CLASSICS OF SCIENCE: Loeb's Fatherless Frogs

Biology

This is the account of the first two tadpoles raised by Dr. Loeb from eggs "fertilized" by a pin-prick. By 1921, from many thousand eggs so treated, he had succeeded in raising more than 20 normal adult frogs. Duplication of his experiments requires, besides endless patience, little more apparatus than a microscope, a platinum needle and a bowl of water.

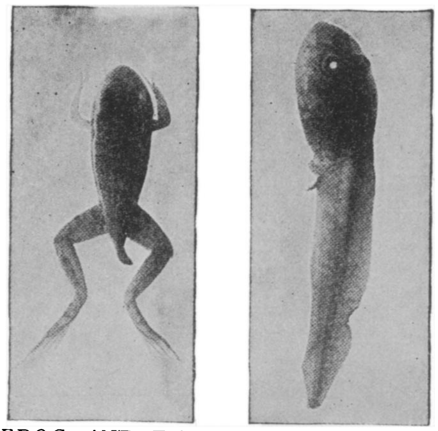
ARTIFICIAL PARTHENOGENESIS AND FERTILIZATION, by Jacques Loeb, member of the Rockefeller Institute for Medical Research. Originally translated from the German by W. O. Redman King, B. A., Assistant Lecturer in Zoology at the University of Leeds, England, supplemented and revised by the author. Chicago, 1913.

Experiments with Invertebrates

The experiments thus far considered have shown that it is possible to imitate the activating effect of the spermatozoon upon the egg of the sea-urchin approximately by submitting the egg to two different processes. The first process consists in calling forth the membrane formation in the egg by a fatty acid (or as we shall see later by a number of other chemicals). This process seems to be the essential feature in the activation of the egg, since it suffices to set in motion the whole apparatus of nuclear and cell division. The second process has only a corrective effect, since the membrane formation alone leads to a rapid disintegration of the egg, unless the temperature is very low. The prevention of this disintegration is brought about by the second process. This second process consists in submitting the egg for a short period to a hypertonic solution containing oxygen (or for a longer period to sea-water free from oxygen).

We will now briefly mention some cytological points worthy of discussion. E. Hindle has investigated the cytological changes in the eggs of *S. purpuratus* which had been treated with butyric acid and subsequently with a hypertonic solution. He found that the changes taking place in such eggs were almost identical with those which take place after the entrance of a spermatozoon. Hindle gives the following description:

The interval (about 20 minutes) between the transference of the eggs from butyric acid to normal sea-water and their subsequent treatment with hypertonic salt solution is characterized by the alterations in the appearance of the cytoplasm and nucleolus, and the subsequent development of a perinuclear zone, as described above. The nucleus then commences to grow and faint radiations can sometimes be seen extending from the perinuclear zone into the surrounding cytoplasm.



FROG AND TADPOLE from eggs fertilized by a pinprick

During immersion in the hypertonic solution there are no apparent changes beyond a slight reduction of the clear zone of hyaloplasm surrounding the nucleus.

After the eggs are put back into normal sea-water the internal changes resulting in the first cleavage follow each other in quick succession. The first change noticed is an increase in the development of the perinuclear zone, followed by further growth of the nucleus. Meanwhile, the meshwork of chromatin becomes coarser and more aggregated together and the nucleolus gradually disappears. This stage is succeeded by a reduction of the perinuclear zone together with its radiations.

About half an hour after transference to normal sea-water, from one pole of the nucleus a definite aster begins to develop, its rays focusing in a more or less indistinct centrosome situated on the nuclear membrane. By division of the centrosome a typical amphiaster is formed in the nuclear area and as it develops the nuclear membrane disappears. At the same time the chromatin assumes the form of a spireme, which subsequently breaks up into about 18 long and slender chromosomes. At this stage it is impossible to clearly distinguish their number, but, as the chromosomes are gradually drawn into the equator of the cleavage amphiaster, they shorten considerably and become quite distinct by the time that the equatorial plate is formed.

At this stage we have made numerous counts of the chromosomes and invariably found it in the neighborhood of 18, which is half the number that is normally present in this species.

This behavior is very similar to the one found in the egg after fertilization by sperm.

In naturally fertilized eggs a distinct aster (cleavage aster) appears at one pole of the nucleus, its rays centering in a clear area which represents a diffuse centrosome. This area divides and the two halves move apart until they come to lie at opposite sides of the nucleus and form the poles of a typical amphiaster which is developed in the nuclear region. Meanwhile the chromatin assumes the form of a spireme, which breaks up into 36 chromosomes that arrange themselves about the equator of this amphiaster to form a nuclear spindle. In the chemically fertilized eggs a nuclear spindle arises in a similar way and the chromatin assumes the form of a spireme preparatory to breaking up into chromosomes, but, instead of 36, only 18 of these latter bodies appear. The subsequent changes are identical in both kinds of eggs. The chromosomes split longitudinally and each half moves along the spindle fibres toward its respective pole. As they approach the poles the chromosomes swell up and eventually fuse together to form a single nucleus in the region occupied by each of the diffuse centrosomes. Meanwhile a cell wall develops between the two nuclei dividing the cytoplasm into two, and finally the spindle fibres disappear. The succeeding processes of development, both internal and external, are similar in both naturally and chemically fertilized eggs, with the exception that at each succeeding division only 18 chromosomes appear in the latter instead of the normal number, 36.

Parthenogenesis in Frogs' Eggs

During the first years of his work and later the writer vainly applied the chemical methods of artificial parthenogenesis to the eggs of fishes and of frogs. The reason for this failure may possibly lie in the relative impermeability of the walls of these eggs for the chemicals used. The walls of the eggs of *Fundulus* are, as long as they are normal, not only impermeable for salts but also for water. It seemed desirable that a method of artificial parthenogenesis for vertebrates should be found since it is so much easier to raise the larvae of vertebrates than of invertebrates. It is under the circumstances no surprise that such a method was found almost accidentally. In 1907 Guyer published a paper in which he reported that by injecting lymph or blood into the unfertilized eggs of frogs he succeeded in starting development and even in obtaining two tadpoles. Considering the importance of these experiments and since they seem to have been overlooked, the writer feels justified in quoting part of Guyer's note:

During three successive springs (1905-7) the writer has experimented on unfertilized frog eggs by injecting them with blood or lymph of either male or female frogs. In all some fifteen hundred eggs have been so operated upon. Shortly before the time for laying, the eggs were taken from the uterus with every precaution to prevent contamination by sperm. Those nearest the cloacal opening were always set aside as a control and in not a single instance (*Turn to next page*)

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did any of them develop. The other eggs were pricked with a very fine-pointed capillary tube which had previously been charged with lymph and corpuscles by dipping it into the lymph or the blood of another frog.

In eggs so treated numerous instances of cell proliferation and embryonic development have been observed, provided the eggs were fully matured and ready for fertilization. Many eggs after six or eight days showed upon sectioning that they had approximated the full blastular and in some cases the gastrular stages, although the condition came about apparently by some sort of internal nuclear arrangement, as no superficial cleavage furrows were observable and no demarkation into cells was visible from the exterior until the third or fourth day, when close inspection showed in some cases numerous small vesicular or cellular outlines.

In some instances definite organs were developed, though frequently distorted and misplaced. Cross-sections of one embryo, for example, showed such pronounced defects as two neural tubes anteriorly. Of the whole number of eggs operated upon only two developed into free-swimming tadpoles and these were apparently normal as far as superficial examination disclosed. They have not yet been sectioned. After sixteen days one died and the other was killed to insure proper fixation for histological study.

Apparently the white rather than the red corpuscles are the stimulating agents which bring about development, because injections of lymph, which contains only white corpuscles, produce the same effect as injections of blood. Whether or not the fluid part of the lymph or blood produced any effect could not be definitely determined from the material at hand.

Guyer thought that probably the cells which he introduced were developing and not the egg. He did not recognize that his experiment was a case of artificial parthenogenesis. This, however, does not detract from the fact that he was the first to cause the development of the unfertilized egg of the frog by puncturing it, that he introduced blood into the egg for this purpose, and that he succeeded in producing two parthenogenetic tadpoles.

Guyer's results were to a large extent confirmed by Bataillon. Bataillon found that mere puncturing of the egg of the frog by a very fine needle could not produce any embryogenesis but that a second factor was necessary, namely, that some of the body liquids (blood of the frog or newt or fish) have to enter the egg. "A considerable percentage of the eggs of *Rana*, touched with the blood and immediately punctured, will develop, while the same eggs squeezed out from the female frog will not develop (when punctured)." Bataillon states that in the eggs thus treated "he found at the beginning of the divisions outside the kinetic figures, chromatin fragments accompanied by asters, which fragments come probably from elements inoculated with the needle." In a recent paper Bataillon reaches the conclusion that it is the leucocytes which cause the development (1913). . . .

While the number of eggs which begin to segment when punctured is not inconsiderable, very few reach the tadpole stage. There is a difference in the response of the eggs of various kinds of frogs to this treatment.

The number of unfertilized eggs which began to segment after puncture was according to Loeb and Bancroft greater in the wood frog than in the leopard frog, and amounted in the most favorable cases to about 40 per cent in the former. Only 2 of about 10,000 punctured eggs of the wood frog reached the tadpole stage, but these died before they were able to swim. The percentage of eggs of the leopard frog which reached the tadpole stage was greater. From 700 punctured eggs of the Southern leopard frog, 13 good morulae were isolated the next day. On the third day, when the fertilized controls were in the gastrula stage, 13 unfertilized punctured eggs were also in the gastrula stage and 4 more eggs were developing abnormally. On the fourth day, 8 of the parthenogenetic eggs had good medullary folds and 4 had irregular folds. On the sixth day most of the fertilized eggs hatched and 8 of the parthenogenetic eggs hatched also. Of these latter, 4 were developing regularly and 4 irregularly. Those that had not hatched were abnormal.

On the eighth day, the larvae arising from the fertilized eggs were swimming. Among the larvae arising from the unfertilized punctured eggs only 3 were normal, and their development was slightly retarded, perhaps one day. In addition, 6 parthenogenetic larvae were abnormal but still alive.

On the thirteenth day, 2 of the parthenogenetic larvae were feeding and these were the only ones which survived definitely. The other parthenogenetic larvae all died during the next few days. Of the 2 surviving larvae, one went through metamorphosis after five months. When it died, the tail was almost completely absorbed (Fig. 76). Its death was probably accidental. The other lived (Fig. 77) a month longer and formed small hind legs, but died in the tadpole stage. A repetition of these experiments by the same authors showed that only the eggs of *Rana sphenocephala* and *R. pipiens* produce tadpoles when punctured, while the unfertilized eggs of *R. silvatica, Chorophilus feriarum,* and of *Bufo americanus* only begin to segment but die before the tadpole stage when they are punctured.

As far as the effect of puncturing the egg is concerned, it may be comparable to the effect of agitation upon the starfish egg. The latter forms a membrane as an effect of agitation and it may be that the destruction of the surface layer of the frog egg is the essential feature in this "traumatic" parthenogenesis. While the spermatozoon causes the destruction of the cortical layer of an egg by a chemical substance (a "lysin"); and while the same can be accomplished in the egg of some starfish and some annelids by gentle agitation, in the egg of the frog it can be done by puncturing the surface layer. Future experiments will decide whether or not the leucocytes play the rôle of the second factor which Bataillon ascribes to them.

Loeb and Bancroft tried to ascertain the sex of the parthenogenetic frog and tadpole which they obtained. The gonads contained eggs in both cases. This at first sight might be taken to indicate that both were females, but the problem is complicated by the fact found by Pflüger, and recently worked out by Kuschakewitsch, that very often in the early stages the gonads of a frog may contain eggs which afterward degenerate. Such "intermediates" may develop into males.

Jacques Loeb was born in Alsace, April 7, 1859, and died in Hamilton, Bermuda, February 11, 1924. He studied medicine at the Universities of Munich and Strasbourg, taking his degree of M. D. in 1884. After teaching in Wurtzburg and Strasbourg, he worked at the biological station at Naples from 1889 to 1891. He then came to America, taught at Bryn Mawr for one year, and joined the faculty of the University of Chicago in 1892. In 1902 he became professor of physiology at the University of California. In 1910 he became head of the division of physiology at the Rockefeller Institute for Medical Research.

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