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The effect of extreme physical factors on the early embryonic development of Asterias vulgaris.

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THE EFFECT OF EXTREME PHYSICAL FACTORS ON THE EARLY EMBRYONIC DEVELOPMENT OF ASTERIAS VULGARIS.

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THE EFFECT OF EXTREME PHYSICAL FACTORS ON THE EARLY EMBRYONIC DEVELOPMENT OF ASTERIAS VULGARIS

INTRODUCTION

Environmental conditions exercise an influence upon the embryology of animals and for the normal course of embryonic development certain factors are requisite in the surrounding medium. Primarily, suitable temperature and salinity of the sea water are requisite, among the necessary factors for marine animals. Within certain limits external conditions may vary without noticeably altering the typical development of the animal form , but an upper and lower limit is always reached beyond which normal development cannot take place. For example, with a rising temperature a degree of heat is finally reached which destroys the vital processes completely and with it the embryonic development, but prevous to this, temperature while not inhibiting development entirely will do so partially with the production of abnormal forms. The same is the case with the lower temperatures, and higher and lower salinities than those requisite for normal development.

During the early part of July 1925, a brief study of the effect of extreme temperatures and salinities on the early embryonic development of Asterias vulgaris was made at the Atlantic Biological Station St. Andrews, N. B. The effect of unfavorable environmental factors, during fertilization, membrane formation, just after formation of the fertilization membrane and during the early cleavage stages, was studied.

METHOD

On being brought into the laboratory the starfish, were placed in clean glass aquaria with running water. They were always used as soon as possible and generally within twenty four hours. The proceedure of Gemmill (1914) was followed throughout. The gonads were examined microscopically to note their condition of maturity and in the case of spermatozoa of activity. The whole animal was washed repeatedly in fresh water and then in clear sea water, and opened on a fresh interradius. One goned from each starfish was removed entire and after several washings was brought into a small syracuse watch glass with about an equal bulk of water and then teased or shredded out. After half an hour dishes containing the ovaries were gently shaken and many ova had separated out by this time and were decanted into other dishes containing a small quantity of sea water and thence into a larger dish with more sea water. In two hours time a drop of the sperm suspension, in sea water was added to the ova.

All solutions used in the experiment were previously prepared from filtered sea water of known salinity, diluted where necessary with aerated distilled water or concentrated by evaporation and placed in clean four-ounze bottles. When the eggs settled in the solutions the supernatant liquid was siphoned off and fresh solution added. This was usually repeated twice. All sea water employed was obtained about thirty feet from shore to insure freedom from shore contamination, and conveyed in glass vessels to the laboratory.

Fairly constant temperatures were maintained at approximately 0°C., 5°C., 10°C., 15°C., by insulation boxes, 20°C approximated by Room temperature, and 25°C by an electric incubator.

EXPERIMENTAL WORK

Starfish with ripe ovaries were readily obtained throughout July, but during the last week the spermatozoa were not very active and the testes were parasitized by minute protozoans.

The sea water outside of the laboratory had an average salinity of 30 (gms per Mille), and a temperature of about 10°C. These were chosen as approximate standards.

The experiments were classed under four headings :-

1. Ova were fertilized at the temperature and salinity at which further development was to be continued.

11. Sperm were added to the ova in water at 10°C. salinity 30 (gms per Mille), 30 seconds before the transference of the ova to the various temperatures and salinities for individual instances

111. Ova were fertilized in sea water (10°C. Salinity 30 (grams per mille)) and transferred to the various temperatures and salinities, after the formation of the fertilization membrane and about the time of the first cleavage.

1V. Ova were fertilized in sea water (10°C.) Salinity 30 (gms per mille), and transferred to the various temperatures and salinities during the 4, 8 and 16-celled stages.

Experiment 1

The Asterias ova were treated as recommended by Gemmill (1914) About one hundred ova were dropped into sea water at temperatures and salinities as follow:- 10°C. salinities 0,(distilled water), 5, 10, 15, 20, 25, 30, 35, 40, 50, gms per mille); 0°C., 5°C., 10°C., 15°C., 20°C., ~~salinity 30°C. salinity 30 (gms per Mille)~~ 25°C., salinity 30 (gms per Mille) To each bottle was added a small drop of the sperm suspension in sea water. The water in each bottle was replenished twice daily.

Table 1

Table 1

Experiment I July 7, 1925.

Observations - indicates no development. + Normal reaction

Bottle	Salinity gms per mille	Temperature °C-highest and lowest limits	Average Temperature °C.	Fertilization Membrane Formation	Cleavage	Blastula formation	Gastrulation	Later development
1	0	9.0-11.0	10.0	-(cytolysis)	-	-	-	-
2	5	9.0-11.0	10.0	-(cytolysis)	-	-	-	-
3	10	9.0-11.0	10.0	-(cytolysis)	-	-	-	-
4	15	9.0-11.0	10.0	+(50%)	unequal (fig 2.)	large cells at one end	few abnormal	-
5	20	9.0-11.0	10.0	+	" (fig4)	"	abnormal	-
6	25	9.0-11.0	10.0	+	normal	normal	normal	reared to bipinnarias
7	30	9.0-11.0	10.0	+	" (fig1,3)	"	"	"
8	35	9.0-11.0	10.0	+	"	"	"	"
9	40	9.0-11.0	10.0	+	"	"	"	died in late gastrula
10	50	9.0-11.0	10.0	+ (25%)	-	-	-	-
11	30	0-1.5	.25	+ (25%)	unequal	-	-	-
12	30	4.0-6.0	5.0	+ (50%)	unequal	abnormal	abnormal	-
13	30	13.7-16.0	14.5	+	normal	normal	normal	bipinnaria
14	30	17.0-23.0	19.0	+	"	"	"	"
15	30	23.5-26.0	24.5	+ 10%	-	-	-	-

5

Experiment 1

Observations See Table 1

Summary

1. To Asterias ova at salinities 0-50 (gms per mille) 10°C., and salinity 30, temperatures .25°C, 5°C, 14.5°C. 19°C. and 24.5°C. a drop of sperm suspension was added.

2. Asterias ova fertilized in sea water at 10°C. salinity 25-35, and at 14.5°C. and 19°C. salinity 30, developed into normal bipinnaria (Comparison made with Gemmill 1914, and Faxon, Agassiz, Fewkes and Mark)

3 Fertilization Membranes separated distinctly in ova fertilized at 0°C. 5°C. 15°C. 20°C. 25°C. salinity 30, and at 10°C. salinities 15 to 50.

4. Normal cleavage and gastrulation took place at 10°C. salinity 25to 40; 14.5°C. and 19°C. salinity 30

5. Unequal cleavage and abnormal gastrulation (heavy yolk laden cells in the basal ectoderm and entoderm See Fig 8) developed at 10°C. salinity 15 to 20, 5~~0~~ C. and 24.5°C. salinity 30.

6. AT 10°C. salinities Oto 10, cytolysis or total disintegration took place shortly after subjection of the ova to the low salinity.

Experiment II.

The Asterias ova were treated as recommended by Gemmill (1914) and ova to which 30 seconds previously spermatozoa had been added (at 10°C salinity 30, gms per mille) were distributed as follow (100 to a bottle):-10°C. salinities 0, 5, 10, 15, 20, 25, 30, 35, 40, (gms per mille), salinity 30, at 0°C, 5°C. 15°C. 20°C, 25°C.

Observations See Table 2

Summary

1. Asterias ova during the process of formation of the fertilization membrane were subjected to temperatures and salinities as follow:- 10°C. salinities 0, 5, 10, 15, 20, 25, 30, 35, 40, (gms per Mille) .3°C. 5.0°C. 15°C. 19°C. and 25°C, salinity 30.

2. Salinities ranging from 0 to 15, at 10°C. and 30 at 25°C. cause a general disintegration of the ova.

3. Normal development of the ova to the bipinnaria stage took place at 10°C. salinities 30to35, and 15°C and 19°C. salinity 30(gms per mille).

4. Unequal cleavage and abnormal gastrulation followed by death resulted at 10°C. salinities 20 to 25, and at .3°C and 5°C. salinity 30(gms per Mille).

Table 2

Experiment 2 July 13, 1925

Observation

Bottle	Salimity gms per Mille	Temperature °C. highest and lowest limits	Average Temperature °C.	Cleavage	Blastula	Gastrulation	Later development
1	0	9.5-11.0	10.0	"	-	-	-
2	5	9.5-11.0	10.0	"	-	-	-
3	10	9.5-11.0	10.0	"	-	-	-
4	15	9.5-11.0	10.0	"	-	-	-
5	20	9.5-11.0	10.0	unequal	abnormal	abnormal	no development beyond gastrulation
6	25	9.5- 11.0	10.0	some normal few unequal	(normal dead) abnormal (fig 6)	abnormal	"
7	30	9.5-11.0	10.0	normal	normal	normal	bipinnaria (normal)
8	35	9.5-11.0	10.0	normal	normal (fig 5)	normal	" "
9	40	9.5-11.0	10.0	disintegrating	-	-	-
10	30	0.0-2.0	0.3	unequal	abnormal	abnormal	dead at gastrulation
11	30	4.0-60	5.0	unequal	abnormal	abnormal	dead at gastrulation
12	30	14.0-15.5	15.0	normal	normal	normal	bipinnaria (normal)
13	30	16.0-20.0	19.0	normal	normal	normal	bipinnaria (normal)
14	30	24.0-25.5	25.0	Disintegrated	-	-	-

b

Experiment 111.

The Asterias ova were treated as previously and after definite membrane formation and in some instances the beginning of the first cleavage about one hundred ova were put in each of a series of bottles at temperatures and salinities as follow:- 10°C salinities 0, 5, 10, 15, 20, 25, and 35 and 40 (gms per mille), 0°C. 5.0°C. 15.0°C 20.0°C. 25.0°C. salinity 30 (gms per mille).
Observations See Table 3

Summary

1. Asterias ova after the formation of the fertilization membrane and up to the first cleavage division were subjected to temperatures and salinities as follow:- 10°C. salinities 0, 5, 10, 15, 20, 25, 30, 35, 40, gms per mille and a salinity of 30 at .3°C. 5°C, 15°C, 1900. and 25°C.

2 The ova disintegrated at 10°C. salinities 0-10, and 25°C. salinity 30.

3. Normal development to the bipinnaria stage took place at 10°C. salinities 25 to 40 (very few at 40) and at 5°C., 15°C. and 19°C., salinity 30 gms per mille.

4. Unequal cleavage, abnormal blastulae and gastrulae developed at 10°C, salinity 20, and at .3°C. salinity 30 (gms per mille). These all died during early gastrulation.

5. At 10°C. salinity 15(gms per mille) and in a few instances at 5°C. salinity 30, the cleavage, was abnormal. Development ceased during cleavage and was followed by death.

Table 3

Experiment 3 July 14, 1925

Observations

Bottle	Salinity gms per mille	Temperature °C. highest and lowest limits	Average Temperature C°	Cleavage	Blastula	Gastrulation	Later Development
1	0	9.5-11.0	10.0	cytolysis	-	-	-
2	5	9.5-11.0	10.0	"	-	-	-
3	10	9.5-11.0	10.0	-	-	-	-
4	15	9.5-11.0	10.0	unequal	Majority dead Rest abnormal	-	-
5	20	9.5-11.0	10.0	Unequal	abnormal	abnormal (fig8)	dead
6	25	9.5-11.0	10.0	Normal	Normal	Normal	bipinnaria
7	30	9.5-11.0	10.0	Normal	Normal	Normal	bipinnaria
8	35	9.5-11.0	10.0	Normal	Normal	Normal (fig7)	bipinnaria
9	40	9.5-11.0	10.0	50% dead 50%Normal	Normal	Normal	bipinnaria(5%) remainder dead
10	30	0.0-20	0.3	50% dead; 50% unequal	25%dead 25%abnormal	abnormal	dead
11	30	4.0-6.0	5.0	90%normal 10%unequal	10%dead 90%normal	normal	early bipinnaria (20%) (70%)→ dead
12	30	14.0-15.5	15.0	normal	normal	normal	bipinnaria
13	30	16.0-20.0	19.0	normal	normal	normal	bipinnaria
14	30	24.0-25.5	25.0	-	-	-	-

Experiment IV

Asterias ova were fertilized and developed to 4.8 and 16-celled stages at 10°C. salinity 30, They were then distributed (about 100 per bottle) at temperatures and salinities as follow-
10°C. Salinities 5,10, 15. 20. 25. 30, 35, 40, 50, (gms per mille)
0°C. 5°C, 15°C. 20°C. 25°C. salinity 30
Observations See Table4.

Summary.

. Asterias ova after the second cleavage and up to the fourth cleavage were subjected to temperatures and salinities as follow:-
10°C. salinities 5, 10, 15, 20, 25, 30, 35, 40, and 50, gms per mille, and 5°C. 4.8°C. 15.0°C. 20.5°C ~~gms per mille~~, 24.5°C. at a salinity of 30 (gms per Mille)

2 The ova disintegrated at 10°C salinities 5, 10, and 50, and at 24.5°C. salinity 30 (gms per mille)

3. Normal development to the bipinnaria stage took place at 10°C salinities 25 to 40 at 15°C and 20,5°C. salinity 30 (gms per Mille).

4. In the majority of instances. apparently normal cleavage took place at .5°C and 4.8°C, salinity 30, at 10°C. salinity 20, but development only proceeded to gastrulation and just to blastula formation at .5°C. salinity 30

5 Abnormal or unequal cleavage of the individual cells took place at 10°C. salinity 15, and the embryos died just previous to gastrulation

Table 4

Experiment 4 July 21, 1925

Observations

Bottle	Salinity grms per mille	Temperature °C. highest and lowest limits	Average Temperature °C	Cleavage (Continuation)	Blastula	Gastrulation	Later Development
1	5	9.0-11.0	10.0	-	-	-	-
2	10	9.0-11.0	10.0	-	-	-	-
3	15	9.0-11.0	10.0	individual cells variable size	lobulated due to difference in cell size	dead	-
4	20	9.0-11.0	10.0	normal	normal	normal and some rather broad	dead at end of gastrulation
5	25	9.0-11.0	10.0	normal	normal	normal	bipinnaria
6	30	9.0-11.0	10.0	normal	normal	normal	bipinnaria
7	35	9.0-11.0	10.0	normal	normal	normal	bipinnaria
8	40	9.0-11.0	10.0	normal	normal	normal	bipinnaria
9	50	9.0-11.0	10.0	-	-	-	-
10	30	0.0-2.0	0.5	Normal few instances of variable size of cells	apparently normal	dead	-
11	30	4.5-5.0	4.8	normal	normal	gastrulae broad at bottom	dead
12	30	14.0-16.5	15.0	normal	normal	normal	bipinnaria
13	30	18.0-22.0	20.5	normal		normal	normal bipinnaria
14	30	23.0-25.5	24.5	no further cleavage; dead		-	- -

General Summary

1. Asterias vulgaris ova before fertilization, during fertilization, after the appearance of the fertilization, membrane and during early cleavage stages were subjected to salinities from 0 to 50 (gms. per mille) at 10°C. and a salinity of 30 (gms per mille) at 0°C. 5°C. 15°C. 20°C. and 25°C. approximately.

11 In general, development to the bipinnaria stage was possible from 10°-19°C. At 19°C development to this point was reached two days sooner than at 10°C. It was possible to attain the early bipinnaria stage at 5°C. salinity 30 though only in cases where cleavage had commenced at a higher temperature (10°C.) This is also true of ova at salinity 40 at 10°C.

111 Abnormal gastrulae, having thickened basal ectoderm and endoderm particularly, developed in each of the four experiments at 10°C. salinity 20 (gms per mille), and 5°C. salinity 30. In the first experiment where the eggs had been fertilized at 10°C. salinity 15 the gastrulae were abnormal. At 10°C salinity 25, and .3°C. salinity 30, in the instances in which the ova were subjected during membrane formation and in the latter case when subjected after membrane formation, the same was true.

1v Unequal or abnormal cleavage, in instances where the ova were fertilized in the same solution in which subsequent development took place with obtained at 10°C. salinity 15and20, and 25°C, and 5°C. with salinity 30. In the second experiment ova subjected during membrane formation showed unequal cleavage at 10°C. salinities 20and25, .3°C. and 5°C salinity 30 (gms per mille). The cleavage in the third experiment in which ova were used after the formation of the fertilization membrane was unequal at 10°C. salinities 15and20and .3°C. and 5°C, salinity 30 In the last experiment ova were used after the

first cleavage divisions and unequal cleavage of individual cells took place at 10°C. salinity 15 (gms per mille), and in a few instances at 5°C. salinity 30.

V Membrane separation (a criterion of fertilization) took place in Sea water at 10°C. salinities 15 to 50 (gms per mille) and at 25°C. 5°C, 14.5°C. 19°C. and 24.5°C. salinity 30.

Vl From section 4 and 5 it is readily seen that:-

l Membrane separation is possible at salinities not permitting normal cleavage

ll Ova subjected to unfavorable conditions (too low salinity or temperature) during membrane separation will not develop even abnormally at as low as salinity as either ova fertilized or subjected after fertilization to the unsuitable environment. This agrees with the findings, of Just (1923) who states that for Echinarachnius parma, "eggs inseminated in dilutions of sea water may separate membranes though they do not cleave," and also "eggs may be inseminated in a dilution that is destructive to the egg which is inseminated in sea water and exposed to this dilution during membrane formation"

Vll In general Asterias Ova cytolyze or disintegrate at 10°C. salinities from 0 up to 15 (gms per Mille) and at 25°C, salinity 30.

Vlll The most obvious variations in structure of ova developing in unfavorable environmental conditions (low salinities at 10°C. and in a few instances at low temperatures 0°C. 5°C) are

a. Unequal Cleavage

b. thickening of the basal parts of the gastrulae

I should like to express my appreciation of Dr. A. G. Huntsman's very kind suggestions and assistance.

Literature Cited

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Echinodermata Plate 7 Embryological

Monographs 1-3

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Biol Bull Vol XLIV P 17.

DESCRIPTION OF PLATE

Early stages of Asterias vulgaris

Figl. Normal two-celled stage (Expt. 1. 7)

Fig. 11. Unequal cleavage, two-celled stage (Expt. 1,4)

Fig. 111. Normal four-celled stage (Expt. 1, 7)

Fig. 1V Abnormal four-celled stage (Expt. 1, 5)

Fig. V. Normal many-celled stage or early blastula (Expt. 11, 8)

Fig. V1. Abnormal ovum at approximately same stage as fig. 5. (Expt. 11 5)

Fig. V11. Normal gastrula (Expt. 111 , 8)

Fig. V111. Abnormal gastrula (Expt. 111 , 5

PLATE I

Fig 1.

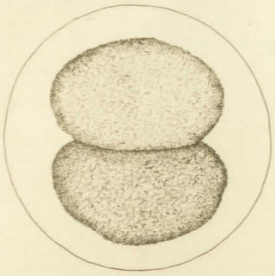

Fig 2.

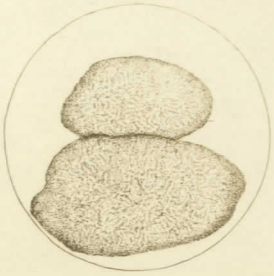

Fig 3.

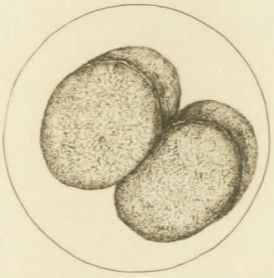

Fig 4.

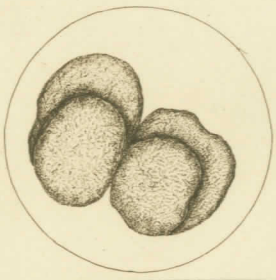

Fig 5.

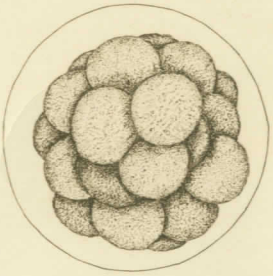

Fig 6.

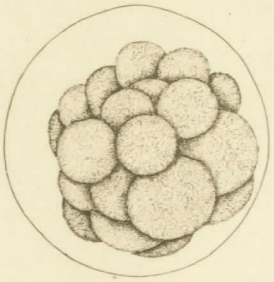

Fig 7.

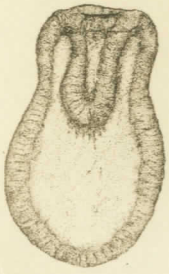

Fig 8.