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THE SURFACE GEL LAYER OF FUNDULUS EGGS IN RELATION TO EPIBOLY

BY J. P. TRINKAUS

OSBORN ZOOLOGICAL LABORATORY, YALE UNIVERSITY, AND MARINE BIOLOGICAL LABORATORY

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There have been several studies relating the properties of the surface gel layers of Amphibian[1] and Teleost[2] eggs to the movements of gastrulation. Teleost eggs, with their remarkable epiboly, offer excellent material for a further analysis of this question. The present report outlines certain preliminary studies on the properties of the surface gel layer of the eggs of *Fundulus heteroclitus*, with special reference to its possible rôle in epiboly.

All studies were made on eggs from which the chorion (or "shell") had been removed. Unless otherwise stated, unbuffered double-strength Holtfreter's solution was the medium employed. It was possible to demonstrate at the outset that Fundulus eggs possess a surface gel layer, by microdissection with glass needles. This gel layer constitutes the outer coat in oviducal eggs, unfertilized eggs and in eggs activated by sea water, as well as in developing eggs at all stages as far as closure of the blastopore. It is continuous, non-pigmented and constitutes the outer layer of both the unsegmented yolk sphere and the blastodisc. The outer surface of this gel layer is non-adhesive. If a blunt instrument is pressed against the egg, the gel layer shows resistance and is thrown into radiating folds. Microdissection of all stages through blastopore closure (Oppenheimer, stage-15)[3] indicates that the surface gel layer is the outer layer of the epiblast to which all epiblast cells are attached. Thus the epiblast tends to come off in sheets when removed. Furthermore, the gel layer of the epiblast is continuous with that of the yolk.

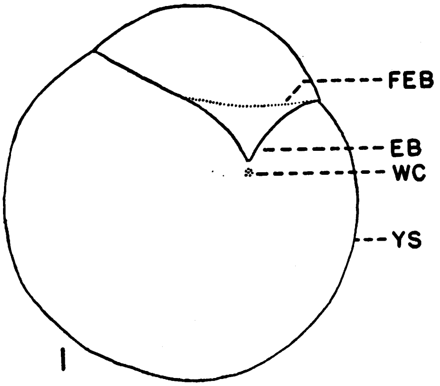
Properties of the Surface Gel Layer.—When a wound is made in the gel layer of the yolk sphere, the aperture first becomes round and expands in size as the gel layer retracts, presumably because of the tension resulting from the turgor of the yolk. This rounding up and expansion of the wound also indicates that the gel layer possesses elastic properties. After this initial increase in size the aperture is closed rapidly, while the relatively fluid yolk continues to ooze during all but perhaps the final stages of the process. This whole process takes from 2–5 minutes depending upon the size of the wound. As the wound closes, folds or stress lines appear in the gel layer, radiating from the wound. The gel layers of oviducal eggs and unfertilized eggs activated by sea water have the same ability to close wounds rapidly. This process takes place in typical fashion when the eggs are in single, double or quadruple-strength Holtfreter's solution, in sea water and in distilled water.

In order to ascertain whether wound closure is actually due to contraction of the surface gel layer, carbon marks[4] were placed on opposite edges of an open wound (prior to the onset of wound closure). As the wound closed, the marks were drawn toward each other until they almost met at the point of wound closure. Such an experiment indicates that wound closure is chiefly the result of the contraction of the edges of an already existing surface layer, rather than the formation of a new membrane from the yolk cytoplasm. The very last stage appears to be a process of protoplasmic "clotting," wherein a small amount of yolk cytoplasm coagulates and forms a plug.

If carbon marks are made at various points on the surface of one hemisphere of the egg and a wound then made in the midst of these marks those marks nearest the closing wound move toward each other but the distance between these marks and marks further away increases. Thus the surface layer stretches or expands as the process of wound closure proceeds. It seems also from this experiment that a large part, if not the total, surface layer of the egg is involved. The integrity of the entire surface layer is not necessary for wound closure, however, inasmuch as two or more wounds in the surface layer will close at the same time, in typical fashion, even though one of the wounds is quite large, e.g., 1 mm. in diameter, which is one-half the diameter of the entire egg.

Wound closure in the surface gel layer of the yolk sphere near the edge of the blastoderm, at all stages from the one-celled blastodisc to the closure of the blastopore, results in a stretching of the blastoderm in the direction of the closing wound, and also in a stretching of individual adjacent cells and periblast nuclei as well. This phenomenon (see Figs. 1 and 2), takes place in a few minutes' time. It demonstrates that the cells at the edge of the blastoderm (and nuclei of the marginal periblast) are attached to the surface gel layer of the yolk sphere in such a manner that tension in this layer exerts a corresponding tension on them, resulting in an appropriate distortion of cell shape.

Experimental demonstration that the surface gel layer is continuous



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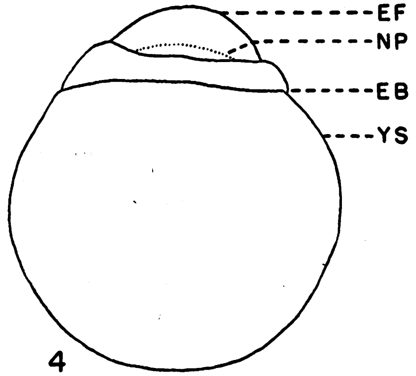

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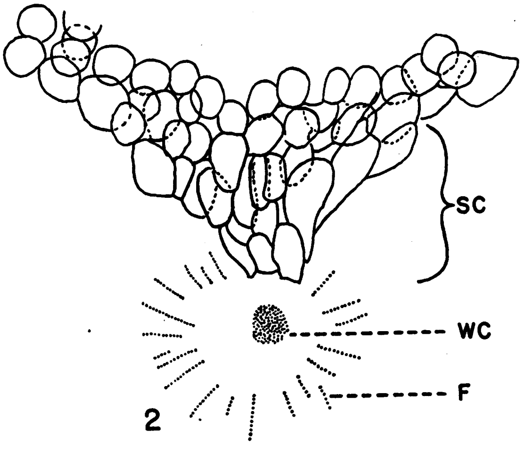

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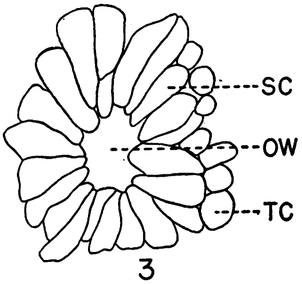

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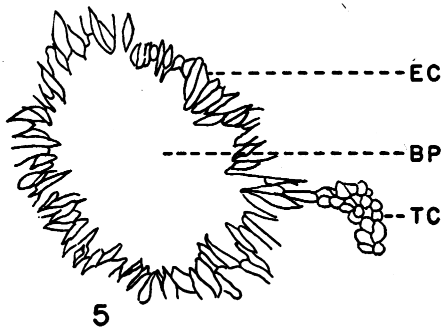

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over the entire surface of the blastoderm is provided by further wound healing experiments. If a group of cells is removed from the epiblast layer in a blastula or gastrula stage, a process of wound closure is initiated, which appears similar to that in the yolk surface layer, although it is slower, taking 7 to 12 minutes or longer. It results in a radial stretching of the cells adjacent to the wound. This stretching becomes apparent when the wound is approximately half closed, and is continually augmented until the completion of the process (see Fig. 3). Such a radial stretching effect is clear evidence that a supra-cellular force, presumably the surface gel layer, is concerned in wound closure in the epiblast cell layer.

A wound in the surface of one of the blastomeres in an early cleavage stage is rapidly closed in a similar manner, with resulting radial folds or lines of stress in the blastomere surface. Apropos of wound healing in early blastomeres, it might be mentioned that it is relatively difficult to wound the surface of a blastomere of the 1- or 2-cell stage. The surface seems tougher than that of the yolk. Furthermore, since little or no cytoplasm pours out of the wound, the cytoplasm of these cells must have a higher viscosity than the yolk.

As a result of these various wound healing experiments it is evident that the outer surface layer of Fundulus eggs is a gel layer possessed of marked elastic properties. In this respect the surface gel layer of Fundulus eggs is like that of Amphibian and trout eggs (cf. Holtfreter[1] and Devillers[2]). However, if rapidity of wound closure be an adequate criterion,

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EXPLANATION OF PLATE

(Drawings based on camera lucida tracings)

FIGURE 1

Stretched edge of blastoderm resulting from wound closure in surface gel layer of yolk sphere in Oppenheimer stage 10. × 20.

FIGURE 2

Stretched cells at the edge of the blastoderm resulting from wound closure in the surface gel layer of the yolk sphere. Stage 8. × 100.

FIGURE 3

Stretched cells of the epiblast after partial wound closure. Stage 11. × 220.

FIGURE 4

Protrusion of exposed floor of blastocoel, after removal of roof of blastocoel. Stage 10+. × 20.

FIGURE 5

Elongate cells at lips of blastopore in normal embryo. Stage 15. × 100.

BP, blastopore; *EB*, edge of blastoderm; *EC*, elongate cells at lips of blastopore; *EF*, exposed floor of blastocoel; *F*, radiating folds in surface gel layer; *FEB*, former edge of blastoderm; *NP*, normal position of floor of blastocoel; *OW*, open wound; *SC*, stretched cell; *TC*, typical epiblast cell; *WC*, point of wound closure; *YS*, yolk sphere.

the elasticity of the Fundulus egg gel layer is considerably greater than that of Amphibian and trout eggs.

The floor of the segmentation cavity or blastocoel was studied in late cleavage and blastula stages to see if it likewise possesses such an elastic surface layer. When a large section of the roof of the blastocoel is removed, the floor of the blastocoel is exposed, consisting at this stage solely of the surface of the yolk sphere. It is not yet covered by periblast. Within a few minutes after such an operation, the exposed surface bulges out as shown by figure 4. This demonstrates both the turgor of the yolk, and the lack of elastic strength in the surface of the yolk at the floor of the segmentation cavity. When a wound is made in the yolk surface at the floor of the segmentation cavity it gapes and remains open. The relative ease with which such a wound is made itself indicates the weakness of this layer. It is thus apparent that the yolk surface at the floor of the segmentation cavity in early stages lacks a surface layer with the same elastic, contractile properties as are possessed by the peripheral surface of the egg. Similar wounding experiments on the floor of the blastocoel in late blastulae and all stages of gastrulae, when the floor is covered with periblast, suggests that the surface at these stages possesses an elastic gel layer, inasmuch as wound closure seems more typical, although often slower (ca. 10 min.).

If an early blastula with an open wound in the floor of the blastocoel remains in the bottom of a dish for an hour or so, all of the yolk oozes out, and all that remains is the surface gel layer lying in the bottom of the dish with marginal blastoderm cells clinging to the edges of the aperture. It has the appearance of an empty cellophane bag. This forms superb material for physicochemical studies. A few preliminary experiments have been done along these lines.

Mechanical and Chemical Studies.—The gel layer, in this isolated condition, will remain intact and apparently in a normal state for at least 12 hours in double-strength Holtfreter's solution. It is non-adhesive on its outer surface (as in the intact egg) and sticky and viscous on its inner surface. If the inner surfaces of 2 pieces are pressed together they will adhere quite firmly. The elastic properties of the material can be demonstrated by stretching pieces between two glass needles. In this manner a piece may be stretched to half again its length, returning to its original length upon release. Small pieces of the gel layer, with cells clinging to them, tend to round up into spherical globules. When the gel layer is emptied of yolk and, therefore, in a slack condition, a wound in it no longer expands into a round opening in the usual manner. Apparently, the normal process of wound closure in the surface gel layer is dependent upon a state of tension in the gel layer, resulting from the normal turgidity of the egg. After a certain small amount of mechanical manipulation, the isolated gel layer completely solates. This is a property of thixotropic gels. It does not return again to the gel state, however, upon standing, as is typical of these gels.

In Ca^{++}-free sea water the surface gel layer of the intact egg dissolves, or better—solates, after the egg has been in the medium for 1–2 hours. In some cases the egg first flattens out on the bottom of the dish like a thick pancake, suggesting a general thinning and weakening of the gel layer, prior to solation. In other cases, the egg retains its spherical shape and its normal wound healing ability for approximately $1^1/_2$ hours. At about this time, however, a wound forms readily without extreme intervention and remains open. All of the surface gel layer then solates in the course of a few minutes. At no time in any of these cases was the outer surface observed to become sticky, as is normal for the inner surface. Both development and wound closure of Fundulus eggs take place normally in distilled water. The effect of Ca^{++}-free sea water is, therefore, not due simply to a lack of Ca^{++}-ions. The positive action of the monovalent ions in the ionically unbalanced solution must also be considered.

The behavior of the surface gel layer of the Fundulus egg in Ca^{++}-free sea water is characteristic of the behavior of the surface gel layers of other eggs in Ca^{++}-free media, e.g., Amphibian. The hyaline plasma layer of Echinoderm eggs also solates in Ca^{++}-free sea water. This latter fact suggests that the similarities of the surface gel layer and the hyaline plasma layer may be more than superficial, a subject which invites further investigation.

Possible Role of Surface Gel Layer in Epiboly.—The properties of the surface gel layer, as outlined in the previous pages, suggest that it may be of some importance in relation to the movements of gastrulation. It certainly seems probable that the surface gel layer, being the outer layer of the epiblast to which all epiblast cells are attached, acts as a coördinating and unifying force in the highly integrated cell movements characteristic of the epiblast in Teleost gastrulation. Furthermore, the stretching effect of the gel layer upon the edge of the blastoderm in wound healing is especially suggestive of a possible causal rôle of the gel layer in epiboly. To test this idea, detailed observations were made of cell shape at the edge of the blastoderm in all stages of epiboly (in *normal* eggs). At the stage when epiboly *seems* to proceed at the greatest rate, namely the final closure of the blastopore (stage-14+ to stage-15), there is a radial elongation of the cells at the lips of the blastopore (see Fig. 5). These elongate cells appear to be stretched toward the point of blastopore closure. This suggests that the blastoderm is being pulled over the surface of the yolk by the contractile tension of the surface gel layer of the yolk sphere. If this be the case, one should be able to cause a uniform stretching of cells at the lips of the blastopore in an earlier stage, e.g., stage-13+ or stage-14, by making a wound in the surface gel layer at the point of future blastopore closure. When this was done, cells at the edges of the blastoderm became stretched in the direction of the point of closure of the blastopore. Furthermore, it was possible by such an experiment to cause precocious closure of the blastopore with the embryo only in an embryonic shield stage (stage-13+).

One further case supports this possible rôle of the surface gel layer in epiboly. In the series of experiments in which the roof of the blastocoel was removed during a late blastula stage (stage-11) one egg remained intact over an 18-hour period. In spite of the fact that the large wound in the roof of the blastocoel remained open, epiboly took place completely with resulting closure of the blastopore. Such a result constitutes supporting evidence for the thesis that epiboly is not an overgrowth, as has often been thought, but is rather the result of the blastoderm being pulled down over the yolk.

If the hypothesis is true that epiboly is due to the contractile tension of the surface gel layer of the yolk sphere on the cells of the blastoderm, it is likely that in the course of epiboly there would be a greater stretching of the blastoderm nearest its edge, with gradually decreasing amount of stretching as one proceeds toward the animal pole, assuming that the surface gel layer of the blastoderm has uniform elasticity. It is certainly true, for example, that, after the closing of a wound in the surface gel layer of the yolk sphere, only the marginal blastoderm cells, nearest the closing wound, show marked stretching.

To check this point, the relative degree of stretching of different regions of the blastoderm was studied by the method of carbon marking. Marks were placed at various points on the surface of the epiblast in an early gastrula stage. Camera lucida records of the positions of these marks relative to one another were made every few hours during the course of epiboly. The distance between carbon marks over the entire blastoderm increased uniformly, as epiboly proceeded. This sort of result suggests that there is a uniform expansion, or spreading, of the epiblast, and, therefore, also of the surface gel layer of the blastoderm, in epiboly. Such a phenomenon indicates that epiboly may not be simply the result of tensions exerted on the edge of the blastoderm by the surface gel layer of the yolk sphere. The situation is more complex, it would seem, than some previous workers have been willing to admit (e.g., Lewis, who, however, quotes no experimental evidence). It is, of course, possible that any tension exerted on the edge of the blastoderm during epiboly is too small to be detected by the carbon marking technique. Nevertheless, these data indicate that the expansion of the surface gel layer of the epiblast must also be considered as a possible causal factor in epiboly.

In spite of these suggestive observations and experimental results, it is still an open question as to whether the surface gel layer actually plays a determining rôle in epiboly in the Fundulus egg. If it is causally important in epiboly, the precise rôle it plays has not yet been determined. Further investigation of the relation of the surface gel layer to the cell movements of early morphogenesis is necessary, for it may possibly shed some light on one of the more obscure aspects of causal embryology, the mechanics of gastrulation.

It should be emphasized that this report has been concerned exclusively with studies on eggs of *F. heteroclitus*. The results are not necessarily representative of Teleost eggs in general. In fact, the surface layers of the eggs of some forms, e.g., *Platypoecilus maculatus* and *Lebistes reticulatus*, seem to have a much lower degree of elastic strength than that of Fundulus eggs. The surface layer of Toadfish eggs (*Opsanus tau*) and Medaka eggs (*Oryzias latipes*), however, appear to be more like that of the Fundulus egg. The properties of the surface gel layer suggest that one of its functions may be to protect the egg from the effects of osmotic changes (as pointed out by Devillers) and mechanical buffeting. The weakness of the gel layers of Lebistes and Platypoecilus eggs thus may be related to the viviparity of these forms, and, conversely, the strength of the gel layers of Fundulus, Opsanus and Oryzias eggs to oviparity.

Summary.—Fundulus eggs possess a surface gel layer, which constitutes the continuous, outer layer of both the unsegmented yolk sphere and the blastoderm. It has striking elastic properties and plays a decisive rôle in wound healing. The contraction of the surface gel layer in wound closure stretches adjacent cells and pulls a large sector of the blastoderm toward the wounded area. At closure of the blastopore in normal embryos, the cells at the blastopore lips elongate. At an earlier stage, this phenomenon is simulated and blastopore closure hastened if the surface gel layer is wounded at the point of future closure of the blastopore, suggesting that epiboly may be the result of contractile tension exerted by the surface gel layer of the yolk sphere. The behavior of the blastoderm, as observed in marking experiments, indicates that the spreading of the surface gel layer of the blastoderm must also be considered as a possible factor in causing epiboly.

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