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CORTICAL CHANGE AT FERTILIZATION IN THE TOAD EGGS¹⁾

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In amphibian eggs the cortical changes at fertilization have been investigated only by Motomura (1952) who studied the cortical granules of *Rana nigromaculata*. This report deals with observations on the cortical granules and the cortical change in the eggs of *Bufo vulgaris*. The observations were mainly done on the materials, fixed with Motomura's fixative (Motomura 1957) or ten per cent formalin solution and sectioned by the paraffin method. To demonstrate the cortical granules, the sections fixed with Motomura's fixative and removed of paraffin were stained with 0.5 per cent aqueous solution of Janus green over a flame until the slides became hot. They were then washed with water, differentiated with methanol, dehydrated with tertiary butanol and mounted in balsam through xylo. In the ripe unfertilized eggs taken from the oviduct, the cortical granules, which stained deeply bluish green with Janus green, were arranged as a single layer in the egg cortex above the pigment layer. The granules were stained with the other basic dyes. They had a strong affinity to basic dyes, gentian violet, Janus black, methylene blue, methyl violet and toluidine blue, but were not stained with weak-basic dyes, malachite green and neutral red, and acid dyes, acid fuchsin, alizarin red and eosin. The cortical granules were stained also in such a method as the slides were immersed in 0.5 per cent aqueous solution of basic dyes without heating.

As in the cases of the sea urchin and fish eggs the cortical granules contain polysaccharides also in the toad eggs. The cortical granules of *Bufo* were positive to periodic acid-Schiff test (P.A.S.) (modified by Lillie) and to Bauer test for polysaccharide; they were not digested with saliva and stained reddish-violet with toluidine blue. Their affinity to toluidine blue decreased less remarkably below pH 4. Therefore, it is certain that the cortical granules contain acid mucopolysaccharide with ester of sulfuric acid, namely sulfomucopolysaccharide.

The cortical granules disappeared from the cortex after fertilization. The perivitelline space of the fertilized egg was filled with the liquid which were P.A.S. positive and stained metachromatically with toluidine blue. Therefore, it is inferred that at the fertilization the cortical granules are extruded out from the egg

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cytoplasm, swelled and formed the perivitelline space. The P.A.S. positive layer covering the whole egg mass was observed in the surface of the fertilized egg more than 30 minutes after the insemination, where the cortical granules disappeared perfectly. This layer corresponds to the surface coat of Holtfreter (1943).

From the results mentioned above, it is certain that the following change occurs in the cortex of *Bufo* eggs at fertilization; stimulation-extrusion of the cortical granules-formation of the perivitelline space-formation of the cortical layer (the surface coat).

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