**More About Cell Sizes: Volumes and Lengths. Nov. 11, 2016**

I) Tissue culture cells shrink if you put them in osmotically hyper-tonic medium.

Their volume can be reduced by as much as half. This also freezes cell locomotion and cell surface protrusions, within a second. Dissolving the sugar sorbitol in tissue culture medium is an easy and non-toxic method for increasing osmotic pressure.

II) If you dilute their culture medium with water, tissue culture cells expand in volume (and will burst in a few seconds if the osmotic pressure of their medium is reduced too much: by 25% or more).

Smaller reductions in osmotic pressure cause plasma membranes to bulge outward in many small hemispherical "**blebs**" (3 to 10 micrometers in diameter). Most of these form near the edges of tissue culture cells, wherever the "front" edges of crawling cells had been. I think this suggests that physical support of the plasma membrane by the cytoskeleton is weaker in the areas where blebs form, and that this weaker connection may have caused these areas to become the front of each cell.

III) Blebs inflate in 10 to 20 seconds, but retract much more slowly, over periods of minutes. Someone should make more careful measurements of these times. During each bleb's formation, the plasma membrane of its outer surface is smoothly hemispherical.

Their surfaces then slowly wrinkle and retract over time periods of a few minutes. During wrinkling and retraction, many blebs move rearward or centripetally away from the edge of their cell and back toward the nucleus.

**Questions to which I don't know the answers:**

a) Do plasma membranes stretch when blebs form?

b) Or is more membrane added or assembled at the places where blebs form?

c) What about physical links between plasma membranes and the cytoskeleton?

Do these links break when blebs form? And vice-versa?

d) Are these links weaker at the leading edges of cells?

e) Is weakening of linkages how protrusions are caused to be concentrated where they are?

f) Neither red or white blood cells form blebs, even when the osmotic pressure of their medium is reduced.

IV) During mitosis, tissue culture cells become spherical, form many blebs, and their plasma membranes get stretched out as long narrow tubes. These tubes are called **retraction fibers**, and they spring back if cut or broken. They are often mistaken for **filopodia**, which are stiff and get rigidly poked out from cell surfaces.

V) When mitosis is completed, cytoplasm flows back into retraction fibers, thereby increasing their diameters. Dozens of scientists have published papers that misinterpret this re-inflation as being the protrusion of filopodia.

VI) If you put any kind of tissue culture cells on a teflon surface, or on any other material to which they do not adhere, which includes agar, they will remain spherical.

Cancerous cells, such as HeLa cells, will grow and divide on such non-adhesive surfaces, and will also grow and divide if suspended within an agar gel.

Non-cancerous cells, such as those cultured from an embryo will not grow (or even survive very long) if located on any non-adhesive surface, nor if suspended in an agar gel. This phenomenon is called "**anchorage dependence**", and is one of the most reliable differences between cancerous and non-cancerous cells. It was discovered "by accident" (by an alert scientist with a well developed curiosity).

VII) If tiny beads, glass rods or spots of metal coating are put on or in teflon sheets or agar gels for cells to attach and spread upon, then non-cancerous cells become able to survive, grow and divide without apparent upper limit. (Forming balls of hundreds of cells around each solid "anchor"). The mechanism isn't known.

There is a surprisingly sharp size threshold in the minimum length of solid anchors sufficient to allow cells to grow and divide. This threshold length is about the same as the length of an individual cell, when fully spread and flattened on a glass surface. What matters is the maximum distance from on end to the other. Donald Ingber did an ingenious experiment in which he made tiny metal islands next to each other in pairs, and close enough for individual cells to bridge from one island to the other. He discovered that distances between these pairs of metal islands determines whether they support growth and survival of non-cancerous cells.

VIII) **Cytochalasins** are a useful family of chemical toxins isolated from fungi that block cell division (cytokinesis) of all kinds of tissue culture cells, whether cancerous or not. In some never-published experiments, I tried to use Cytochalasin B to cause formation of giant tissue culture cells, simply by long term culture (for weeks) of cells in culture media containing a high enough concentration of cytochalasin to prevent any cell divisions.

I expected (and hoped) that each cell would continue to grow without limit, and continue to have mitotic divisions, but no cytokinesis. That would have produced giant syncytia, which did happen with some cancerous cells. Non-cancerous cells, however, stopped growing when they had reached about five times normal size (that's a very rough guess - lack of a suitable method for size measurement was the main reason I never published this work). They also stopped further mitoses. Please try to invent one or more hypotheses that might explain why some cells can grow without dividing, but others can't (or won't). Maybe the cause is related to anchorage dependence? Or to Contact Inhibition?

IX) **Cartilage cells** live embedded in special gels, whose function is to suck up water by a form of osmosis that does not involve semi-permeable membranes. The effect of this pressure is the same as if each cartilage cell were immersed in a hypertonic salt solution. The volumes of individual chondrocytes vary at least ten-fold from one part of a cartilage to another.

Many scientists believe (= assume) that the growth of cartilage is driven by mitosis and growth (enlargement) of cartilage cells. This pressure is supposedly strongest in the parts of growing cartilages where these cells are largest, and where these cells line up parallel to the direction of growth, mitosis and cytokinesis.

I suspect that theory is wrong: that the real expansion force is osmotic pressure, and that this pressure is strongest where the chondrocytes are smallest, weakest where they are biggest, and that the directionality of cartilage elongation is caused by weakening of resistance to osmotic pressure.

Please try to figure out how to test these alternative (and opposite) theories.