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Artificial Parthenogenesis in Rana Pipiens

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GENERAL THEORY OF ARTIFICIAL PARTHENOGENESIS

Experiments in artificial parthenogenesis are based on, and help to prove a fundamental postulate of physiology, namely, that all vital phenomena may be explained by the laws of physico-chemical science.

Previous to the first artificial parthenogenesis experiments fertilization was believed to be effected by the combination of the male and female pro-nuclei. This belief told us nothing of the nature of the actual activating force, for the function of the sperm could be viewed from a chemical, physical, or mystical standpoint with no proof in favor of any one, but with the general acceptance of the latter.

When the development of eggs was initiated by a hypertonic solution of sea water the old theory of fertilization being caused by something inherent in the sperm, probably connected with chromosomes, was overthrown and fertilization was viewed in a very different light--that of a chemical stimulation. However, when frog's eggs were caused to develop by a simple pricking with a needle, we are forced to acknowledge that development may be initiated by a purely mechanical force.

The object of this paper is to prove that development of a frog's egg may be initiated by a mechanical force alone (a needle), that this development is materially aided by a chemical agent (blood), and most important, to suggest the possible significance of each step in a light that will aid and stimulate further investigation.

METHOD OF INDUCING OVULATION IN RANA PIPIENS

To get the eggs to leave the ovaries, enter the oviducts and reside in the uterus as mature ova, the following technique, based on the method of Rugh, was followed.

A female frog was incapacitated by vigorously bumping its head on a table. It was then decapitated and the lower jaw cut off. The top of the cranium was cut off by making a longitudinal cut with scissors and then the bone was picked off with forceps thus exposing the brain. The brain was then cut dorso-ventrally anterior to the optic lobes and then deflected back. The anterior lobe of the pituitary was taken out. This lobe occurs just posterior to the optic chiasm and is easily recognized by its vasculation.

The gland was then placed immediately in one cc of a .6 N NaCl solution, teased, macerated, and injected with a hypodermic needle into the lower right quadrant of the coelom of the frog in which ovulation is to be induced.

If the pituitary is not to be used at once it may be kept indefinitely in absolute alcohol but must be dissolved in .6 N NaCl for injection. When injecting the frog care must be taken to penetrate the body wall and yet not go so far as to injure the intestine. The frog to be injected should measure at least 74 mm from snout to cloaca to insure its being mature. Two glands were injected the afternoon of the first day and one the morning of the next day. The eggs should be in the uterus and ready for stripping by twenty-four hours following the time of the first injection. During this twenty-four hours the frog should be kept at room temperature.

After the frog was injected it was kept in a container partly filled with water and after the first stripping the water was changed as the frog laid eggs from day to day. If more eggs are desired for pricking after the first stripping the frog must be kept at a temperature of five degrees until needed.

The following is a description of the apparatus and conditions necessary for successful stripping and pricking: six slides, six Syracuse watch glasses, large and small mouthed pipette, six petri dishes, a moist chamber, plenty of water (either pond water or tap but it must be free of chlorine and sperm), and dissecting instruments.

The needles must be no larger than 30 mu in diameter at the point and

may be of glass or platinum or mangenese wire. Glass needles may be made by heating the ends of one-eighth inch glass rods together in a micro burner until they get just below red heat, then stick the two ends together and quickly pull them apart. A short but sharp point should be produced. If the point is too long it will break off in the egg. The needles should be examined under a 16 mm objective. Platinum needles should be used if they are available as the glass needles break off in the eggs rather easily, and at least six should be on hand when pricking the eggs.

When pricking and observing the eggs use a 20 x binocular scope with a spotlight focused on the eggs. Caution must be taken or the heat from the lamp might injure the eggs.

The only important condition to observe throughout the experiment is absolute sperm sterility of water and frogs. For this purpose heating to 70 degrees or the use of seventy per cent alcohol is sufficient.

To obtain the maximum development of the eggs they were smeared with frog's blood before pricking in the following way: A female frog (to avoid sperm contamination) was incapacitated by knocking its head on a table. It was immersed in seventy per cent alcohol for three minutes to kill any sperm adhering to its skin, then placed on a wet towel in a moist chamber. The frog was dried off and the abdomen opened. Next the gastrocnemius muscle was dissected out. The frog was left in this condition until the blood was needed for smearing at which time the tip of the ventricle was cut, the muscle held by forceps was dipped in the pericardial cavity and the adhering blood smeared on the egg.

The stripping was done in the following manner: The frog with eggs in uteri was dried and held in the right hand with its head toward the wrist and the left hind leg held between the fourth and fifth finger.

The first and second fingers covered the abdomen just posterior to the sternum. The frog's right hind leg was held with the left hand. A gentle pressure was exerted on the ventral and lateral sides of the abdomen and eggs emerged in a double row--one from each horn of the uterus. They were allowed to fall on a glass slide resting on an inverted Syracure watch glass; two double rows of eggs were stripped on each of two slides at one time. The frog was then put in its container and the eggs smeared with blood from the frog previously prepared. The microscope was then focused on the eggs and they were pricked in this way: each egg was pricked once as shallowly as possible but still penetrating the egg. The animal pole was always pricked in preference to the vegetal pole when the former could be reached but it was pricked slightly off the center to avoid injury to the pro-nucleus of the egg. As soon as the eggs on one slide were all pricked it was placed in a petri dish three-fourths filled with sperm-sterile water. The other slide was then treated in a similar manner. Four more slides of eggs were stripped, smeared with blood, and pricked. Two slides at a time were used to avoid desiccation of the eggs while waiting to be pricked. As the eggs were pricked they were counted and the number and time of pricking recorded on the cover of the petri dish. Fifteen minutes after pricking the gelatinous covering of the eggs was loosened from the slide with a scalpel and allowed to adhere only at the ends of the strip, and the strip cut across the middle to avoid bucking as swelling progresses. This was done to avoid pressure on the eggs from the swelling gelatinous capsules which would give abnormal cleavages and thus complicate the results. After the first thirty minutes the water was changed in each dish. Two and one quarter hours after pricking, the eggs began to cleave and the results from then on were recorded

in a chart. It was necessary here to distinguish between superficial furrows and puckering of the eggs and true cleavages. The true cleavages when first formed were deep and clear cut. As soon as the living eggs reached the many celled stage they were separated from the others with a sharp scalpel and needles and transferred with a wide mouthed pipette to another petri dish conveying as little water as possible as it may be polluted by the dead eggs.

From now on, the eggs were removed as they died and the water changed from time to time.

RECORD OF EGGS PRICKED

Record of first set	Record of second set
Species of frog---Rana pipiens	Rana pipiens
Type of water-----Aquarium	Aquarium
pH of water-------7.95	7.95
Temperature-------23	22
Number of eggs----71	483
Time pricked------3 p.m. 3/20/40	3 p.m. 4/3/40
Per cent of eggs reaching cleavage- 21 per cent	see below

The eggs of the first set were all dead after a few reached the many celled stage. They were all smeared with blood before pricking. These results are not very informative as the number of eggs pricked was too small to eliminate experimental errors.

Of the 483 eggs of the second set 304 were pricked with blood using a sharp needle. Thirty-three eggs or 10.8% reached the many celled stage.

One hundred and twelve were pricked with blood using a jagged needle previously broken off in an egg. Several eggs reached first and second

cleavage but only one reached the many celled stage.

Sixty-seven were pricked with a sharp needle but without blood. Most showed first and second cleavages but none went any further.

Of the thirty-four eggs reaching the many celled stage, thirteen which had formed a yolk plug were fixed in Bouins fixative for sectioning. Of the remaining twenty-one, twenty died and one formed a tadpole which lived for ten days after hatching.

The following account is of normal and abnormal features observed in the developing eggs:

NORMAL

About one-half hour after the egg is pricked, if it still lives, the second polar body is extruded. It appears as a whitish speck on the black animal pole.

At one and a half hours after pricking a flattening of the animal pole to form one face of a disc is noticeable. The two polar bodies are found on this disc.

At two and one quarter to two and a half hours the first cleavage furrow appeared on the flat disk and spread over the egg--the sides of this furrow are wrinkled.

The third division is horizontal and cuts the first two--not at the equator of the egg but in the animal pole or on the border of animal and vegetal.

After eighteen hours the blastopore appears. A horizontal line of pigment in the white cells marks the beginning of the archenteron.

After twenty-four and a half hours the yolk plug stage is reached, the lips of the blastopore form a circle with white cells protruding.

ABNORMAL

Several changes in the eggs were noted, the significance of which is, however, vague. This fact is certain: inhibition may take place at any stage of development and is probably caused by any one of many conditions. Some of these are: (1) the damaging effect of handling the eggs which includes abnormal pressure in stripping, (2) desiccation when on the slide in preparation for stripping, (3) injury caused when applying the blood, (4) the actual pricking which is probably the greatest factor as it includes the place and depth of puncture and the size of the needle.

As the rate of development is also different, we may conclude that much variation occurs in chromosomal processes.

Some of the eggs underwent the first and second cleavage divisions then stopped cleaving and the animal and vegetal poles exhibited an intermixing and presented a swirled effect. Others showed a predominace of one pole over another.

SUMMARY

In reviewing the work done in a light that may give the probable explanation of the factors involved it will not be necessary to include all the various theories which are so admirably presented in the references. To summarize existing theories is not the purpose of this paper nor is it to advance any new explanation. The thought here is to point out new paths of experimentation to seek the explanation of the old problem of the true nature of fertilization as a physico-chemical phenomenum.

The explanation probably rests on the relation of the physical stimulus with the chemical medium. The function of the blood is at present unknown. Does it affect the tonicity, does a leucocyte enter the egg and act like a male pronucleus, would blood from other animals act in the same way, and what would be the effect of centrifuged blood? It is the answers to these questions that should be sought in order to throw light on what is now a mere observed fact.

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