OXIDATION OF THE OVUM

OF

THE WHITE RAT

By

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HISTORICAL.

It has long been assumed by some prominent
Zoologists that there is a definite relation be-
tween the time of ovulation and the sex ratio.
T. 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 preponder-
ance of males. Some fifty years later his results
were partially confimmed by R. Pearl and H. M.
Parshley. 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 fe-
male. 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 regu-
lating 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 defin-
ite 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 act-
ivity in a great variety of organisms and indi-
vidual cells. The greater the rate of metabol-
ism the more rapid was the process of oxidation
in solutions of oxidizing agents such as potass-
ium cyanide, weak acids, etc.

To determine whether such a relation exists
between the age of the ovum and its rate of dis-
integration 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 rapid-
ity with which they reproduce in captivity make
them especially desirable as laboratory speci-
mens. 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 per-
iod of gestation.

J. A. Long, of the University of Cal-
ifornia, in collaboration with others, has
found the complete cycle to occupy approxi-
mately 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 consti-
tution as the medium by which it is normally
supplied. This was accomplished by a simple,
yet quite efficient circulation slide, Fig.
I. 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
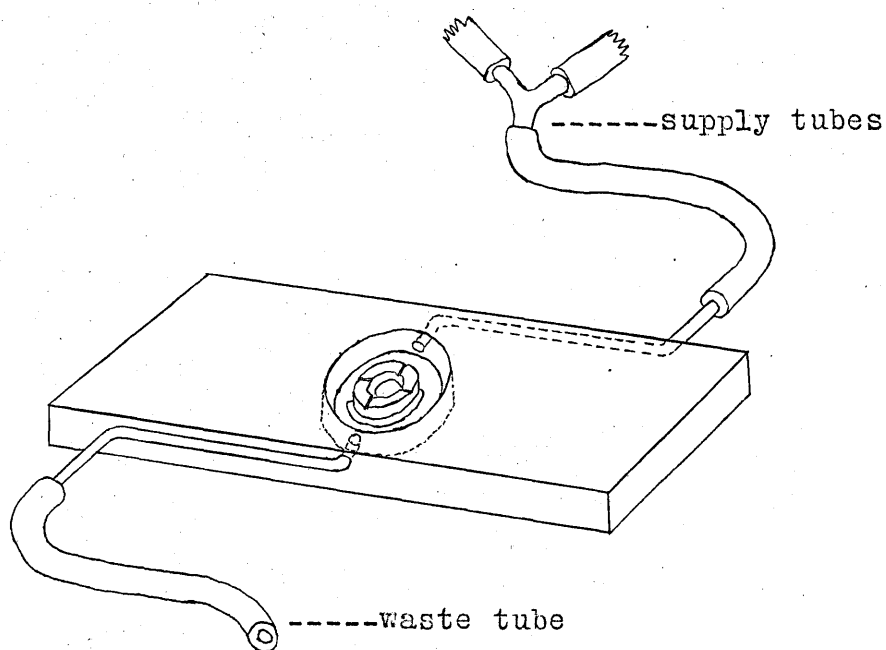connected with a waste bottle. A small circular

------supply tubes

------waste tube

Fig. I. The circulation slide showing the
chamber in place within the cavity of the
slide, also the tubes leading to the re-
agent 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.01 % 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 be-
cause 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 ster-
ile 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 subject-
ed to the oxidizing solution.

In older cases the eggs did not appear in
clusters but were scattered along the entire ovi-
duct. 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 secret-
ions in the oviduct, fat globules, loose follicle
cells, and blood corpuscles.

After the egg had been placed in the circula-
tion 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-
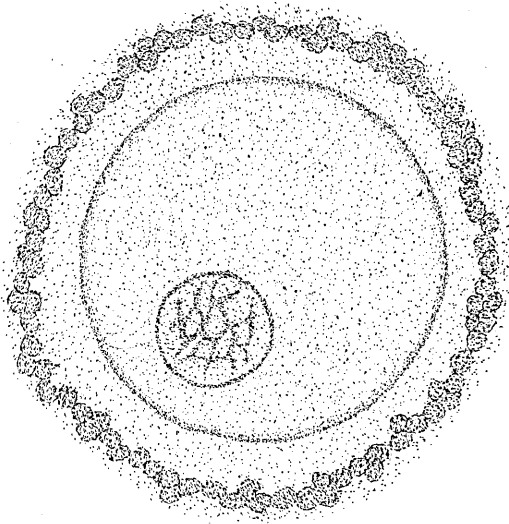
Fig. II.   The mature ovum as it appears
when removed from the oviduct.

plete disintegration. This was first noticed
in the region of the nucleus and from there the
disintegration extended from within to the out-
side 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 devel-
opment took place the portion first to disinte-
grate 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 be-
ginning and the end of the process was easily de-
tected.

As a control measure two ova were removed
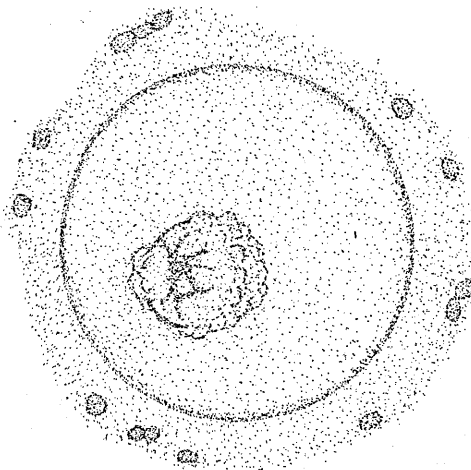from a female which had given birth to a litter

Fig. III. Diagram of the ovum showing
the process of disintegration beginning
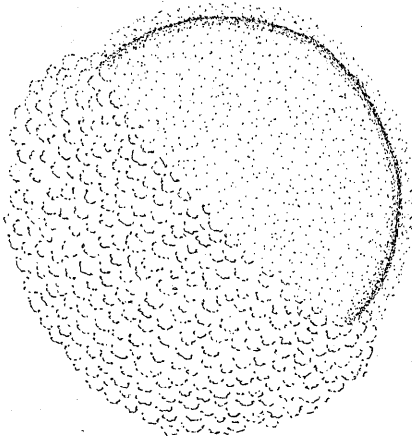in the region of the nucleus.

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 similiarly
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 op-
timum time at which eggs might be secured. Fail-
ure in these cases was very probably due to inabil-
ity 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 ob-
tained 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 ob-
tained from thirteen cases taken at various inter-
vals in the period in which the eggs might be ob-
tained.

Case	Time elapsed	Disintegration time
1	18 hours	3 min. 49 sec.
2	18	3:34
3	18	3:42
4	21	4:52
5	16	2:38
6	16	2:54
7	23	5:14
8	23	5:28
9	17	2:51
10	16	2:44
11	23	5:43
12	16	2:14
13	23	4:57

Average disintegration time for 16 hour cases   2:37.5

"	"	"	"	17	"	"	2:51
"	"	"	"	18	"	"	3:41.6
"	"	"	"	21	"	"	4:52
"	"	"	"	23	"	"	5:20.5

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 dis-
charged from the ovary and bearing the greater
electrical charge may upon fertilization have a ten-
dency to attract a spermatozoon bearing the great-
er electro-negative charge which will be one bear-
ing 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 po-
tential 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 de-
creases 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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