in the vaginal epithelium, which amounts to the addition of from 10 to 16 new layers of cells in 48 hours, and results in a cornification process in this tissue. This rapid growth in the vagina is correlated with a corresponding growth and secretion in the uterus.

Therefore, an active extract substitutes for this ovarian secretion instead of merely influencing the growth and secretion of the intact ovaries. This is the point we wished to make in attempting a distinction between this hormone as the "causative" mechanism and other possible "regulatory" factors in the growth changes in the female genital tract.[7]

146 (2669)

Observations on intravitam staining of centrifuged marine eggs.

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That living protoplasm cannot be stained has long been known but is not as yet generally appreciated. Because of the numerous cellular inclusions (granules, globules and vacuoles of various kinds) which do take up the dye, a cell stained *intravitam* may appear stained as a whole, but upon separating the inclusions from the protoplasmic matrix the latter will be found free from the dye. The relatively large eggs of certain marine invertebrates furnish excellent objects for demonstrating this fact, since by centrifugation the formed elements may be separated from the protoplasmic ground substance. If *Arbacia* eggs are centrifuged, the cell contents separate into four well-defined zones; the lipoid globules are massed at one pole, the pigment granules at the opposite pole; adjoining the pigment zone is a layer of granules of varying sizes, and between this and the mass of lipoid globules, a band of optically empty, homogenous cytoplasm. The width of these four zones depends, to a certain extent, upon the

[7] *Am. J. Anat.*, 1924, xxxiv, 161, 164.

length of time the eggs have been subjugated to centrifugal force. In the egg of the clam, *Cumingia*, only three zones are formed (the pigment and granular zones do not separate).

Freshly obtained unfertilized *Arbacia* eggs were centrifuged in small haematocrit tubes; the zoned eggs were then placed in a 1:40,000 solution of neutral red or brilliant cresyl-blue in seawater. It was found that only the pigment and granular zones took up the dye; the clear cytoplasm and the lipoids remained unstained. Identical results were obtained when the eggs were first stained and then centrifuged. Examination with dark field illumination showed the zoning with particular clearness, and brought out the neutral red stained granules with great brilliance. *Arbacia* eggs stained and centrifuged could be fertilized, and many developed to the gastrula stage.

An apparent exception to the general observation that living cytoplasm does not stain was found to occur when the centrifuged eggs were exposed to the dye for so long a time that the cytoplasm became very finely granular, that is, underwent a granular degeneration. The apparent staining of the protoplasm was probably due to coloration of the innumerable, closely packed, extremely minute granules in the injured protoplasm.

In an effort to determine the reaction of the protoplasm and its various inclusions, *Arbacia* or *Cumingia* eggs were placed in weak solutions of dibromthymolsulphonephthalein (brom thymol blue)[1] in seawater. The indicator penetrated slowly but many eggs were eventually colored a deep yellow; however, on centrifuging them they failed to separate into layers, indicating that they had been killed, and that no information was afforded as to the reaction of the cell while living.

These experiments constitute further evidence and a ready method for demonstration that living protoplasm cannot be stained by such relatively non-toxic dyes as neutral red or brilliant cresyl-blue; the method also furnishes means for determining whether an alleged vital dye has injured or killed the cell by coagulating the cytoplasm.

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[1] Crozier had found this compound non-toxic for such cells as Paramecium and Opalina whose cytoplasm stained diffusely yellow, thereby indicating an acid reaction. PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 58.