SUPERFICIAL GEL LAYERS OF CELLS AND EGGS AND THEIR ROLE IN EARLY DEVELOPMENT*

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GEL LAYERS

Every cell and egg and ameboid organism has a superficial gel layer and less viscous endoplasm. Gel layer and endoplasm are different states of the same cytoplasm which more or less readily changes from one to the other especially in the region where they grade into one another. Gel layers are flexible, stretchible, more or less adhesive and above all contractile.

Thickness and viscosity are roughly proportional to cell size. Undivided amphibian eggs, diameter 2 to 3 mm. have thicker and more viscous gel layers than zebra fish ones, diameter 0.65 to 0.75 mm. The latter in turn are thicker and more viscous than gel layers of echinoderm eggs, diameter 0.06 to 0.10 mm. The gel layers of adult somatic cells, diameter 0.010 to 0.030 mm. are much thinner than those of eggs. The great thickness and viscosity of the gel layers of eggs is correlated with the necessity of maintaining the spherical shape of the eggs by contractile tension against the flattening effect of gravity. The efect of gravity on small somatic cells is negligable.

The gel layer of every cell is derived from the original gel layer of the undivided egg. Cells are never without a gel layer. Gel layers of undivided eggs become greatly modified after they have been

* Aided by a grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council and by funds from Dr. Henry Winsor, Philadelphia.

stretched by being pulled into the cleavage furrows and subjected to new environments. Since gel layers are part of the cytoplasm they also become modified as it differentiates.

SOL-GEL CHANGES

There are indications that some factor in the external environment is responsible for the superficial gelation and that it gels until it is balanced by an internal factor which tends to decrease viscosity. The assumption that something in the external environment has a gelating effect on cytoplasm was suggested long ago by Rhumbler (1898) for amebae. As gel layers are stretched over adjacent surfaces of daughter cells there is probably a compensatory gelation on their inner aspect. It also seems probable that when a gel layer contracts and tends to thicken its inner aspect becomes less viscous, as though the external gelating factor can act to a certain depth only.

FLEXIBILITY

The flexible quality of the gel layers is a passive one but essential. If gel layers were rigid changes of cell form would be impossible.

STRETCHIBILITY

Gel layers are stretchible but not necessarily elastic. Gel layers are stretched during every cleavage as the gel layer is pulled into the furrow by the contracting constriction of the equatorial cleavage band (Lewis '39). The gel layer is also stretched during pseudopod formation and whenever cells and eggs are distorted by external pressure or gravity. Stretching is always antagonized by the contractile tension of the gel layer of the stretched part. When gel layers are stretched artificially, as with needles, they contract back by virtue of their contractile property, not elastic property which is negligable.

ADHESIVENESS AND SURFACE TENSION

Adhesiveness is presumably due to a modification of the superficial aspect of the gel layer when it comes into contact with other cells or a substratum. Surfaces become fluid or semifluid. Surface tension forces cause the cells to spread out on one another or on a

substratum. If this semifluid layer sets into a firm gel (cement substance) surface tension forces cease to exsist and firmly adherent cells are held tightly together until this substance becomes less viscous. Cement and sticky substances are probably identical in composition but of different viscosities. Both consist of cytoplasm derived from the superficial aspect of the gel layer and the fluid in its meshes.

Surface tension which forces adherent cells to spread on one another or on a substratum is opposed by the contractile tension of the gel layer, which usually tends to make cells spherical, and by the viscosity of the endoplasm. Surface tension cannot cause cells to advance and migrate. A cell may be forced to spread into a thin plate on the cover glass by surface tension but not to advance in any direction beyond the limits of the spreading.

CONTRACTILITY

Contractility is the outstanding property of all gel layers. It plays the leading mechanical role in changes of cell form, cell locomotion, cleavage of cells and eggs and early morphogenesis. This property is so obvious that it scarcely needs experimental proof. Experiments on the gel layers of fish and amphibian eggs, which will be given in detail further on amply reveal its contractile property. One can actually see the posterior ends of amebae and of slime-molds contract, also the superficial ends of surface cells of Amblystoma during gastrulation. Holtfreter has shown how isolated necks of amphibian bottle cells contract into balls or if not cut off to the cell body.

Wound healing experiments reveal (1) that gel layers are always in a state of contractile tension, (2) that they exert continuous contractile tension and (3) that they probably solate as they contract. These properties are also revealed during the normal behavior of cells and eggs.

The strength of the contractile tension which gel layers exert presumably depends on viscosity, thickness and the chemical constitution of the cytoplasm. The velocity of contraction presumably depends on the chemical constitution of the cytoplasm. Other factors modify strength and velocity.

LOCAL INCREASES AND DECREASES OF CONTRACTILE TENSION

Gel layers tend to exert uniform contractile tension in all directions. Without local changes movements would be impossible. Local

increases and decreases of thickness and/or viscosity and hence contractile tension of gel layers are produced by unknown factors in the internal and external environments of gel layers. Local changes of contractile tension exerted by gel layers play essential roles in changes of cell form, cell cleavage and when combined with adhesiveness in cell locomotion. Many early developmental processes such as blastodisk formation, cleavage and epiboly of fish eggs, gastrulation, neural tube formation, invaginations of lens, eye, ear vesicles and other morphogenic events of amphibian and presumably of all other types of eggs result from local changes of contractile tension.

ELASTICITY

Elasticity has been much confused with contractility. Contractility is due to an intrinsic property or force of the gel layer itself. Elastic tension develops when a solid body is stretched or bent or distorted by some outside force applied to it. About the only way a gel layer can develop elastic tension is for the endoplasm to swell and stretch the gel layer.

It is obvious that elastic tension, if such a state does exist in intact eggs or cells, plays no part in the following type of experiment. If a small puncture is made in the gel layer of a zebra fish egg which already has a large wound, the small wound will enlarge, constrict and heal before the large one heals. Any elastic tension which might possibly have existed before the large wound was made would have disappeared long before the small wound was made.

ENDOPLASM

Endoplasm is the less viscous part of the cytoplasm. The gradation of gel layers into endoplasm may be abrupt as in amebae, slime-molds and white blood cells or gradual as in fibroblasts where the endoplasm is in a gel or semigel state. The gradation is subject to wide fluctuations in the same cell as during mitosis, locomotion, and perhaps other activities. When endoplasm is in the gel or semigel state it is contractile. Seifriz ('43) states that "fluid protoplasm is contractile. Usually the contracting regions of a plasmodium are of a high viscosity but protoplasm need not be so to exhibit contractility." It does not seem possible for streaming endoplasm of the slime-molds or amebae or fish eggs to possess contractility at the time of streaming.

AMEBOID LOCOMOTION

Since ameboid locomotion plays the leading role in the mechanics of amphibian gastrulation, a clear understanding of it is of great importance. Furthermore, although it may seem far fetched at first glance, the same mechanical principles are involved in fish blastodisk formation and in fish and amphibian epiboly as in ameboid locomotion.

Ameboid locomotion is due to oriented variations of the contractile tension of the gel layer and to the adhesion or temporary adhesion of the cell to a substratum. During locomotion the gel layer at the posterior end forces endoplasm forward and stretches the weak area into a pseudopod. An increase of contractile tension at the posterior end of a cell would have the same effect as a decrease at the anterior end. As the posterior end contracts it shortens and solates and mixes with the forward moving endoplasm. Adjacent gel layer is pulled to the shortened posterior end where it in turn contracts, shortens and solates. This continues as long as the cell or ameba migrates. The posterior end is continually shortening. At the anterior end endoplasm gels as it comes into contact with the anterior edge of the lateral gel wall. This extends the gel wall forward as rapidly as it contracts, shortens and solates at the posterior end. As the posterior end shortens it assumes a more and more forward position.

The posterior end is the oldest, the most viscous and the most contractile part of the gel layer. The lateral wall exerts enough contractile tension to prevent it from bulging. As the anterior end continues to expand it is prevented from becoming too weak and rupturing by continuous gelation on its inner aspect.

In order for a cell to move forward it must acquire temporary adhesion to a substratum at or near its anterior end. As the anterior end advances new adhesions are acquired and the posterior part of the old adhesion is released.

Mast ('26) assumed that the anterior pseudopod is expanded by internal pressure on the endoplasm as a result of elastic contraction of the plasmagel layer. My concept is active contraction of the gel layer. Holtfreter ('46c) has a different idea. The forward streaming of endoplasm is, if I understand Holtfreter correctly, due to active intrinsic expansion of the cell membrane of the pseudopod. In other words the endoplasm is sucked forward by a potential vacuum, an inconceivable phenomenon with such a flexible membrane. The gel layer of the

pseudopod is, like the gel layer of the rest the cell, always exerting contractile tension. If so, it cannot be actively expanding itself at the same time. It may be passively expanded.

CONSTRICTION RINGS

A constriction ring usually develops at the base of the pseudopod of a lymphocyte after each forward expansion of the anterior end. It serves as a marker since it does not move as the anterior end advances and the posterior end shortens (Lewis '39). It reveals that during locomotion, the part of the cell in front of the ring lengthens and the part posterior to it shortens. When the posterior end shortens to the level of the ring the latter constricts to form the tail.

Holtfreter's ('46) idea concerning the constriction rings of isolated amphibian cells differs from mine. These cells scarcely progress at all and the constriction rings move backwards. He suggests that they correspond to the waves produced by passing a closed finger along a rubber tube. The constriction rings move backward, according to my view, because although these elongated cells are not migrating due to lack of proper adhesion they are undergoing the usual expansion and gelation at the anterior end and contraction, shortening and solation at the posterior end, that a migrating cell undergoes. The contraction, shortening and solation at the posterior end pulls adjacent gel layer toward the posterior end and with it the constriction rings which are part of the gel layer.

ZEBRA FISH EGG GEL LAYERS

The gel layer of the undivided egg is continuous over yolk and blastodisk. It is convenient to use disk gel layer for disk part and yolk gel layer for yolk part. During cleavage the disk part becomes divided and subdivided and its cells become more or less adherent.

The gel layers exhibit the usual properties of gel layers, flexibility, stretchibility, adhesiveness and contractility.

WOUND HEALING

The contractile property of gel layers is revealed by wound healing experiments. The gel layer of the undivided egg tends to exert uniform contractile tension in all tangential directions. The following events are

common to small wounds which heal; (1) rapid retraction of the wound edge and enlargement of the wound, (2) constriction of the wound edge and closure of the wound, (3) oozing of endoplasm and yolk from open wounds, (4) formation of radial folds from near the constricting wound edge and their disappearance after healing, (5) depression of the wound area during constriction and its disappearance afterwards, (6) reshaping of eggs into smaller spheres.

Rapid retraction of the wound edge indicates that the gel layer is always exerting contractile tension in manifold tangential directions.

Constriction is attributed to an increase of contractile tension of a thickened circumferential band in the edge of the gel layer immediately around the wound which is of sufficient strength to overcome the retraction pull of the surrounding gel layer.

Oozing of endoplasm and yolk can be attributed to the disintigrating effect of water on exposed endoplasm and to the general contractile tension of the gel layer over the surface of the egg which exerts pressure on the interior. Exposed surface of the endoplasm does not gel.

Radial folds indicate that constriction occurs more rapidly than the surrounding gel layer can accomodate itself to the rapidly decreasing circumference of the constricting wound edge. The folds do not involve the constriction band. It does the puckering but does not pucker itself. Similar folds are often produced during early cleavage of amphibian, fish and echinoderm eggs on the sides of the furrow which is produced by the contracting constriction of the equatorial band of the gel layer responsible for cleavage.

Depression of the wound area is attributed to the loss of yolk and endoplasm and to contraction of semigelated endoplasm which exerts an inward pull on the gel layer and a constricting action also.

The reshaping of eggs into smaller spheres is atributed to the tendency of the gel layer to exert uniform contractile tension in all tangential directions.

The behavior of large wounds which fail to heal reveals not only the contractile property but also the almost unlimited ability of the gel layer to contract. If the undivided blastodisk is separated from yolk in water, each part will undergo the usual series of wound healing events. Both parts reshape themselves, after considerable loss of endoplasm and yolk, into small spheres, except for a depressed area around a small open wound. As endoplasm and yolk are squeezed

through the hole by continuous contraction of the gel layers, the latter decreases in size until the preparation becomes too small to survive.

This apparent unlimited ability of the gel layer to contract and presumably to solate on its inner aspect, for it does not seem to increase greatly in thickness, is not limited to wound healing. It occurs at the posterior end of migrating amebae and cells and during gastrulation of the fish egg. A gel layer will continue to contract as long as it is unopposed or weakly opposed by another part of it or until it forms a small gel sphere or until it solates.

BLASTODISK FORMATION

At the time of fertilization the gel layer, which completely surrounds the mixed endoplasm-yolk mass, exerts approximately uniform contractile tension in all tangential directions. This tends to keep the egg spherical. A few minutes after fertilization the disk begins to appear at the animal pole. It slowly increases in size. At about 22 minutes after fertilization, according to Roosen-Runge ('38), endoplasm begins to stream at a more rapid rate from yolk into disk and the latter enlarges rapidly. The flow stops during cleavage and is resumed again after division. Endoplasm continues to flow into the disk in diminishing amounts in the intervals between the first 6 cleavages, until nearly all of it has been squeezed into the disk. The yolk globules then become compressed into closely fitting polyhedra and the whole mass becomes transparent owing to loss of light refraction around the former spherical globules.

Streaming of endoplasm is attributed to decreased viscosity of disk endoplasm and its gel layer. This permits the contraction of the yolk part of the gel layer to squeeze endoplasm out of the yolk into the disk and expand it (Lewis and Roosen-Runge, '42). The interruption of streaming during the cleavages is due to increase of viscosity of disk gel layer and endoplasm and hence of contractile tension. Decrease of cytoplasmic viscosity during prophase and increase during cleavage always occur during mitosis.

It is evident that the yolk-endoplasmic mass must have a structure which permits endoplasm to be squeezed out and holds back yolk globules. It is also obvious that the yolk mass must be attached to the yolk part of the gel layer and not to the disk part, otherwise the whole yolk mass would surge to the animal pole.

After considering the possibility that there might be some sort of sieve membrane like Mast's hypothetical plasmagel sheet, an alternative hypothesis has been adopted. It seems probable that each yolk globule is encased in a thin film of gelated cytoplasm and that the globules become adherent to one another to form a sponge-like structure attached to the yolk gel layer. In early stages the channels are large and numerous. As endoplasm is squeezed out the channels become smaller and smaller and adhesion of globule to globule more marked. In motion pictures one can see streams of endoplasm in irregular channels between yolk globules which remain fixed in position presumably because they adhere to one another.

GASTRULATION

Gastrulation involves several processes. The spreading of the disk over the yolk with concomitant decrease of its thickness and increase of its area is one with which we are especially concerned.

The late blastula consists of a giant yolk cell and attached blastodisk. The gel layers of the yolk and periblast form a continuous layer. Periblast endoplasm with its nuclei is continuous with yolk endoplasm and forms part of the giant yolk cell. The blastodisk consists of epiblast and blastema (presumptive meso-entoderm). The epiblast cells are firmly adherent to one another. Its periphery is firmly adherent to the yolk gel layer.

Until the beginning of gastrulation the contractile tension exerted by the yolk gel layer is balanced by that of the disk and periblast. Gastrulation begins when this balance is upset, presumably by a decrease of the contractile tension of the gel layers of the adherent epiblast cells and/or periblast.

Contraction of yolk gel layer pulls edge of disk toward vegetal pole and at the same time exerts pressure on the yolk mass and thrusts it with equal force against periblast and disk and expands them (Lewis '43, '44). The yolk mass acts as a hydrostatic cushion. Pressure is exerted equally in all directions, against yolk gel layer as well as disk. The weakest part, the disk, is stretched and expanded. The adherent epiblast cells are stretched into thin plates with great increase of total surface area.

Yolk gel layer contraction continues until, in the course of 4 to 5 hours, the blastopore is closed. As the yolk gel layer contracts its inner aspect solates into yolk endoplasm.

AMBLYSTOMA PUNCTATUM GEL LAYERS

The gel layers of undivided eggs and of the outer aspect of all surface cells of cleavage and later stages are unusually thick and viscous. These outer gel layers are greatly modified after they have been stretched during cleavage. They become thinner, less pigmented and more or less adhesive in the new environment. The great viscosity and thickness of the outer gel layers can be attributed to the peculiar quality of amphibian egg cytoplasm, which reacts in this particular manner to the external environment. Its reaction to the interior environment is quite different. The two environments act differently even on the cytoplasm of the same surface cell.

According to Holtfreter ('43a, '48), cells facing the outer world have a "surface coat" which is firmly attached to the underlying cell membrane. He considers it a syncytial layer which covers and binds the surface cells together. His many experiments, according to my interpretation, and many of my own, indicate that the "surface coat" is part of the gel layer and not a separate entity or a syncytial layer. When the "surface coat" is stretched to cover interior cells during cleavage it is not lost or cast off.

PROPERTIES

The gel layers of amphibian eggs and cells exhibit all the usual properties of gel layers. The flexibility and stretchibility can easily be tested with needles. All changes of shape and all cleavages are possible because gel layers are flexible and stretchible.

ADHESIVENESS

The adhesiveness of cells can be roughly tested by dissecting living gastrulae in water. Yolk cells are weakly adherent and rapidly fall apart when exposed to water. The surface cells on the other hand are strongly adherent especially where their exceedingly viscous outer gel layers abut one another.

CONTRACTILITY

Contractile tension of gel layers can be tested by wound healing experiments. They show that the gel layer is always in a state of contractile tension.

WOUND HEALING

Holtfreter's experiment and many of my own show that small wounds behave like fish egg ones. There is first retraction and increase of wound size. This shows that the gel layer is exerting contractile tension in all tangential directions. The wound edge then constricts and closes the wound. This indicates that the gel layer bordering the wound thickens into a constriction band. Radial folds do not involve the constriction band. It puckers but not pucker itself. The same series of events occur in healing of epithelial wounds. Owing to the strong adhesion of the cells they behave as though their gel layers were continuous.

Holtfreter's explanation of wound constriction is quite different from mine. He states ('43a), "Everywhere the gliding movements of the individual cells were evidently directed by a common centripetal force represented by the expansion of the syncytial surface layer"; "That the stretching of the dye marks or of cells in the circumference of a wound is really due to active spreading of the coat and not, as might be assumed at a first glance, to being stretched by an actively constricting wound edge....." Since the gel layers of undivided eggs and adherent outer gel layers of late stages are always in a state of contractile tension they cannot at the same time indulge in active intrinsic expansion. Weaker parts can be expanded by outside forces against decreased contractile tension as when the ectoderm is expanded by internal pressure and by pulls of the contracting surface of the vegetal hemisphere.

The superficial gel layers of the surface cells are always in a state of contractile tension. Since the late blastula is approximately spherical the superficial end of each surface cell must be exerting the same amount of tension in all tangential directions otherwise owing to their firm adhesion to one another, a cell with a weaker gel layer would be stretched and a cell with a stronger gel layer would contract and pull or stretch neighboring cells toward the contracting end.

MECHANICS OF GASTRULATION

Three forces are responsible for the mechanics of gastrulation; (1) continuous contractile tension exerted by the gel layers of cells and local variations of it; (2) adhesiveness of cells, a surface tension force and variations of it and (3) hydrostatic pressure produced by cell secretions into archenteron.

As a result of increased contraction of the superficial ends of surface cells they are forced to elongate and/or migrate inward. This is active movement. As outer cell ends contract adherent neighboring and remote cells are pulled and stretched toward the contracting ends. This is passive movement.

Invaginations (Lewis '47) which are due to simultaneous increased contraction of the superficial ends of groups of adjacent adherent epithelial cells are active movements. They also exert pulls on neighboring cells and produce passive movements.

Hydrostatic pressure, produced in the archenteron cavity by secretions of its entodermal cells or the swelling of these secretions, is responsible for stretching the entoderm and for other passive movements. It will not be considered further.

Adhesion of cells is obviously essential for all these active and passive movements. As an active surface tension force it increases adherent surface areas of cells and forces them to flatten against one another thus producing active movements. Neighboring cells are passively pulled toward such areas.

In addition to the pasive movements due to pulls as cells ingress, invaginate and adhere, the contraction of vegetal hemisphere areas exerts increased pressure on the interior of the egg. This thrusts the yolk cell mass against the blastocoele and surface layers. The weakest part of the surface, the ectoderm, is stretched. The inner push is equal to the surface pull and plays an equally important part in epiboly. It is antagonized by the contractile tension of the ectoderm.

The usual maps of the presumptive areas at the beginning of gastrulation show entoderm, mesoderm, chordaderm and ectoderm. Differences of cell behavior in Amblystoma indicate that entoderm may be subdivided into large vegetal or polar, dorsal entoderm and ventral entoderm areas.

The complicated series of movements of the different areas have been traced by Vogt and others with the use of dye marks. All these movements result from the 3 forces involved. Their peculiarities depend on the many factors involving time and topography.

Although the different movements of gastrulation exhibit considerable independence they are evidently coordinated in time. The coordinating factor is probably cytochemical differentiation which proceeds steadily throughout gastrulation, at different rates in different types of cells. As cells differentiate one reaction of cytoplasm to external egg environment is increased contractile tension of the

superficial ends of surface cells. This forces them to elongate inward and in many cases to migrate from the surface.

Vegetal area cells are the first ones to show increased contractile tension of the gel layers on their superficial ends. These large gray cells are forced to elongate inward into long necked bottle cells. Many migrate from the surface. Neighboring and remote cells are pulled toward this contracting area.

While this is in progress small dorsal entoderm cells begin to ingress over an arc of 180°, into bottle cells which do not become detached from the surface. As more cells are involved a mixed ingression and invagination form a long shallow blastopore. Some neighboring vegetal cells are usually involved to form the floor of the shallow blastopore pit. The length of the blastopore is greatly reduced as vegetal and dorsal entoderm areas are reduced. Neighboring and remote cells are pulled toward this contracting area.

Meantime ventral entoderm cells elongate inward a little. This reduces the area as it is pulled toward the vegetal pole and floor of the blastopore pit (archenteron). The active decrease of this surface area pulls neighboring and remote cells toward it.

After all dorsal entoderm has ingressed and invaginated, the now smooth dorsal lip consists of chordaderm. This area has been converging and elongating. Continuation of this process is partly responsible for dorsal lip advance and involution of notochord around its edge. As dorsal lip advances over ventral entoderm archenteron is elongated posteriorly. The respective roles of the gel layer contractions and adhesiveness are uncertain. Schechtman ('42) has shown that chordaderm possesses the property of intrinsic elongation.

Mesoderm cells begin to migrate from the surface at the time when dorsal lip begins to advance. The superficial ends of the cells at and near the meso-endodermal border contract and force the cells to elongate into bottle cells and then to migrate from the surface. As this process progresses around the meso-entodermal border a constriction circle is formed which involves outer wall of dorsal lip (notochord). Its contracting constriction probably plays a role in dorsal lip advance and constriction of the blastopore. As mesoderm cells ingress neighboring and remote cells (ectoderm) are pulled toward the mesoentodermal border. After all mesoderm cells have ingressed and chordaderm has involuted the blastopore is constricted to a slit by contraction of the mesoderm constriction circle.

SUMMARY

Every cell and egg has a superficial gel layer and less viscous endoplasm. They are different states of the same cytoplasm. Gel layers always exert contractile tension. This is the motive force for ameboid locomotion, for blastodisk formation and gastrulation (epiboly) of fish eggs and for most movements of amphibian gastrulation. Movements occur when one part of the gel layer increases or decreases in viscosity (contractility).

Adhesion of cells to one another is essential for the active movements of locomotion and invagination of cells during gastrulation and for all passive movements due to pulls and pushes which result from the active movements.

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