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Cell Guidance by Alterations in Monomolecular Films

Abstract. *Monolayers of stearic and behenic acids were transferred to quartz slides by the Blodgett technique. Troughs of different depths were cut into these multilayers (substrata), and additional monolayers were superimposed. When cultured and fresh embryonic cells were grown on these substrata, the cells were entrapped within troughs whose depths were as small as 60 angstroms. The results demonstrate cellular responses to molecular changes in contact surfaces.*

The classical experiments of Harrison (*1*) were among the earliest demonstrations of cell guidance. He showed that cells in tissue culture would follow the threads of a spider web. Weiss (*2*) found that spindle cells in a blood plasma clot would orient in the direction of the lines of tension of the coagulum. He suggested that the guiding cue consisted of submicroscopic oriented aggregations of fibrin molecules. Since that time Weiss and coworkers (see *3*) have carried out a systematic study of the effects of physical configuration of the contact substratum on the morphology and behavior of cells. He has grouped these phenomena under the conceptual heading "contact guidance."

The research data and analysis presented below resulted from experiments designed to elucidate this phenomenon, especially the role of molecular or submicroscopic mechanisms. The experiments were suggested by my earlier work (*4*) regarding the interactions between tissue culture cells and multimolecular layers of fatty acids, wherein it was found that the rates of cellular attachment and spreading are functions of the number of monolayers underlying the cells. In these earlier experiments, monolayers of barium stearate–stearic acid were transferred by the Blodgett technique (*5*) to quartz slides from a Langmuir trough such that nonpolar methyl groups faced the cell surface. Increments in thickness of these substrata could be made as small as 50 Å. In general, the time necessary for attachment and spreading of cells was increasingly lengthened as the number of subjacent monomolecular layers was increased. Similar results have since been obtained for behenic acid–barium behenate multilayers.

From these results it was predicted that if a random population of cells were grown on substrata of varying thickness a statistically significant sample should migrate to and be entrapped in the nether regions. To test this prediction a predetermined number of monolayers both of stearic and behenic acid was transferred to quartz base slides. Troughs 10 to 100 μ wide were cut into the layers and additional monolayers were superimposed. The resulting substrata had troughs 25 to 1000 Å deep, whose floors were 25 to several thousand angstroms distant from the quartz slide. Single cell suspensions were randomly dispersed on these hydrophobic surfaces. The cells then attached, spread and migrated in a random manner on these surfaces. Figure 1 illustrates the entrapment of these cells in a groove in the form of a cross, roughly 180 Å deep, in a multimolecular film of behenic acid. Cells have been entrapped in similar depressions only 60 Å deep.

This marked sensitivity of cells to alterations in substrata led to some further studies. Dissociated cells were grown for 10 hours in standard culture medium on grooved substrata of barium stearate–stearic acid. The distances to the top and bottom of the troughs or grooves were varied to determine the physical conditions under which alignment similar to that shown in Fig. 1 could be observed. Figure 2 summarizes the results for three cell types, a tissue culture strain of human conjunctiva cells, fresh embryonic chick heart (10 to 11 days) cells, and fresh embryonic chick lung (10 to 11 days) cells. The distance to the top of the trough is plotted as the ordinate, and the distance to the bottom as the abscissa.

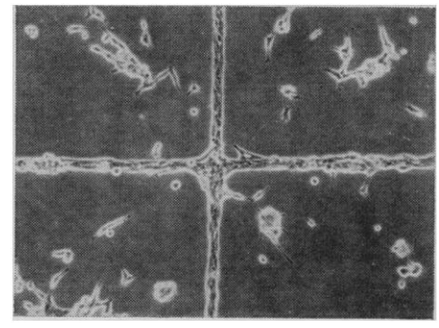

Fig. 1. Alignment of tissue culture cells within a trough cut in multilayers of behenic acid.

When the distance to the top and bottom of the trough satisfied conditions indicated by coordinates to the left of the straight-line graph, cell alignment was observed; otherwise, no entrapment or alignment occurred. As shown in Fig. 2, the depth of the trough must be increased as the height of its floor is increased. In addition, the results are suggestive of differential sensitivities of cells of different origins.

Under conditions favorable for the differential response of cells, the trough serves as a trap. Cells adhere and spread more rapidly within the trough and accommodate to its dimensions. In narrow troughs, spreading is polarized and individual cells demarcate the region by lengthwise alignment and

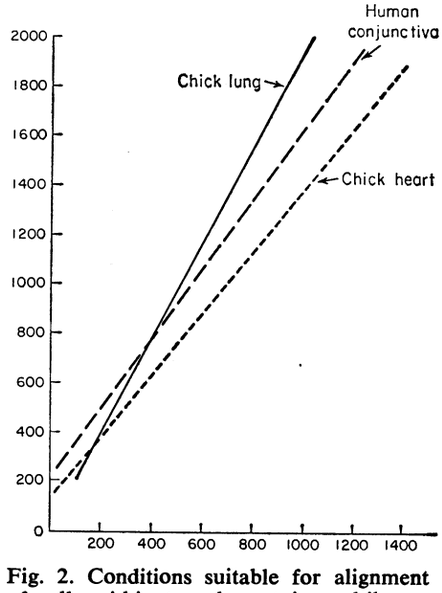

Fig. 2. Conditions suitable for alignment of cells within troughs cut in multilayers of stearic acid. *Abscissa*: Distance in angstroms from surface of slide to bottom of trough. *Ordinate*: Distance in angstroms from surface of slide to top of trough.

orientation. In wide troughs, spreading is symmetrical, except for a bias at the edges, and the trough is demarcated by the selective accumulation of groups of cells.

In the case of substrata composed of stearic acid–barium stearate, the interaction between serum components, especially albumin fractions, skeletonizes the multilayers by the selective solubilization of stearic acid molecules residing in the upper five to ten layers. This "ripping off" reaction has been described by Sher and Sobotka (*6*). No such reaction occurs when behenic acid–barium behenate is used as the substratum. Consistent with the absence of this reaction for behenic acid is the observation that the minimum trough depth for alignment was 60 Å in behenic acid multilayers and 200 Å in stearic acid multilayers.

The experiments point to the change in physical thickness of the multimolecular films and concomitant alterations in the adhesivity and spreading rates as the principal factor underlying this phenomenon. Similar phenomena should be observed when cells contact any surface whose molecular composition in either a spatial or temporal sense provides regions of variegated cell adhesivity. Thus cells are trapped within troughs even on chrome-plated glass if the floor of the trough reaches down to the glass, to which cells adhere more readily.

The mechanism whereby cells sense alterations in their substrata as small as 60 Å remains obscure. Though the diameter of the cells studied was roughly 200,000 Å, many cell types are known to possess surface projections or "microfibrils" roughly 1000 Å in diameter (*7*). These or similar microprotrusions may serve as sensing elements capable of responding to small changes in the physical structure of molecular carpets in contact with cells. Additional experiments are necessary to determine their function. However, regardless of the mechanism underlying this highly sensitive interaction between cell and substratum, it has been demonstrated that the molecular composition of surfaces can play a salient role in directed cell contact, aggregation, and movement (*8*).

MURRAY D. ROSENBERG
Rockefeller Institute, New York 21

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Selective Sensitivity to Direction of Movement in Ganglion Cells of the Rabbit Retina

Abstract. *Among the ganglion cells in the rabbit's retina there is a class that responds to movement of a stimulus in one direction, and does not respond to movement in the opposite direction. The same directional selectivity holds over the whole receptive field of one such cell, but the selected direction differs in different cells. The discharge is almost uninfluenced by the intensity of the stimulus spot, and the response occurs for the same direction of movement when a black spot is substituted for a light spot.*

The great sensitivity of retinal ganglion cells to movement of a pattern of light over the retina has been recognized since Hartline's work on the frog (*1*). Hubel and Wiesel presented evidence that certain cells in the cat's cortex respond, not to any movement, but only to movement in a particular direction (*2*). Reports of similar directional selectivity have been made on the retina and optic tectum of frog (*3*), on the cortex of cat (*4*), on the lateral geniculate of rabbit (*5*), and most recently on the retina of pigeon (*6*) and the tectum of rabbit (*7*). The extraction of information as to direction of motion is a surprisingly complex task for a few synaptic layers to perform, and some doubt remained in our minds as to whether a simpler explanation of apparent directional selectivity had been adequately excluded.

Hartline showed that "off" units in the frog responded to any diminution in the total contribution from all parts of the receptive field to the ganglion cell (*1*). As well as responding to dimming of a uniform light, they also responded to movement of a spot of light away from the most sensitive central zone of the receptive field. In such a unit, if a stimulating spot is moved to and fro over the edge of the receptive field one may easily obtain records showing a discharge to movement in one direction, and not in the opposite direction. It would be misleading, however, to call this directional selectivity, for if other regions of the receptive field are explored the direction of movement giving the maximum discharge will not be constant; it will always tend to lie on a line away from the center of the receptive field. On the other hand, a unit showing true selectivity for direction should show the same direction of preference in all parts of its receptive field. A similar argument applies to contrast; a unit which is genuinely selecting out direction of motion should show the same preference regardless of contrast, whereas Hartline showed that the frog's "off" units discharged when a shadow moved towards the center of the receptive field, not away from it as with bright stimulus spots.

The receptive fields of most of the cells for which directional sensitivity has been reported are more complex than those of the "off" units investigated by Hartline, and we originally felt that an explanation in terms of a change in the pooled excitatory and inhibitory contributions from all parts of the receptive field had not been excluded except for certain cortical neurones (*2*). However, we have found that about one-third of the units isolated in the rabbit's retina show a movement sensitivity in which the direction of preferential response is invariant in different parts of the receptive field, and is invariant for changes in contrast. We think this excludes simple explanations of the type outlined above, and shows that retinal units can be genuinely directionally selective.

Single retinal units were isolated by a technique similar to that of Kuffler (*8*). The rabbits were lightly anesthetized with urethane, or in some cases decerebrated under ether. Figure 1 shows the response of a unit to movement of a spot of light all the way across its receptive field. It is clear that movement in a posterior-anterior direction evokes a vigorous discharge, whereas movement from anterior to posterior evokes none. We were sure