THE EFFECTS OF TREATMENT WITH ANTI-EGG-JELLY SERUM UPON CLEAVAGE AND DEVELOPMENT OF FERTILIZED FROG EGGS

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ABSTRACT

THE EFFECTS OF TREATMENT WITH ANTI-EGG-JELLY SERUM UPON CLEAVAGE AND DEVELOPMENT OF FERTILIZED FROG EGGS

by Eva Eleanor Cerny

It has been established that, in the frog egg, treatment with serum against the jelly coat will result in an inhibition of cleavage and growth of frog embryos. Similar results have been obtained by Tyler and Brookbank (1956) upon treatment of fertilized sea-urchin eggs with antifertilizin serum.

Frog eggs dejellied in a solution of potassium cyanide and treated with the gamma globulin fraction of anti-jelly serum cleaved in significantly lower numbers than eggs treated with either the gamma globulin fraction of control serum, aerated water, or one-tenth per cent full strength Holtfreter's solution. In addition, in two out of the three serum samples fractionated, either a cytoplasmic streaking was evident, or a cortical reaction accompanied by an elevation of the vitelline membrane. The third serum sample did not exhibit a one-hundred per cent lethality, but even

here a definite decrease in the number of eggs going on to cleavage and further development was noted.

A time threshold has also been established, in that eggs treated during a certain period of time after fertilization recuperate completely, but if this period is exceeded, treated eggs will undergo cytolysis. This sensitive period could be due to an appearance, at this particular stage in development, of a new antigenic substance. The possibility of some mucopolysaccharide (e.g. inter-cellular matrix or even some intra cellular changes) being involved is discussed.

The jelly coats of eggs are soluble in potassium cyanide only in a hydrated form. The biological importance of molecular rearrangements which may occur during the hydration of jelly from fertilized frog eggs may reside in the well-known refractoriness of eggs to sperm entry after they have been immersed in fluid for some time.

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By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF PLATES

INTRODUCTION

It has long been suspected that the jelly coats of Amphibian eggs, as well as those of Echinoderm eggs, play an important role in the process of fertilization. The interaction of Echinoderm jelly coats with spermatozoa has been extensively investigated, especially by workers such as Lillie (1919), Tyler and Brookbank (1956), and Vasseur (1952). Tyler (1959) has recently reviewed the subject, and presented an immunological model of sperm-egg interactions.

An early observation indicating the importance of the jelly coat of Amphibian eggs in fertilization was that jelly-less eggs of the Anuran species are not generally fertilizable (Bataillon 1919). His results have since been confirmed by several workers (Rugh, 1935; Tchou-Su and Wang, 1956; Shaver and Barch, 1960).

Kambara (1953) made the discovery (verified in 1956 by Tchou-Su and Wang upon Bufo bufo asiaticus) that jelly-less eggs of the toad Bufo vulgaris formosus are not fertilizable, but can be inseminated successfully when re-enveloped in a jelly coat. Since dejellied eggs coated with gelatin were also fertilizable, he concluded that the jelly coat acts

as a substrate for the thigmotactic response of the spermatozoa.

In 1956, Tyler and Brookbank found that antisera developed in rabbits against the jelly-coat material of sea-urchin eggs can block cell division in the early cleavage stages and can also inhibit the development of the embryos treated at later stages. A series of similar investigations employing the frog Rana pipiens (Shaver and Barch, 1960) demonstrated that a pre-treatment of either the eggs or the spermatozoa with anti-egg-jelly serum significantly reduced the number of eggs going on to cleavage. It was concluded that antibodies against jelly components either acted as a block to the fertilization reaction, or inhibited some post-fertilization phenomenon leading to cleavage.

Shaver and Barch and Shivers (1962) next established the fact that the antigenic components identified in the egg-jelly are found only in the tissues concerned with the production and secretion of the jelly: (i.e., oviducal tissue). At the same time they also postulated the existence in the anti-jelly serum of a molecular species complementary to a component coming directly from the egg, or located between the egg and the inner jelly coat, which is exuded into the jelly coats.

It is well-known that the hydration of the jelly which occurs when the frog egg is immersed in fluid prior to fertilization will cause the egg to become impenetrable by sperm (cf Rugh, 1951). Similar changes in the consistency of jelly coats of Echinoderm eggs (Paspaleff, 1927; Runnstrom, Tiselius and Vasseur, 1942) seem to have the opposite effect, inasmuch as an unhydrated, compact jelly coat will form a barrier to the sperm, which disappears when the swelling of the jelly occurs. Vasseur (1952) believes the swelling of the jelly coat to depend upon an increased rate of hydration (water absorption) of the jelly.

Runnstrom (1948) established that the surface of the egg is likewise changed as well as the jelly coat, and suggested the probability of cytoplasmic alterations as well (1949), concluding that the improvement in fertilization in the sea-urchin egg, noted by the previous investigations, is not due solely to the swelling of the jelly.

Motomura (1952) believes that the decrease in fertilizability of eggs of Rana nigromaculata upon immersion in fluid is primarily caused by the breakdown of the cortical granules of the unfertilized eggs which activates them to the point where they no longer can be inseminated.

In view of the fact that pretreatment of eggs with antisera against their jelly coats leads to an inhibition of

the development of the treated eggs, the possibility exists that the antiserum could exert its inhibitory action by a formation of a cross-linked lattice among the jelly molecules, which could act as a mechanical barrier to the fertilizing sperm. (Metz, 1961).

This explanation was tested by C. A. Shivers and C. B. Metz (1962), who found that even a non-precipitating, univalent antibody (which had been rendered univalent by digestion with the proteolytic enzyme papain) would block fertilization, and therefore concluded by saying that "a direct blocking of receptor sites in the egg jelly that perform a significant role(s) in fertilization" seemed probable.

It has been the endeavour of the present investigation to attempt to localize the action of anti-jelly serum on developing eggs, and to determine during which time periods after fertilization the effects which have been observed occur.

MATERIALS AND METHODS

A. General Procedure for Treating Eggs.

Female Rana pipiens of either Wisconsin or Vermont stock were injected with pituitary glands two or three days prior to the experiment in order to induce ovulation. The eggs were then squeezed onto clean slides and fertilized with sperm suspension obtained by maceration of two testes of R. pipiens in 10 mls of one-tenth per cent of full strength Holtfreter's solution. After ten minutes, the excess sperm suspension was decanted, and the eggs placed in a finger bowl half filled with aerated water. They were left here until the jelly became fully hydrated (25 minutes to one hour).

The jelly coats were then removed according to the method described by Bataillon (1919), as follows: the eggs were dislodged from slides and placed in a large finger bowl containing about 175 mls of potassium cyanide solution (0.8% - 1.2%). The egg masses were then separated into clumps of approximately equal size, and the bowl was then gently rotated for anywhere from ten to twenty minutes, until the

time when, if the bowl was tipped, the few eggs which were caught on the upper dry side would show no light-reflecting jelly coat surrounding them.

Following this, as much of the cyanide solution as possible was poured off, and the finger bowl was covered by gauze and was placed under a tap with a slow, steady stream of water running into it. The eggs were thus washed for thirty minutes.

After washing was completed, a medicine dropper with a fairly large opening was selected, and this was then swirled around in the finger bowl for about five minutes, in order to free the eggs from each other and from the bottom of the finger bowl.

The eggs which have had their jelly coats dissolved tend to aggregate with no intervening spaces and can be recognized at a glance. The dejellied eggs were removed from the finger bowl with the aid of the medicine dropper and placed in a small dish. They were then examined under a binocular miscroscope, and damaged eggs (exhibiting either surface pigment streaking or any other pigment disarrangement) were discarded.

The undamaged, jelly-less eggs were next placed in dishes containing different sera. In each experiment, a control batch of eggs was placed into a sample of aerated

water, and another into one-tenth percent of full strength Holtfreter's solution. By far the greater percentage of experiments was carried out using a 1:1 concentration of non-immune rabbit serum (diluted with one-tenth per cent of full strength Holtfreter's solution) and a 1:1 dilution of rabbit anti-jelly serum. A few experiments were conducted with antisera against other tissues, and these were also used in a 1:1 concentration. The only exceptions were the experiments in which the effects of the period of treatment was tested, where full strength sera were used.

B. General Procedure Used for Fractionating Sera.

Blood for control serum was withdrawn from rabbits prior to the injection of antigen. The anti-jelly serum was obtained by injecting the animals intraperitoneally with antigen which had been prepared by blending ten mg of lyophilized jelly (untreated, in all cases) per ml of 0.85% sodium chloride, buffered with Na_2HPO_4 / KH_2PO_4 at a pH of 7.2. All sera were dialyzed against 0.65% sodium chloride for 24 hours before using.

The gama globulin fractions were obtained by the method of Horesji and Smetana (1956). This was accomplished by adding 3.5 parts of an 0.4% Rivanol (ethoxy-diamino acridine lactate) solution to one part serum, adjusting the pH to 8.0,

(by adding $NaHCO_3$), and decanting the fluid globulin fraction. The excess Rivanol was removed from the filtrate by adding an excess of activated charcoal. The charcoal-Rivanol complex was then removed by filtering the solution through Whatman's number one filter paper, or vacuum filtration through an L-6 Seitz filter.

The filtrate was lyophilized until dry, resuspended in the original volume of fluid, dialyzed against one-tenth per cent of full strength Holtfreter's solution for 48 hours, and frozen until ready for use.

The gamma globulin fractions were tested for the presence of antibodies (in the case of anti-jelly sera only) by means of the Ouchterlony double-diffusion technique (1949). Only those anti-jelly sera in which the presence of antibodies was indicated were used.

Embryos were cultured in the same sample of serum until the termination of each experiment.

RESULTS

The results are represented by Graph 1. It can be observed that the controls exhibit approximately a twenty-five per cent death rate by the time the blastula stage has been reached, assuming a hundred per cent rate of fertilization. This was judged to be due to cyanide damage to eggs which were not visibly affected at earlier stages.

The gradual increase in mortality among the controls is somewhat harder to explain, but this, again, is probably due to a delayed, or, perhaps, an unobservable effect of the cyanide solution on the eggs. Another factor which might be of considerable importance is the fact that the eggs are without their usual protective jelly coat, making them more susceptible to any environmental change. However, the differences in mortality at all stages between the control embryos and those treated with anti-jelly serum are evidently due to specific action of the antiserum.

The three different serum samples that were fractionated yielded results as tabulated in Table number 1. It therefore appears from the results as though every rabbit differed in its antibody-forming responses. It is pertinent

Table 1.--Results obtained after culturing eggs in sera.

			Developmental Stage Reached					
			Blastulae		Neurulae		Tailbuds	
		# eggs	#	%	#	%	#	%
Serum Sample 1	Control Serum	235	204	87	150	64	75	32
	Anti-jelly Serum	207	152	74	24	12	14	7
Serum Sample 2	Control Serum	360	266	74	206	58	75	21
	Anti-jelly Serum	352	0	0	0	0	0	0
Serum Sample 3	Control Serum	102	82	80	45	44	30	29
	Anti-jelly Serum	101	0	0	0	0	0	0

in this connection to mention the fact that serum sample number 1 was obtained from a single rabbit, while serum samples two and three were mixtures of sera obtained from three different rabbits apiece.

The usual appearance of eggs whose cleavage had been blocked right at the start of development by treatment with a 1:1 dilution of anti-jelly serum was indicated by the appearance of either blisters (see figure A, Plate I.) or of white spots (figure B, Plate I.) The former were accompanied by an exaggeration of the vitelline membrane; in the latter, an extreme amount of cytoplasmic streaking was evident.

A dilution of 1:5 and 1:10 (fractionated anti-jelly serum diluted with one-tenth per cent full strength Holtfreter's solution) yielded development up to the blastula stage, with the embryos appearing as shown in figures C and D in Plate II. The eggs treated in a 1:5 dilution, when serum sample number two was employed, were rather obviously affected (this can be noted by the uneven pigment distribution among the individual cells), while those treated with a 1:10 dilution appeared completely normal. However, embryos treated with either dilution failed to continue in their further development, and eventually started cytolyzing at the animal poles.

Table 2.--Results obtained after treating eggs with sera two hours post-fertilization for different time periods.

Time of Treatment in Mins.	Treatment		Developmental Stage Reached				
			Eggs	Blastulae	Gast.	Neur.	Tbds.
		#	16	10	10	10	9
	Control Serum	%	100	62.5	62.5	62.5	56.25
2 M		#	18	11	10	6	6
	Anti-jelly Serum	%	100	61.2	55.5	33.4	33.4
		#	18	11	11	8	5
	Control Serum	%	100	61.1	61.1	44.4	27.8
7 M		#	19	10	10	7	4
	Anti-jelly Serum	%	100	52.6	52.6	36.8	21.0
		#	18	11	11	7	7
	Control Serum	%	100	61.1	61.1	38.9	38.9
15 M		#	19	10	7	5	3
	Anti-jelly Serum	%	100	52.6	32.8	26.3	15.8
		#	14	9	8	8	8
	Control Serum	%	100	64.3	57.1	57.1	57.1
30 M		#	15	11	5	1	1
	Anti-jelly Serum	%	100	73.3	33.3	6.7	6.7

Gast. = Gastrulae
Neur. = Neurulae
Tbds. = Tailbuds
# = number
% = percentage

Results obtained with serum sample number three were essentially similar to those of sample two, but the data in Table 1 indicate that serum sample 1 produced less effect on development than the other two.

The results just presented were obtained from experiments in which eggs had been placed in antiserum as soon as possible after removal of the jelly by the cyanide solution, (ca. 1 1/2 - 3 hours after fertilization), and left in the antiserum until the end of the experiment. Therefore, a series of experiments was done, in which eggs were introduced into antiserum and control serum two hours or two hours and forty five minutes after fertilization, and treated for various periods of time ranging from two minutes to thirty minutes. At the end of treatment, the eggs were removed, washed and placed in one-tenth per cent full strength Holtfreter's solution to develop. Since only a very small number of eggs was treated, the results allow only a very general comparison. The results obtained do seem to indicate, however, that the duration of the treatment itself may not be as important as the time which has elapsed between treatment and the actual time of fertilization.

Graphs 2 to 5 indicate the results. In each set of graphs, the bottom one represents eggs placed in the sera two hours after fertilization, and the top graph represents

those treated two hours and forty-five minutes after fertilization. The data are presented in percentages of embryos reaching the stage indicated at bottom of graphs (ordinates) plotted against the duration of treatment (abscissa). In the eggs treated two hours after fertilization, a treatment of two minutes was employed, which was not included in the experiment begun two hours forty-five minutes after fertilization.

It can be seen that the differences in development between embryos treated with control and anti-jelly serum were much more pronounced in those in which treatment was begun two hours and forty-five minutes after fertilization. In these embryos, treatment for sixteen minutes and for thirty minutes produced greater effects on later development, than treatment for five minutes. The same general results are noted in eggs where treatment was begun two hours post-fertilization, but the magnitude of the effects is not nearly so great.

An attempt to obtain a reversible blocking of cleavage resulted largely in failure, since a blockage was always followed by cytolysis. Eggs were treated in a 1:1 dilution of antiserum and control serum about two-and-one-half hours after fertilization, and left in the sera until control eggs were in first cleavage. Both experimental and

Table 3.--Results obtained after treating eggs with sera two hours and forty-five minutes post-fertilization* for different time periods.

Time of Treatment in Mins.	Treatment		Developmental Stage Reached				
			Eggs	Blastulae	Gast.	Neur.	Tbds.
		#	22	19	19	19	19
	Control Serum	%	100	86.4	86.4	86.4	86.4
6 M		#	22	16	12	10	7
	Anti-jelly Serum	%	100	72.7	54.5	45.4	31.8
		#	15	15	15	15	14
	Control Serum	%	100	100	100	100	93.3
16 M		#	22	5	1	1	1
	Anti-jelly Serum	%	100	22.7	4.5	4.5	4.5
		#	18	18	18	17	16
	Control Serum	%	100	100	100	94.4	88.9
30 M		#	22	0	0	0	0
	Anti-jelly Serum	%	100	0	0	0	0

*Abbreviations as in Table 2.

control eggs were then returned to one-tenth per cent full strength Holtfreter's solution. The control eggs continued to develop in the usual numbers, but, in most cases, the antiserum-treated eggs did not develop (in one batch it appeared as though some "recovery" had occurred, but this observation could not be duplicated.)

Graph I.--Results obtained after culturing eggs in aerated water, 0.1 per cent Holtfreter's solution, control serum and anti-jelly serum.

Vertical axis represents the percentage of eggs treated which reached a particular developmental stage.

The horizontal axis represents the particular stage reached; i.e., B=blastula stage, G= gastrula stage, N=neurula stage, T-tailbud stage.

H_2O refers to eggs cultured in aerated water

Holt refers to eggs cultured in one-tenth per cent full strength Holtfreter's solution

CS refers to eggs cultured in control sera

AJS refers to eggs cultured in anti-jelly sera.

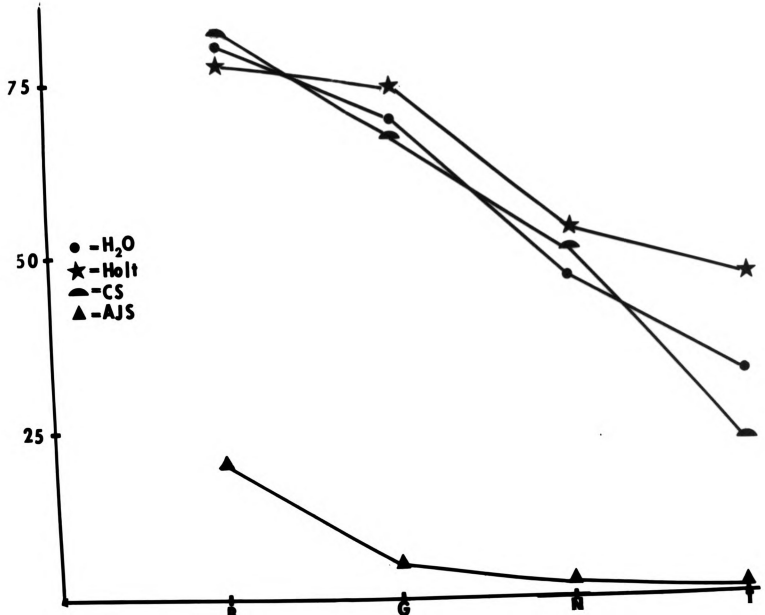
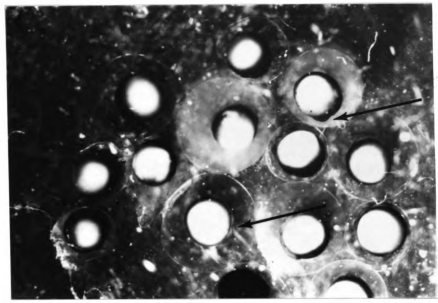

75
50
25
● -H2O
★ -Holt
◗ -CS
▲ -AJS
G
R
T

Plate I.--Appearance of eggs after culturing in anti-jelly serum.

A. Appearance of blisters as noted in about 50% of the cases of eggs treated with anti-jelly serum. Note the raising of the vitelline membrane. The raising of the cortex is indicated by arrows. (Serum sample 2 used - See Table 1).

B. Appearance of white spots, caused by treatment with anti-jelly serum. (Serum sample 2 - See Table 1).



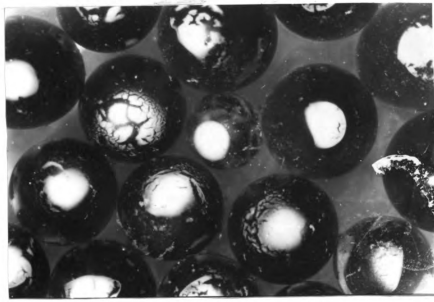
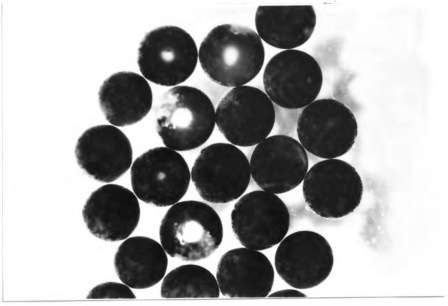
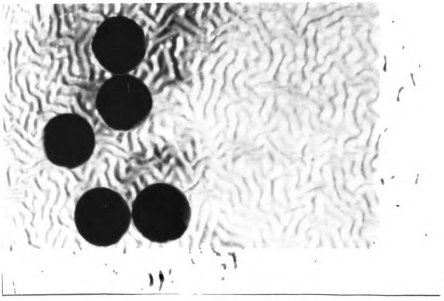


Plate II.--Appearance of eggs after culturing in different dilutions of anti-jelly serum.

C. Blastulae obtained by treating the eggs with a 1:5 dilution of the anti-jelly serum. Note that some embryos are showing signs of cytolysis (white areas in animal hemispheres). (Serum sample 2 was used in this experiment - See Table 1).

D. Appearance of eggs upon treatment with a 1:10 dilution of the anti-jelly serum (sample 2). Here the blastulae appear normal, but further development has been blocked.





Results of treating eggs with control serum and with anti-jelly serum for varying lengths of time.

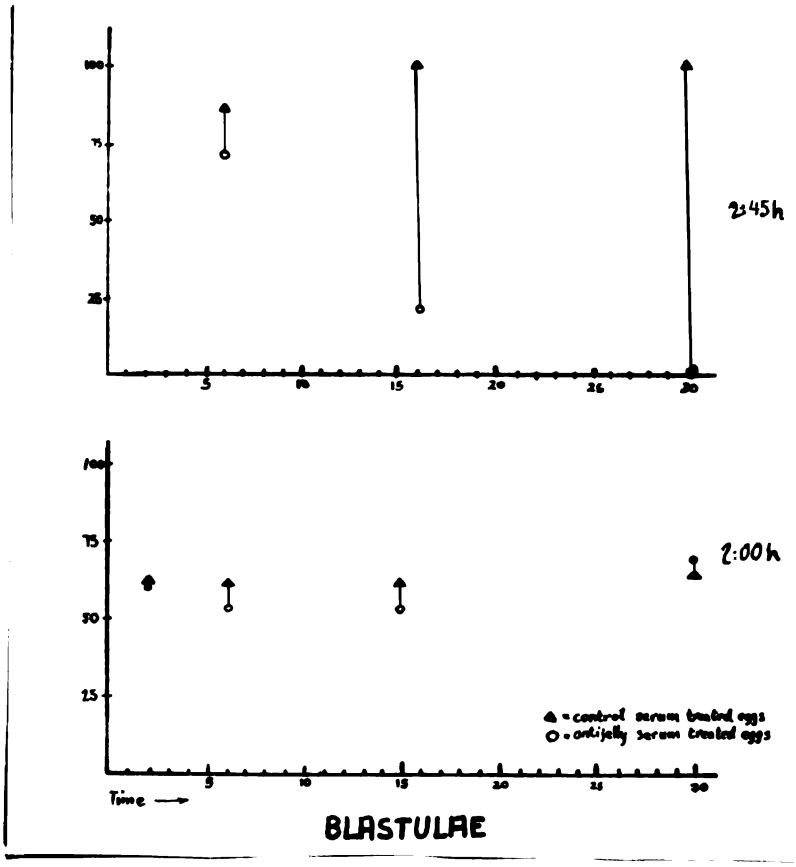
Graph II.--Eggs treated with control serum and with anti-jelly serum for varying lengths of time.

A graph of the time effect of treatment. The vertical axis represents the percentage of surviving eggs at the blastula stage, while the horizontal axis represents the duration of time (in minutes) of the treatment. The lower half of the graph represents eggs treated 2 hours after fertilization, while the eggs in the upper graph were treated 2 hours and 45 minutes after fertilization. The important thing to note here is the difference between the control serum treated eggs (triangles) and the anti-jelly serum treated eggs (circles). The differences are represented by the straight lines joining each triangle and its corresponding circle. Thus it is obvious that eggs treated at 2 3/4 hours after fertilization exhibit greater differences than eggs treated at 2 hours after fertilization.

Graph III.--The same experiment as in graph II at a later stage in development. (gastrulae). Note the increase in the magnitude of the effects as later and later developmental stages are reached.

Upper figure--treatment 2 hours 45 minutes post-fertilization.

Lower figure--treatment 2 hours post-fertilization.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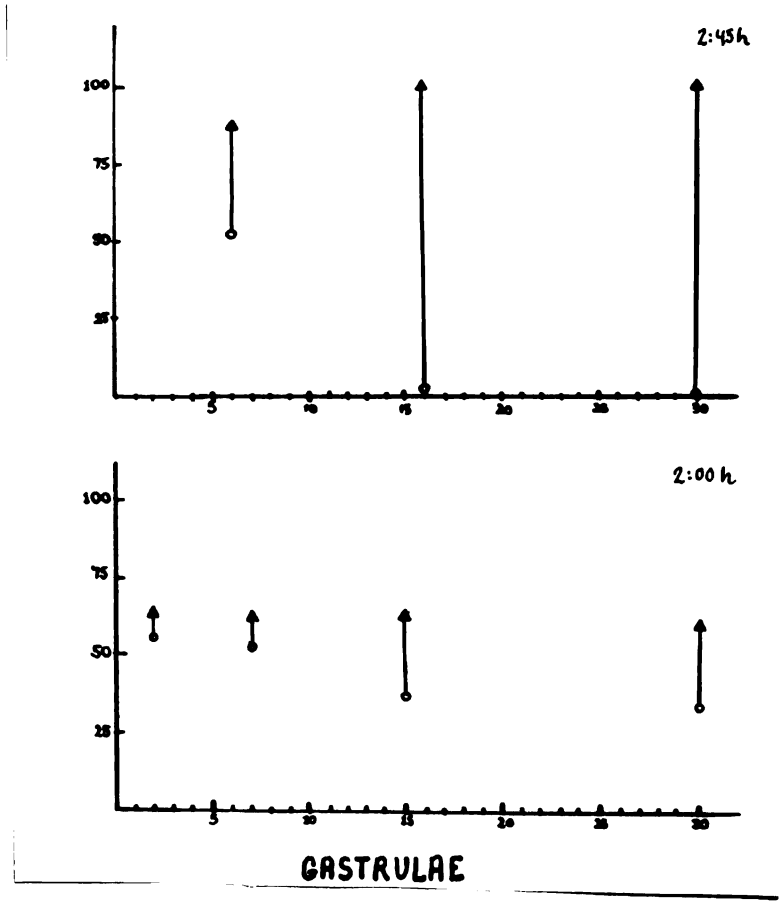
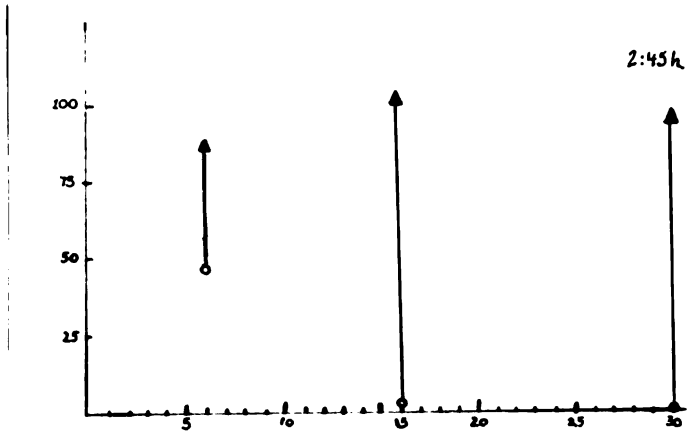
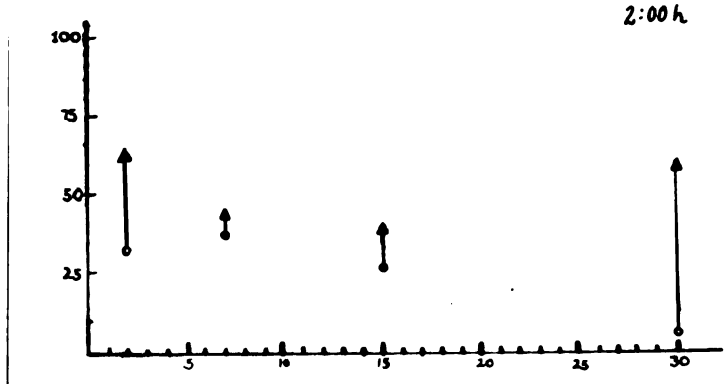
2:00h
▲ = control serum treated eggs
○ = antijelly serum treated eggs
Time
BLASTULAE


2:45h
2:00h
GASTRULAE

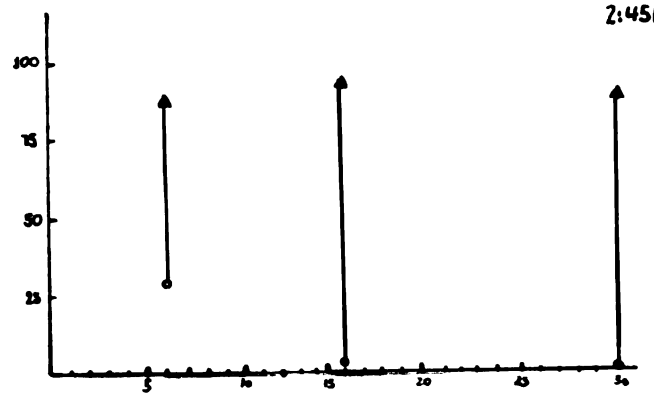
Graph IV.--The same experiment as in graph III at the time of neurulation. Lower and upper figures as in graph III.

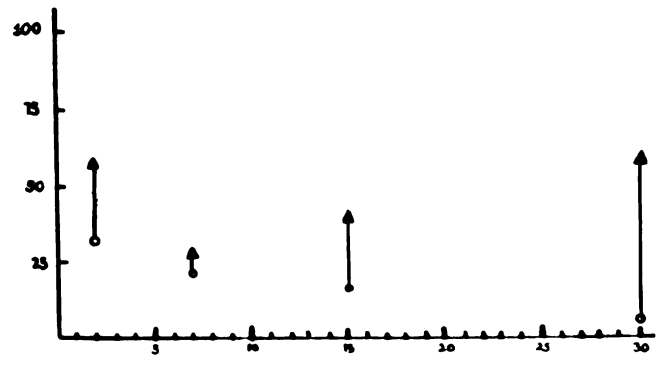
Graph V.--Same experiment as in graph IV, at the tailbud stage. Here the differences between the control serum treated specimen and the anti-jelly treated specimen are at their maximum. Yet, even here, the differences in the upper graph are larger than in the lower graph. Lower and upper figures as in graph IV.





NEURULAE





TAILBUDS

DISCUSSION

A. Effects of Cyanide on Egg-jelly and the Possible Subsequent Effects on Development.

The observation that fully hydrated frog egg-jelly could actually be dissolved in a potassium cyanide solution, but that the jelly will not dissolve if it is not fully hydrated led to attempts to dissolve the jelly at different times following fertilization (prior to complete hydration). Treatment with potassium cyanide solution prior to full hydration not only failed to dissolve the jelly, but the jelly tended to become sticky and lose its firm framework, without actually undergoing any dissolution. The eggs within this gum-like structure would undergo cytolysis accompanied by pigment streaking. A similar unsuccessful result had been obtained once previously, when an 0.08% potassium cyanide solution was accidentally used.

Either concentration used to dissolve the jelly (0.8% - 1.2% potassium cyanide) would be sufficiently strong to inhibit the oxygen consumption of the egg, since, according to Brachet's (1950) estimation, a 0.001 molar potassium cyanide solution will block 90% of the oxygen

consumption of a frog egg. It is possible, therefore, that a certain concentration (ca. 1.0%), and under particular conditions, the potassium cyanide can dissolve the jelly, while at other concentrations, it will alter the structure of the jelly without dissolving it. The altered jelly might then allow the potassium cyanide to penetrate into the egg and there produce cytotoxic effects perhaps related to those observed by Brachet (1950).

From the data indicating differences in solubility of unhydrated and hydrated jelly coats a rearrangement of the molecular lattice work of the jelly coat seems to be suggested. This possibility may be connected with two previously noted phenomena:

(1) The jelly visibly swells upon being left in water for some time (15-30 mins).

(2) Eggs whose jelly coats are fully hydrated will become impermeable to sperm (Rugh, 1951).

Such a change in the molecular arrangement of the jelly may alter some chemical bonds in such a way that they would be disrupted by the cyanide solution.

Regarding the effect of cyanide on frog eggs themselves, the observation that all the control embryos showed a progressive mortality as development proceeded, suggests some delayed effects of the cyanide treatment.

While a number of studies has been made on the effects of lower concentrations of cyanide on both frog eggs and sea-urchin eggs, practically no work at all has been done on the effects of higher concentrations of cyanide (at least, not in the case of the frog egg).

An experiment by Immers and Runnstrom (1958) on the effects of different concentrations of cyanide on fertilized sea-urchin eggs yielded a number of interesting facts:

(1) There exists a direct correlation between the cyanide concentration and the degree of development.

(2) Exposure to cyanide brings about a certain delay both of the development of the surface hyaline layer and of the normal cytoplasmic structure.

(3) Upon a prolonged exposure to cyanide, a separation of different cytoplasmic phases occurs. This separation becomes more pronounced when the eggs are transferred to a hypertonic sea-water after a previous treatment with cyanide, and hyaline blisters form at the periphery of the egg, giving a blister like appearance.

(4) The cytoplasm of the unfertilized egg does not exhibit the same separation into different phases as that of the fertilized egg.

The above-mentioned authors come to the conclusion that,

apparently, the most highly-organized cell components are the least susceptible to the treatment with potassium cyanide, such that at a cyanide concentration which still allows the division of the centrosome and the chromosomes, the coordinated movements of the cytoplasm (which are necessary for cytoplasmic cleavage) are evidently blocked.

In the present experiments, frog eggs that remained in the cyanide solution for a longer time than it takes for the jelly to dissolve exhibited definite pigment streaking and cytoplasmic disarrangement. A definite pattern was followed, in that, first, a hardly-noticeable wrinkling of the cortex occurred, after which a cytoplasmic streak, usually grey in color, appeared. Next, the whole cytoplasm would become disarranged, and a rapid lysis would soon follow.

Thus, during the initial stages of experimentation, the cortical wrinkling plus other minor effects went unnoticed, and this was what, in all probability, accounted for the high (ca. 25%) mortality rate in the controls before the blastula stage was reached.

The "delayed" effects of cyanide (exhibited by a slow but constant decrease in the number of individuals going on to develop to successively later stages) may be due to some unobservable, toxic effects involving the cytoplasm which,

with growth and cleavage, would affect processes where integrity is necessary for successful development.

B. Effect of the Anti-jelly Serum on the Cleavage and Development of the Frog Eggs.

In recent years, developmental processes such as fertilization, cleavage, and especially, differentiation, have been attacked with the methods of immunochemical analysis. The concept of developing systems as embodying the interactions of complementary molecular configurations has been stressed particularly (cf. Tyler, 1957; Weiss, 1955.)

The initial phases of development particularly have attracted the attention of investigators interested in such immunological models of development. One of the earliest investigators of interacting substances of gamates, Frank Lillie, (1919), especially emphasized the importance of complementary groups in the fertilization reaction: ('fertilizin' of eggs and 'anti-fertilizin' of spermatozoa.) More recently Tyler and his students have published extensively on the effects of antisera prepared against adult tissue as well as the effect of eggs and spermatozoa of sea-urchins on fertilization and subsequent development. The experiments of this laboratory of most pertinence to the

present work are those reported by Tyler and Brookbank (1956), in which sea-urchin eggs, after fertilization, were treated with antisera against various constituents of spermatozoa, eggs, and certain adult tissues. The results of these experiments indicated that an antiserum against fertilizin of the jelly coat of the egg was especially active in blocking cleavage, and this phenomena suggested the present investigation on the effects of the anti-jelly serum on developing frog eggs.

During the course of this experiment, cleavage blocking caused by anti-jelly serum was followed by a very rapid cytolysis, an event that gave rise to a question as to whether cleavage was being blocked due to a cytotoxic effect, or whether the cytolysis merely followed the blocking of cleavage. In other words, doubt arose as to whether cleavage inhibition is a 'primary' or a 'secondary' effect.

This question was investigated by attempting to see whether a reversible inhibition of cleavage could be obtained. The results obtained suggest that the blocking of cleavage may be a secondary effect, since once the antibodies exert their effect (an event which requires anywhere from thirty to forty-five minutes), the egg will proceed on to cytolysis. Yet the possibility that cytolysis and inhibition of mitosis might occur simultaneously, or almost simultaneously, cannot be discarded.

The fact that eggs in the early blastula stage required far less time for a much more cytotoxic effect to become apparent, could be explained on the basis of the fact that, during the early blastula stage, very many cleavage furrows are being formed. Since it is known that, for the Urodele, the permeability of the embryonic cells is highest in the region of the cleavage furrows, (Løvtrup 1960), it is possible that the interactions of antibodies and cell surfaces are also highest in this region.

This possibility seems to be supported by the fact that even eggs treated with control serum at this particular stage exhibited an unusual number of abnormalities in further development. Therefore, it appears that a mere subjection of the embryo to an osmotic shock at this susceptible stage is enough to cause a great deal of damage. It should be noted here that these embryos are minus their natural protective coat of jelly as well, and would thus respond more drastically to adverse conditions. However, embryos treated with antiserum were invariably more affected than those treated with control serum, indicating an effect of antibodies in addition to a general reaction to osmotic change.

It is possible that the antibodies may be attaching to the constituents of the hyaline layer and/or the intercellular matrix, which would be expected to be exposed in the

area of the cleavage furrows. Since the intercellular matrix is probably a mucopolysaccharide of some sort, it seems possible that antibodies against the egg jelly (which is also a mucopolysaccharide) would react with the antigen of the former. This would explain why the antiserum effect was not as drastic in the gastrulae as in the early blastulae, since, in the former, only a minimal amount of intercellular matrix would be exposed, owing to the closer packing of surface cells. However, the possibility also exists that the effects of permeability alone are the cause of these different susceptibilities of blastulae and gastrulae, since Løvtrup's (1960) findings indicate that, shortly before gastrulation, the permeability of the embryo is decreased (his conclusions being based on findings that the rate of swelling of the embryo due to water uptake will gradually decrease with development, ceasing completely between Harrison stages 9 and 13.)

Perhaps both mechanisms operate simultaneously to cause the rapid cytolysis. Yet, due to the behavior of the anti-serum treated organisms, the permeability factor is evidently not as specific as the action of the antibodies.

The very curious phenomenon resulting in the appearance of the "blisters" in about one-half of the eggs

treated with anti-jelly serum was accompanied by the following characteristics:

(1) the vitelline membrane was raised

(2) no cytoplasmic streaking was observed

(3) the cortical pigment layer was raised at different loci on the cortex of the egg, in a manner suggestive of blisters

The embryos treated with control serum did not, at any time, exhibit any effect similar to this.

Another type of cytolysis, in which blisters did not appear, was characterized by pigment streaking which eventually ended with the appearance of white spots in the animal hemisphere. This type of surface change is quite characteristic of the first phases of cytolytic breakdown in frog eggs. The production of this effect by anti-jelly serum indicates, again, the susceptibility of the egg surface to the molecular configurations complementary to the jelly coat.

The similarity in behavior of the frog and the sea-urchin egg to respective treatments with anti-jelly and anti-fertilizin sera was further exhibited by the dilution experiments, during the course of which a direct correlation between the strength of the antiserum and the degree of development was noted.

In summary, it can be stated that the anti-jelly serum probably contains antibodies against some vital substance(s) necessary in a developing embryo. Antibodies against these substances, when present in a sufficient concentration, will cause a rapid death of the embryo.

SUMMARY

1. The jelly around fertilized eggs, when hydrated, will dissolve in a solution of 0.8 - 1.2% potassium cyanide, without causing an immediate or delayed mortality in most (ca. 60%) visibly undamaged eggs. This will not happen if the jelly coat is not fully hydrated.

2. Anti-jelly serum will not only inhibit the cleavage of frog zygotes, but will also cause their cytolysis, accompanied by an extreme amount of cytoplasmic streaking, and, sometimes, by the appearance of blisters, which do not exhibit cytoplasmic streaking, but, rather, cortical bulges and a raising of the vitelline membrane. In either case, cytolysis is very rapid.

3. The time factor is important, as egg-treatment of short duration will not affect the eggs, but once a time threshold has been passed, cytolysis will set in. The actual duration of time that the eggs are treated does not appear to be as important as the time that has elapsed between fertilization and treatment. Also, a delayed effect is well exemplified here, in that

deleterious effects increase proportionately with further stages of development.

4. The cleavage inhibition appears to be irreversible, in that once a cleavage is blocked, cytolysis will inevitably follow.

5. The results obtained agree in general with the results obtained by treating sea-urchin eggs with antiserum against fertilizin. (see Tyler and Brookbank, 1956).

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