Analysis of Growth of the Chick Marginal Blastoderm

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The spread of the blastoderm over the yolk of the avian egg is being investigated in connection with the significance of yolk structure. Carbon powder and vital dye marking of the marginal cells of the blastoderm indicate that growth occurs actively from the edge and not by cell proliferation in the more proximal and central regions, with resulting passive transport of the margin. Marks placed at the edge were found to remain behind as the blastoderm continued its growth over the yolk.

The presence of free nuclei in the periblast of teleost eggs was reported by Ryder (*1*) and Agassiz and Whitman (*2*). This same situation was found to exist in pigeon eggs by Harper (*3*). The marginal cells of the blastoderm were found to be open peripherally, with a syncytial region extending into the periblast (Fig. 1). A more detailed study was carried

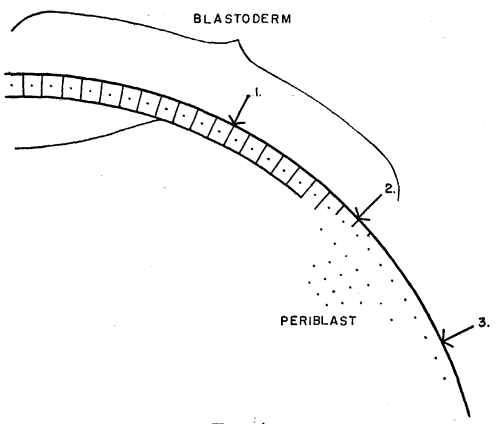

FIG. 1.

out by Blount (*4*), and more recently an essentially similar situation has been described for the hen's egg (*5*).

Blastoderm growth is considered to take place by the continual advance of these free nuclei accompanied by subsequent cytoplasmic compartmentalization. The blastoderm would, in effect, cut the marginal periblast into cells while maintaining a zone of free nuclei for further expansion.

If this situation exists, then it should be possible for the blastoderm margin to continue its migration around the yolk following complete isolation from previously established tissue. To test this, a series of isolations was performed at the sites indicated in Fig. 1. These sites, labeled 1, 2, and 3, respectively, are in the blastoderm proper, at the zone of junction where the open marginal cells merge with the peri-

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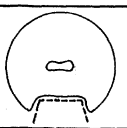
AGE AT TIME OF OPERATION (HOURS)	SITE OF CAUTERY	APPEARANCE AFTER 24 HOURS OF INCUBATION
10		
24		
24		
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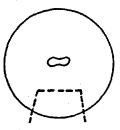
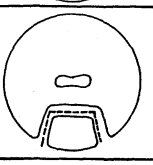
48		

FIG. 2.

blast, and in the periblast itself. Isolation was accomplished by cautery with a fine, resistance-heated wire applied to the yolk surface through a window cut in the shell. This results in a clearly visible local coagulation of albumen and yolk that makes further identification marking unnecessary. The width of the visible injury was 1.5 mm, and vital staining with methylene blue and neotetrazolium chloride showed cell destruction for an additional 0.5 mm on both sides of the line of cautery. The windows were sealed, and the eggs incubated for 24 hr. The results are summarized in Fig. 2.

The isolated marginal portion of the blastoderm grows at approximately the same rate as the unoperated region. Since the isolated portion cannot possibly be pushed forward by more proximal cell proliferation, and since it progresses over the yolk in an apparently normal fashion, growth must take place at the blastoderm edge. This growth is not influenced by the separation of the marginal region from previously established tissue. Cauterization at site 2, the marginal region, halts all subsequent growth, and cauterization at site 3 results in a cessation of growth when the marginal cells reach the injured region. The fact that no growth of cells occurs distal to the cauterization at site 3 indicates that the isolated syncytial periblast is not capable of cell formation but evidently requires continuity with the marginal cells to undergo compartmentalization.

The growth of the isolated marginal blastoderm has

been described in *Fundulus* eggs by Trinkaus (*6*). Removal of the blastocoel roof in this form has no effect upon further epiboly of the remaining peripheral portion of the blastoderm. Trinkaus has provided evidence for the role of the contractile tension developed by the surface gel layer which "pulls the blastoderm down over the yolk." The operation of a similar mechanism in the hen's egg is doubtful in view of the following observations. Small defects created in the path of the advancing blastoderm demonstrate that growth is not limited to one direction. In addition to downward spread, the blastoderm, after bypassing the defect, is capable of both lateral and upward growth. Furthermore, since cauterization at site 3 does not cause an immediate cessation of growth (as would be expected if contractile tension were pulling the blastoderm down) and, furthermore, has no effect upon growth until the blastoderm margin actually touches the damaged region, it is clear that contractile tension does not influence blastodermal growth in the hen's egg. Recent evidence (*7*) indicates that the role of the surface contractile tension in *Fundulus* epiboly is not a major one.

References

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On the Nature of the So-Called Background Material in Estrogen Fractions of Extracts Prepared from Hydrolyzed Urine[1]

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In 1948 Friedgood, Garst, and Haagen-Smit (*1*) reported an essentially new micromethod for the extraction and partition of crystalline estrone, estradiol, and estriol, and for their quantitative assay by ultraviolet spectrophotometry. In the course of applying this method to the extraction of the natural estrogens from hydrolyzed urine, to their subsequent separation from urinary phenols and neutral steroids, and to their partition from one another, it was found that the so-called background material (*2–8*) interfered significantly (*9–11*).

We have tried various methods for the separation of the "background" material from the urinary estrogens: as, for example, modification of the procedure for the steam distillation of the phenolic fraction, various washes of the extracts, changes in the solvents for equilibration, and variations of the pH at which the extractions were done. Although some of these attempts produced considerable reduction in the amount of the interfering ultraviolet-absorbing material in the final extracts, some interference persisted. At that point in the investigation a number of observations from a variety of experiments indicated that the "background" material might consist of a phenolic steroid or steroids which developed a quinone structure during the extraction procedure. A fundamental analysis done on a partially purified sample lent further support to this interpretation of the data (Table 1). A large part of this steroidlike mixture has been found to exhibit physical and chemical properties similar to those of estriol when studied in the Craig distributor and by rubber chromatography according to the method of Nyc, Maron, Garst, and Friedgood (*12*). Moreover, comparison of the ultraviolet spectrum of this steroidlike material with that of a synthetic estrogen derivative (*13*) indicates a possible structural relationship of the steroidlike material to the natural estrogens (Fig. 1).

TABLE 1

FUNDAMENTAL ANALYSIS OF STEROIDLIKE MATERIAL: COMPARISON WITH VALUES FOR THEORETICAL ESTRIOL DERIVATIVES

	Carbon (%)	Hydrogen (%)
Steroidlike material	71.5	7.6
Theoretical estriol catechol	71.1	7.9
Theoretical estriol quinone	71.6	7.3

This steroidlike material is found consistently in the urine of both males and females; and it is increased in amount about threefold during pregnancy. The excretion values in nonpregnancy urines are rather constant; they are of the order of 3–4 mg/24-hr sample.

A further study of this steroidlike material is now in progress in order to achieve its complete identification, as well as its separation from the natural urinary estrogens.

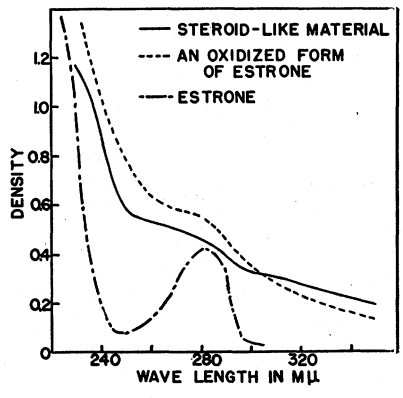

FIG. 1. Comparison of the ultraviolet absorption of the steroidlike material with that of an oxidized form of estrone. The latter was produced by exposing a mixture of estrone and 10% of its weight of riboflavin to 100 ft-c of light for 3 days.

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