

PSEUDO-CLEAVAGE OF THE FROG'S EGG

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During a study suggested by Dr. Waldo Shumway on the fertilization of coelomic eggs of the frog *Rana pipiens*, it was found that these eggs exhibited a surface phenomenon which resembled normal cleavage and is termed pseudo-cleavage. Inasmuch as the writers have found no reference of this phenomenon for amphibian eggs in the literature it has seemed proper to record its appearance in this paper.

Method.—Coelomic eggs (without jelly) were obtained by the pituitary-ovulation method of Rugh ('41). The coelomic eggs were removed after opening the body cavity and placed in a watch glass or finger bowl containing fresh sperm suspension or sperm-free salt solution. The eggs were periodically observed under binocular magnification for changes in the external appearance and for the formation of polar bodies. Observations were continued until the eggs cytolized or ceased to undergo surface changes. The sperm suspensions were made up in biological (chlorine-free) water. The sperm-free salt solutions used were as follows: biological water, NaCl, LiCl, frog Ringer's and Holtfreter's solution at normal and at reduced concentrations.

Experimental.—Inseminated coelomic eggs were not fertilizable and did not develop into embryos. Coelomic eggs inseminated or exposed to sperm-free salt solutions underwent cortical changes which gave an external appearance similar to cleavage planes. This phenomenon is referred to as pseudo-cleavage since cytological study showed that none of the eggs observed had complete cleavage planes which cut through the entire egg. In one case a blastomere-like segment was cut off tangentially through the upper part of the animal pole, but could not be called a true blastomere.

The order of events during the process of pseudo-cleavage were as follows. A few hours after insemination, or exposure to sperm-free salt solutions, the cytoplasm of the egg sunk away from the animal

pole and became separated from the vitelline membrane which continued to maintain its original spherical shape. The space between the flattened cytoplasm and the vitelline membrane occupied, in some eggs, as much as one-fifth of the egg's diameter. Whether a fluid was present in this space was not determined. Diffusion of water or salts in solution from the surrounding medium, or dehydration of the egg cytoplasm, could supply liquid to fill this space. In some cases the first polar body was given off.

Pseudo-cleavage began as a constriction of the plasma membrane either at the center or at the periphery of the flattened cytoplasm. Deepening and increase in length of the constriction gave it the appearance of a primary cleavage furrow of the normal egg with the exception that the path of the furrow was not straight across the egg but deviated either to the right or left. In most cases secondary and tertiary furrows were formed by additional constrictions which cut off from the primary furrow or began at the periphery and met it at the center. In a few isolated cases pseudo-cleavage commenced in the animal pole near the equator of the egg, yet the cytoplasm had sunken at the apex of the animal pole.

Several hours later the egg rounded up as additional constrictions were cut off on the animal pole so that the egg appeared to have many surface blastomeres. These were of variable size and number. As many as sixteen were formed on the animal pole of one egg. These blastomere-like configurations after a few hours became flattened and the surface of the egg became relatively smooth. Cleavage furrows in most cases did not penetrate beyond the equator of the egg into the yolk material, yet the surface of the vegetal pole on some eggs was marked off with thin white lines which resembled cleavage planes. Pseudo-cleavage occurred in all of the experimental solutions except the sperm-free NaCl (it

occurred in inseminated NaCl.) The extent of pseudo-cleavage in the various salt solutions used was variable, the low concentrations producing the effect as well as the normal concentrations. The rate of pseudo-cleavage was not constant for all eggs but seemed to vary with the egg and the medium.

Discussion.—The phenomenon of pseudo-cleavage in the frog's egg probably is caused by several factors. Experimental evidence to demonstrate these factors is lacking. Osmotic pressure differences between the cytoplasm and the medium or between the cytoplasm and the liquid in the space below the vitelline membrane may cause shrinkage of the cytoplasm and consequent surface configuration. Since both normal and lowered concentrations of salt solutions caused pseudo-cleavage it may be that a permeability gradient existed which permitted a certain concentration of water, which was conducive to pseudo-cleavage, to be established in the cortex layer of the cytoplasm. From a chemical standpoint, the permeability may have permitted certain cations or anions to enter

or leave the egg and cause the cortical cytoplasm to stiffen and constrict in a cleavage pattern. Calcium may well be the ion involved. The phenomenon may be an expression of an aging process. Schechter ('41) working with the unfertilized clam egg, *Macra*, in sea water found that when the egg aged an indentation was formed on the egg. The surface of the egg became crumpled and polar bodies were sometimes given off. He thinks that these changes are caused by nuclear material released by the aged germinal vesicle and that the process may be an "abortive attempt at parthenogenesis before the cell dies."

Conclusions.—Coelomic eggs upon insemination or exposure to salt solutions undergo surface changes which give the external appearance of cleavage. This phenomenon is termed pseudo-cleavage.

REFERENCES

- Rugh, R. Induced ovulation and artificial fertilization in the frog. 1934 Biol. Bull., 66, 22-29.
 Schechter, V. Experimental studies upon the egg cells of the clam, *Macra solidissima*, with special reference to longevity. 1941 Jour. Exp. Zool., 86 (3), 461-479.