CHAPTER II

POLAR BODIES AND FERTILIZATION

WHETHER the egg leaves the ovary by means of its own activity, or by some other mechanism, we do not know. That the egg itself takes some part in the process seems possible from the fact that it is set free only at a particular moment of its maturation, *i.e.* at a time when certain processes have taken place in its interior. This same process takes place simultaneously in all the eggs in the ovary. The separation of the egg from the ovary is not dependent upon the act of copulation, for several cases are on record in which isolated females were found to have eggs in the body-cavity and oviducts.

The egg set free in the cœlomic cavity is covered by an extremely thin membrane, the egg-membrane or vitelline membrane. The egg itself is very soft and easily broken if handled. Later, when in the oviduct, the protoplasm seems to become more firm.

The egg shows a white and a dark hemisphere. The relative distribution of superficial pigment in the egg determines the extent of the white and dark surfaces. The outer layer of pigment in the black hemisphere seems to be in close contact with, or fixed to, the vitelline membrane, but the pigment lying in the protoplasm beneath the outer layer is free to move with any movement of the protoplasm (Figs. 8, 9). The relative extent of surface of the egg that is black or white is variable in different species, and even in different females of the same species; but all the eggs from one female show approximately the same distribution of pigment.

EXTRUSION OF THE FIRST POLAR BODY AND EGG-LAYING

Just prior to the extrusion of the egg into the body-cavity of the frog, the nucleus undergoes a remarkable change, so that in place of a large watery nucleus only a small mass of chromatic substance, lying in the protoplasm, is present. An achromatic spindle appears, and the chromatin in the form of granules is arranged at the equator of the spindle. The spindle lies at the surface of the egg near the centre of the black hemisphere (Fig. 11, A). It lies also in the centre of the fovea, which is found on the surface of the egg. The fovea marks the former position of the large ovarian nucleus, and although the nuclear membrane of the original nucleus has disappeared, and its watery cavity has been encroached upon by the surrounding protoplasm, yet the pigment has not penetrated very deeply into this region. The eggs pass in this condition from the body-cavity into the oviducts. Newport ('51) believed that, owing to the close attachment of the oviducts at their inner openings to the walls of the pericardium, at each contraction of the heart the slit-like openings of the oviducts would gape open, and any eggs in the vicinity might be forced into the mouths of the tubes. Also, he thought that owing to the muscular movements of the body, and the resulting shifting of the internal organs, the eggs sooner or later pass near the openings of the oviduct, and are then carried into the tube. At any rate, there seems to be not much ground for the older statement that the mouths of the oviducts actually grasp the eggs by a muscular movement like that of swallowing. According to Nussbaum ('95), the eggs, when set free from the ovary into the body-cavity of the frog, are carried into the open mouths of the oviducts by the motion of the cilia of the cœlomic epithelium. These cilia drive anteriorly any bodies lying free in the body-cavity. If, for instance, eggs taken from one frog be placed in the vicinity of the openings of the oviducts in the body-cavity of another frog, they will be carried into the open mouths of the oviducts by the action of the cilia in that region.

The cilia do not cover the entire surface of the cœlomic epithelium, and there are certain recesses in the body-cavity destitute of cilia. The eggs that accumulate in these recesses will be sooner or later forced out into the general cavity as a result of the alternate contractions and expansions of the ventral musculature of the body-wall, as well as by the changes produced by the filling and emptying of the lungs, and by the movements of the heart.

Swammerdam's account in 1737 describes the passage of the egg from the ovaries to the oviducts by way of the cœlomic space. Spallanzani in 1785 observed that the females of Bufo igneus, isolated before union with the male, could still lay their eggs. One of the tree-frogs has its eggs in the uterus before it unites with the male. On the other hand, Spallanzani stated that females of the stinking toad if isolated while the eggs are still in the ovaries will retain their eggs, but if separated after having paired will then deposit their eggs. According to the evidence of several authors, Rana temporaria when isolated will, in certain cases at least, set free its eggs.

It has been suggested that the embrace of the male is mechanically necessary in order that the eggs may pass from the ovary into the oviducts, but this is certainly not always the case, and if not necessary in one form is probably not necessary in others. The sexual excitement set up by the tight embrace of the male may however be necessary in some species for the successful performance of egg-laying. The eggs pass one by one down the length of the oviducts, ultimately to reach the lower portion of the tube, the so-called uterus, where the eggs accumulate. If a frog is killed at the height of the breeding season, free eggs are often found in the body-cavity, and a series of eggs passing individually down the ovarian tubes, as well as an accumulation of eggs in the uteri. In their passage through the oviducts the eggs undergo certain internal changes and receive also their egg-coats. In the tubes of the oviducts the nuclear spindle divides, so that half of the original chromatin goes

FIG. 10.—Egg in jelly. (After Schultze.)

to one pole of the spindle, and half to the other. The spindle has assumed, during this time, a radial position with respect to the egg, so that we may speak of a distal and of a proximal or central end (Fig. 11, A). The distal end pushes out into a protrusion of protoplasm that has simultaneously formed at this

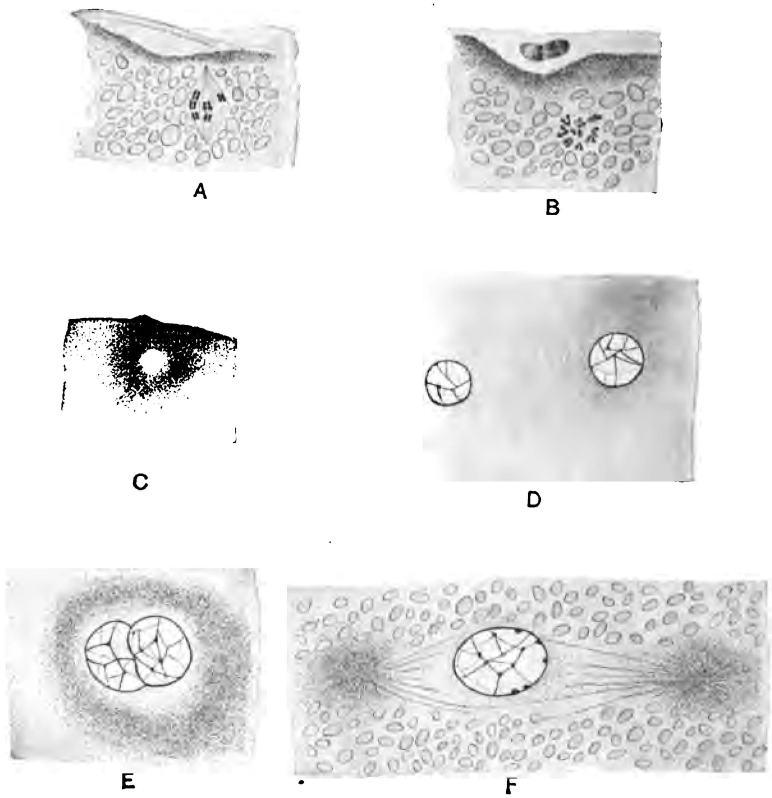

FIG. 11. — Extrusion of first polar body and fertilization of egg of Toad. (From preparations made by Helen D. King.) A. First polar spindle. B. First polar body extruded; second polar spindle present. C. Entrance of spermatozoön. D. Male and female pronuclei. E. Apposition of two pronuclei. F. First segmentation-spindle.

point of the surface of the egg. This protrusion of protoplasm with its enclosed half of the nucleus gradually pinches off from the surface of the egg, and there is thus formed the first polar body (Fig. 11, B). The egg gets a thin layer of gelatinous substance around it soon after entering the oviduct, *i.e.* before it has reached the first part of the convoluted portion. This is the so-called chorion, — a thin investing membrane which adheres closely to the vitelline layer around the egg. During the remainder of the passage through the oviducal tube the egg gets two other distinct gelatinous layers (Fig. 10). The middle layer of the three is, according to Newport, a watery layer of considerable thickness. The outer gelatinous covering is also thick and serves to stick the eggs together in a bunch, and even to stick the bunches of eggs, when laid, to surrounding objects.

The spawning of certain species of frogs takes place very rapidly, and by a single effort. Newport says that the process takes place in a few seconds or less than a minute, and that all the eggs that have accumulated in the uteri are laid at once. When laid, the egg-cluster forms a rounded mass which is, at first, scarcely as large as a walnut. The eggs then seem to consist almost entirely of dark-colored "yelks" with thin gelatinous envelopes. "Up to about this period the ova remain undisturbed in the water in a mass as they are expelled, and lie indiscriminately, some with the dark and some with the white portion of the yelk uppermost or horizontal. But during the time that has passed since the ova have been in contact with the water, the envelopes have imbibed fluid and expanded until these investments of the yelk have a thickness equal to about two-thirds of the diameter of the yelk itself."

"The yelks, that have remained up to this time with their white surface uppermost, now change their position spontaneously by a partial rotation of the whole mass of each on its axis, within the vitelline membrane, until the dark surface of the whole is placed uppermost. Whether this change of position is merely the result of expansion of the vitelline membrane at this period, or whether it be also connected, as we may fairly believe, with changes going on in the interior of the yelk, I am not prepared to decide."

THE JELLY OF THE EGG, AND THE SECOND POLAR BODY

The jelly around the frog's egg serves, no doubt, as a protection to the egg. The soft eggs are kept in spherical shape and protected from injury from without. The slime protects them from water-snails that will eat the eggs if they are shelled out from the jelly. The jelly may also protect them against water-birds. The eggs and young tadpoles seem, however, in themselves to be distasteful to certain crustacea (Bernard and Bratuschek, '91).

This jelly has the physical peculiarity of allowing the sun's rays to pass through, but hinders reflection of the rays from the interior to the outside. The result is that in the sunlight the mass of eggs is at a higher temperature than the surrounding water, and as the eggs of many frogs are laid in the early spring, when the water is quite cool, this property of the jelly helps to hasten their development.

Hertwig ('77) thought that a change takes place in the interior of the egg after fertilization, so that a difference in the specific gravity of different parts of the egg is brought about. Schultze ('87), however, pointed out that at this period the egg contracts slightly from its vitelline membrane, and between the egg and its membrane a fluid collects, that is probably squeezed out of the egg itself. The egg, freed from its innermost coat which held it in place, then rapidly orients itself with respect to gravity. Unfertilized eggs will also, after a time, slowly rotate, and in these it can be seen that the separation of the egg from its membrane is less perfect than in fertilized eggs. "At the *moment* when the ovum is expelled from the body, the envelope is merely a thin gelatinous layer, its entire diameter being equal only to about one-sixth of the diameter of the yelk. After it has been *one minute* in water, and begun to imbibe and expand, it is then equal to about one-fourth of the diameter of the yelk. At the end of *two minutes* it is enlarged to one-third, and in *three minutes*, to one-half the diameter of this body. In four minutes, it exceeds three-fifths, and in six minutes, two-thirds, and it continues to imbibe fluid and expand at the same rate, until, at from ten to fifteen minutes, it very nearly equals in thickness the whole diameter of the yelk; and at half an hour it is one-fourth greater than this. At the end of three hours the membranes have acquired nearly their full size."

"The expansion of the envelope is greatly retarded at the end of the third or fourth hour, until after cleavage of the yelk has taken place, when it again proceeds, but much more slowly than at first."[1]

In Rana fusca the extrusion of the *second* polar body takes place one half-hour after fertilization, and the process can be seen under a low magnifying glass or even with the naked eye. A whitish speck appears in the black hemisphere near the point at which the first polar body was extruded. It is necessary, however, to make sections of the egg to discover the further changes that are taking place. Schultze ('87) has given a careful description of the process. The nucleus that remains in the egg after the extrusion of the first polar body assumes once more a horizontal position, but *does not go into a resting-stage* (Fig. 11, B), *i.e.* the chromatic loops or threads do not re-fuse into a network nor does a nuclear membrane form. The chromatin arranges itself on a new spindle. The latter then assumes a more or less radial position, and the second polar body is extruded half an hour after the egg is laid. It is probable that the second polar body is not extruded under normal conditions until after a spermatozoön has entered the egg.

One and a half hours after the egg is laid, another change may be seen taking place. Near to or at the apex of the black pole the egg is seen to flatten, and an accumulation of fluid is found here between the egg and its vitelline membrane (Fig. 10). At or near the centre of this flattened portion one may see the fovea, and near or in it the polar bodies appear on the flattened disc. This chamber formed between the flattened egg and the inner membrane was seen by Newport and called the "respiratory chamber." It may ultimately be as large as one-sixth the diameter of the whole egg. Schultze points out that it lies somewhat excentrically with respect to the egg-axis (Fig. 10). The clear fluid in this chamber has been supposed to be the watery contents of the original large nucleus of the egg, which has been squeezed out of the egg. Very little evidence has as yet been given to support this view. Some of the older embryologists thought that this fluid represented

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[1] Newport ('51), p. 193.

the original egg-nucleus itself, which was squeezed out of the egg at this time. Now, however, since we know the complete history of the nucleus during this period, the suggestion of its entire loss by the egg does not call for serious criticism.

Entrance of Spermatozoön and Copulation of Pronuclei

The sperm of the male is poured out into the water, and probably over the eggs themselves at the moment when they are laid, and the spermatozoa begin at once to bore into the jelly of the egg-mass (Fig. 10).

Kupffer has described the entrance of the spermatozoön into the eggs of Bufo variabilis. When the head of a spermatozoön touches the egg-membrane, the protoplasm of the egg draws back slightly at the point of contact, but quickly returns again to its first position. The period of penetration of the spermatozoön from the moment of contact of the sperm-head until the spermatozoön disappears into the egg, lasts in some cases from one to one and a half minutes, in other cases only three-fourths of a minute. Several spermatozoa were observed by Kupffer to enter each egg.

Other spermatozoa reach the egg-membrane, but do not seem to be able to enter the egg. In the regions where these spermatozoa lie, the surface of the egg rises up in small protuberances. This process occurs about fifteen minutes after the first spermatozoa have entered, and lasts about one or two minutes, after which the protuberances sink back into the egg. The spermatozoa in the regions of the protuberances are left outside the egg-membrane. This peculiar phenomenon is described by Kupffer as a counter demonstration of the egg against those spermatozoa that have not been able to enter. Eggs that have been artificially fertilized show, when cut into sections, that one hour after fertilization a dark pigmented streak is formed, reaching from the pigmented coating of the egg into the yolk-mass. The process takes place in the upper or dark hemisphere, and regularly at one side of the centre of the dark field near to the edge of the white border. The streak takes a somewhat oblique course toward the centre of the egg. At the central end the dark streak is rounded, and encloses a clear spot.

In this clear region one sees a distinct pronucleus about nine microns (μ) in diameter. Eggs one and a half hours after fertilization show that the pigmented streak has penetrated deeper into the egg, and in the frog the male pronucleus has enlarged to 32 by 22 μ (Fig. 11, D, for the toad).

At this stage another nucleus is present in the frog's egg, and this lies not far from the end of the pigmented streak (Fig. 11, D). This measures 22 μ, and has the same structure as the male nucleus. These two nuclei are undoubtedly the male and female pronuclei. We now know that the female pronucleus has come directly from the original egg-nucleus, which has, after extruding its two polar bodies, penetrated once more deeper into the egg. The complete history has not been traced in the frog, but there can be no reasonable doubt as to what takes place. In the newt (and in the toad) the history has been followed, and it is found that the female pronucleus arises from the egg-nucleus after the extrusion of the polar bodies.

In the next half-hour Hertwig has found that the nuclei approach more nearly to each other, and the pigment-streak penetrates deeper into the egg, the swollen end enlarges, and the two large oval male and female pronuclei are then found together in the swollen end of the streak (Fig. 11, E). In a preparation of an older stage both nuclei have increased in volume to 35 μ, and have flattened against each other. *They then fuse into one nucleus* which measures 44 μ (Fig. 11, F, toad). The resulting nucleus, the segmentation-nucleus, is surrounded by clear protoplasm and then by a pigment-coat. From the segmentation-nucleus a streak of pigment extends to the dark surface of the egg and marks the path of entrance of the spermatozoön. All preparations after two and a half hours showed the union of the two pronuclei.

If the jelly be examined after the eggs have been laid, several or many spermatozoa can be seen boring their way through the jelly toward the egg. Some will have reached the inner layers, and still others lie in the outer coats (Fig. 10). It is probable that after one spermatozoön has succeeded in forcing its way through the inner coat and *into* the egg, changes then take place in the egg that prevent or make difficult the further entrance of other spermatozoa. The contraction of the egg, noted above, may possibly have something to do with the process. If, however, two or more spermatozoa should reach the surface of the egg at about the same moment, it is not improbable that more than one might enter.[1] Both may then pass toward the female pronucleus, but in the frog it is probable that after one male pronucleus has fused with the female pronucleus, the further progress of other male pronuclei that happen to get into the egg is stopped.

It is sometimes said that the female pronucleus *attracts* the male pronucleus, but the approach of the two may be due to changes in the protoplasm; for the migration of the pronuclei through the egg is probably in most cases brought about by the protoplasm of the egg under the influence of the pronuclei, and the pronuclei themselves are merely passively carried along.

In the newt (Jordan, '93) it seems to be usual for more than one spermatozoön to enter, but only one of these fuses with the female pronucleus. The others subsequently degenerate and go to pieces. In the eggs of other animals, as the starfish, polyspermy, or the entrance of more than one spermatozoön into the egg, brings about disastrous results, causing irregular division of the nucleus and subsequent irregularities in the segmentation of the egg. In these eggs the field of action is small, and the male pronuclei or their centrosomes mutually influence one another and the female pronucleus. In large eggs with much yolk, such as those of the Amphibia and of the Sauropsida, the spermatozoa may be too far apart to affect one another or the segmentation-nucleus, and after the fusion of one male pronucleus with the female the movement of the other male pronuclei towards the female pronucleus seems to stop.

The head of the spermatozoön enters the egg to become the male pronucleus. The tail of the spermatozoön is left at the

[1] It is probable that Kupffer's ('82) account does not apply to eggs under normal conditions.

surface of the egg, or if a part enter the egg it takes no share in the subsequent changes. The middle piece of the spermatozoön is now known to contain a body that plays a most conspicuous part in many animals in the division or cleavage of the egg. The middle piece enters with the head of the spermatozoön. It contains the centrosome, which divides, and around each centre an elaborate system of rays develops. The two centrosomes migrate to opposite sides of the segmentation-nucleus, and between the two appears the spindle of the first cleavage. In the frog the history of the middle piece and centrosome, and the origin of the segmentation-spindle have not yet been worked out.