THE RELATIVE EXTENSIBILITY OF CELL SURFACES

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ABSTRACT

Observations have been made on the response, *in vitro*, of cultured and freshly dissociated cells to mechanical deformation. Large numbers of individual cells were studied by means of a special culture chamber bounded by two parallel glass coverslips whose spacing could be reduced from 140 to 2 microns in steps of roughly 0.5 micron. The degree of deformation required for herniation of the cell surface was measured. These measurements lead to the definition of a statistical index characteristic of the extensibility of cell surfaces. This index has been shown to be distinctive for several types of cells; to alter with certain stages of embryonic development; and to be stable with respect to the culturing of cells and certain alterations in the method of cell culture.

INTRODUCTION

The technique of cell distortion has been used by investigators for varying reasons. Driesch (1891) was one of the earliest when he observed that sea urchin eggs compressed between glass plates could deform plastically with little development of internal stresses. Danielli (1952) studied the division of flattened eggs, and Rappaport (1960) by hanging small glass beads from cells studied cleavage in sand dollar eggs distorted by a constant tensile stress. In a different vein, Goldacre, Easty, and Ambrose (1957), and Davies, Wilkins, and Boddy (1954) used cell distortion to improve measurements in interference microscopy and microspectrophotometry. Ashton (1959) used a modified Du Nouy tensiometer for the study of the surface precipitation reaction in sea urchin eggs and ameba. The measurements presented below demonstrate how cell distortion can be used also to obtain a statistical index characteristic of the relative extensibility of cell surfaces. A fairly simple and serviceable technique is described for studying the reactions of cells to mechanical constraint and distortion.

MATERIAL AND METHODS

Cells for these experiments were obtained from stock cultures of human conjunctiva strains or from freshly dissociated tissues of chick embryos. The stock culture monolayers were detached by enzymatic treatment with trypsin or by removal of divalent cations with sodium versenate at 37°C for 3 to 5 minutes. The trypsinized cells were centrifuged at 500 RPM for 5 minutes and resuspended in various growth media. Cells treated with versene did not require centrifugation since the chelating agent could be removed directly. The fresh embryonic tissue was dissociated by treatment with 1 per cent trypsin-pancreatin for 15 to 30 minutes at 37°C.

The test chamber, illustrated in Fig. 1, consists of a lower tissue or cell compartment (*l.c.*) and an upper air compartment (*u.c.*). The lower one (*l.c.*) is formed by two parallel coverglasses (*a* and *b*) separated by a distance of 90 to 140 microns and can be perfused with liquids or gases via two communication channels. The upper compartment (*u.c.*) is formed by the central coverglass (*b*) and a heavier parallel glass slide (*c*). Its internal air pressure is controlled accurately *via* a single channel.

The operation of the test chamber is as follows.

The suspension of cells under investigation is placed in the cell chamber either on the central (*b*) or lower coverglass (*a*). The compartment (*l.c.*) is maintained at atmospheric pressure while being perfused. The air pressure within the upper chamber (*u.c.*) is gradually varied so as to bend the central coverglass (*b*). Since glass bends in accordance with the displacement equations for a plate rather than a membrane, fairly large pressure increases are needed to produce small displacements, as shown by the experimental displacement-pressure curves in Fig. 2. In this manner, the center of the central coverglass can be displaced a distance of 150 microns in 0.5 micron steps. Reduced pressure in the tissue compartment will have similar effects, except that the cellular environs will no longer be at atmospheric pressure. In these ways, it is possible to flatten at various rates fairly large populations of cells and to assess the effects of this physical change. The technique has the advantage of being applicable to the study of cells whose diameters vary from but a few to over 100 microns, as well as being useful for several significant measurements and observations, such as inhomogeneities of cell surfaces, surface precipitation reactions, and determination of the relative extensibility of cell surfaces.

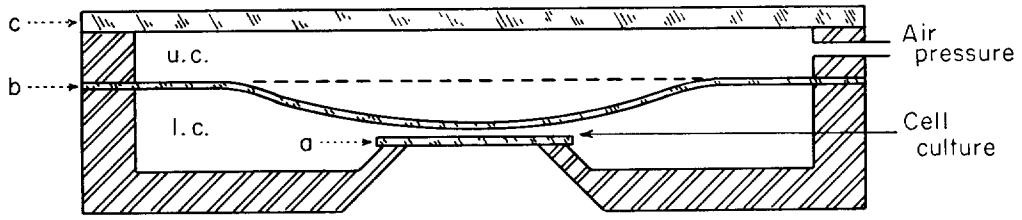

FIGURE 1

Test chamber for distortion of cells.

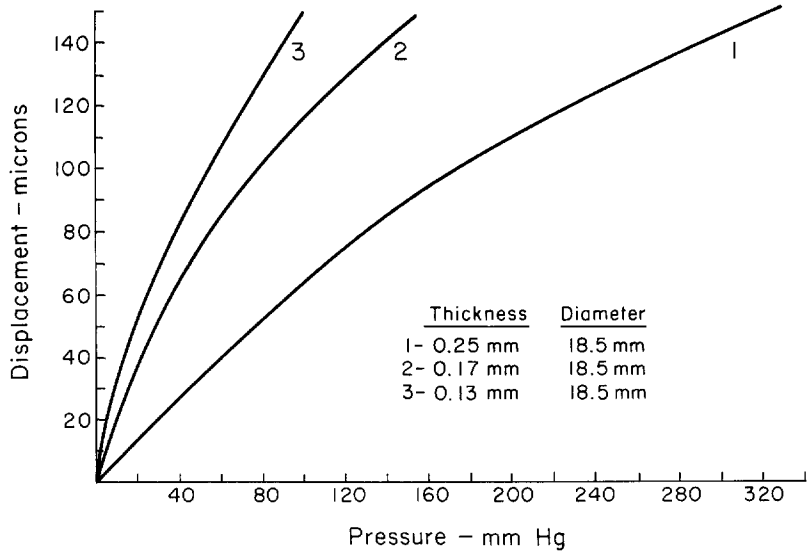

FIGURE 2

Pressure-displacement curves for coverglasses, clamped edge.

To determine the relative extensibility, recently dissociated cells were allowed to attach to a glass substratum for 15 to 30 minutes. During this brief period the cells do not spread but maintain their spherical shape as shown in Fig. 3 *a*. The diameter of the spherical cell is measured. Upon application of a distorting force at a fairly constant rate, the cells assume the shape of oblate spheroids (Fig. 3 *b*), and

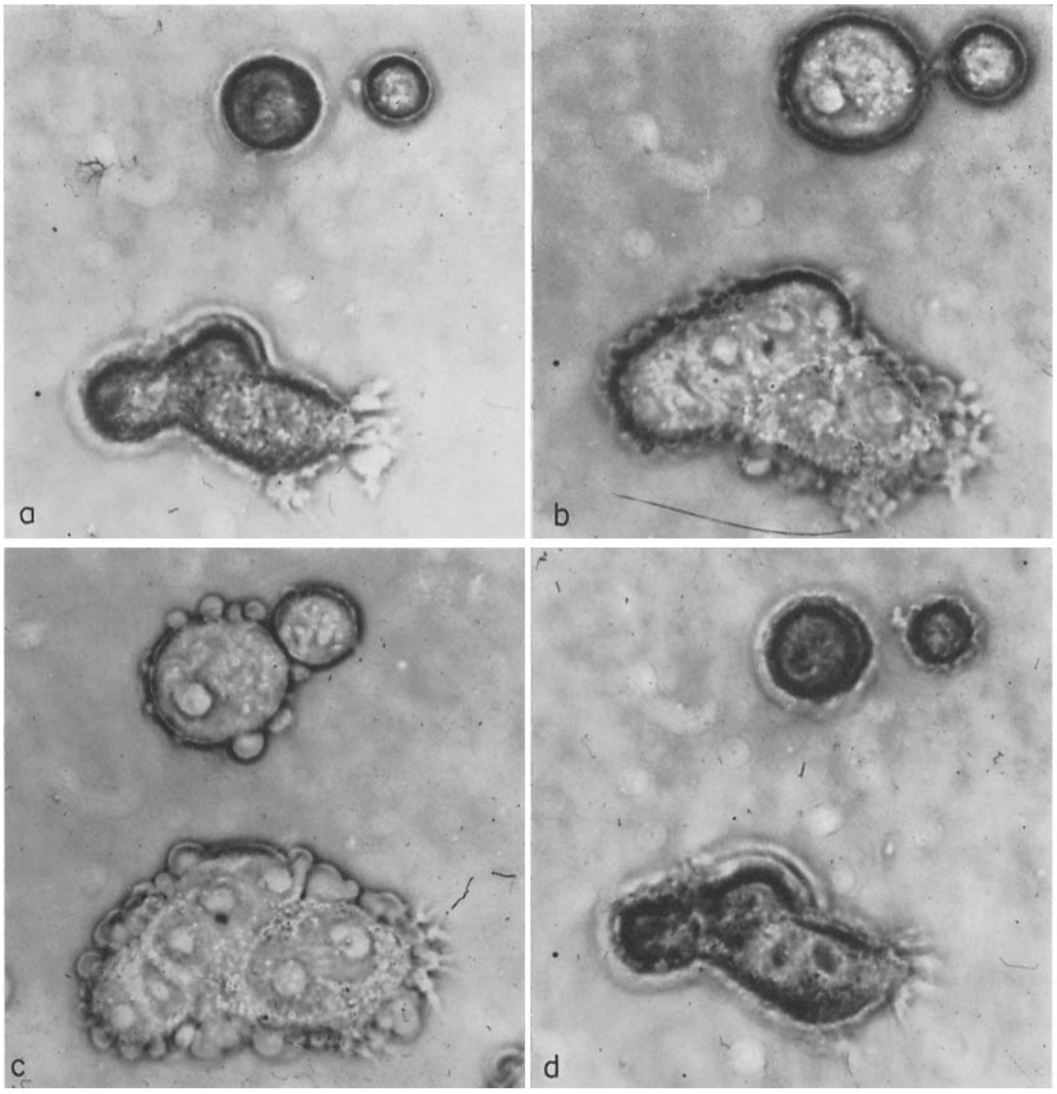

FIGURE 3

Changes in cell shape with distortion: *a*. Spherical cell; *b*. Mild distortion; *c*. Appearance of herniations; *d*. Five minutes after release of the distorting force. × 750.

with increasing distortion, herniations of the cell surface appear (Fig. 3 *c*). The diameter of the cell is again measured upon first appearance of a protrusion, and on the assumption that the distorted cell is spheroidal in shape, it is possible to calculate the curvature and area of the surfaces. The appearance of herniations is a reversible phenomenon, and within 5 minutes after removal of the distorting force the cell again assumes the normal appearance shown in Fig. 3 *d*.

MEASUREMENTS

1. *Extensibility as a Function of Cell Type*

Measurements were made on three distinct cell populations: fresh chick embryo heart cells, fresh chick embryo liver cells, and a tissue culture strain originally derived from human conjunctiva. The homogeneity of each cell group was determined with respect to cell size shortly after dissociation

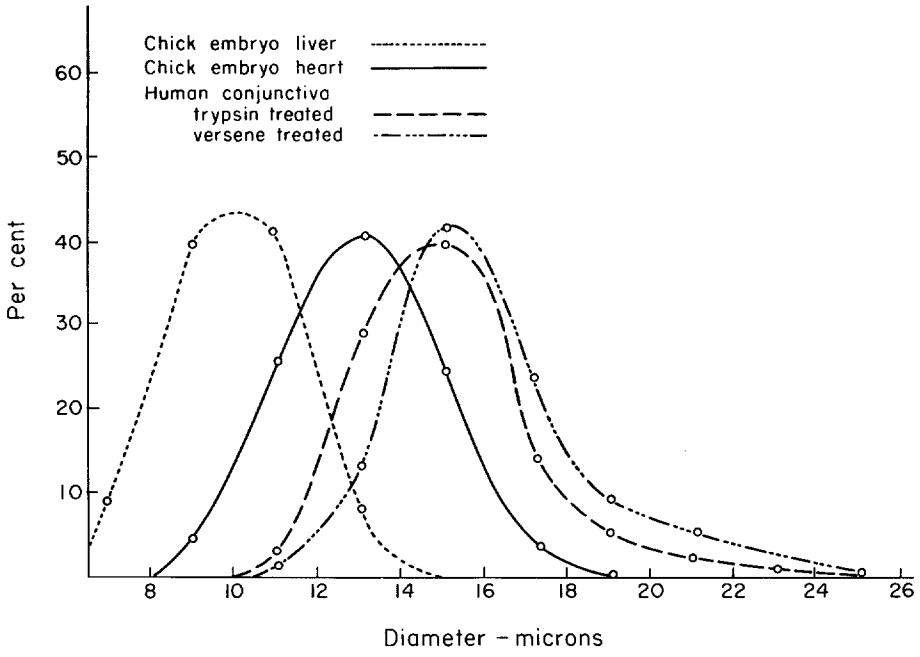

FIGURE 4

Initial distribution curves of cell diameters for various cell types.

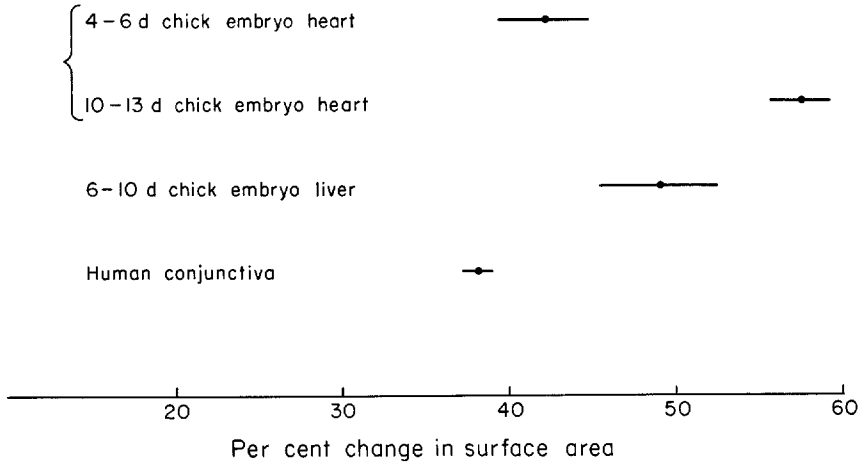

FIGURE 5

Relative index of surface extensibility plotted as per cent change in surface area for different cell types.

of the tissue or culture. The size-distribution curves are shown in Fig. 4. The mean diameter of each cell type within one standard deviation is as follows: chick embryo liver cells, 10.0 ± 1.6 microns; chick embryo heart cells, 13.1 ± 1.9 microns; trypsin-treated human conjunctiva cells, 15.2 ± 2.4 microns; versene-treated human conjunctiva cells 16.3 ± 2.8 microns. The initial diameter and volume of each cell type followed a normal distribution.

The extensibilities of the surfaces, plotted as the average per cent change in surface area required

to produce the first observable herniation, are illustrated for these cell types in Fig. 5. The 95 per cent confidence limits, a more stringent statistical criterion than standard error, are indicated for each mean value. Data are presented for 4 to 6 day chick embryo heart, 10 to 13 day chick embryo heart, 6 to 10 day chick embryo liver, and human conjunctiva cells. All mean values differ significantly. The older heart cells withstand an average increase in surface area in excess of 55 per cent in comparison with the average increase of 38 per cent obtained for the cultured human conjunctiva cells. The older heart cells withstood much larger

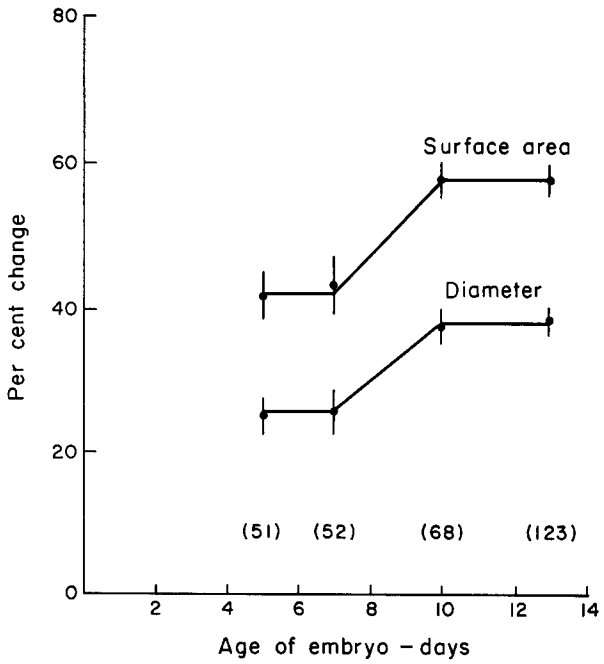

FIGURE 6

Relative index of surface extensibility as a function of age of embryo: chick embryo heart cells. Number of measurements in parenthesis.

increases in surface area than the younger ones, suggesting a possible change in surface structure with development of the embryo.

2. *Time Course of Extensibility with Embryonic Development*

Changes with the age of the chick embryo were studied in further detail, and the results are illustrated in Figs. 6 and 7. Indexes of the surface extensibility are presented for fresh chick embryo heart cells obtained from 5, 7, 10, and 13 day embryos and for fresh chick embryo liver cells obtained from 6 and 10 day embryos. An abrupt increase in surface extensibility of the heart cells was found to occur between the 7th and 10th days of embryonic development. During a similar period, the surface extensibility of the liver cells remains relatively constant. Developmentally, the only gross change in the heart during this period is the appearance of septa that delineate the cardiac chambers.

3. *Time Course of Extensibility with in vitro Culture*

There is considerable evidence that the conditions of tissue culture either preselect certain cell strains from a given population of fresh tissue or that the cells lose irreversibly or reversibly many original characteristics and specializations in adapting to an artificial environment. As an additional test of these proposals, heart cells obtained from chick embryos at different stages of development were maintained in tissue culture for 2 to 3 weeks and the surface extensibility measured as a function of the age of the culture. During the entire period the microscopic appearance of the cells was unchanged. The cells kept their striking spindle shape and several continued to beat during the first 10 days. The indexes of surface extensibility are shown in Fig. 8, where mean values and 95 per cent confidence limits are plotted. The val-

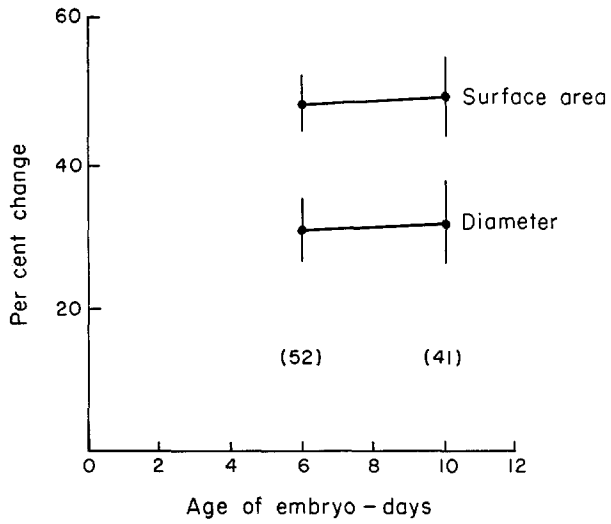

FIGURE 7

Relative index of surface extensibility as a function of age of embryo: chick embryo liver cells. Number of measurements in parenthesis.

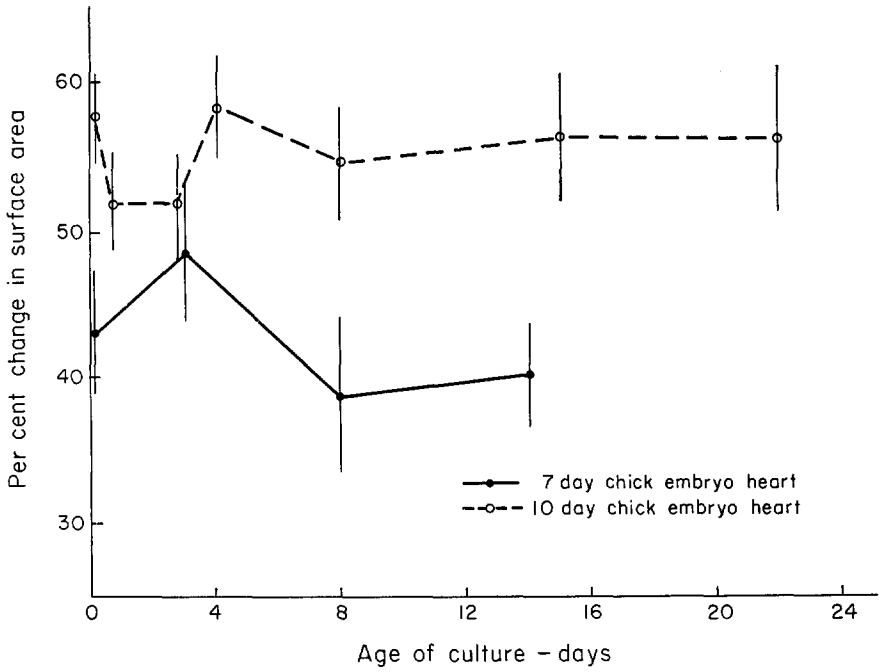

FIGURE 8

Relative index of surface extensibility as a function of age of culture: chick embryo heart cells.

ues were highly erratic during the first 4 days of culture, but later exhibited far greater stability. During the later stable period, the extensibility did not differ significantly from that obtained for the cells dissociated from fresh tissue.

4. *Extensibility as a Function of Cell Treatments*

Cells dissociated by enzymatic digestion of cell-substratum and intercellular bonds have been

known to differ in several characteristics, such as dry weight and exudate production, from cells which could be dissociated by chelating agents or gentle mechanical agitation. Monolayer cultures of human conjunctiva cells were dispersed in several ways and suspended in various media to learn whether differences could be detected in the indexes for surface extensibility. The results are illustrated in Fig. 9. Dissociation by 0.05 to 1.0 per cent trypsin or 0.05 per cent versene produced no significant differences in these indexes. Similarly, the presence or absence of either calcium or serum resulted in no appreciable statistical differences. In all these experiments, the indexes were relatively constant.

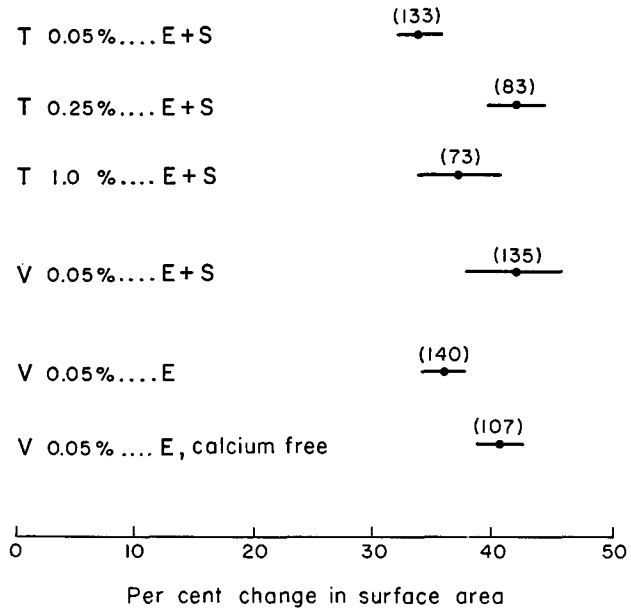

FIGURE 9

Relative index of surface extensibility as a function of cell treatment or absence of calcium or serum from medium. Number of measurements in parenthesis.

DISCUSSION

Cells proliferating, developing, or in a resting state *in vivo* or *in vitro* are normally subject to several stresses and forces of distortion. The inner cells of a group or organ system can be distorted by the outer layers of cells or the presence of a capsular membrane. Contractile tissues distort adjacent cells. Cells transported *via* blood or lymphatic vessels of small bore often undergo distortion. Zeidman (1961) has shown that the fate of circulating tumor cells is, in part, determined by the flexibility of their surfaces. Such phenomena are common occurrences, and the ability of cells to withstand mechanical distortion is of considerable importance.

The observations described in this report bring out several points of interest. As discussed above in the section "Materials and Methods," herniations resulting from the distortion of cells recede quite rapidly with decompression of the cell. The cell reassumes a normal appearance, as observed by phase microscopy, and can migrate and contact other cells in an unmodified manner. Except for two experiments, cells later underwent unaltered mitosis. In the exceptional cases, mitosis was arrested during telophase when full cleavage did not take place.

Excessive or prolonged distortion often resulted in the death of the cell. Many cells, however, seemed to adapt to prolonged deformation. The herniations receded within an interval of roughly 30 minutes, and the surface contour once again appeared smooth. These cells were so confined that they could not round up to commence mitosis and died in prophase.

When multiple herniations appeared, the effect of one upon another could be readily observed. Numerous demonstrations indicated that the initiation and enlargement of one herniation did not prevent the development of others. At first glance, this observation appears to be contrary to that of Weiss and Garber (1952). These investi-

gators made quantitative studies on the shape transformation of cell bodies in response to the texture of plasma clots, and observed that the emergence of an advancing process tended to prevent the emergence of another process around its base by competitive drainage. The experimental situation of Weiss and Garber, however, differs from the one described in this paper. Their experiments focused on the competitive interaction between cell and environment, and the emergence of pseudopodia was a slow equilibrated process in part directed by the inhomogeneous structure of the medium. In my experiments, this interaction has been greatly reduced and the herniations emerge very rapidly without time for the gradual internal equilibration of disturbances to the cell membrane or underlying ectoplasm.

Though the state or physical properties of the surface seem to be variable spatially and temporally, this variation is within sufficiently confined limits that a meaningful statistical index, characteristic of a cell population, can be determined. Statistically significant differences have been obtained among fresh embryonic chick liver and heart cells and a tissue culture strain of human conjunctiva cells. The heart cells of the chick embryo exhibit a striking increase in extensibility between the 7th and 10th days of embryonic development. No such change was noted for the liver cells of the embryo. In addition, measurement of this variable has proved useful in assessing the effects of *in vitro* growth conditions. There is considerable evidence that the conditions of tissue culture either preselect certain cell strains from freshly dissociated tissue or there is a "dedifferentiation or modulation" of cells with subsequent loss or lack of expression of certain characteristics. When embryonic heart cells were maintained *in vitro* for several weeks, no striking changes in microscopic morphology were observed, although during the first 4 days of culture the relative extensibility was observed to be erratic, after which stable determinations were found. The time course for fluctuations is similar to that observed by other investigators such as Holtzer, Abbott, Lash, and Holtzer (1960) in their studies on the changes in chondrocytes under tissue culture conditions. As regards the index of extensibility, there is no evidence for preselection or modulation since the final values do not differ from the initial ones. Moreover, the cultured strains of heart cells and of human conjunctiva cells still differ significantly in their relative indices (Figs. 5 and 8). It is difficult to ascertain why the surface extensibility of cells should fluctuate during this period of adaptation and whether the fluctuations result primarily from changes in the membrane or in underlying structures.

An additional point of interest was the heterogeneous structure and activities of the cell surface. These measurements are in line with studies demonstrating, by means of light and electron microscopy and biochemical methods, that parts of the surfaces of some cells appear differentiated. Fawcett (1958) summarized much of the data on morphological specializations such as microvilli, desmosomes, and other interdigitations or contact regions. Danielli (1941) found that a small fraction of the membranes of certain cells may be more permeable and suggested that in such regions the bimolecular lipid film was exposed. Rothstein (1954) demonstrated that specialized enzymatic activities may be limited to certain regions of the cell surface. The numerous observations on the location and development of herniations when cells were distorted point not only to spatial heterogeneities in the extensibility of the cell surface, but also to a temporal factor. The location of herniations was unpredictable. This unpredictability continued even when the surface response of a cell was studied by means of successive applications and releases of the deforming force. The extensibility thus varied over the surface of the cell and its distribution changed with time.

The measurements of relative extensibility are useful in comparing cell populations or the effects of time or physical and chemical agents on a population. Measurements of surface deformation in response to a measurable force have been limited to more manageable preparations such as individual marine eggs of fairly large diameters. Cole (1932) deformed marine eggs with a small gold beam, and Harvey (1931) obtained similar data by means of a centrifuge microscope. Their early experiments resulted in values for the so called "surface tension" or "tension at the surface" of 0.08 to 0.2 dynes/cm. More detailed summaries of their work can be found in reviews by Harvey and Danielli (1938) and Harvey (1954). More recently Mitchison and Swann (1954) devised the "cell elastimeter" consisting of a pipette roughly 50 microns in bore and connected to an accurate control for pressure reduction. Upon application of the pipette to the surface of the sea urchin eggs,

small bulges or protrusions were formed and tension-displacement curves obtained. On the basis of such curves, they concluded that the cell membrane undergoes deformation in a manner similar to that of a sphere with a thick wall, possessing both stiffness and internal tension. These experiments together with others by Mitchison (1956) led to the concept of a "surface in depth," that is, a surface whose mechanical properties are determined by not only the cell membrane, 70 to 120 Angstroms thick, but also the contiguous ectoplasmic gel, 1.0 to 1.5 microns thick. Estimates for Young's modulus ranged from 1×10^4 to 2×10^4 dynes/cm^2 with a resting pressure within the egg ranging from 0 to 95 dynes/cm^2. It is difficult to ascertain from these data whether these values were variable with respect to time or location on the surface of the egg.

The technique and measurements described in this paper demonstrate the feasibility of defining an index that provides a predictable statistical value representative of a variety of cell types. Measurements have been made on large populations of individual cells under controlled environmental conditions. The method can be used to determine whether the frequency of herniations is in any way specific for cell types. These data were not assembled for the present study. The technique described may likewise prove useful in determining alterations in surface extensibility associated with cell-to-cell and cell-to-substratum interactions. For these studies careful control of substratum structure will be required. In this manner it may be possible to obtain additional information regarding the interdependencies of these highly complex systems.

The physical-chemical alterations associated with the formation of herniations in deformed cells remain obscure. Mechanical distortion brings about an increase in surface area and results in a complex strain pattern throughout the cell. One cannot state whether a herniation represents the flow of cytoplasmic contents into an unfolding region of membrane or flow through a disruption in the continuity of the membrane, or whether the cause lies in expansion of subjacent ectoplasmic gel. All that can be said at present is that the surface herniations resulting from mechanical distortion can be looked upon as statistical indices of the intactness of heterogeneous cell surfaces, where the cell surface is considered as being composed of both the outer membrane and its underlying ectoplasm.

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