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The Cortical Alveoli of Salmon Egg [1)]

With 10 Text-figures

Mamoru KUSA

Department of Biology, Yamagata University, Yamagata
(*Communicated by* T. YAMAMOTO)

In a previous work on the unfertilized eggs of the stickleback (Kusa, 1953b), it was stated that a certain polysaccharide substance, which is positive for the Hotchkiss test, is contained in the cortical alveoli, and that an essential feature of the breakdown process of the alveoli, occurring upon both fertilization and artificial activation, consists in an actual discharge of the whole alveolar system from the egg surface. The present investigation is particularly concerned with the chemical properties of the contents of the alveoli, as determined by means of various staining tests in the unfertilized eggs of the two salmon species, *Oncorhynchus keta* and *O. nerka* (landlocked form).

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OBSERVATIONS

The cortical alveoli in the unfertilized eggs of the dog salmon, *O. keta*, have been observed only with fixed material (Kanoh, 1950; Yamamoto, K., 1951). To the eggs of the red salmon, *O. nerka*, a similar technique can also be successfully applied in ascertaining the presence of an alveolar system in the peripheral cytoplasm. In these species, however, the cortical alveoli of the intact eggs can hardly be seen from

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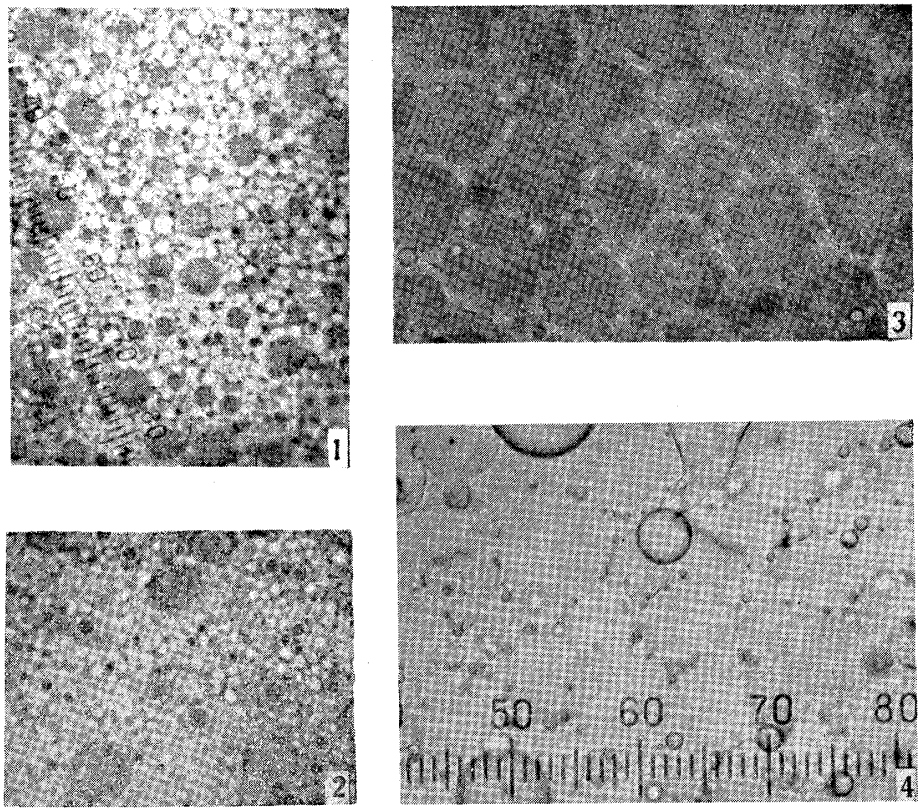
outside. This may be attributed, at least in part, to the large quantity of yolk contained in the cytoplasm, since the chorion of these eggs is not so opaque as to hinder the inspection of the egg interior. In order to overcome this difficulty, a large cut was made with fine scissors or scalpel on a fresh egg in Ringer's solution; and as soon as the yolky fluid from the cut was dissolved in the Ringer, a small piece of the chorion was carefully taken out, and observed under both ordinary and phase contrast microscopes. The microscopical inspection of this piece reveals that there exists a peripheral cytoplasm, lining the chorion and being densely packed with cortical alveoli (Fig. 1). These alveoli, each



Figs. 1–4. Inner surface of the chorion excised from the fresh unfertilized egg of *O. keta* in Ringer's solution. Explanation are given in the text. Figs. 1 and 2, ca.×100; Figs. 3 and 4, ca.×600.

with a diameter of 3 to 30 micra, seem somewhat refractive and appear as bright spots, when placed slightly out of focus (Fig. 2). Under phase contrast illumination (dark contrast), the alveoli appear dark (Fig. 3). On a closer examination, some droplet-like inclusions are found in the interstices among the alveoli (Fig. 4). These inclusions are 1 to 1.5 micra in diameter; their refractive index seems somewhat different from either alveoli or oil droplets.

Next, the eggs, all fixed with Bouin or Allen-Bouin fluid, were examined cytochemically with staining tests. The material within the alveoli reacts intensely to the Hotchkiss test for polysaccharide substance (Glick, 1949) (Figs. 5 and 6). The application of Bauer's CrO_3-Schiff method (Lison, 1936; Ries, 1938) results in an intense violet coloration of the alveolar contents, and demonstrates the presence of polysaccharide[2]. The coloration of alveoli with Bauer's reagents, however, appeared more intensely in dog salmon than in red salmon eggs.

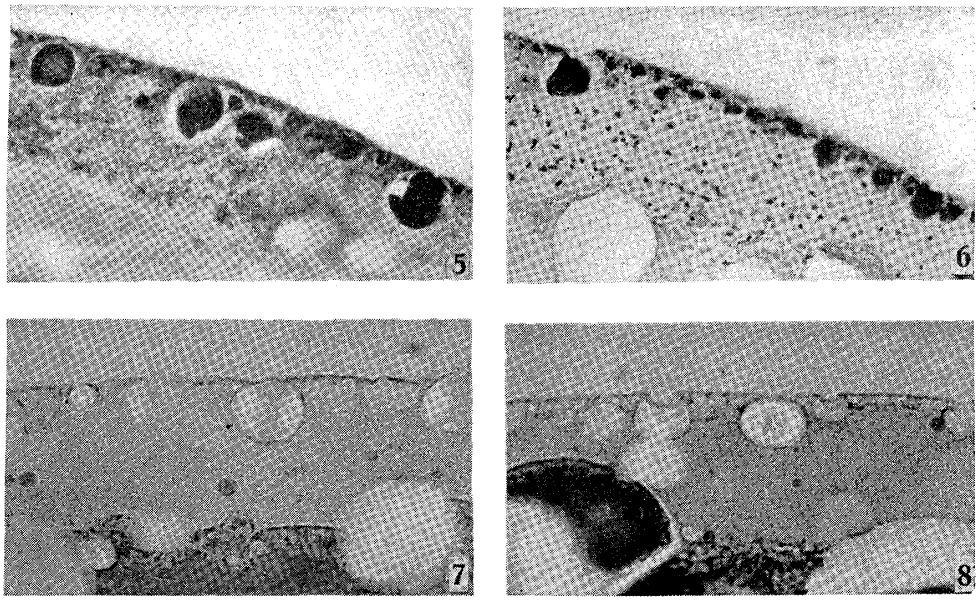


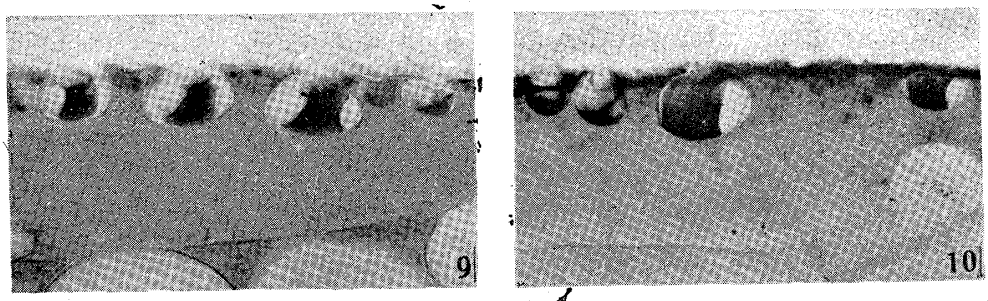
Fig. 5–8. Cytochemical tests of the alveoli. ca.×450. 5. Dog slamon egg, with Hotchkiss test: 6, Red salmon egg, tested as in Fig. 5; 7, Dog salmon egg, with protein test; 8, Red salmon egg, tested as in Fig. 7.

Recently Yasuma and Ichikawa (1951) have developed a cytochemical stain for protein with their ninhydrin-Schiff's reagent. With this test, the alveolar contents of these salmonid eggs still show a positive reaction, although this is rather weak compared with the intense coloration of the yolk mass (Figs. 7 and 8). This suggests that some protein substance is also contained within the alveoli.

In fixed eggs, the alveolar substance is stainable with some basic dyes, such as thionin, neutral red, toluidine blue, safranin O, dahlia violet, Janus green B and Delafield's hematoxylin. The first four of these produce a more distinct stain than the others. The contents also become colored, as shown in Figure 10, with pyronin B in watery solution

2) With reference to the Bauer-positive polysaccharides, Ries's handbook of histophysiology (1938, p. 180) reads as follows; "Ausser Glycogen reagieren positiv: Paraglycogen, Stärke, Cellulose, Tunicin, Galactogen; nicht hingegen Inulin".

by prolonged standing (about 50 days at room temperature), although they are scarcely stained by the freshly prepared dye solution. In marked contrast to their stainability with Delafield's hematoxylin, the alveoli show little affinity to Heidenhain's iron-hematoxylin. The stainability with these basic dyes does not decline much even after 2 to 5 hours of treatment with hyaluronidase (Sprase, Mochida preparation. 5000 V.U.M. in 100 cc of Ringer) at a temperature of 37°C. With some



Figs. 9–10. Alveoli of dog salmon egg, stained with basic dyes. ca.×450. 9, With neutral red; 10, With pyronin B.

basic dyes, marked metachromatic shades are exhibited: brown with neutral red, reddish purple with both thionin and toluidine blue. However, in general, the alveoli have little affinity for acid dyes, such as eosin, acid fuchsin and orange G, except for aniline blue in Mallory's triple stain.

GENERAL CONSIDERATIONS

As indicated in the foregoing observations, the content of the cortical alveoli in these salmonid eggs is positive for the Hotchkiss test. The cytoplasmic constituents, which become visible with this test, are regarded as glycogen and other compounds containing polysaccharide, such as mucoprotein, cerebrocides and hyaluronic acid, but the idea of the presence of hyaluronic acid in the alveoli is hardly tenable, as proved in the digestion-test with hyaluronidase. The alveoli are also colored metachromatically with some basic dyes. The metachromatic staining is a characteristic feature of mucoprotein compounds containing polysaccharide esters of sulfuric acid (Lison, 1936; Ries, 1938). Consequently, it may be concluded that mucoprotein forms a principal constituent of the cortical alveoli. The alveoli are also stained with an aged solution of pyronin B. This may substantiate the idea of the chemical nature of the alveolar substance as inferred from its metachromatic character, since a strong affinity for an aged pyronin solution is another feature of a mucoprotein esterified to sulfate (Monné and Hårde, 1950). Besides

the strong basophilic character, the cortical alveoli exhibit affinity to acid dye, as proved with Mallory's triple stain. This coloration may depend upon the presence of amino sugar in the alveoli. It has been pointed out that the alveoli are also reactive to Bauer's test. Although the chemical nature of this substance in the alveoli has not been fully determined, the question remains as to whether this Bauer-positive feature is characteristic of the Hotchkiss-positive polysaccharide esterified to sulfate, or whether it is based on some other substances in the alveoli. The alveoli, as shown above, are slightly positive to a certain protein test. However, it still seems difficult, at present, to determine whether this staining is caused by a protein component linked with polysaccharide or by free protein molecules. In short, all these facts indicate that a polysaccharide esterified to sulfate is a component characteristic of the cortical alveoli in these fish eggs.

Based upon similar studies of some sea urchin eggs, it has been shown that the cortical granules consist basically of a polysaccharide substance combined with sulfuric acid residues (Monné and Hårde, 1950). Thus, apparently, the cortical alveoli of salmonid eggs have much in common with the cortical granules of sea urchin eggs, not merely in chemical composition, but also in structural aspects (e.g., *cf.* Kusa, 1953a and b). In the unfertilized eggs of *Nereis limbata*, the intraovular jelly precursor substance disappears at the time of fertilization or artificial activation, and changes into the jelly layer external to the egg (Lillie, 1911; Novikoff, 1939; Costello, 1949). The chemical analysis of this jelly material has proved the presence of a carbohydrate-containing uronic acid (*cf.* Costello, 1949). Recently, T. Yamamoto (1952) has established the matachromatic character of the cortical granules in the unfertilized eggs of a polychaete worm, *Tylorrhynchus heterochaetus*. On activation of the egg of the ascidian, *Cynthia roretzi*, there also separated from the egg surface the so-called "test-cells" (Hirai, 1941), in which the presence of a certain mucin-like substance was determined later (Hirai, 1949). These facts all suggest that the unfertilized eggs of several animal species are similar in posessing, within the peripheral cytoplasm, certain carbohydrate components, which are completely discharged at the time of egg activation.

Summary

Examination was made of the cortical alveoli in the unfertilized eggs of the salmons, *Oncorhynchus keta* and *O. nerka*. The alveoli, 3 to 30 micra in diameter, of the fresh dog salmon egg are highly refractive,

but appear dark under phase contrast illumination (dark contrast). The cytochemical observations lead toward the conclusion that the alveolar material of these fish eggs is chiefly composed of mucoprotein containing polysaccharide esters with sulfuric acid; and the alveoli may also contain amino sugar. In this connection, the close similarity with regard to the chemical character of these cortical structures in animal eggs of several species is pointed out.

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