisely defined regions of the oviduct is the lack of information on the exact extent of the regions of the oviduct which secrete the different layers of jelly around eggs. Barch & Shaver (1963) showed that, in addition to components restricted to the upper or lower regions of *R. pipiens* oviducts, some components quite clearly were coextensive between upper and middle oviducal regions, while others were coextensive between the middle and lower regions. In the present experiments, the uncoiled oviducts, after dissection, were arbitrarily cut into thirds, from which the regional antigens were prepared. Considering that the three layers which are visible around the egg after hydration of the jelly differ in their dimensions, it seems unlikely that the upper, middle and lower thirds of the oviducts would contain cells secreting only the inner, middle and outer layers of jelly respectively. Furthermore, recent histochemical observations (D. E. Williams, unpublished results; Lee, 1968) indicate that there may be five or more layers of materials around *R. pipiens* eggs which differ in their composition of acid and neutral mucopolysaccharides, and in sulfated groups.

Thus, while the species-specific components visualized by Shivers in the outer and middle layers of egg jellies may have been predominantly produced in the lower oviducal regions, it cannot be concluded that all of this type of component originated there. Likewise, all of the jelly components shared by *R. pipiens* with other ranid species may not originate in the upper regions of the oviduct. For example, the data resulting from the experiments in which antisera to various oviducal regions of *R. pipiens* had been absorbed with whole oviducal materials from other species, before treating eggs (Fig. 4), indicate variations in the locations of components shared with other species. Absorption of antisera to *R. pipiens* upper oviducal material with whole *R. clamitans* oviducal antigens removed significant amounts of inhibiting antibodies, but absorption of the *R. pipiens* upper oviducal antisera with whole oviducts of *R. catesbeiana* did not significantly reduce the inhibition of fertilization. From this, it could be concluded that *R. clamitans* oviducts contain materials complementary to antibodies prepared against *R. pipiens* upper oviducal materials, whereas the *R. catesbeiana* oviducts do not. However, when antisera to materials from the lower segments of *R. pipiens* oviducts are absorbed with materials from *R. clamitans* and *R. catesbeiana* oviducts, it is the latter which neutralize inhibiting antibodies, indicating that components common with the lower *R. pipiens* oviduct are present in *R. catesbeiana*.

Thus, if the species-specific and common components of the egg jellies of ranid species play significant roles in the fertilization process, there appear to be differences in the sites where these components would operate. Spermatozoa
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would presumably encounter component(s) shared between *R. pipiens* and *R. clamitans* in the inner and middle layers of egg jelly; the components shared by *R. pipiens* and *R. catesbeiana*, on the other hand, would be present in the outer and middle layers. Consequently, the hypothesis presented by Shaver (1966) should be altered to the extent that components in the egg jellies of any ranid species which are shared with other ranid species are not localized only in the inner and middle layers. This would, in turn, necessitate a reconsideration of the mechanisms whereby fertilization would occur in ranids with sperm of homologous and heterologous species. It is possible, of course, that the localization in the jelly layers around eggs of the different types of components after hydration of the jellies has little or no correlation with the situation immediately after the egg has been laid. At that time the different components in the jelly may be quite close to each other. Spermatozoa might then react preferentially with species-specific components, in the case of homologous inseminations, or with shared components, in the case of heterologous inseminations, without regard to the placement of the components.

The inhibition of fertilization in both *R. pipiens* and *R. clamitans* eggs to an equal degree by antisera to whole or regional oviducal antigens of all the ranid species tested was an unexpected result. It would have been expected that species-specific jelly components would play a more important role in fertilization than other jelly components, on the basis of Shaver's (1966) suggested model. For example, antisera against whole oviducal materials from *R. pipiens* should have been more inhibitory to fertilization of *R. pipiens* eggs than antisera against material from *R. clamitans*. It may be that species-specific components, whose presence has been demonstrated, play no more leading a role in fertilization reactions, in the species being considered, than the components shared with other species. Alternatively, there may be interactions involving the species-specific components in fertilization that are undetectable with the methods used in these experiments.

It seems apparent that the complexity of the oviducal secretions, as demonstrated histochemically (Lee, 1968; Humphries, 1966; D. E. Williams), will require more precise separation and characterization of antigenic materials from the jelly coats of amphibian eggs. Hedrick, Gussek, Oliphant & Wolf (1969) have shown that there are at least five macromolecular species present in solubilized jelly-coat material of *Xenopus laevis* as demonstrated by electrophoretic and ion exchange chromatography techniques, and that each jelly layer contains distinct macromolecules. It is clear, however, from the evidence to date (1) that some components in the jelly coats are essential for fertilization, (2) that these include both species-specific configurations and components shared with other species of the same genus (*Rana*), and (3) that there is a regional distribution in the oviduct of these components. The exact nature of the role of the jelly components in fertilization remains to be ascertained.
RÉSUMÉ

Relations interspécifiques de matériaux provenant de l'oviducte en rapport avec la fécondation chez les Amphibiens

Dans ces expériences, les œufs de l'espèce Rana ont été traités, avant la fécondation, par des anti-sérum anti-matériaux d'oviducte de Rana, Bufo et Ambystoma. Ces antisérum ont été employés dans trois types de traitement.

Des œufs de Rana pipiens et R. clamitans ont été traités avec des antisérum préparés contre les matériaux de l'oviducte entier, ou contre les segments antérieur et postérieur de l'oviducte, de R. pipiens, R. clamitans, R. sylvatica, R. catesbeiana, Bufo américense, B. marinus et Ambystoma mexicanum.

Des œufs de Rana pipiens ont été traités avec des sérums préparés contre les matériaux de l'oviducte entier de Rana pipiens ou de la partie antérieure, moyenne ou postérieure de cet organe, et préalablement absorbés par les matériaux des oviductes entiers des espèces désignées.

Des œufs de Rana pipiens ont été traités avec des antisérum préparés contre les matériaux des oviductes entiers ou segments antérieur, moyen ou postérieur de l'oviducte de Rana pipiens, et qui ont été absorbés par les matériaux provenant d'oviductes entiers ou de segments antérieur, moyen ou postérieur d'oviductes de l'espèce Rana.

Les résultats indiquent que le traitement avec des sérums dirigés contre les matériaux de l'oviducte de Rana, inhibe généralement la fécondation des œufs de Rana, alors que le traitement avec des sérums dirigés contre les matériaux des oviductes d'autres espèces d'Amphibiens n'a pas d'effet.

Les expériences avec les antisérum absorbés indiquent que les oviductes de Rana pipiens sécrètent des constitutants, qui sont également présents dans d'autres espèces de Rana, et qui sont localisés dans des régions déterminées de l'oviducte.

Les relations entre les sécrétions des oviductes d'Amphibiens et la fécondation sont discutées en rapport avec la complexité des enveloppes qui entourent l'œuf, complexité démontrée histochimiquement.

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