[9] T. Hinton, D. T. Noyes, and J. Ellis, *Phys. Zoöl.*, **24**, 335, 1951.
[10] E. S. Gersh, *Drosophila Information Service*, **20**, 86, 1946.
[11] G. Zimm, *J. Exptl. Zoöl.*, **116**, 289, 1951.
[12] R. B. Goldschmidt, A. Hannah, and L. K. Piternick, *Univ. Calif. (Berkeley) Publs. Zoöl.*, **55**, 67, 1951.

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*ANTISERA THAT BLOCK CELL DIVISION IN DEVELOPING EGGS OF SEA URCHINS**

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As part of a program of investigation[1, 2, 3] of problems of fertilization and early development, the senior author has prepared antisera against various materials obtained from eggs, sperm, embryos, and adults of several species of sea urchins. In the course of the tests on developing eggs, it was observed that certain of the antisera blocked cleavage very effectively. The various antisera produced against fertilizin were found to be particularly effective cleavage-blocking agents. This seemed of special interest, since fertilizin is known[1–5] to be the sperm-receptor substance that constitutes the surface coat of the egg and is now chemically rather well defined. The cleavage-blocking action was therefore further investigated. In the present paper the results of a survey of the action of various kinds of antisera are presented.

MATERIAL AND METHODS

Antisera were prepared in rabbits against materials of the sea urchins *Strongylocentrotus purpuratus, S. franciscanus, Lytechinus pictus* (Newport Bay, California), and *Arbacia punctulata* (Woods Hole, Massachusetts). The rabbits were first bled for control sera several days before injection. The immunization schedule generally consisted of two intravenous and one intra-abdominal injections per week for three weeks. The rabbits were usually bled on the fifth and seventh days and sometimes on the ninth day after the last injection. The nitrogen content of the total amount of antigen that was injected ranged from about 2 to 2.5 mg., except for the fertilizin preparations, which were 0.8–1 mg. The preparation of fertilizin from the eggs, antifertilizin from sperm, and antifertilizin from eggs was according to previously described[1, 2] methods which, in the case of fertilizin and antifertilizin from sperm, yield highly pure solutions. For the preparation of sea-urchin "blood" the coelomic fluid of unripe animals was bled through a puncture in the peristome, and the clotted cells were centrifuged, washed in sea water, and then homogenized. For epidermal antigen the sea-urchin tests were thoroughly washed, all soft tissue scraped from the inside, and the portions containing the rows of tube feet were discarded. The remainder was ground in diluted (30 per cent) sea water, centrifuged at about 1500 × g for 15 minutes, and the sediment discarded. For antigens of the various developmental stages the fertilized eggs were cultured, with precautions to insure uniformity. The embryos were centrifuged at the desired stage, washed

in sea water, and then homogenized in 1 per cent NaCl in the cold. The suspensions of embryos in swimming stages were made 0.0001 molar in NaCN to immobilize the embryos so that they could be more readily sedimented. Motility returned to these after centrifugation and washing.

For the tests on the developing eggs the antisera and control sera were dialyzed in the cold against sea water. Between tests the sera were stored in the frozen state. Both unheated sera and sera heated at 56° C. for $^1/_2$ hour, to inactivate complement, were tested. The eggs to be tested for cleavage block were demembranated 1–2 minutes after fertilization by drawing them up into a syringe through a 20-gauge hypodermic needle; they were then washed, and 0.1 ml. of the suspension, containing about 100 eggs, was added to 0.1 ml. of the full-strength serum or dilutions thereof, at the specified times after fertilization. They were cultured at 17° C.

RESULTS

Table 1 lists the number of rabbits that produced or failed to produce cleavage-blocking antisera in response to immunization with the various antigens. The

TABLE 1

PRODUCTION OF ANTISERA THAT BLOCK CLEAVAGE OF EGGS OF *Strongylocentrotus purpuratus* AND *Lytechinus pictus*

IMMUNIZING ANTIGEN	NO. OF RABBITS	NO. PRODUCING ANTISERA THAT BLOCK CLEAVAGE OF *S. purp.* eggs	NO. PRODUCING ANTISERA THAT BLOCK CLEAVAGE OF *L. pictus* eggs
None	53	1	1
S. purpuratus:			
Fertilizin	10	10	10
Univalent fertilizin	3	3	2
Fertilizin-treated sperm	3	1	1
Whole sperm	5	0	0
Antifertilizin from sperm	6	1	1
Nucleoprotein from sperm	1	0	0
Antifertilizin from eggs	6	3	3
Extract of fertilized eggs	1	..	1
Extract of hatched blastulae	1	1	1
Extract of gastrulae	2	2	2
Extract of prism larvae	2	2	2
Extract of pluteus larvae	2	2	2
Blood	3	0	0
Extract of epidermis	2	0	0
L. pictus:			
Fertilizin	5	3	3
Fertilizin-treated sperm	2	2	2
Whole sperm	2	0	0
Antifertilizin from sperm	6	0	0
Antifertilizin from eggs	1	1	1
Extract of fertilized eggs	1	..	1
Homogenate of 16-cell stage	1	1	1
Extract of hatched blastulae	2	2	2
Extract of gastrulae	1	1	1
Extract of prism larvae	1	1	1
Extract of epidermis	2	0	0
S. franciscanus:			
Fertilizin	2	2	2
Whole sperm	1	0	0
A. punctulata:			
Fertilizin	2	0	0
Antifertilizin from sperm	1	0	0
Antifertilizin from eggs	2	0	0
Blood	1	0	0

tabulation is based on whether or not one or more of the serum samples of the rabbit blocked cleavage, two or three different bleedings having been tested, each two or more times. The criterion for blocking was whether or not development was stopped before the 32-cell stage when the eggs and full-strength sera were mixed at 10–15 minutes after fertilization.

In the control tests (pre-injection bleedings) one serum was encountered that blocked *S. purpuratus* eggs and one that blocked *L. pictus* eggs. These two sera were small samples that were left over from other kinds of experiments. Unfortunately, there was only enough for one test with each, and no check could be made on possible abnormal conditions in these sera. In view also of the lack of blocking effect of control sera from the other 51 rabbits, it appears that little weight need be given to the exceptions.

Of the 10 rabbits injected with *S. purpuratus* fertilizin, all produced antisera that blocked cleavage of the homologous eggs and of those of *L. pictus*. Of the 5 rabbits that were immunized with *L. pictus* fertilizin, 3 produced similarly cytotoxic antisera. Two rabbits that were injected with *S. franciscanus* fertilizin both produced active antisera, but 2 that received *A. punctulata* fertilizin did not. Fertilizin that had been rendered nonagglutinating ("univalent") by heat treatment[6] retained its ability to induce the formation of cytotoxic antisera, as indicated in the table. Of 22 rabbits that received whole sperm, the antifertilizin preparation, or nucleoprotein from sperm of these four species, only 1 produced a cleavage-blocking antiserum, and its effect was comparatively weak. Injection of 8 rabbits with blood or with extract of epidermis of these sea urchins failed to yield blocking antisera. Extracts of the unfertilized, jellyless eggs (antifertilizin), of fertilized uncleaved eggs, and of developing embryos at various stages up to the pluteus stage were effective in most cases, as the table shows, but in general the antisera had weaker action than had those produced by immunization with fertilizin.

When the developing eggs are cultured in increasing dilutions of a blocking antiserum, the blocking effect and ensuing cytolysis are progressively delayed. The following data exemplify this relationship in a test of the degree of development attained in 3 days at 17° C. when *S. purpuratus* eggs, 15 minutes after fertilization, are added to serial twofold dilutions of an antiserum against *S. purpuratus* fertilizin.

	Serum Dilution						
	2× to 16×	32×	64×	128×	256×	512×	1,024×
Stage at 72 hr.	1-cell†	2-†	4-†	16-†	32- to 64-†	Blastula†	Prism to plutei

The eggs and embryos in the dilutions up to 512× had cytolyzed, as indicated by the dagger (†). Those in the 1,024-fold dilution of this serum were alive but retarded with respect to the controls. In many other similar titrations appropriate dilution of a cleavage-blocking antiserum resulted in blocking of development at practically any later stage. When swimming embryos are introduced into undiluted cleavage-blocking antisera at various later stages of development, they are almost immediately immobilized. Cytolysis ensues in about the same time as for the uncleaved eggs, and dilution of the antisera delays the effect correspondingly. Cross-reaction occurs also between embryos and antisera of *S. purpuratus* and *L. pictus*.

Antisera that had been heated to 56° C. for $^1/_2$ hour to inactivate complement were compared with unheated antisera in 12 tests and showed no significant reduction in cleavage-blocking action.

DISCUSSION

It has long been known that antisera prepared against cells or cell extracts of various organisms can inhibit division of the cells and can induce other cytotoxic effects. In recent years cytopathogenic sera have been studied primarily by workers in the field of tumor research, where efforts have been directed toward the development of a tumor-specific antiserum. Among current investigations in this field may be mentioned the work of Nungester and Fisher,[7] Imagawa *et al.*,[8] and Mountain.[9] The new feature of the present work is that the block to division can be obtained with an antiserum produced against a cell constituent that is chemically rather well defined and whose primary location is known. In sea urchins fertilizin constitutes the gelatinous coat and surface of the unfertilized egg.[1-3] It is a glycoprotein containing roughly equal amounts of sugars, amino acids, and sulfate. In the species thus far examined, there are usually two kinds of sugars and some fourteen kinds of amino acids in the molecule.[2, 3, 10] Preparations are readily obtained that are electrophoretically and ultracentrifugally homogeneous and whose purity can be further demonstrated by tests of absorption with homologous sperm. Its molecular weight, in different species, is near 300,000, with an axial ratio of 20:1.[5]

Biologically, fertilizin represents the specific receptor substance for the attachment of the sperm and is largely responsible for the tissue- and species-specificity of fertilization.

In the cleavage-blocking tests the gelatinous coat and fertilization membrane are removed from the eggs, the action of the antisera being very much retarded if these are present. The fact that the fertilizin-antisera act on the denuded eggs implies that the surface of the latter has antigens in common with fertilizin. This may be related to the cytofertilizin reported by Motomura[11] and may also account for the re-fertilizability of fertilized eggs.[12] The later stages presumably also retain fertilizin-like antigens on the surface. These common antigenic structures are evidently not simply "species antigens," since the antisera are effective on the embryos of a heterologous species of sea urchin.

Experiments on cell-division-blocking antisera have generally been performed with whole cells, homogenates thereof, or particulate extracts as antigens. The present results point to the desirability of employing, as immunizing antigen in such experiments, cell-surface constituents obtained by mild extraction procedures. An indication that such methods may also be effective with cells of higher animals appears in experiments by Billingham and Sparrow,[13] who noted that saline washings of dissociated epidermal cells of rabbits could induce an iso-immunization, as evidence by accelerated incompatibility reaction to skin grafts.

SUMMARY

Cell division in developing sea-urchin eggs can be blocked by rabbit antisera against homologous fertilizin (the surface coat of the unfertilized egg) and, somewhat less effectively, by antisera against other extracts of the eggs and embryos at various stages of development. Antisera against sperm are ineffective in this regard as are also antisera against blood and epidermal tissue of the adult. Cross-reaction occurs with related species of sea urchins. Inactivation of complement does not reduce the blocking action of the antisera. Upon dilution of the fertilizin-antisera, development is blocked at progressively later stages of development.

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[1] A. Tyler, *Physiol. Revs.*, **28**, 180–219, 1948.
[2] A. Tyler, *Am. Naturalist*, **83**, 195–219, 1949.
[3] A. Tyler, *Analysis of Development* (Philadelphia: W. B. Saunders Co., 1955), pp. 556–573.
[4] A. Tyler and C. B. Metz, *Pubbl. Staz. zool. Napoli*, **27**, 128–145, 1955.
[5] A. Tyler, *Exptl. Cell Research*, 1956 (in press).
[6] A. Tyler, *Biol. Bull.*, **81**, 190–204, 1941.
[7] W. J. Nungester and H. Fisher, *Cancer Research*, **14**, 284–288, 1954.
[8] D. T. Imagawa, J. T. Syverton, and J. J. Bittner, *Cancer Research*, **14**, 1–11, 1954.
[9] I. M. Mountain, *J. Immunol.*, **75**, 478–484, 1955.
[10] E. Vasseur, *Acta chem. Scand.*, **2**, 900–913, 1948; **4**, 1144–1145, 1950; **6**, 376–384, 1952.
[11] I. Motomura, *Exptl. Cell Research*, **5**, 187–190, 1953.
[12] A. Tyler, A. Monroy, and C. B. Metz, *Biol. Bull.*, 1956 (in press).
[13] R. E. Billingham and E. M. Sparrow, *J. Embryol. Exptl. Morphol.*, **3**, 265–285, 1955.

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*INHIBITION OF DIVISION AND DEVELOPMENT OF SEA-URCHIN EGGS BY ANTISERA AGAINST FERTILIZIN**

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In the previous paper[1] it was reported that rabbit antisera prepared against fertilizin of sea urchins were effective in blocking cell division and development of the fertilized eggs. The results of some further investigations of this action are presented here. The materials and testing methods were the same as previously described, except where otherwise noted.

EXPERIMENTS

Development in Immune Sera.—As noted in the previous paper, rabbit antisera prepared against the fertilizins of the sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus* can block division before the first cleavage when added to the demembranated eggs a short time after fertilization. The amount of developmental progress attained is a function of the strength of the antiserum. An antiserum that blocks division before the first cleavage when used full strength will, upon dilution, permit development to proceed to progressively later stages of cleavage and embryonic development.

Cytological studies have been made of eggs that have been treated with strong antiserum at various times in the division cycle. These have shown that nuclear as well as cytoplasmic division is arrested and that mitosis may be stopped at practically any stage of the cycle, there being no evidence of a specially sensitive phase. The strongest antisera so far available, permit, when used full strength, an amount of development equivalent to approximately one-fifth to one-fourth of a division cycle. Thus, for example, eggs of *Lytechinus* that take 95 minutes for the first division at 17° C. will make about 20 minutes' developmental progress in an undiluted