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The Dispensability of Complement for Antidevelopmental Action of Antisera Against Fertilizin in Sea Urchin Eggs.* (29970)

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Antisera that are prepared in rabbits against the purified fertilizin of sea urchin eggs have been previously shown(1-6) to have cytotoxic effects. When added to unfertilized eggs such antisera inhibit fertilization and, after prolonged treatment, cause visible blistering and cytolysis. When added to fertilized eggs, in various stages of development, the antisera can block cleavage abruptly, nuclear as well as cytoplasmic division being inhibited. Visible cytolysis follows some time later.

According to present evidence(5) fertilizin is a primary constituent of the plasma membrane of the egg and extends through the overlying vitelline membrane in the form of attenuated micelles that constitute the gelatinous coat of the egg. Antisera that are prepared against purified fertilizin, derived from the gelatinous coat, may be expected to react with the plasma membrane of the fertilized (deprived of vitelline membrane) as well as the unfertilized egg. It is, then, understandable that such antisera can block development of the fertilized egg. In fact the evidence(5) shows that antibodies directed against internal constituents of the egg (*e.g.*, prepared by absorbing antiwhole egg antisera) exert very little if any cytotoxic action on the intact unfertilized, or fertilized, egg.

In the previously reported(1-3) studies there was evidence that the heat-labile components (C'1 and C'2) of complement are not needed for the cleavage-blocking action of the antisera *vs* fertilizin since such sera remained active after being heated to 56°C for ½ hour. Since, however, complement has

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been so generally found to be involved in cytotoxic or cytolytic effects of antibodies on cells of various kinds (see reviews by Osler (7), Winn(8), Stetson(9), Swisher(10)) and since there have been very few reports (*e.g.*, Amos and Wakefield(11), Gabourel(12), Ehrlich and Halbert(13), Boyse *et al*(14)) of such action of antibodies occurring without complement it seemed desirable to explore further the question of the extent to which complement may be involved in the present system. For this purpose we have (a) done extensive titrations of heated and unheated antisera for cleavage-blocking action, (b) depleted the antisera of complement by means of a foreign antigen-antibody reaction and (c) titrated heated and unheated antisera for cleavage-blocking action in presence of various amounts of added complement in the form of fresh guinea pig serum.

Materials and methods. Eggs and sperm of the sea urchins *Lytechinus pictus* and *Strongylocentrotus purpuratus* were obtained by the method of KCl-induced spawning(15). An artificial sea water(16) was used both for keeping the animals and for culturing the developing eggs. The antisera were prepared as previously described(1-3). Primarily those prepared against purified fertilizin preparations were used in the present experiment. Some experiments were also run with antisera prepared against whole egg homogenates, as noted below. The antisera, and the control sera from pre-injection bleedings, were dialyzed against sea water before use.

The tests of blocking action were done with eggs from which the fertilization membranes were removed. The demembranation was done within one to three minutes after fertilization by passing the eggs rapidly through a pipette or syringe and needle with a bore of approximately 1 mm. The tests were done in glass hemagglutinating slides, with 0.1 ml each of antiserum dilution and of egg suspension (ca. 100 eggs/0.1 ml).

Results. (a) Table I summarizes tests of the effect of heating at 56°C for one hour on the antidevelopmental action of 36 antisera derived from 10 different rabbits. The tests were made with serial 2-fold dilutions of the antisera. Pre-injection sera served as controls. Titers were determined as the dilutions (interpolated where necessary) giving 50% inhibition of first cleavage, and also as 50% inhibition of development to swimming blastulae. The figures in the second and third columns of the Table give the number of tests in which the ratio of titers of heated to unheated antisera fall within the values listed in the first column. The absence of any significant effect of heating is indicated by the facts that the majority of the tests gave ratios in the range close to unity (0.8 to 1.2) and the remaining tests gave ratios that are about equally distributed above and below this range. It seems safe to assume, then, that the latter divergences represent chance variations.

TABLE I. Effect of Heating (56°C, 1 Hour) on Antidevelopmental Action of Antisera *vs* Fertilizin of Sea Urchins (*S. purpuratus* and *L. pictus*).

Ratio of titers for 50% inhibition: Heated/Unheated	No. of separate experiments	
	Scored at 2-cell stage	Scored at blastula stage
0 – .4	1	0
.4– .8	8	6
.8–1.2	17	19
1.2–1.6	7	7
1.6–2.0	3	4

The 36 antisera represent different bleedings of 10 rabbits tested along with preinjection controls. Their 50% inhibition titers ranged from 8× to 64× for cleavage block and 32× to 256× for blastula block.

(b) Table II presents results of experiments in which complement was removed by an unrelated antigen-antibody system; namely, hen's ovalbumin reacting with the homologous rabbit antiserum. Antisera *vs* fertilizin were incubated with the precipitate that was obtained by reacting 0.2 ml of heated antiovalbumin serum with 0.2 ml of a solution of ovalbumin (0.335 mg/ml) previously found to precipitate the antibody at equivalence. The precipitates were washed twice in 0.9% NaCl before use and incubation with the fertilizin-antisera (0.5 ml) was for 30 minutes at 37°C and 16 hours at 4°C. When this procedure was followed with fresh guinea pig serum the complement titer (50% hemolysis of 2% suspension of sensitized sheep erythrocytes) was lowered from 1/256 to 1/32.

As the results in Table II show, the removal of components of complement by this method had no significant effect on the cleavage-

blocking ability of the fertilizin-antisera.

(c) Table III gives results of tests of the effect of adding complement-containing guinea pig serum to heated, cleavage-blocking antisera. One of the tested antisera was prepared against whole egg homogenate; the other against purified fertilizin. With both antisera the addition of complement had no significant effect on the titers for blocking of cleavage or of inhibition of later development.

Discussion. The present and previously reported results show that the antisera against fertilizin exert their antidevelopmental effect even when deprived of complement and that

TABLE II. Effect of Removing Complement by Coprecipitation with an Unrelated Antigen-Antibody System (Egg Albumin-Anti-Egg Albumin) on Blocking Action of Antisera *vs* Fertilizin. Eggs of *Strongylocentrotus purpuratus* placed in serum dilutions at 15 min after fertilization.

		% in indicated stages at:					
		100 min		200 min			
Serum	Dilution	1 cell	2 cell	1 cell	2 cell	4 cell	Cyt.
Normal rabbit	1/4	20	80	15	10	75	0
Antiserum RFB2 *vs* fertilizin	1/4	100	0	100	0	0	0
	1/8	100	0	85	15	0	0
	1/16	100	0	85	15	0	0
	1/32	50	50	60	25	15	0
	1/64	50	50	50	25	25	0
Antiserum RFB2 decomplemented	1/4	95	5	95	5	0	0
	1/8	90	10	90	10	0	0
	1/16	90	10	85	15	0	0
	1/32	75	25	85	15	0	0
	1/64	50	50	60	25	15	0
Antiserum S IIIB *vs* fertilizin	1/4	90	10	90	10	0	0
	1/8	100	0	90	0	0	10
	1/16	20	80	25	25	50	0
	1/32	20	80	25	25	50	0
	1/64	20	80	25	50	50	0
Antiserum S IIIB decomplemented	1/4	50	50	50	25	25	0
	1/8	50	50	50	25	25	0
	1/16	20	80	25	25	50	0
	1/32	20	80	25	25	50	0
	1/64	20	80	25	25	50	0

TABLE III. Effect of Addition of Complement on Blocking Action of Heated Rabbit Antisera *vs* Fertilizin of *Lytechinus pictus*. Eggs introduced at 15 min after fertilization. Figures represent percentage reaching 2 cell, blastula or pluteus stages at indicated times.

		Unheated guinea pig serum*			Heated guinea pig serum†		
		2 cell	Blastula	Pluteus	2 cell	Blastula	Pluteus
Serum	Dilution	1 hr	6 hr	24 hr	1 hr	6 hr	24 hr
Normal rabbit	1/5	80	95	50	80	95	25
Antiserum 2B *vs* egg homogenate	1/5	20	0	0	20	0	0
	1/10	50	25	0	50	20	0
	1/20	80	75	15	80	75	0
	1/40	80	95	40 (20)‡	80	95	25 (10)‡
Antiserum 7A *vs* fertilizin	1/5	0	0	0	0	0	0
	1/10	15	0	0	15	0	0
	1/20	50	40	0	50	25	0
	1/40	80	95	25	80	95	10 (15)‡

* Guinea pig serum was reconstituted lyophilized preparation (Hyland Labs) used at a dilution of 1/20. Complement titer 1/160 for 50% hemolysis of sensitized sheep RBC in a suspension of 2.5%.

† Heat treatment 30 min at 56°C.

‡ Figures in parentheses represent exogastrulated embryos.

the effect is not enhanced by addition of complement. There are relatively few cases that may be cited from the more recent literature in which antisera have been shown to have cytotoxic effects without the evident participation of complement.

One such example appears in experiments by Boyse *et al*(14) with diffusion chambers which are known to be relatively impermeable to complement(11). In this situation leukemic cells were destroyed by antibody. Other cells, however, were found to be resistant. The results indicate that destruction of the leukemic cells requires little, if any, added complement. Similar findings have been reported by Gabourel(12) for L-fibroblasts in diffusion chambers in immunized mice. Another example of apparently complement-independent cytotoxic action is contained in experiments by Ehrlich and Halbert(13) with antilens serum.

It should, of course, be noted that in most experiments in which reactions of living cells to antibodies are examined the tests are generally of some direct cytolytic effects, rather than of interference with proliferation. Quite possibly when more systems are investigated with respect to the latter property a similar lack of removal or addition of complement may be found. It is pertinent to record here tests of the ability of antiserum-treated sea urchin eggs to exclude the dye eosin Y. The inhibition of cleavage is obtained without any initial effect on dye-exclusion. Later, when visible signs of cytolysis appear the eosin Y enters the eggs.

One reservation may be made with respect to the interpretation of the present experiments as indicating absence of any role of complement in the antidevelopmental action of these anti-sea urchin antibodies. This is the possibility that the eggs themselves may contribute one or more of the components of complement. Earlier experiments(17) had shown that the antifertilizin of sperm is related to C'4 of guinea pig complement, in that it can release this component after it had been bound by fertilizin. It is, then, possible that the antifertilizin within the egg, which also has the property of reacting with fertilizin of the egg's plasma membrane, may provide effectively the equivalent of complement action in the antiserum-treated eggs.

Summary. The present experiments add further evidence that serum-complement has no effect on the ability of rabbit antisera prepared against the fertilizin of sea urchin eggs to block cleavage and development of the fertilized eggs.

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