THE EFFECTS OF ANTI-EGG JELLY SERUM ON COELOMIC FROG EGGS

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ABSTRACT

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ON COELOMIC FROG EGGS

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Treating normal uterine frog eggs with anti-egg jelly serum will reduce the percentage of eggs that will cleave (Shaver and Barch 1960). This is similar to the effects noted by Tyler and Brookbank (1956) using sea urchin eggs treated with a serum made against fertilizin (the jelly coat).

The present experiments are concerned with the effects on the fertilizability and cleavage of Rana pipiens Schreber coelomic eggs treated with anti-egg jelly serum before the deposition of the jelly coat.

Appropriate doses of pituitary glands were injected into females which previously had had their oviducts removed. The coelomic eggs thus obtained were treated with either egg jelly antiserum, non-immune rabbit serum, or full strength Holtfreter's solution. The treated eggs were transferred into ovarectomized, pituitary stimulated hosts for trans-

port down the oviducts and the deposition of the jelly coat. The treated eggs were removed and inseminated. The results were recorded as percentages of eggs which cleaved.

The data suggest an effect like that obtained when normal uterine eggs were treated with egg jelly antiserum. That is, a significantly lower percentage of cleavage was obtained from the coelomic eggs when treated with the anti-egg jelly serum than with either non-immune serum or full strength Holtfreter's solution.

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By

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INTRODUCTION

The jelly coat around Amphibian eggs plays a major role in the fertilization process. Jellyless eggs, either before the jelly coat has been put on or after the jelly has been removed, either by chemical or mechanical means, are incapable of being fertilized (Bataillon, 1919; Rugh, 1935; Kambara, 1953; Tchow-Tu and Wang, 1956).

Kambara (1953), using the toad Bufo vulgaris formosus, found that the second layer of jelly from the egg surface, the C layer, is necessary for fertilization. Glick and Shaver (1963) demonstrated in Rana pipiens that the farther the egg proceeds down the oviduct, and thus the more jelly the egg has collected, the more likely it is able to be fertilized. They have interpreted this result as indicating that species-specific components, put down in the lower oviduct, play a major role in the fertilization reaction.

However, jellyless eggs will develop partheno-genetically after inoculation with a cellular constituent (Bataillion, 1919) or by the introduction of a blastula nucleus (Rubtelny and Bradt, 1961). The later investigators observed that coelomic eggs developed

more normally, after the nuclear transfer, when they were inserted into a jelly envelope taken from a uterine egg. Also, Chivers (personal communication) has demonstrated that body cavity eggs of B. pipiens can be fertilized if inseminated with a sperm suspension that was in previous contact with normal jellied eggs. He obtained about 20 percent cleavage.

Kambara (1953), working with B.v. formosus, has suggested that the chemical composition of the jelly may not be an important factor; he claims to have obtained fertilization after inseminating dejellied eggs re-enveloped in a coating of 6 percent gelatin, indicating that at least one of the functions of the jelly may be to act as a substrate for a thigmotatic response for the sperm. There is no evidence of this in any other species. Katagiri (personal communication) has not been able to repeat Kambara's results even with the same species of toad. He has however obtained some data indicating that fertilization is possible when only a small amount of the jelly coat is present around the eggs.

Good and Daniel (1947) obtained a small percentage of cleavage when they treated jellyless newt (Taricha torosus) eggs with a very dense sperm suspension.

Some investigators have suggested the possibility that there may be interacting substances of eggs and sperm necessary for fertilization. Tyler (1956) has suggested models for the fertilization reaction involving complementary sites on the gametes that act much the same as antigen-antibody reactions. It should be noted that any interference with these "active sites" or neighboring sites would affect fertilization and / or development.

Probably the first to suggest that fertilization may be similar to a serological interaction was Lillie (1919). On the basis of his sperm-agglutination experiments, he introduced the concept of fertilizin (of the egg) and antifertilizin (of the sperm) interacting in fertilization. Other workers have shown similar results in species of the Phyla Annelida, Mollusca, and Chordata (Metz 1957).

Glaser (as cited in Shaver and Marsh 1960) reported agglutination of R. pipiens spermatozoa in egg water of the same species. Numerous attempts to repeat this were unsuccessful (Bernstein, 1952; Shaver (personal communication). However Bernstein (1952) has reported irreversible agglutination of Rana claritans sperm in egg water of the same species.

It is thought that this is due to the fact that R. claritans jelly is more soluble than R. pipiens. No agglutination was noted however in "egg water" from jellyless eggs. Thus it would seem that the agglutinating molecule in R. claritans is present in the jelly components. In sea urchins, the main source of fertilizin is the highly soluble jelly coat (Tyler, 1957). However, Motomura (1950) has demonstrated that the egg produces some fertilizin, which he calls cytofertilizin.

It has been demonstrated (Tyler and Brookbank 1956) in the sea urchin that antisera produced against fertilizin will inhibit fertilization and cleavage. It may be incorrect, however, to say that fertilizin antibodies are indeed blocking specific sites necessary for fertilization. They may, in fact, be attached to adjacent sites and be physically blocking the operative ones.

Shaver and Barch (1960) noted similar effects when they treated uterine eggs with anti-jelly serum prior to fertilization. They postulated a barrier to sperm penetration produced by the reaction between the jelly coat and the serum. Later Shivers and Metz (1962) rendered the antibody univalent by treatment

with papain, which eliminated the precipitation reaction on the jelly coat noted by Shaver and Barch. However making the antibody univalent did not affect the inhibitory properties of the antiserum. Shivers and Metz conclude that the inhibition is due to blocking a receptor site in or on the jelly. Whether the blocking is due to an immulogically specific antigen-antibody reaction is still a question.

The location of the primary receptor site for the sperm, affected by the antiserum, may be on the outer layer of the jelly. Shivers (1962) has shown by fluorescent-antibody staining that components shared in common with other species are located in the innermost layer of jelly, and that the antisera most effective in inhibiting fertilization are the species-specific ones made against the outer layers of jelly.

From later work by Shaver, Barch, and Shivers (1962) there is some evidence that at least one component found in the jelly coat is similar to one in the egg. Also, it appears that chemically dejellied, fertilized eggs when treated with antijelly serum will cease cleavage (Cerny, 1963). The question arose

whether the treatment of R. pipiens eggs with anti-egg jelly serum, before the jelly coat is deposited on them, would inhibit fertilization and cleavage, either by occupying a site on the egg surface complementary to the antibody directed against the egg component of the jelly, or by reacting with sites on the egg surface complementary to jelly configuration.

MATERIAL AND METHODS

Two techniques have been used to affect interferale transfer of coelomic eggs. Lavin (1963) injected into host females vitally stained coelomic eggs which had been treated with various sera (e.g. anti-large oocyte, non-immune rabbit serum, etc.) before being transferred.

The procedure used in this study was developed by Arnold and Shaver (1962). This technique consists of stimulating an oviductectomized donor female with pituitary glands to ovulate eggs into the coelome, treating the eggs obtained, and transferring them into a host ovarectomized female.

Although the technique devised by Arnold and Shaver takes longer, due to the surgery required, and is a bit more difficult, the period of time in which eggs are outside the body cavity of the animal is less than in Lavin's technique, which requires staining and washing of the eggs. The extra manipulations, required for these procedures, are also eliminated.

Specimens of Rana pipiens Schreber obtained from dealers in Wisconsin and Vermont were used.

A. GENERAL PROCEDURE FOR REMOVING OVIDUCTS AND OVARIES

To remove the oviduct, the donors were ether anesthetized in a pint canning jar. They were removed when movement ceased and a folded water-soaked paper towek, held in place with a rubber band, was wrapped around their hind legs. This was done to keep the frogs immobilized since some recovered from the ether quite rapidly, sometimes before the operations were completed. A small incision about 6 mm in length was then made through the skin and the musculature dorsal to the forearm and posterior to the head. The oviduct was grasped with a pair of forceps and gently pulled through the opening. The anterior end, including the ostium, was pulled from its attachment, the mesentery attaching the oviduct to the body wall and kidney was cut along the entire length, and the posterior end was sectioned in the region of the uterus. The muscle layers and skin were then separately sewn using 5-0 silk surgical sutures. The procedure was repeated on the opposite side. However, before the second side was sutured, whole *R. pipiens* pituitary glands were introduced into the body cavity

to evoke ovulation, the number of glands being varied according to the season. Three glands were injected per day for two days dur'ng the winter months and two per day for two days as normal ovulation time (spring) approached.

Forty eight hours later the donors were pithed and their body cavities opened. In the majority of females close to 100% of the eggs had been ovulated.

The hosts were anethethized in the same manner as the donors. An incision about 10 mm long was made on the abdominal wall over the area of the ovaries but just lateral of the median line, so as not to injure the ventral abdominal vein. Both ovaries were then pulled through the same incision; blood vessels were tied off and cut distal to the ligation.

Most of the host females were prepared shortly prior to the time of transfer. A few were prepared two to three days in advance to determine if the shock of having ovarectomy at the time of transfer inhibited the transferred eggs from reaching the oviscs.

The treated eggs from the donor were then injected into the body cavity of the host, through the same

incision from which the ovaries had been removed, with the aid of a syringe and a blunt number 12 needle. Pituitary glands were then added, the number varying according to the season, to stimulate the cilia of the coelomic wall and of the oviducts to move the eggs up to the ostia and down the oviducts. Twenty four hours later the eggs were stripped onto glass slides and inseminated with a sperm suspension made up of two macerated testes per 10 ml. of 0.1% Holtfreter's solution. The eggs were treated with the sperm suspension for 10 minutes, after which it was decanted. The eggs were then placed into large finger bowls partly filled with aerated tap water. The eggs were kept at room temperature and 8 to 10 hours after fertilization they were checked for the percentage of cleavage.

After the eggs were removed, the hosts were pithed and the body cavities were inspected for remnants of ovaries. They were found only once and the results of that experiment were discarded.

B. GENERAL PROCEDURE FOR THE TRANSFER AND TREATMENT

The actual transfer process consisted of scooping

the eggs from the exposed body cavity of the donor and placing them in full strength Holtfreter's solution. All of the eggs were removed quickly since, if taken as needed they would be trapped in the donor's coagulating blood. They were divided into three batches, each of which received a different treatment and was injected into a different host.

The eggs were treated with; 1) full strength Holtfreter's solution, 2) non-immune serum, and 3) an antiserum prepared against egg jelly. In the preliminary experiments (Table I) anti-oviduct serum, anti-lower oviduct serum, and anti-upper oviduct serum were also used. In all cases the sera were diluted 1:1 with .1% Holtfreter's solution prior to use.

The eggs were left in the sera, at pH 7.4 to 7.5, for 8 to 10 minutes. They were then washed either with full strength Holtfreter's solution or 0.1% Holtfreter's solution and transferred. The controls, treated with Holtfreter's solution alone, were handled as many times as the other two groups.

The order of treatments was varied to minimize any deleterious effects that might result from being in the Holtfreter's solution for a prolonged period of time. Lavin (1963) claims that body cavity eggs can be held in this way for up to twelve hours with only minor loss of viability.

C. GENERAL PROCEDURE FOR TESTING AND PREPARING ANTISERUM

The antiserum was checked for antibodies by means of a modified Ouchterlony double diffusion method (Shaver, 1961). The presence of three distinct constituents, as indicated by precipitation lines between the anti-egg jelly serum and its homologous antiserum of the usual antibody titre. Precipitation lines were absent when the non-immune serum was reacted against jelly antigens. Three lines were chosen to demonstrate the activity of the jelly antiserum because there are at least that many separate components identifiable in the jelly taken from eggs (Shaver and Marsh, 1962). (See Plate I.)

The egg-jelly antigen was prepared by blending 10 mg. of lyophilized egg jelly per ml of 0.85% NaCl, buffered with Na_2HPO_4/KH_2PO_4 at pH7.2. Then 1.5 ml. of a mixture of the antigen and Freund adjuvant was injected into rabbits by the subscapular route. Three to four weeks later the animals were bled for antiserum. All sera were frozen until needed after being dialyzed against Amphibian saline (0.65%) for 48 hours. Non-immune serum was obtained before the injection of the antigen.

RESULTS

The results of the experiments are presented in Tables I, II, and III, and in Graph I. Table I contains the data obtained from preliminary experiments, in which eggs from one donor were transferred to only one host, after one of the seven different treatments employed. These results were not statistically analyzed, because it was felt that individual variations among donors, as well as among hosts, would be too great for accurate analysis. Therefore, the next experiments were designed to eliminate the variations among donor eggs in any one experiment. This was accomplished by subdividing the eggs from one donor into three batches and subjecting each batch to a different treatment, and transferring each batch to a separate host. Table II presents the results of all experiments performed in this manner. Of the thirty eight experiments performed, the results of only sixteen could be used for statistical analysis, since in ten cases, no cleavage occured in the eggs treated either with Holtfreter's solution or with non-immune

serum, and in twelve cases at least one of the three hosts died before the eggs could be removed. Table III presents the results of the sixteen experiments which were subjected to an analysis of variance. This was performed to detect heterogeneity among the results of the various treatments. The statistical design of the analysis took into account the variations in the eggs from different donors. Variations between host females receiving eggs of the same donor could not be analyzed by this procedure. The probability of physiological differences among the hosts having significant effects on the donor eggs seems rather slight, however. The F value obtained from the analysis was 3.57 and the table value (Table 10.5.3 in Snedecor, 1956) for the appropriate degrees of freedom (2 and 30) was 3.32 at the 5% level. This indicates that there was significant heterogeneity due to the effects on the eggs of the three different treatments.

In an attempt to determine which difference was significant a sequential G test was used. The actual difference between the means of the effects of the full strength Holtfreter's solution and the non-immune serum was 6.9 (see appendix) units less than the

computed difference, D (D is the product of $\bar{x}$ and a factor C taken from table 10.6.1 in Snedecor, 1956). This definitely shows that there is no significant difference between these two groups. The difference between the effects of the other two pairs of treatments were, however, significant (see appendix) at the five percent level. The value calculated for the actual difference between the means of the effects of the full strength Holtfreter's solution and the anti-egg jelly serum was 1.26 units more than the corresponding computed D and the actual difference between the non-immune serum and the anti-egg jelly serum was 0.87 unit more.

In comparing the percentages of cleavage in Tables I, II, and III (also see Graph I), it can be seen that a general trend occurs. That is that, regardless of the transfer procedure used (one donor-one host or one donor-three hosts), eggs treated with non-immune serum had higher percentages of cleavage than those treated with the anti-egg jelly serum. Since the analysis of variance detected heterogeneity among treatments and the Q test showed that the differences between the control and the experimental groups were significant, the results demonstrate that the antibodies produced against egg-jelly, in the concentrations used, had a definite effect in lowering the fertilizability of the

treated eggs. Lavin (1943) obtained similar results employing other types of antisera on coelomic eggs, but the number of experiments she reported was quite small, and no statistical analysis was indicated in her report.

TABLE I *

Preliminary Data (not included in statistical analysis)
shewing the number of eggs inseminated and the number that cleaved after being treated with various substances before the deposition of jelly in the host animal

Treatments	.1% Holt.		Non-immune serum		Fract. non-immune ser.		Anti-egg jelly ser.		Anti-ovid serum		Anti-upper oviduct		Anti-lower oviduct	
	#eggs	#Cl.	#eggs	#Cl.	#eggs	#Cl.	#eggs	#Cl.	#eggs	#Cl.	#eggs	#Cl.	#eggs	#Cl.
	408	28	242	39	247	1	641	12	133	20	551	103	472	120
	448	22	642	166	623	397	240	0	428	28	397	87	214	8
	563	2	183	7	154	0	268	1	239	6				
	97	5	62	0	182	0	318	0	412	63				
	245	0	343	8	338	0	240	36	364	35				
	235	9	161	0			316	27	627	17				
	152	22	167	27			458	198	353	0				
			87	27			621	126	223	0				
			134	0			478	9	87	3				
									539	1				
Totals	2169	88	2750	760	1544	403	3620	319	3411	173	949	190	686	128
Percentage of cleavage of total	4.1		27.6		26.1		8.8		5.1		20.0		18.7	

* Each set of numbers involve one doner one host transfer.

TABLE II x

Total of all experiments using one donor-three host technique showing the number of treated eggs inseminated and the number that cleaved

	Full St. Holt.		Non-immune Serum		Ant. Egg Jelly Ser.	
	#eggs	#Cl.	#eggs	#Cl.	#eggs	#Cl.
	[illegible]	59	[illegible]	59	[illegible]	4
	170	48	102	16	67	5
	21[illegible]	165	[illegible]	133	[illegible]	202
	312	36	317	110	32	0
	[illegible]	[illegible]	[illegible]	1	[illegible]	0
	3[illegible]7	214	169	56	313	0
	216	32	[illegible]	7	[illegible]	23
	534	111	305	10	276	4
	1[illegible]3	49	[illegible]	21	272	24
	258	11	255	45	395	20
	[illegible]	173	375	216	[illegible]	143
	368	55	507	205	421	170
	2[illegible]3	16	349	184	167	54
	170	30	562	115	310	0
	273	46	[illegible]	1	269	7
	218	9	94	4	56	2
*	80	0	-	-	-	-
	127	0	[illegible]	49	-	-
	-	-	260	39	157	7
	-	-	34	0	-	-
	-	-	354	126	305	1
	-	-	178	0	64	0
	19	0	39	1	-	-
	-	-	65	0	-	-
	-	-	352	16	211	49
	-	-	214	4	[illegible]	41
	-	-	215	9	-	-
	92	0	-	-	[illegible]	21
-	[illegible]	0	127	1	104	0
	194	54	16	0	40	1
	210	0	220	0	9	0
	14	0	12	0	135	4
	[illegible]	0	243	1	66	0
	387	16	127	0	361	22
	337	2	171	0	2[illegible]3	2
	25	0	234	17	170	44
	1[illegible]9	2	53	0	231	0
	198	30	25	0	131	4
	6426	1169	78[illegible]4	1526	[illegible]	[illegible]54
%Cleavage of total	18.2		19.4		12.2	

* at least one host died before removal of eggs (total-12)

\- no cleavage in either control group (total-10)

x each of the 38 rows represent eggs taken from one donor

TABLE III

(Data used in statistical analysis)
Showing the number of eggs treated the number cleaved and the percentage of cleavage for each experiment
Each of the 16 rows represent eggs taken from a single donor

Full Strength Volt.			Non-Imm. Ser.			Anti-Egg Jelly Ser.		
#eggs	#Cl.	%Cl.	#eggs	#Cl.	%Cl.	#eggs	#Cl.	%Cl.
398	50	12.6	550	59	10.7	475	4	0.8
170	48	28.2	102	16	15.7	67	5	7.1
218	163	74.8	290	153	52.8	290	202	69.7
312	36	11.5	377	110	29.4	32	0	0
141	131	92.9	83	1	1.2	152	0	0
387	214	55.3	169	56	33.1	313	0	0
216	32	14.8	108	7	7.5	184	23	12.5
534	11	2.1	305	10	3.1	276	4	1.5
183	40	21.9	330	81	24.6	272	24	8.8
258	11	4.3	255	45	17.7	395	20	5.1
353	173	49.0	375	216	57.6	344	143	41.6
368	55	15.0	507	205	40.4	421	170	40.4
243	16	6.6	349	184	52.7	167	554	32.3
170	30	17.6	562	115	20.6	310	0	0
273	46	16.6	108	1	0.9	269	7	2.6
218	9	4.1	924	4	4.2	56	2	3.5
4442	1065		4574	1263		4023	658	

% cleavage of total	23.9	27.5	16.3

GRAPH I

Represents the total percentage of cleavage of all sets of data

FSH Full Strength Holtfreter
NIS Non Immune Rabbit Serum
AJ Anti-Egg Jelly Serum
.IH One Tenth Percent Holt.

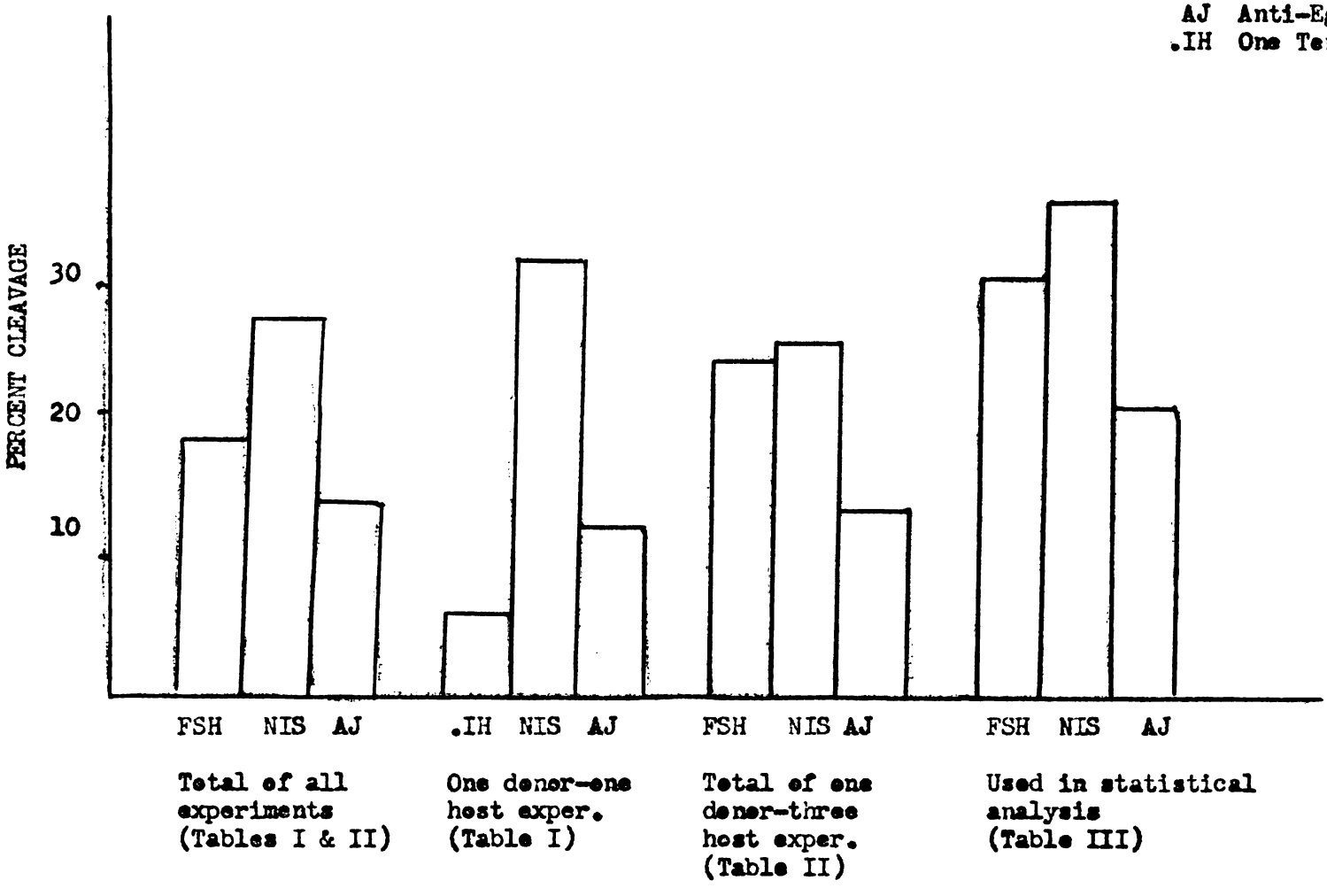

PLATE [illegible]

I Diagram of an Ouchterlony Plate showing at least three distinct lines (1, 2, & 3) between two of the [illegible] jelly [illegible] [illegible] [illegible] jelly material [illegible]. [illegible] represents [illegible].

II Diagram of an Ouchterlony Plate showing [illegible] line between two of the [illegible] [illegible] [illegible] [illegible]. [illegible] represents [illegible] [illegible].

DISCUSSION

The basic question that arises in considering the results of these experiments concerns the nature of the inhibitory effects of the antisera used, on the surface of eggs before jelly is deposited. The purpose of the treatments of jellyless eggs was to determine if antibodies against components in egg jelly could inhibit fertilization and cleavage of treated eggs, after they had passed down the oviducts of the host animal. The antisera employed in these tests contained antibodies directed against components identical with egg material, as well as against components directed against egg jelly (see Plate II). This confirms the observation of Shaver, Rarch, and Shivers (1962), who have discussed the origin of the material in egg-jelly that reacts with ovarian egg material. Although it is possible that contamination of the jelly material with egg components, in the process of removing the jelly from uterine eggs, is the source of the egg antigens, this seems unlikely, in view of the care taken in the dejellying process and of the thorough washing of the jelly. Shaver, Rarch and Shivers (1962) consider it

more likely that material is extruded from the egg into the jelly, during the period of hydration of the jelly coat. This explanation was suggested by the observation that "egg water"(water in which jellied eggs have been standing for some time), when lyophilized resuspended, and reacted with either anti-jelly serum or with anti-ovary serum, was found to contain components from both jelly and from the eggs. There was some evidence from the tests made by these investigators, that the amount of egg material in the jelly coat and in "egg water" may vary with the length of time the eggs have been immersed in water. This factor may be the reason why precipitation reactions on gel diffusion plates are quite variable, when anti-egg jelly serum and ovarian material are reacted.

The inhibition of fertilization observed, in the present experiments, in eggs treated with anti-egg jelly serum before jelly deposition could thus be explained in part on the basis of the blockage of sites on the egg surface which would be complementary to antibodies in the antiserum directed against egg components. That this hypothesis does not explain the action of anti-egg jelly sera on uterine (jellied) eggs, however,

is indicated by some observations of Shivers (1961). This investigator noted that anti-egg jelly serum, when absorbed with ovarian material, prior to treating uterine eggs with it, still retained inhibitory effects on fertilization. Thus in addition to the interaction between the sites on the jellyless egg surface and antibodies directed against egg material, postulated above, which could also occur at the surface of jellied eggs, one would have to postulate interactions between components in the jelly coat of uterine eggs and antibodies directed against them, such as would have been contained in Shiver's absorbed antiserum. Both types of components would be contained in the anti-egg jelly sera used in the present experiments, and the question arises as to the role of each of them in the fertilization of normal (jellied) eggs.

Some observations of Shivers (personal communication) may contribute to an understanding of this problem. He obtained cleavage of jellyless R. pipiens eggs which had been inseminated with a sperm suspension that had previous contact with uterine eggs. This suggests that the attachment of the spermatozoon to the egg surface involves a two-step process. The

first step may be an interaction between the sperm and the jelly to "activate" the male gamete. It may be at this point where inhibition occurs when uterine (jellied) eggs are treated with anti-jelly serum (Shaver and Marsh 1960). The second step may involve the interaction of the "activated" spermatozoön with sites on the egg surface. This step may be the one affected when jellyless eggs react with antibodies in anti-egg jelly serum directed against the egg material in the jelly. Additional evidence that two or more sites on the egg surface or in the jelly coat are involved in the fertilization reaction, is the fact that antiovary serum will show precipitation lines when reacted against sperm antigens, while jelly antiserum does not (Shaver, Marsh, and Shivers, 1962). Assuming that the lines between the antiovary serum and the sperm represent components involved in fertilization, one can see that in the frog two separate sites, one on the jelly and one on the egg surface, could be postulated.

In the sea urchin, based on electron microscope studies showing that the plasma membrane of unfertilized eggs extends through the vitelline membrane,

Tyler (1963) suggests that the fertilizin molecules of the jelly are an extension of the plasma membrane fertilizin. As a component of the plasma membrane, polyspermy after fertilization and removal of the fertilization membrane (Tyler, Monroy, and Metz, 1956). Tyler also states that the vitelline membrane does not contain fertilizin molecules. It is possible that in frog eggs two sites for the location of jelly molecules exist such as suggested above for the sea urchin egg, one in the jelly coat and the other at the egg surface. Kemp (1956), in an electron microscope study, has described, in developing R. pipiens oocytes, protoplasmic processes (microvilli) projecting through the zona radiata or vitelline membrane intertwining with microvilli of follicular cells. Wheter these egg cell processes in the frog contain "fertilizin" like molecules is unknown. Unlike the sea-urchin egg, however, where the source of the jelly-coat is still in question (Metz, 1957), all of the jelly layers around frog eggs are secreted by oviducal cells. Thus, analogous functions in fertilization of sea urchin and frog eggs, may be accomplished by

substances of different origin.

It is known that the eggs of a variety of species can be activated parthenogentically by treatment with hypertonic or hypotonic salt water, by temperature change, irradiation and a number of other agents (Tyler, 1941). Perlmann (1959) claims that, in sea urchins, unfertilized eggs can be activated by treatment with antijelly and anti-ovary sera. Most of the activating material, called antigen "A", is located in the egg, Tyler (1939) has been unable to activate eggs of a different species of sea urchin with antisera similar to those used by Perlmann. He suggested (1963) that the changes observed by Perlmann may be due to cytolytic action of the antisera. None of the phenomena associated with activation such as the lifting of the vitelline membrane or extrusion of the second polar body was noted in this study either after the eggs were treated with antisera, or before they were fertilized. Lavin (1963), using eight different antisera on jellyless frog eggs, also observed no artificial activation.

Perlmann (1954) and Tyler and Brookhart (1956) described wrinkling, blistering, and cytolysis when normal and dejellied sea urchin eggs were treated with

strong anti ora against fertilisin. Perry (1943) states that antisera against frog-egg-jelly, when applied to dejellied fertilized eggs caused cytolysis. In the present experiments general cytolysis was noted after treating some batches of eggs with all reagents used, including Holtfreter's solution. This suggests that manipulations of the eggs caused the cytolysis and not the antisera.

Osmotic shock may have been involved in lowering the percentage of cleavage. This is thought to be the reason why (see Table I) such a low percentage was obtained in the eggs treated with .1% Holtfreter's solution. After the change to full strength Holtfreter's solution was made, the percentage of cleavage greatly increased (Tables II and III). It should be noted that the eggs lacked the protection of their normal jelly coat and thus would be more sensitive to slight variations in osmolarity. However, since full strength Holtfreter's solution is in physiological balance with Amphibian eggs and embryos and since the ova used has been dialyzed against physiological saline solution before use, it seems unlikely that the effects of any treatment were due to osmotic shock.

Tyler (1939) states that it is possible that

antibodies against fertilizin and egg components may actually enter the sea urchin egg and exert their effects on some internal process inhibiting nuclear as well as cytoplasmic divisions. However, later, finding that antibodies against internal components of the sea urchin egg do not block development, he (Tyler, 1963) restates his earlier theory. "The antibodies (against internal components) evidently do not manage to get inside the egg, or the cells of the embryo, in sea urchins". Flickinger and Nace (1952) demonstrated that frog egg cell membrane is not readily permeable to antibodies. Thus, if the antiserum were used here produced its inhibitory effect by combining with a specific site, it probably did so on the egg surface and not be combining with some internal site.

Shaver and March (1960) observed a precipitation reaction on the jelly coat when uterine eggs of L. pipiens were exposed to full strength antijelly serum. Later Shivers and Metz (1962) noted that, when egg-jelly antibodies were made univalent no reduction in the inhibitory effect was noted, even though the precipitation reaction was no longer observed.

Perlman (1957) describes an antigen "J" in the jelly coat of sea urchins, antibodies against which are responsible for the precipitation reaction. Although the antibodies in the present study were multivalent, no precipitation on the egg surface was noted. It may be that the number of reactive sites complementary to anti-egg jelly serum on the egg surface is too small for noticeable precipitation or that the titre of antibodies against the egg exudate in the anti-egg jelly serum was insufficient to cause an interaction. Since the antibody used was multivalent, it may be expected that clumping of anti-serum treated eggs would occur, as in sea-urchin eggs treated with antifertilizin. However no such effect was noted.

It is also possible that mechanical trapping of the antibodies by attachment to similar but not identical antigens, forming a cross-linked lattice among adjacent sites, could be blocking sperm-egg contact. This possibility could have been tested by using univalent antibody (Rivers and Metz, 1962). There seems little doubt that the effect of the anti-egg jelly serum does lower the percentage of cleavage. However, with the present information it can not be stated whether the anti-egg jelly serum blocked a site on the egg

necessary for normal interaction of egg and sperm, but the data obtained are not inconsistent with this interpretation.

CONCLUSIONS

The anti-egg jelly antisera used in this study, when applied to body cavity (jellyless) eggs before the deposition of the jelly coat in the host animal, did significantly reduce the percentage of eggs that cleaved. Disregarding unspecific effects of the antisera it is thought that the specific antigen antibody combination of jelly material and / or egg material did inhibit cleavage.

SUMMARY

1) Coelomic eggs were obtained from oviductectectorized donors, treated and transferred into ovarectorized hosts.

2) The treatments consisted on an anti-egg jelly serum, a non-immune serum and full strength Holtfreter's solution.

3) After the host jelly was deposited around the donor's eggs they were removed and fertilized. Results were tabulated as the percentage of eggs that cleaved.

4) The data obtained agrees with the work of Shaver and Marsh (1960) on uterine (jellied) eggs that anti-egg jelly serum treatment prior to insemination reduces the number of eggs that cleaved.

5) The results of these experiments are discussed with relation to and comparison with sea urchin work.

APPENDIX

Summary of Q test

Treatments	$\bar{x}$	$\bar{x}$-17.92	$\bar{x}$-28.23
Full St. Holt.	31.26	13.34 (12.07)	3.03 (10.02)
Non-Imm Serum	28.23	10.89 (10.02)	
Anti-Egg Jelly Serum	17.29		

( ) indicate calculated D values

d.f. based on error term (30 d.f.)

$D = S\bar{x}\ Q$

$$S\bar{x} = \sqrt{\frac{\text{M.S. error}}{\text{no. of exp.}}}$$

$$S\bar{x} = \sqrt{\frac{193.11}{16}}$$

$$S\bar{x} = 3.47$$

Comparison of various treatments

	Q	$S\bar{x}$	D(cal.)	D(obtained)	Dc-Do
FSH to AEJS	3.48	3.47	12.07	13.34	-1.26
NIS to AEJS	2.89	3.47	10.02	10.89	-0.87
FSH to NIS	2.89	3.47	9.89	3.03	-6.99

FSH=full strength Holtfreter's solution
NIS=non-immune serum
AEJS=anti-egg jelly serum

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