OXIDATION OF THE OVUM

OF

THE WHITE RAT

By

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B. H. Kettelkamp.
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OXIDATION OF THE OVUM OF
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HISTORICAL.

It has long been assumed by some prominent zoologists that there is a definite relation between the time of ovulation and the sex ratio. Thury in 1863 pointed out that cows which were fertilized at the beginning of the heat period produced relatively many female progeny while cows served late in the period produced a preponderance of males. Some fifty years later his results were partially confirmed by R. Pearl and H. M. Parsley. Although they did not come to the far reaching conclusion that Thury did, they did find a definite relation between the time of ovulation and the sex ratio. It might be pointed out that the discrepancies in the results obtained may be due to the fact that the exact time of ovulation has never been satisfactorily identified for cattle.
Neither has the length of the oestrous cycle been definitely determined.

The author's interest in this connection was aroused several years ago in the breeding of a Spitz bitch. The oestrous cycle in the bitch occupies some fifteen to eighteen days, the longest period known among mammals. She was first bred as soon as any signs of heat were noticeable. A litter of six puppies was born of which four were females and two were males. The second time she was bred no attempt was made to regulate the copulatory act and of a litter of seven three were females and four were males. In the following spring she was bred exactly fifteen days after the first signs of heat were noticeable. A litter of five puppies was born of which all were males.

Although a generalization cannot be reached from this one incident it was sufficient to arouse the author's interest and this paper is the result of his attempt to determine if this phenomenon occurs regularly and if so to suggest a possible cause.

Thury suggested the cause as being due to a freshness or staleness of the ovum. The fertilization of a fresh ovum, one which had recently been
discharged from the ovary, gave rise to a female. A stale ovum, one which had been for some time in the Fallopian tube, when fertilized gave rise to a male. He offered no further solution than this. Stock breeders, although they make no attempt to explain it, have firmly believed that they can control the sex of their breeds by regulating the time of fertilization but failure to recognize the exact beginning of the period of heat is no doubt responsible for the fluctuations in their results.

Assuming for the time that there is a definite relation between ovulation and the sex ratio the author has attempted to determine whether there are any changes in the ovum at different periods in the oestrous cycle.

STATEMENT OF PROBLEM.

A modification of the method of Child (1915) for determining metabolic gradients seemed the most logical procedure. If there are changes in the ovum at different periods in the cycle they must be due to the metabolic activity or intracellular gradient. Child found that the rate of oxidation
was in direct proportion to the metabolic activity in a great variety of organisms and individual cells. The greater the rate of metabolism the more rapid was the process of oxidation in solutions of oxidizing agents such as potassium cyanide, weak acids, etc.

To determine whether such a relation exists between the age of the ovum and its rate of disintegration and the relation of this to the sex ratio, if there is any, is the purpose of this paper.

MATERIALS.

The white rat, Mus Norvegicus albinus, was chosen as the most suitable animal. The ease with which these animals are kept and the rapidity with which they reproduce in captivity make them especially desirable as laboratory specimens. Furthermore, an abundance of work has been done on this animal and definite data have been compiled by several investigators concerning breeding habits, definite periods in the oestrous cycle, the exact time of ovulation, and the period of gestation.
J. A. Long, of the University of California, in collaboration with others, has found the complete cycle to occupy approximately 4.8 days and he has also developed a technique for removing eggs from the oviducts in the living condition. A modification of his technique was used in this work.

For the examination of live material it is necessary to keep it bathed at all times in a solution of nearly the same chemical constitution as the medium by which it is normally supplied. This was accomplished by a simple, yet quite efficient circulation slide, Fig. 1. A culture slide, 1 inch by 3 inches by $\frac{1}{4}$ of an inch in thickness was used. Holes were drilled through each edge of the slide into the chamber. Fine glass tubes were bent to fit into the holes and were securely cemented in place. One tube was connected by means of a Y-tube with two supply bottles, one containing the nutrient solution and the other the oxidizing solution. The tube from the other side of the slide was connected with a waste bottle. A small circular
Fig. I. The circulation slide showing the chamber in place within the cavity of the slide, also the tubes leading to the reagent and waste jars.
chamber was made to fit into the cavity of the slide. The purpose of this was to prevent the currents of solution coming into the slide from carrying the material to be examined out of the waste tube. The tubes were connected by rubber tubes to the supply and waste bottles and after a thick cover glass was cemented in place over the chamber of the slide, the clamps on the tubes could be released and a constant flow of the solution over the material to be examined could be very easily controlled.

All work was done in a constant temperature box large enough to contain the microscope with the slide in place, the supply bottles, and to allow for free movement of the hands. The temperature was kept at 37 degrees Centigrade at all times.

The nutrient solution was prepared according to the formula of Locke and contained salts in the following proportions:

- NaCl  .09
- CaCl  .024
- KCl  .042
- NaHCO  .01
To this was added 0.1% of dextrose. The solution was then autoclaved for fifteen minutes at fifteen pounds pressure and carefully plugged to prevent bacterial contamination.

The potassium cyanide solution contained 200 mg. per 100 c.c. of distilled water.

A very close watch was kept over all pregnant females in order that the exact time of parturition might be determined. This was essential because ovulation in the rat is known to occur at a fairly definite interval following parturition. All females were isolated several hours before the litter was expected to be born.

Ovulation was found to occur approximately sixteen hours after the litter was born. Sixteen hours was the least time after parturition that the eggs were found to have been discharged from the ovary and the greatest time after the litter was born that they were still found in the oviducts was twenty three hours.

When the desired length of time had elapsed after parturition the animal was killed by severing the spinal cord between the cranium and the
atlas. The use of anesthetics was avoided because of a possible effect on the blood supply of the ova. The body cavity was quickly opened and one ovary and oviduct removed to the constant temperature box. The entire organ was placed in a sterile watch crystal and was covered with sterile Locke's solution.

In positive cases when too long a time had not elapsed the eggs could be seen in a fold of the oviduct. They are arranged in a more or less irregular cluster and all are surrounded by an abundance of follicle cells. When the eggs were found the oviduct was teased apart and the eggs allowed to flow out into the watch crystal. By means of a sterile pipette they were then removed to the circular chamber within the cavity of the circulation slide. This operation occupied from six to ten minutes.

It is supposed that the several eggs produced by one ovary are discharged nearly simultaneously. At any rate, the time of discharge of eggs from one ovary was not sufficient to cause any appreciable difference in the age of the ova. For this reason
only one or two eggs from each ovary were subjected to the oxidizing solution.

In older cases the eggs did not appear in clusters but were scattered along the entire oviduct. Some had very probably passed on down into the horns of the uterus because in only a few cases were more than two ova found in females that had gone twenty three hours after parturition. In younger cases it was not uncommon to find as many as seven ova in one fold of the oviduct. In cases that had gone more than twenty three hours no ova were found.

In these older cases the entire oviduct was teased apart and the eggs, when present, readily flowed out. Eggs of this age had usually lost most of their follicle cells and were quite bare. This rendered them difficult to observe because their presence might easily be obscured by secretions in the oviduct, fat globules, loose follicle cells, and blood corpuscles.

After the egg had been placed in the circulation chamber with a few drops of Locke's solution to prevent drying the cover glass was cemented in
place with a coat of vaseline. The entire chamber was then filled with Locke's solution by releasing the clamp on the supply bottle until all air bubbles were expelled. This tube was then closed and the second clamp on the potassium cyanide bottle was released. At the same instant that the cyanide solution was introduced a stop watch was set in motion in order that the exact time required to oxidize the egg might be recorded. The solution was allowed to flow over the egg until the process of oxidation was complete.

The mature, unfertilized egg of the white rat varies from approximately 0.12 mm. to 0.16 mm. in diameter. It is spherical in shape and is surrounded by a clear girdle, the zona pellucida. Outside of this girdle are several thicknesses of follicular cells. The nucleus stands out quite distinctly and is nearly spherical in shape. Fig. II.

The cyanide solution was introduced into the chamber and the follicular cells soon lost their attachment and were washed free from the egg. This was followed immediately by the beginning of the oxidation of the egg which was evidenced by a com-
Fig. II. The mature ovum as it appears when removed from the oviduct.
plete disintegration. This was first noticed in the region of the nucleus and from there the disintegration extended from within to the outside and continued until the entire ovum had lost its spherical shape and remained as a scattered mass of unorganized material. Figs. III and IV.

Child found in the oxidation of the eggs of the sea-urchin that disintegration began where the nucleus lay nearest the surface. When development took place the portion first to disintegrate was that which normally gave rise to the head of the embryo and the axis along which the disintegration proceeded was destined to become the major axis of the developing embryo. Whether or not this condition exists in the ovum of the rat can at this time only be conjectured. However, disintegration in all the observed cases seemed to begin in the region of the nucleus and then to proceed throughout the entire egg. Both the beginning and the end of the process was easily detected.

As a control measure two ova were removed from a female which had given birth to a litter
Fig. III. Diagram of the ovum showing the process of disintegration beginning in the region of the nucleus.
Fig. IV. Diagram of the ovum in the process of disintegration, a later stage than Fig. III.
of young eighteen hours previously. These ova were removed under the same conditions as those which were treated with the potassium cyanide solution. They were removed and quickly placed in the circulation chamber. Locke's solution was allowed to flow gently over them for a period of six minutes, a time considerably longer than that necessary to oxidize any of the eggs similarly treated with potassium cyanide. However, at the end of that time no signs of disintegration were noticeable.

RESULTS.

Eight females were killed before any ova were found. In each of these cases the time elapsed after parturition had been eighteen hours, the optimum time at which eggs might be secured. Failure in these cases was very probably due to inability to observe the eggs in the living oviduct. In the ninth case the entire oviduct was teased apart and one ovum was secured. No accurate data was obtained however, until the eleventh female had been killed. After it had been learned where the eggs
might be found, what was their appearance, and a technique had been developed by which they might be removed, it was a relatively simple procedure to remove them.

In all the cases recorded below the females were isolated several hours before the litter was born and the time after parturition was reckoned from the time the last of the litter was born until the female was killed.

The following is a summary of the data obtained from thirteen cases taken at various intervals in the period in which the eggs might be obtained.
<table>
<thead>
<tr>
<th>Case</th>
<th>Time elapsed</th>
<th>Disintegration time</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>18 hours</td>
<td>3 min. 49 sec.</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>3:34</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
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<tr>
<td>13</td>
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</table>

Average disintegration time for 16 hour cases 2:37.5
CONCLUSIONS.

It is quite evident from the above data that there is an intracellular change in eggs of different ages of this particular animal. There is a difference of two minutes and forty three seconds in the rate of disintegration in an ovum of sixteen hours and one of twenty three hours. The younger the egg the more rapid is the process of disintegration. According to the observations of Child (1915) the more rapid the process the greater is the metabolic activity, and accordingly the greater the electrical potential. The cause of this phenomenon and the constancy with which it seems to occur can only be conjectured. It appears to be a change of an electro-chemical nature, a decrease in electrical potential due to a slowing down of the metabolic activity within the ovum. As the time after parturition increases the electrical potential of the egg decreases. It is known, in some cases at least, that after fertilization the metabolic activity of the egg is markedly increased and that this acceleration is responsible in some way for the initiation of development.
It has been pointed out by R. S. Lillie, (1913) that the direction and speed of the electrical migration of living cells are chiefly dependent upon the electrical characteristics of their constituent colloids. Sperm solutions and cells containing an abundance of nucleic acid moved with great rapidity and uniformity with the negative stream, while cells with voluminous cytoplasm, such as leucocytes, flowed with much speed toward the cathode. This difference in the direction of electrical convection presumably implies a difference in the electrical charge carried individually by these two kinds of cells.

Assuming that the egg which contains a large amount of cytoplasm bears an electro-positive charge and the sperm, being composed chiefly of nuclear material, bears an electro-negative charge, it may be suggested that there may be some relation between the electrical characteristics of the two gametes that is responsible for the initiation of development and indirectly for the determination of the sex of the resulting individual.
It is quite possible that an egg recently discharged from the ovary and bearing the greater electrical charge may upon fertilization have a tendency to attract a spermatozoon bearing the greater electro-negative charge which will be one bearing an X-chromosome. The result, in animals in which the female is homozygous for a sex factor, will be a female.

As the age of the ovum increases and the potential decreases there will come a period when the chances for fertilization by either type of spermatozoon are equal and offspring of both sexes will appear in equal numbers. Finally, as the age of the ovum increases and its potential decreases there will be a greater tendency for it to attract a spermatozoon with a lesser charge which will be one not having an X-chromosome and the resulting individual will be a male.

Although highly hypothetical this seems a possible explanation for the phenomenon described by Thurry in 1863. Until more work is done and more data obtained relative to the phenomenon of
fertilization no definite statement can be made which, without doubt, will account for the determination of sex at the time of fertilization.
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