PRELIMINARY OBSERVATIONS ON THE EFFECT OF FERTILIZATION ON THE GOLGI BODIES IN THE EGGS OF *ACENTROGOBIUS NEILLI* (*GOBIUS NEILLI*, DAY).

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Introduction.

In recent years though much attention has been focussed on the form and function of the Golgi apparatus, no serious attempt has been made to formulate any general theory regarding its function, irrespective of the nature of the cell in which it is found. There appears also a tendency on the part of the investigators to confine themselves to literature bearing on their particular field of inquiry, instead of that bearing on the particular inclusion with which they are dealing. It is also rather surprising that an inclusion which has been shown to be universal in occurrence should be attributed special functions in certain cell groups, forgetting that it may have a general function in all cells.

The secretory hypothesis postulated by Nassonov (1923 and 1924) was extended by Bowen (1922, 1924, 1926 *a*, *b*, *c* and *d*) to include the acrosome formation in spermatogenesis and also to the formation of secretory products in all kinds of secretory cells. The secretory function has also been emphasised by a number of other workers such as Brambell (1923 and 1925), Cramer and Ludford (1925, 1926 and 1929), Ludford (1925), Rau and Ludford (1925) in a variety of cells though the possibility of such a function in Oogenesis has been lost sight of by most workers. In a previous paper (Subramaniam, 1935) the author has demonstrated in *Clibanarius* that fatty and albuminous yolk arise in relation with the Golgi apparatus and it is the aim in this paper to demonstrate that the apparatus may have a mucous secreting function in the developing egg.

While working on the Oogenesis of *Acentrogobius neilli* (Subramaniam and Gopala Aiyar*) it was found that some of the unmodified Golgi elements formed a concentration below the zona radiata. It was further observed

* In course of publication.

(Gopala Aiyar†) that within fifteen minutes after fertilization the zona was converted into a mucilaginous boat-shaped envelope attached to the substratum by mucilaginous threads.

The present work was undertaken under the kind suggestion of Professor R. Gopala Aiyar, Director, University Zoological Research Laboratory to study the effect of fertilization on the Golgi bodies lying below the zona radiata which apparently play no part in Oogenesis. I have great pleasure in thanking him for the material as well as for his valuable advice and criticism.

Methods and Material.

Gobius neilli is a brackish water fish common in the waters of the Adyar river and fertilizations were carried out in dishes containing the river water. The pH of the water varied between 8·4 and 8·6 and the salinity was 19. The temperature of the dishes was kept almost constant between 24 and 26° C.

Eggs from two minutes up to six hours after fertilization were fixed in various fixatives such as Mann Kopsch, Nassonov and Da Fano. Fixatives containing osmic acid failed to impregnate the fertilized eggs properly even though they were pricked with a sharp needle. Material fixed in Da Fano (12–16 hours in Cobalt Nitrate Formalin mixture and 2–3 days in 1·5% Silver Nitrate) gave very good results. Unfertilized and undeveloped eggs present in egg clusters afforded a test for the accuracy of the fixations. The observations given below are based entirely on such Da Fano material which were toned and later counterstained in Alum Carmine.

Golgi Bodies in the Mature Oogonium.

In order to understand the effect of fertilization a brief resumé of the behaviour of the Golgi in Oogenesis would be useful. The apparatus occurs in the youngest oocytes as an irregular mass which by breaking up gives rise to a number of granules which lie scattered in the cytoplasm. The majority of these bodies enlarge and secrete fatty yolk—yolk containing a large quantity of fatty or lipoidal matter—inside their interior. The unmodified elements slowly begin to migrate to the periphery of the cell, where just below the zona radiata they form a concentration (Fig. 1A. *g.p.c.*).

Changes Undergone by the Zona Radiata on Fertilization.

The mature oocyte has a uniform zona attached to which may be seen under higher powers of the microscope a layer of follicle cells. The first effect of fertilization is an expansion of the zona radiata which when fixed gets

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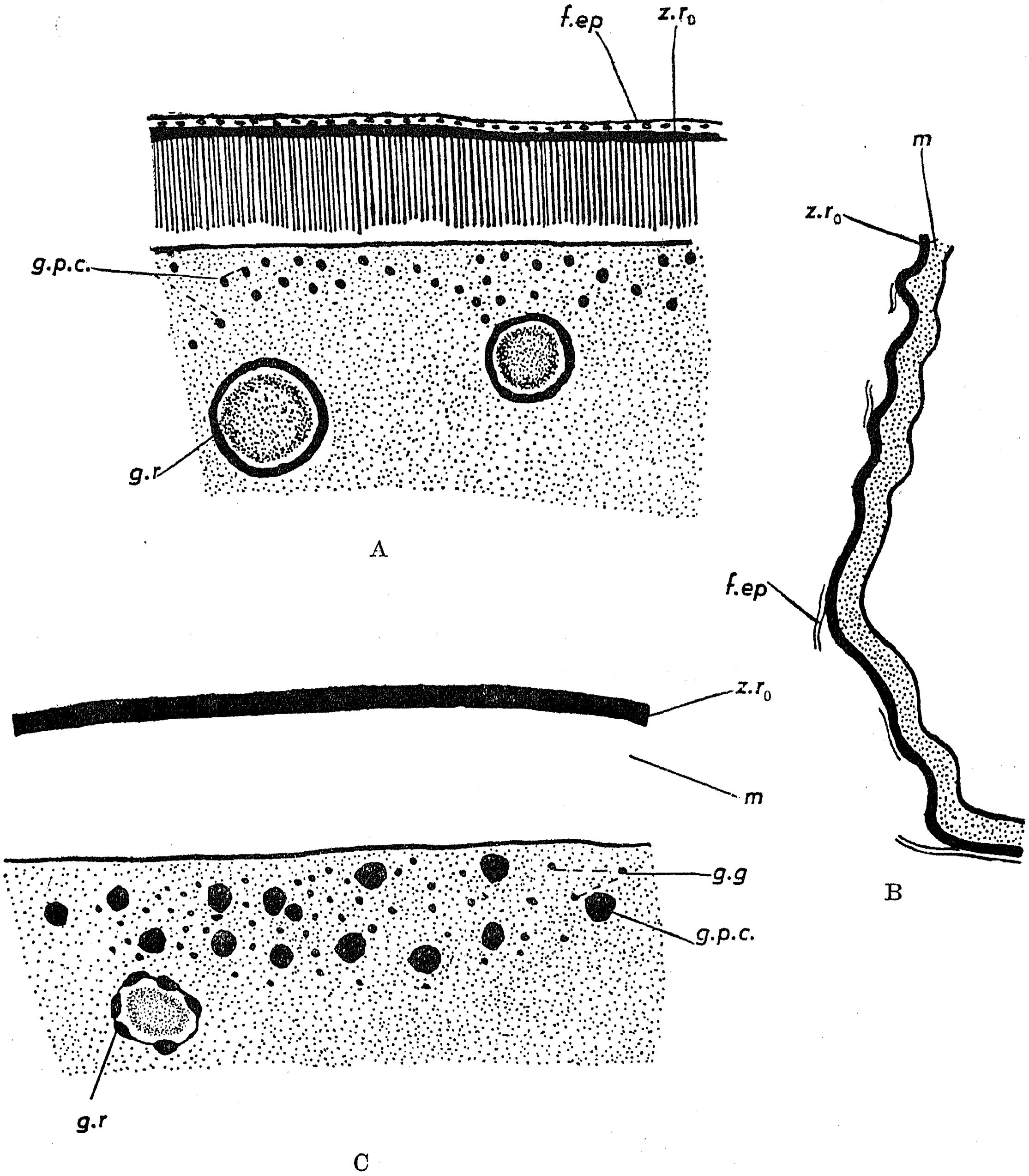

FIG. 1.

A.—*Zona radiata* and the Golgi peripheral concentration as seen in an unfertilised egg. ×2500.

B.—Appearance of the *zona radiata* five minutes after fertilization. ×700.

C.—*Zona radiata* and the Golgi peripheral concentration five minutes after fertilization. Some of the Golgi granules formed by the breaking up of the Golgi rims of the fatty yolk droplets are also seen. ×2500.

f.ep.—Follicular epithelium. *g.g.*—Golgi granules formed by the breaking up of Golgi rims of fatty yolk droplets. *g.r.*—Golgi rims. *f.y.d.*—Fatty yolk droplets. *m.*—Mucous. *z.r.*—*Zona radiata.* $z.r_0$.—Outer membrane of the *zona radiata.*

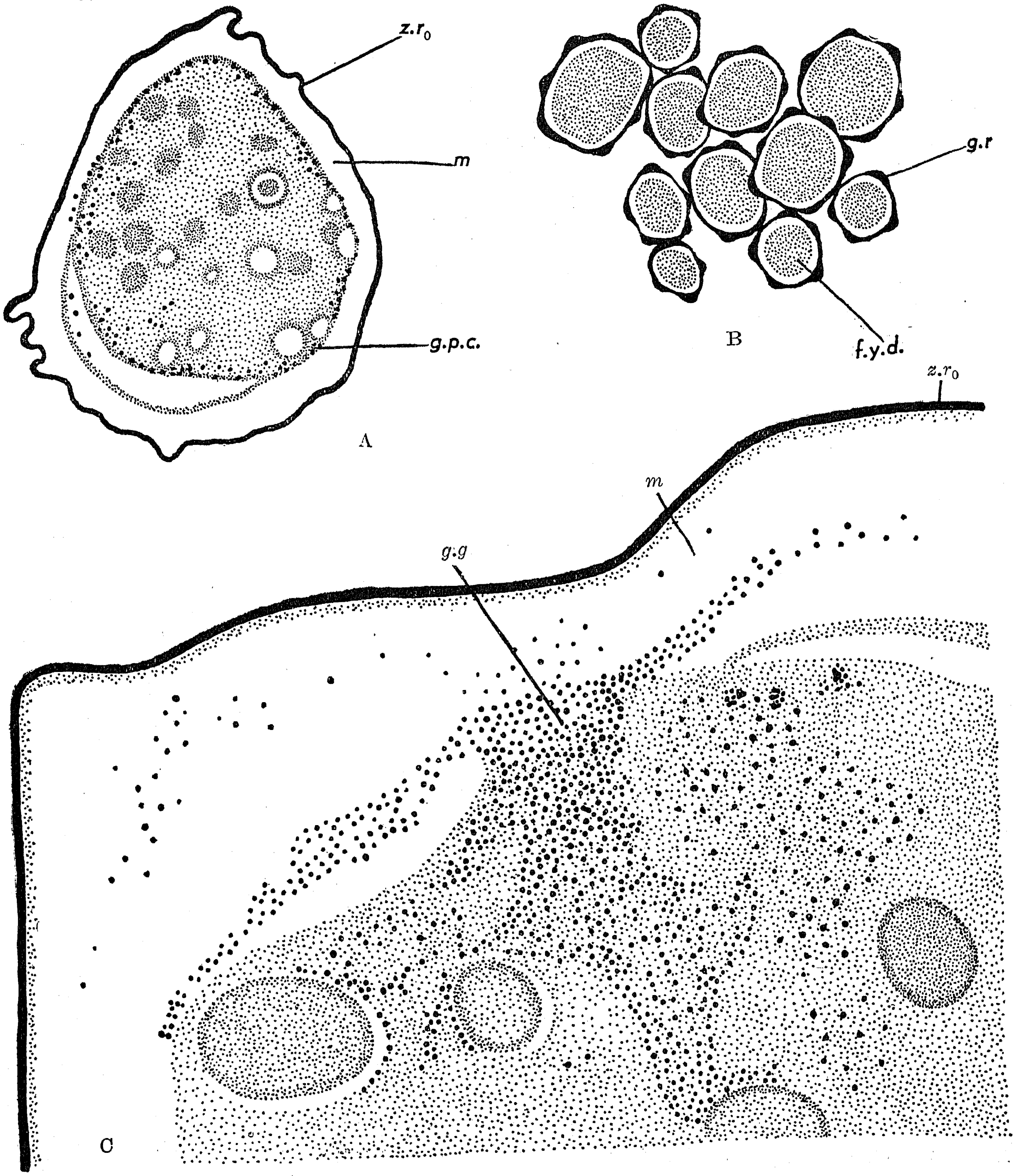

FIG. 2. A.—Oocyte showing the conversion of the *zona radiata* into mucilage. The *zona radiata* has elongated and is lobed. The fatty yolk droplets have begun to run together into large droplets. The Golgi peripheral concentration is very clear. ×260.

B.—Illustrates the manner of the breaking up of Golgi rims into granules. ×2500.

C.—A developing egg in which mucilage formation has been completed. The Golgi granules are being extruded into the mucous. ×2500.

g.g.—Golgi grains formed from the breaking up of the Golgi peripheral concentration (*g.p.c.*) and Golgi rims (*g.r.*) of the fatty yolk droplets (*f.y.d.*). $z.r_0$—Outer membrane of the *zona radiata*. *m.*—Mucous.

thrown into blunt lobe-like structures (Fig. 1B and Fig. 2A). The follicular cells become torn up and disintegrate. The expansion of the zona is accompanied by a reduction of its thickness and a loss of its striations (Fig. 1B). Side by side can also be seen a transformation of the inner portions of the zona (Fig. 2A and C. *m.*) into mucilaginous matter.

Now the fertilized egg undergoes further development suspended in the middle of the mucilage which has an outer definite limiting membrane (Fig. 2A. $z.r_o$).

Changes in the Golgi Bodies.

On fertilization the Golgi bodies lying below the zona (Fig. 1A. *g.p.c.*) seem to enlarge as can be seen from Figs. 1A and C. Fig. 1A is that of an unfertilized mature oocyte drawn under 2,500 magnification and Fig. 1C that of a fertilized one under the same magnification.

Accompanying this increase in size some changes have been observed in the rim of the fatty yolk droplets also. The Golgi rim of these vacuoles which are uniform before fertilization (Fig. 1A. *g.r.*) become beaded (Fig. 1C). With further development the beads separate and divide and give rise to minute irregular Golgi bodies. The majority of these migrate to the periphery of the cytoplasm and become arranged below the zona among the hypertrophied Golgi grains (Fig. 1C). After this, secretion of mucous seems to be more rapid. The process of transformation of zona into mucous is not uniform as can be seen from the figures. When the mucous envelope is fully formed the hypertrophied grains break up into small granules and seem to be extruded into the mucous along with the other grains lying below the zona radiata (Fig. 2C). As can be seen from the figures extrusion is also irregular, the granules where mucous formation has been completed being extruded first.

Discussion.

The sequence of changes in the behaviour of the Golgi bodies synchronising with the conversion of the zona into a mucilaginous envelope strongly suggests that the Golgi apparatus is concerned in the process. (1) The migration of the unmodified granules in the unfertilized egg to the periphery, (2) the formation of a concentration below the zona radiata, (3) the migration of the granules formed by the breaking up of the rims of fatty yolk vacuoles and their arrangement below the zona, (4) the enlargement of the initial grains lying near the periphery of the cytoplasm, and (5) their final extrusion make it highly probable that they are concerned in the transformation of the zona. This view is further strengthened by (6) the occurrence of a more rapid secretion of mucous after the appearance of the smaller grains at the periphery and their extrusion in places where mucous formation has already been completed.

Now the question arises whether the transformation of the zona can be considered as an act of secretion. Bowen (1926*a*) while discussing the meaning of the term secretion says that "the act of 'secretion' is clearly that by which the secretory granules are produced, the granules themselves being to the cytologist a secretion". Even in secretory cells Bowen remarks that materials for the synthetic operations of the Golgi generally come from the cytoplasm and that in the production of these substances the mitochondria, the nucleolus and even the nucleus may take part. Further he suggests that 'the materials for the secretory granules would be elaborated primarily in the Golgi apparatus and thence transferred to the granules'. The secretory granules are of various types such as mucous, serous, lipoidal, etc., and hence instead of the literal acceptance of Bowen's hypothesis it appears that the synthetic function of the Golgi may be extended to mean the production of intra-cellular enzymes in which sense it seems to have been used by Brambell (1923), and particularly by Gatenby and Ludford (1921), Cramer and Ludford (1925, 1926 and 1929), Ludford (1925), and Rau and Ludford (1925). Bowen himself throws out the suggestion that "It would be a most engaging hypothesis to extend this synthetic activity of the Golgi apparatus to the production of intra-cellular enzymes whose presence can be proved chemically, but which have thus far escaped morphological inquiry; that in other words, the source of the visible extra-cellular enzymes is likewise the source of the invisible intra-cellular enzymes." But at the same time he does not support the suggestion fully, as he terms it pure speculation.

If the theory that the primary function of the Golgi bodies is the production of various enzymes which act in different ways on available materials, is accepted, then the change of the zona radiata into a mucilaginous envelope could be easily understood. According to the suggestion put forward the secretory products are only secondary in origin, the primary secretions being enzymes having different functions in different cells.

One is tempted to extend this theory to the production of deutoplasmic inclusions during Oogenesis, as the evidence presented in this paper—where the Golgi bodies initially secreting fatty yolk are later concerned in the secretion of the mucous envelope of the oosperm—warrants the suggestion that the function of the Golgi apparatus in Oogenesis is only secretory, the nature of the secretion being incidental to the needs of the hour. (See Subramaniam, 1934.)

Summary.

1. The Golgi elements lying below the zona enlarge on fertilization.

2. The rims of the fatty yolk droplets break up into irregular granules, the majority of which also form a concentration below the zona radiata.

3. Concurrent with these changes the inner portions of the zona become converted into a mucilaginous envelope with an outer definite membrane.

4. When the mucous envelope is completely formed, the majority of the Golgi are extruded.

5. The function of the Golgi in Oogenesis is only secretory, the nature of the secretion being only secondary in importance.

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