6-1938

The Ovary of the Frog

Wyman O. Bergner

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THE OVARY OF THE FROG

My thanks are especially due to Father Carroll for the suggestion of the subject, the suggestion of the writing, and for the instruction in the use of methods. Dr. Seel of the Biology Department is thanked for the suggestions given in the preparation and staining of the histological studies.

WYMAN O. BERGNER

A Thesis Submitted to the Faculty of Marquette University in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science

Milwaukee, Wisconsin
June 1938
My thanks are especially due to Father Carroll for the suggestion of the subject, the supervision of the writing, and for the correction of the final copy. Dr. Steil of the Botany Department is thanked for the suggestions given in the preparation and staining of the histological studies.

Part I. Reproductive System and Ovulation

Part II. The Frog Egg

Part III. Relation of the Pituitary to Ovulation

Part IV. Effect of Colored Light and Temperature on the Development

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Introduction

All living organisms, under normal conditions, are able to reproduce an organism of its own kind by the natural function of its reproductive system. Except in such cases where reproduction is asexual, there are always two individuals, male and female, which produce sex elements called the ovum and the spermatozoon. In reproduction, the sperm unites with the ovum to form the new individual. This fertilized ovum is commonly referred to as the zygote, which produces the organism ultimately formed.

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Introduction.

All living organisms, under proper conditions, are able to reproduce an organism of its own kind by the natural function of its reproductive system. Except in such cases where reproduction is asexual, there are always two individuals, male and female, which produce two elements called the ovum and the spermatozoa. In the vertebrate kingdom, the sperm unites with the ovum to form the new individual. This fertilized ovum is commonly referred to as the zygote, which produces the embryo from which the new individual is ultimately formed.

In this work the ovary is studied from several viewpoints: 1) anatomical; the size, location of the ovary, and the organs connected with it. 2) physiological; the ovary is discussed as an egg and hormone producer. 3) histological; an attempt is made to interpret several microscopic sections of the ovary. 4) embryological; there is a brief consideration of the development of the ovum in the ovary.

It is true that comparatively little work has been done on this phase of the life processes, but what work has been completed is thorough and conclusive. As far as the anatomy and function of the ovary are concerned, all writers are agreed. It will be noticed that the greatest amount of work concerning hormone effects, effect of light, heat and grafting experiments have been done
only within the past twelve years. Wills, 1936, stated that there remains much experimental work to be done.

shape vary greatly depending on the season of the year. After ovulations and resumption of the egg, the ovaries are shrunk to a very small size. Before

Material and Methods

A preserved female specimen of Rana catesbiana was dissected in order to study the location, size, and attachments of the ovary. The ovary of a freshly killed female Rana pipiens was also dissected out, but this one was fixed in Bouin's solution, and prepared for sectioning according to standard histological methods. The slides were stained with Eosin.

Microphotographs of the histological preparations were made under three different magnifications. (1) A lobule of an ovary was taken with a magnification of 30x. (2) A portion of this lobule was taken with a magnification of 125x. (3) A part of a single ovum was taken with a magnification of 30x.

The ovaries are almost always distended by small shot-like projections which are the ova being formed in the ovary. These ovaries, on the ventral surface of the kidneys, are found enveloped in folds of peritoneum. This peritoneum extends dorsally from each ovary to the dorsal body wall where it becomes continuous with the peritoneum of the body cavity. This membrane which is double, is known as the mesovarium.
Part I

The ovaries of the frog are located on the ventral surface of the kidneys. (Plate 1) Their size and shape vary greatly depending on the season of the year. After ovulation and dehiscence of the ova, the ovaries are shrunken to a very small size. Before this dehiscence of the ova, while they are still in the ovaries, these organs fill nearly the entire body cavity, the remaining vital organs being quite crowded toward the ventral surface. After the eggs have left the ovaries by means of a rupture of the follicles, they enter the oviducts. These oviducts are convoluted tubules which extend through nearly the entire body length. The anterior end, found near the base of the lungs, is open. It is through these openings that the ova enter the oviducts and by means of these structures are carried to the uteri from which they leave the body cavity, going to the outside.

The surfaces of the ovaries are almost always distended by small shot-like projections which are the ova being formed in the ovary. These ovaries, on the ventral surface of the kidneys, are found enveloped in folds of peritoneum. This peritoneum extends dorsally from each ovary to the dorsal body wall where it becomes continuous with the peritoneum of the body cavity. This membrane which is double, is known as the mesovarium.
This structure serves to suspend the ovaries and to hold them in their place in the body cavity. It also supplies the ovaries with the necessary blood vessels and nerves. From the region of this mesovarium, the oogenic cells of each saculated portion of the ovary begin to proliferate for the reproduction season. Each ovum is covered by cuboidal cells that elaborate the yolk which is later added to the cytoplasm of the ova. Around these nurse cells, a sheath or theca of connective tissue is added.

The inner part of the ovary is lined with flat epithelial cells which arise as outgrowths of the kidney. The middle part of the ovary varies in thickness at different times and in different places. It is composed of follicle cells and eggs in various stages of development. The developing eggs lie within these small chambers or follicles as they are called. After the egg has reached its full development, it ruptures the wall of this follicle cell and gets into the body cavity. After all the developed ova have reached the body cavity, the ovary has shrunk to a very small size, but already contains the ova for the next year's production. These eggs may remain in the body for a short time. Eventually, however, they are swept toward the openings of the oviducts which are at the anterior end and lie near the esophagus.
These oviducts are a pair of white twisted tubules with thick gelatinous walls. They start with open ends at the front end of the body cavity as noted before, near the esophagus and close to the lung roots. These oviducts lead posteriorly, becoming larger and much convoluted. The posterior ends are greatly dilated, but have thinner walls than the anterior portions. These dilated parts are the ovisacs or uteri. The ova are stored in these sacs just previous to being liberated.

Unlike the male of the species, the female has genital ducts which are distinct from the ureter. The eggs are moved into the mouth of the oviducts by ciliary action, and they are moved down the oviduct by action of cilia on the ridges of the inner walls. It is here, in the oviduct, that the eggs receive their coating of jelly-like yolk. It is interesting to note the differences in the structures found around the ova of the various vertebrates and the sources of these structures. In this chart, what structures are formed in the ovary, and what structures are deposited by the oviduct are given for six types of vertebrates. (Chart I)

The time that the eggs remain in the uterus depends upon the presence or absence of a male. If a male is present, it clasps the female with its forelegs. Then as the eggs pass out from the cloaca, they are fertilized
by the sperm which is discharged over them. If, however, the male is not present, the female emits only a few eggs at a time so that not all of them will be wasted. Nussbaum (1915) found one female late in the summer which had a great deal of jelly present in the uterus. The ova, however, had all degenerated. This showed that in the absence of a male, the female will not let the eggs out to be wasted in the water.

Chart I.

<table>
<thead>
<tr>
<th>HOMOLOGIES OF STRUCTURES AROUND OVA OF VERTEBRATES.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formed in Ovary</strong></td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Birds</td>
</tr>
<tr>
<td>Amphibian</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Reptile</td>
</tr>
<tr>
<td>Monotreme</td>
</tr>
<tr>
<td>Marsupial</td>
</tr>
<tr>
<td>Eutheria</td>
</tr>
</tbody>
</table>
In tadpoles of 10 mm. in length, which is just after the mouth opens, a pair of longitudinal ridge-like thickenings of peritoneum appear along the dorsal surface of the body cavity close to the mesentery and along the inner borders of the developing kidneys. These genital ridges, as they are called, are present in all tadpoles, since there is no sex differentiation until much later. These ridges, at first, are due to slight modifications in the shape of the peritoneal epithelial cells which elsewhere flattened, here become cubic or slightly columnar. The ridges become especially prominent at the anterior end. This growth is due to the increasing by division of the epithelial cells to form a layer several cells in thickness. It is also partly due to an ingrowth of an axial core of connective tissue from the basal membrane of peritoneum along which the blood vessels gain access to this ridge. The ovary or testis, as the case may be, is formed from the posterior two-thirds of this genital ridge.

The anterior one-third undergoes degenerative changes and forms fat bodies. At an early stage, certain of the epithelial cells of the genital ridge become very conspicuous by their larger size and more spherical shape. These enlarged cells are the primitive ova. Around the primitive ova are many smaller epithelial
cells which will form the capsules or follicles. These follicles cause projections to be formed on the surface of the genital ridge. The primitive ova multiply rapidly, being formed either from the surface epithelium or from the already existing ova.

At the time of metamorphosis, sexual differentiation occurs. In the female, this change consists of a great increase in size of the genital ridge which sooner or later develops into the ovary. The change also consists in the formation of permanent ova. These permanent ova are derived from the primitive ones. In some cases each primitive ova is converted directly into a permanent one. The permanent egg is enclosed in a follicle too, the same as the primitive ones are, but the former differ from the latter in three ways: (1) the permanent ova are larger, (2) the permanent ova contains food yolk, and (3) there are several important changes in the nucleus.

In the primitive egg, the nucleus is small, granular, and consists of an elastic capsule. This membrane is filled with a fluid and is traversed by a protoplasmic reticulum enlarged at its nodes. These nodes will later form the germinal spots. When this permanent egg has reached a size of .5 mm. in diameter, an investment, the vitelline membrane, forms around it within the follicle. It is not actually known, but it is generally agreed that
this membrane is formed or layed down by the ovum itself. Later yet in the development, a black pigment is formed by the ovum and appears over the entire surface. Still later it becomes restricted to only one-half of the ovum. It is known that the ovum produces this pigment, but the actual formation or function is not known. The black portion is known as the animal hemisphere, while the remaining white portion is called the vegetative hemisphere. The black pigment, covering approximately one-half of the sphere, is situated in the upper part when it is free to move. According to Wieman, 1930, the nucleus becomes situated near the animal pole.

When the egg has been laid, this nucleus has completed the maturation process as far as the metaphase of the second maturation division. The nucleus will not divide beyond this phase unless it is fertilized by the male spermatozoon. The head and middle piece of the sperm enters the egg. The point of entrance of the sperm may lie in any meridian, but it is usually about forty degrees from the animal pole. The cleavage process begins almost immediately. Roux, 1887, showed that the plane of entrance of the sperm plays a very important part in the establishment of future embryonic axes. He showed also that the entrance of the sperm at the boundary of the black and white zones in the egg, is followed by a
shifting of the black hemisphere toward the point of entrance, forming the gray crescent. It is in this area that the dorsal lip of the blastopore is formed. The gray crescent also marks the dorsal region of the egg, the plane which bisects the gray crescent forming the median plane of the embryo. By controlling the point of entrance by applying the sperm by hand, Roux showed that the entrance point determines the posterior pole of the embryo, the entrance being in the ventral region extending downward to the posterior region.

The frog egg is somewhere between the meiolecithal egg of Amphioxus which has little or no yolk, and the hen's egg, the telolecithal type, which has a large amount of yolk. In the frog egg there are three definite layers or regions. These layers are coats of jelly. The egg shown in Plate 4 was still in the ovary, and the structure of the egg and follicle wall can be seen quite clearly. This follicle wall is made up of squamous epithelium and it is possible to distinguish the nuclei of these cells. The small dots which can be seen in the central portion of this egg are nuclear granules, for the nucleus of the egg is not yet completely formed. Plate 2, a diagrammatic drawing of the frog a short time after laying and fertilization, shows the path taken by the sperm which has fertilized the egg.
After the egg has been fertilized, it will undergo the various stages of cleavage. According to Shumway, (1935) the cleavage of the frog's egg is of the unequal, holoblastic type. Holoblastic cleavage is shown when the entire egg divides, the cleavage being unequal when, after the eight-cell stage, the divisions which occur are not equal either in size or rate. Cleavage will not be discussed in this paper, but several figures are included. (Plate 3) In Fig. 7 of this plate, notice where the invagination will later occur. This invagination will form the gastrula stage, and it is from this curving inward that the diploblastic tadpole eventually arises.

Plate 5 shows several eggs in various stages of development. Plate 4 is an enlargement of one of the ova shown in this Plate 5. Notice in both cases that the three layers of jelly and the nuclear material in the inner layer show very well. Plate 4 also shows the nuclei of the follicle wall cells, and while they show up in Plate 5 also, this figure shows the mesovarium which supports the entire ovary in the body cavity.

It was deemed advisable to include among these plates a cross section of the ovary. This section shows several ova in different stages of development although they are all very young. The follicle cells which surround the ova show very plainly. (Plate 6.)
Beside the necessary presence of the male frog, there is some importance attached to the pituitary gland, and it has been shown that there is a definite relation between this hormone and ovulation which is the discharge of the eggs from the ovary. Rugh, 1937, wrote, "Much work is needed on this matter of the pituitary-ovulation relation. It has been shown that even with a standard dose of two female adult pituitaries, frogs do not always give a reaction, nor is the reaction identical in any two cases. Yet, it is known that the degree to which an ovary empties is dependent upon the amount of anterior pituitary hormone which has been injected into the animal."

Rugh concluded from this therefore, that either the dose of the injected hormone varies or else the recipients vary in their susceptibility to the hormone. Rugh performed all his experiments over a long period of time. He collected frogs in November, when all egg formation and fertilization were definitely finished, and again in February when the eggs were nearly ready for ovulation, but before any had begun.

The following then, is a summary or conclusion drawn from the work he has done along this line. As has been pointed out before, those frogs up to 70 mm. in length are sexually immature. Rugh found however,
that in the next stage, 70 to 74 mm. frogs, there was a definite weight relation in the anterior pituitaries of the male and female frogs, and that the glands in the two sexes showed correlating differences. From the November crop of frogs, it was found that the largest animals were usually females. It was noted too, that while the largest males were 5.7 mm. shorter than the females on the average, the anterior pituitaries of these males were 28% heavier than those in the largest females.

The relation was somewhat different in the February group of frogs. Again, the average female was longer than the average male, and the larger females had bigger pituitary glands than the males. However, it was found that in animals of equal size, the larger gland was found in the male. During the hibernation, both male and female show a decrease in the relative weight of the pituitary as compared with body length, this reduction being as much as 29% in some females. During this period the weight changes in the ovary and in the pituitary are correlated, since the weight reduction of the ovary ranged from 7% to 32%, and the body weight was found to be reduced 8.6%.

While the largest females in both groups tend to have the largest ovaries, it was found that there is no
definite relation or rigid correlation between body length and ovarian weight. The heaviest ovaries which were found were 24 gm. in weight, while the smallest were only 5 gm. These small ones however, were immature.

It was noticed and can be shown that the larger ovaries, either in the November group or in the February group, are more susceptible to equal doses of the hormone. A dose of 4 mg. of the hormone from a male will induce 7.7% ovulation in November, while an equal dose from a female of the same group will induce about 34% ovulation. In February, these same doses, from either male or female will always induce 100% ovulation. The potency of the female pituitary is not reduced in the least even after the animal has been induced to ovulation by the injected hormone. This injection seems to be comparable to the liberation of the host's hormone in a normal breeding, and the host's gland is unaffected by this injection.

It is interesting to note here that 5 mg. of male pituitary is equal to about 3 mg. of female gland tissue in respect to inducing ovulation in November. Although the male gland is about 16% heavier than that of the female, it is only 60% as potent as the female gland per unit of weight. However, as is shown in Chart II, larger doses of the hormone from either male or female of either group will tend to induce greater ovulation.
As has been pointed out, increasing doses of the hormone from either male or female donor tends to induce increasing ovulation in animals. (Page 12) From this chart however, it is seen that while both sexes can cause ovulation to increase, the female glands very obviously are more potent. Notice that 5 mg. of female pituitary more than doubles the percentage of ovulation brought about by 8 mg. of the male hormone.

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**Chart II**

<table>
<thead>
<tr>
<th>Size Range of Recipient</th>
<th>Females used</th>
<th>Donor of Pituitary</th>
<th>Dosage of Pit. (mg.)</th>
<th>Per cent. Ovulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>74-94 mm.</td>
<td>5</td>
<td>Male</td>
<td>2</td>
<td>0.0</td>
</tr>
<tr>
<td>78-93 mm.</td>
<td>5</td>
<td>Male</td>
<td>4</td>
<td>7.7</td>
</tr>
<tr>
<td>79-94 mm.</td>
<td>5</td>
<td>Male</td>
<td>5</td>
<td>27.00</td>
</tr>
<tr>
<td>76-92 mm.</td>
<td>5</td>
<td>Male</td>
<td>6</td>
<td>36.60</td>
</tr>
<tr>
<td>74-91 mm.</td>
<td>5</td>
<td>Male</td>
<td>8</td>
<td>41.40</td>
</tr>
<tr>
<td>77-92 mm.</td>
<td>5</td>
<td>Female</td>
<td>2</td>
<td>0.0</td>
</tr>
<tr>
<td>83-97 mm.</td>
<td>4</td>
<td>Female</td>
<td>3</td>
<td>23.70</td>
</tr>
<tr>
<td>77-98 mm.</td>
<td>5</td>
<td>Female</td>
<td>4</td>
<td>34.20</td>
</tr>
<tr>
<td>77-92 mm.</td>
<td>5</td>
<td>Female</td>
<td>5</td>
<td>88.00</td>
</tr>
</tbody>
</table>

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Yung, 1878, after a great deal of work on growing tadpoles under various colored light rays, announced that he found the various colors affected differentially the growth of these tadpoles. The results which he had obtained from his work showed a descending order of effectiveness as follows: violet was the best light for growth, followed by blue, yellow, white, dark, red, and green which was the noorest for growth. Rugh, 1935, claimed that Yung was in error, and produced the following as proof of his statement. Yung in his experiments, used filters of double thickness. These filters were at fault, because while they did not reduce the luminous transmission on the tadpoles, they did reduce the energy transmission by 14%.

On the basis of the chart concerning the growth rate of tadpoles under different parts of the spectrum, the following can be validly concluded. "In the limits of the solar spectrum, the quality of radiation does not show any differentiated effect on the growth rate of the frog egg or tadpole, if the radiant energy is equalized at the different spectral regions." (See Chart III.)

A study of the temperature effect on the early development of Rana pipiens was made and reported by Atlas in 1935. He found that while the cleavage rate remains constant in any temperature up to the 64-cell stage, the
Chart III

GROWTH RATE OF TADPOLES UNDER DIFFERENT PARTS OF THE SPECTRUM, USING EQUAL RADIANT LIGHT

<table>
<thead>
<tr>
<th>Color</th>
<th>Radiant Energy (Min./day)</th>
<th>Length (mm.) 15 days</th>
<th>Length (mm.) 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunlight</td>
<td>125</td>
<td>15.56</td>
<td>22.10</td>
</tr>
<tr>
<td>Blue</td>
<td>125</td>
<td>15.52</td>
<td>22.7</td>
</tr>
<tr>
<td>Green</td>
<td>125</td>
<td>15.50</td>
<td>22.14</td>
</tr>
<tr>
<td>Yellow-red</td>
<td>125</td>
<td>15.52</td>
<td>22.10</td>
</tr>
<tr>
<td>Red</td>
<td>125</td>
<td>15.54</td>
<td>22.7</td>
</tr>
<tr>
<td>Infra-red</td>
<td>125</td>
<td>15.60</td>
<td>22.12</td>
</tr>
<tr>
<td>Darkness</td>
<td>125</td>
<td>15.50</td>
<td>22.7</td>
</tr>
</tbody>
</table>

On the basis of information from the above chart, it would seem that Yung was in error when he announced that the growth rate is affected by colored light. Under the red light, the tadpoles grew least, gaining only 6.46 mm., while under the green which Yung had said was the poorest, the tadpoles grew most, gaining 6.64 mm. This is a difference of only .18 mm. and is not significant.

After Rugh.
rate of development after that or at least from gastrulation on, has been found to be a constant determined by the temperature. It was also found that the relation of gill growth and body growth is changed by temperature in such a way that the maximum size attained decreases as the temperature decreases. The increase in relative velocity of development with increase in temperature over lower temperatures, is identical for different stages of development. At higher temperature ranges, there is a less rapid increase in relative velocity for earlier stages as compared with the later stages.

It is also important perhaps, to notice that the change in susceptibility to injury by heat has been studied for the cleavage stages. An increase in tolerance to heat proportional to the number of cleavages has been found to occur.
As early as 1902, Pflueger had taken ovaries from several frogs which had been collected from three different localities. He found that in this collection of animals picked at random, the percentage of males varied greatly in the various localities. The males of the species were always predominated in number by the females. From some areas however, the male-female ratio was about even. He concluded from this observation that under most conditions the male mortality rate must be higher than the female death rate.

Later on however, he concluded that some females change over to males after a certain period of growth. From a large group of males he selected four animals, and in one of the four he found evidence of a Graafian follicle, which is the envelope of cells in which the egg or ovum is enclosed before ovulation. On this basis he then concluded that there were three types of animals, the male, the female and the third type which was at first female but which later in development changed into a male.

In 1908, Schmitt-Marxcell advanced the theory that the hermaphrodites of Pflueger were not hermaphrodites at all, but that the bi-sexual animal was merely an intermediate form which at first had all the female characteristics which later changed and grew into normal testes. So, in one way Pflueger had been right. There was a division of the
animals besides the division by sex, but not in the way he had described. The division was into differentiated and undifferentiated races. The differentiated races are those which form definite sex character early in their development, while the undifferentiated races are those which do not show definite testes until late in the larval life and then are formed or laid down by the pro-gonad. The undifferentiated is one in which the sex is determined late in the development metagamically. It is distinguished from the true female by the poor development of the ovaries.

The data of Schmitt-Marcell indicated a progressive rise in the percentage of male animals with the increase in age, so the chances that this conclusion is correct is quite reasonable. Now, what is the explanation for this phenomenon? This question has not yet been fully answered. Why do the differentiated races go directly to the ultimate, while the undifferentiated races go through a series of changes before they reach their full development? Although several hypotheses have been advanced, this second question has been a ground for argument for a long time. No theory has been adequate to account for all the facts.

The direction of the solution however, has been pointed out by Herwig, 1912. He found that when
differentiated males and females were crossed, only morphologically sexually differentiated progeny resulted, and the sex ratio was very close to 1:1. However, when the undifferentiated animals were crossed, only larvae which showed indirect gonial development resulted, and the first filial generation were sexually indifferent larvae.

He found too, that the differentiated races develop in all races of frogs, but the undifferentiated type varies in different races. This would seem to indicate that no doubt the phenomenon is in some way connected with heredity. It may be due to some mutation which occurred in certain races of frogs, and it is probable that the answer to the whole question will be found by someone working along this line.

Chart IV shows the comparison of ovaries and testes in undifferentiated races.
### Chart IV

<table>
<thead>
<tr>
<th><strong>COMPARISON OF OVARY &amp; TESTES IN UNDIFFERENTIATED RACES</strong></th>
<th><strong>OVARY.</strong></th>
<th><strong>TESTES.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Morphologically undifferentiated glands. First migration of the sex cord cells.</td>
<td>1. The same condition exists here as in the female.</td>
<td></td>
</tr>
<tr>
<td>2. The germ cells remain in the germinal epithelium which thickens greatly.</td>
<td>2. The germ cells remain in the germinal epithelium which thickens in some races but not in all.</td>
<td></td>
</tr>
<tr>
<td>3. There is a formation of germ cell nests.</td>
<td>3. There is a formation of germ cell nests which are the fore-runners of the progonad lobules.</td>
<td></td>
</tr>
<tr>
<td>4. The germ cells may start maturation, but get only as far as the diplotene stage, and then start second period of growth.</td>
<td>4. The germ cells of some races begin maturation and may go through all the stages up to the anaphase of the first spermatocyte division. A few giant spermatids are formed, but they degenerate and very few enter second period of growth.</td>
<td></td>
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<tr>
<td>5. The germ cell nests are obliterated by the growing oocytes.</td>
<td>5. The germ cell nests persist until metamorphosis and many of them become part of the definitive testes.</td>
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<tr>
<td>6. The second growth of the sex cord never occurs.</td>
<td>6. There is a formation of the definitive sex cords, the cross tubules, and passage of residual cells of the progonad lobules into the sex cords.</td>
<td></td>
</tr>
<tr>
<td>7. The oocytes grow slowly, probably for several seasons after metamorphosis. The exact period when the eggs are mature is unknown.</td>
<td>7. The maturation cells of the progonads degenerate: definitive gonads are formed and the germ cells undergo a second maturation cycle forming ripe sex products. This may occur in some animals shortly before the metamorphosis, and in others shortly after this important transformation.</td>
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From W. W. Swingle.
Geddes and Thompson (1899) set forth the theory that the sex of the individual animal was determined by the metabolic rate of the individual. This theory was known as the Metabolic Theory. Riddle (1927, 1931, 1932) supported this idea, and set about to prove conclusively its validity. He was never able to establish definite proof for the idea, but contrary facts have been set forth.

Wills (1936) published the following results from experiments performed with the leopard frog and four other closely related animals. He found that during the time of sexual differentiation and even shortly after, there was no significant difference in the respiration rates in males or females of the species investigated. He also found that although there was no significant respiration difference in the immature animals, there was a 12% higher rate in 27 mature males than in 25 adult females.

Besides these facts, he concluded from his experiments several other new ideas. The sex differentiation is independent of the metabolic rate of the developing organism. Significant difference in the metabolic rates of the two sexes is not established absolutely until the individuals have reached sexual maturity. Therefore, it was concluded that the difference in the metabolic rates is a secondary sex character, and not one of primary importance, and also the metabolic theory of sex determination is not sustained by established facts.
Closely allied to this Metabolic Theory of sex determination is the physico-chemical interpretation of sexuality which was reviewed and set forth by Joyet-Laverigne. (1931)

These writers held that the male and female of any species, whether plant or animal, had different chemical compositions. This chemical difference, they claimed, constituted a primary sex difference or sex character which expressed itself in different metabolic levels. This may explain the difference noted in the rates in the adults, but of course, argues neither for nor against the theory of Geddes and Thompson. In the light of this additional evidence and with what has gone before, it seems logical to conclude that the metabolic rate of an animal has nothing to do with its sex determination.

Another theory which has been advanced as an explanation for the determination of sex is the Hormone Theory. Advocates claimed that in any one animal a certain hormone may be present. This hormone may act differently on different animals, or its chemical composition may vary in different cases. At any rate, it may cause development along a certain line, and so in one animal the male organs may form, and in another animal the female organs may arise. This idea has been shown to be false, too.
Emil Witschi (1927) in experiments in testis grafting showed that the theory of hormone sex determination is inconsistent. This worker used local races of the European frog, Rana temporaria. In seventeen sexually indifferent tadpoles he introduced pieces of testis from an adult frog. The grafts became attached in various places in the body cavity, and though the behavior was slightly different in each of the seventeen cases, all parts of the testes proved capable of normal development and function. The development and sex determination of the host's gonads were in no way affected by these grafts. From these results it seems that the theory of hormone sex determination has been proved to be in error.

Among the writers in this field who have published findings in these magazines, two names stand out above the others. These are Swingle and Rush. The most important writing of the former was on the sex changes and reversals which have been observed in the free tadpoles. Rush has shown the relation between ovulation and the amount of anterior pituitary hormone present in the body of the female. He also has worked on the growth rate of the tadpoles under different colored lights. His work is very conclusive and presented in an understandable manner.
Conclusion

Since most of the work on the ovary and reproduction has been done in comparatively recent years, most of the reading was done from articles which appeared in scientific periodicals. It will be noted from the bibliography that all the articles used were those which have been published within the last twelve years.

The textbooks mentioned were used for anatomical and structural studies only. As would be expected, the texts covered only the all ready known facts concerning the ovary. Such things as structure and function were very nearly the same in all. It was therefore, from the articles in the more recent publications that all the information concerning experimental work was taken.

Among the writers in this field who have published findings in these magazines, two names stand out above the others. These two are Swingle and Rugh. The most important writing of the former was on the sex changes and reversals which have been observed in the frog tadpoles. Rugh has shown the relation between ovulation and the amount of anterior pituitary hormone present in the body of the female. He also has worked on the growth rate of the tadpoles under different colored lights. His work is very conclusive and presented in an understandable manner.
However, the work which was presented in all cases was very conclusive, and backed with sufficient scientific proof. As was pointed out before, little work has been done on this phase of the study of the frog, and although all the structures and their functions are known, much experimental work needs to be done on hormone relation, and also on the effects of physical changes on the function of the ovary.
Key:
1. Infundibulum
2. Oviduct
3. Ovary
4. Kidney
5. Ureter
6. Uterus
7. Cloaca

Plate 1. (A short time after laying and fertilization.)
Plate 2.

FROG EGG (A short time after laying and fertilization. Notice the swollen egg membranes.)

Key: P, pigmented penetration path of the sperm; C, presumably the chorion or egg envelope formed by cells of the ovarian follicle; R, the polar bodies; 1, 2, and 3, the outer, middle, and inner layers of egg "jelly".

From McLewen, from Ziegler after Schultze.
Key: 1, 2-cell stage; 2, 8-cell stage; 3, 8-cell stage passing into 16-cell stage; 4, 32-cell stage; 5, later cleavage stage; 6, early stage of the blastocoel, A showing the segmentation cavity; the stippling marking approximate position of the gray crescent material; 7, section of the later blastula.

From Wieman.
Key:

A. Follicle wall cells. Notice nuclei.
B. Scattered nuclear material of the egg.

Notice the three layers of egg jelly, referred to on Page 8.
Plate 5.

Key:

A. Portion of the mesovarium, the structure which supports the ovary in the body cavity.
B. A developing ovum.
C. Portion of the yolk which will later surround the ova.
Key:

A. Young ovum developing in a follicle cell.
B. Follicle cell wall.
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