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# Loss of Fertilizing Power of Sea-Urchin and Urechis Sperm Treated with "Univalent" Antibodies vs. Antifertilizin.*

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The substance on sperm that reacts with the natural sperm agglutinin (fertilizin) has been termed[1] antifertilizin. It has been extracted and purified[2] and its role in fertilization examined[3] by a study of the fertilizing capacity and respiration of spermatozoa from which it had been partially extracted. It has also been shown[3] to be an active antigen capable of eliciting antibody formation in rabbits. This suggested the use of rabbit antisera for obtaining further information concerning its role in fertilization. The results of the present investigation show that such antisera, treated so as to render them non-agglutinating, destroy to a great extent the fertilizing capacity of the spermatozoa without appreciably affecting their motility.

*Material and Methods.* Antifertilizin solutions were prepared from sperm of the sea-urchin *Lytechinus pictus* and the gephyrean worm *Urechis caupo* by extraction with pH 3-4 sea water and purified by dialysis, salting out with saturated ammonium sulfate and isoelectric precipitation in the absence of salts. Examined electrophoretically the preparations showed only one component. The solutions of antifertilizin used for immunization contained 0.2 to 0.3 mg N/ml. These solutions titered to 128 to 256 in the egg-agglutination reaction. The rabbits (4 for Lytechinus and 2 for Urechis) were each given an alternate-day series of 6 to 10 injections with a total of 12 to 22 ml of the solutions. The antisera, obtained 4 to 8 days after the last injection agglutinated homologous sperm, the titers with 1% sperm suspensions ranging from 256 to 2048.

*Experimental.* Sea-urchin or *Urechis* sperm treated with homologous antiserum is found to be relatively ineffective for fertilization. This might well be expected on the basis simply of a mechanical effect of the agglutinin which ties up the sperm in clumps. To test for specific effects of antibodies on fertilizing capacity of sperm, it is desirable, then, to avoid the agglutinating action. In a preliminary previous test[3] this was done to some extent by the use of dilute sperm with excess antiserum and an inhibiting effect on fertilizing capacity was obtained. More recently, a fairly effective method, photo-oxidation, has been found[4] for converting antibodies into a non-agglutinating, but still specifically reactive form, termed "univalent." This provided an opportunity for further investigation of the problem.

Samples of the antisera, having agglutinative titers of 512 to 2048, were subjected to photo-oxidation as previously[4] described using 0.2% eosin as photo-sensitizer. The amount of photo-oxidation required to destroy agglutinating action in different samples of antisera varied from $O_2$ uptakes of 200 to 500 $mm^3$/ml. Since conversion of the antibodies to the univalent form evidently[4] involves a general interchange of parts of the various serum proteins, the variation may

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2 Frank, J., *Biol. Bull.*, 1939, **76**, 190; Tyler, A., *Proc. Nat. Acad. Sci.*, 1939, **25**, 317; Tyler, A., *West. J. Surg. Obst. and Gyn.*, 1942, **50**, 126; Tyler, A., *Proc. Nat. Acad. Sci.*, 1942, **28**, 391; Hartmann, M., Schartau, O., and Wallenfels, K., *Biol. Zentralblatt*, 1940, **60**, 398; Runnström, J., Tiselius, A., and Lindvall, S., *Ark. f. Zool.* (Stockholm), 1945, **36A**, No. 22, 1.

3 Tyler, A., and O'Melveny, K., *Biol. Bull.*, 1941, **81**, 364.

4 Tyler, A., *J. Immunol.*, 1945, **51**, 157; Tyler, A., *J. Immunol.*, 1945, **51**, 329; Tyler, A., and Swingle, S. M., *J. Immunol.*, 1945, **51**, 339.

TABLE I.
Fertilization of Lytechinus and Urechis Eggs by Homologous Sperm Treated with "Univalent" Antibodies.

Percentage fertilization of *ca.* 400 eggs in 5 ml of sea-water by sperm treated 15 minutes with photo-oxidized rabbit-antisera vs. antifertilizin of Lytechinus (L1 and L2) and of Urechis (U1 and U2) and normal rabbit-serum (N1), using 1 vol. of 1% sperm to 9 vols. of half-strength serum adjusted to sea water salinity. Photo-oxidation: 325 mm³ $O_2$ uptake per ml serum for L1, U1, N1; 410 mm³ for L2, U2. Original agglutinative titers: 512 for L1 and L2; 1024 for U1; 2048 for U2. Titers for inhibition of agglutination: 6 for L1; 9 for L2; 8 for U1; 12 for U2.

| Ml of serum-treated sperm (0.1%) used for insemination | Eggs and sperm of Lytechinus | | | | | | | | | | | | | Eggs and sperm of Urechis | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Test 1 | | | Test 2 | | | Test 3 | | Test 4 | | Test 5 | | Test 6 | | Test1 | | Test 2 | | Test 3 | |
| | L1 | N1 | U1 | L1 | N1 | U1 | L1 | N1 | L1 | U1 | L2 | U2 | L2 | U2 | U1 | L1 | U2 | L2 | U2 | L2 |
| 0.013 | — | — | — | — | — | — | 0 | 10 | 0 | 35 | — | — | — | — | ½ | 20 | — | — | 0 | 2 |
| 0.025 | 0 | 0 | 0 | 0 | 10 | 15 | 0 | 15 | 0 | 60 | 0 | 25 | 0 | 50 | 2 | 55 | 0 | 60 | 0 | 10 |
| 0.05 | 0 | 0 | 0 | 0 | 30 | 35 | 0 | 45 | 0 | 95 | 1 | 70 | ½ | 90 | 5 | 95 | 0 | 90 | 0 | 30 |
| 0.1 | 0 | 10 | 4 | 0 | 85 | 75 | 0 | 75 | 0 | 100 | 1 | 75 | 5 | 100 | 12 | 100 | 1 | 90 | 0 | 45 |
| 0.2 | 0 | 25 | 50 | 0 | 95 | 99 | ½ | 85 | 0 | 100 | 2 | 90 | 15 | 99 | 15 | 99 | 0 | 85 | ½ | 80 |
| 0.4 | 0 | 65 | 85 | 0 | 100 | 100 | 1 | 99 | 2 | 100 | 5 | 100 | 15 | 99 | 30 | 100 | 5 | 90 | ½ | 100 |
| 0.8 | 0 | 95 | 99 | 1 | 100 | 99 | — | — | — | — | 20 | 100 | 40 | 99 | — | — | 10 | 90 | 1 | 100 |

be due to differences in their relative amounts and properties in the different samples. After photo-oxidation beyond the point where agglutinating action was lost, the samples of antisera were tested for the presence of "univalent" antibodies by the inhibition method.[4] Several samples were thus obtained that had inhibition titers (amount of treated serum required to prevent the agglutinating action of one volume of untreated, agglutinating, serum) ranging from 4 to 12 and these were employed for treatment of the sperm.

Several things complicate tests of this sort with sperm. It is well known that the fertilizing capacity of sperm decreases with age of the suspension and that the rate of decrease varies inversely with the concentration. Also, it is obvious that the use of too little or too much sperm would obscure possible effects on fertilization and it is important that the range of amounts of sperm used for insemination cover or approximate the minimum at which the control gives 100% fertilization. At the same time it is desirable to treat the sperm with an excess of antibodies. Further, sperm from different animals may vary considerably in fertilizing capacity. Although it was possible to standardize sperm suspensions, eggs and procedure to some extent, only one-fourth of the tests covered the proper range.

In the tests one volume of 1% sperm suspension (1 vol. dry sperm plus 99 vols. sea-water; turbidity checked photoelectrically) was mixed with 9 volumes of serum that had been adjusted to the salinity of sea-water by the addition of an equal volume of concentrated (1.73-fold) sea-water. After 15 minutes various amounts of the sperm-serum mixtures were added to dishes containing aliquot samples of freshly washed eggs (ca. 400 eggs per dish) in a volume of 5 ml. The eggs were examined after ½ hour for membrane elevation and after 2 hours for cleavage.

The results of 6 sets of experiments with Lytechinus and of 3 with Urechis are given in Table I. In these tests the control sera consisted of similarly photo-oxidized heterologous antisera, *i.e.*, U1 and U2 (anti-Urechis) for Lytechinus and L1 and L2 (anti-Lytechinus) for Urechis as well as N1 (normal rabbit-serum). The variability mentioned above, is illustrated in the results of the different tests in which the same serum and sperm concentrations were employed. However, for the present purpose, comparisons need only to be made within each test. On this basis, it may be seen from Table I that the spermatozoa treated with homologous serum give considerably less fertilization than do those treated with control serum. In certain of the tests (Lytechinus tests 5 and 6, Urechis tests 1 and 3) the smallest amounts

of control serum-treated sperm gave percentages of fertilization that approximated those obtained with the largest amounts of homologous antiserum-treated sperm. From these and from the other tests by extrapolation an estimate may be made of the reduction in fertilizing capacity of the sperm due to treatment with antiserum. This reduction ranges from 32-fold in 3 of the tests (Lytechinus 5 and 6, Urechis 1) to considerably more than 64-fold in the others. The difference apparently correlates with differences in antibody-titer (based on both original agglutinative titers and inhibitive titers) but more extensive tests would be necessary for the determination of any quantitative relation. The present results suffice to demonstrate that treatment of sperm with antisera can effect a greater than 64-fold reduction in fertilizing power.

In each of the tests the spermatozoa were examined microscopically for possible effects of antiserum on their motility. No noticeable differences from the activity of the spermatozoa in the control sera were observed, about 70 to 90% of the sperm being highly active in both.

*Discussion.* Since the antibodies employed in these tests were produced as a result of immunization of rabbits with antifertilizin obtained from the spermatozoa, it would appear that the inhibiting effect on fertilization furnishes further support of the view that the antifertilizin is intimately involved in the fertilization process. It does not, however, necessarily follow that the specific groups or structures of the antifertilizin molecules that may be involved in the initial reaction of sperm and egg are the same as those that serve as the active antigenic determinants in producing antibodies in the rabbit. It is quite conceivable that the antibodies are directed against other specific groups, and their presence on the surface simply blocks the action of those involved in the reaction with fertilizin, etc. Tests that are planned with closely related, difficultly cross-fertilizing, species may help decide this point.

In the present experiments the antibodies were first converted to a non-agglutinating "univalent," form in order to eliminate the mechanical effect of the clumping. It may be noted that such "univalent" antibodies may provide a useful tool in various types of immunological investigations involving enzymes, hormones, bacterial antigens, viruses, etc., where it is desirable to avoid the agglomerating action of the ordinary, "multivalent," antibodies.

*Summary.* Antisera were produced in rabbits by immunization with purified antifertilizin of Lytechinus and of Urechis and were photo-oxidized so as to convert the antibodies into the "univalent" (non-agglutinating) form. Sperm treated with such antisera were found to possess less than 1/64 of the fertilizing capacity of the controls although their activity was not noticeably affected. The bearing of the results on the significance of antifertilizin in the fertilization reaction is briefly discussed and the possible general use of "univalent" antibodies is mentioned.

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## Influence of Amino Acid Feeding upon Antibody Production in Protein Depleted Rats.*

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In previous publications from this laboratory the deleterious effects of prolonged protein depletion upon antibody formation in rabbits and rats have been reported.[1,2] In

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