GRAVITY AND THE CELL: INTRACELLULAR STRUCTURES AND STOKES SEDIMENTATION

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ABSTRACT

Plant and certain animal embryos appear to be responsive to the gravity vector during early stages of development. The sensing of gravity of individual cells could be based upon convection of particle sedimentation. Various intracellular particles have been proposed as gravity sensors in the cells of developing plants, and the participation of amyloplasts and dictyosomes has been suggested but not proven. An exploration of the mammalian cell for sedimenting particles reveals that their existence is unlikely, especially in the presence of a network of microtubules and microfilaments considered to be responsible for intracellular organization. Destruction of these structures renders the cell susceptible to accelerations several times g. Large dense particles, such as chromosomes, nucleoli, and cytoplasmic organelles are acted upon by forces much larger than that due to gravity, and their positions in the cell appear to be insensitive to gravity.

INTRODUCTION

Space Biology Research was originally designed to answer the question, Is Space Safe?, and the next phase of research is designed around the use of the conditions of space flight as a biological research tool. The latter phase is designed to answer such questions as, Can We Learn Something of Fundamental Significance by Performing Experiments Under Space Flight Conditions and Obtain Biological Insights that Cannot be Acquired on the Ground? At the inception of space research some 20 years ago, there was concern in both the U.S. and the Soviet Union about the effects of weightlessness on living things. It needed to be known in particular whether the absence of gravity had no effect or a catastrophic effect on biological systems under space flight conditions. It was easy to solve problems introduced by the space environment by the use of engineering to protect against the lack of an atmosphere and the presence of radiation, but engineering against weightlessness and its possible biological effects proved to be extremely difficult. Fortunately, early experiments indicated that the biological effects of zero G was certainly not catastrophic and the 84-day Skylab mission suffered no catastrophes as a consequence of the absence of a gravitational field.

In view of the conclusion that the absence of gravity has no catastrophic effect on man in space, future research is directed at the basic study of what we presume to be gravity dependent environmental responses. In other words, space flight conditions are to be made available for basic science experiments. Due to volume limitations and other limitations on spacecraft, it is logical to begin with research at the cellular level.

Although we know of many biological phenomena affected by gravity, their connection to molecular and physical concepts are extremely poorly understood. In this sense, the effect of gravity is paradoxical because the cell is the basic structure of living things, and the organisms' properties depend upon cells. Yet it is much easier to think of gravity as acting on larger systems as cells are at the limit of size and mass which is influenced by the gravitational field.

DEVELOPING SYSTEMS

The effect of abnormal gravitational exposure upon embryonic development was noted during the previous century (1). The most remarkable gravity-dependent phenomena in

developmental biology include the obvious polarization of amphibian egg cell division at early stages and the reliable upward growth of coleoptiles and downward growth of roots in germinating plant seedlings. It should be no surprise that these phenomena have been the favorite subjects of investigations of the effects of gravity compensation and weightlessness (2,3).

Amphibian Embryos

The inversion of embryos of Rana sp. before they reach the 4-cell stage can lead to the formation of double embryos (1,4). Gravity compensation and centrifugation can lead, under appropriate conditions, to similar effects (4,5). Evidently, very soon after fertilization, events occur which orient the egg and establish the planes of further cell divisions and the ultimate symmetry of the organism. The gravity sensing mechanism in this system is thought to be associated with a density gradient in the materials of the yolk.

Attempts to induce developmental abnormalities in weightlessness during orbital flight of Rana eggs yielded negative results (6,7,8), presumably because exposure to weightlessness did not adequately coincide with the gravity-sensitive period of orientation or possibly because Rana pipiens, used in orbital experiments, is not as sensitive to orientation as Rana fusca, which was used in classical experiments (1). There was also no microscopic evidence for the redistribution of morphological structures during orbital weightlessness (8).

Plant Geotropism

Cytological studies on the distribution of amyloplasts in wheat seedlings flown on Biosatellite II led to the conclusion that these granules were distributed at random under weightlessness,as in seedlings grown on a clinostat, rather than being clumped on the lower cell wall as in erect control seedlings (9,10). Fixation experiments indicated that these plastids return to their normal position in the cell in less than 4 hr. These organelles were observed because they are thought by some (11,12), but not others (13) to play a role as "statoliths"--intracellular indicators of the gravity vector. The identification of "statoliths", however, depends on the ability of the plant physiologist to distinguish between cause and effect. It remains to be determined whether the elongation plant cell responds to sedimenting amyloplasts or positions its amyloplasts in response to a metabolic gradient formed by activities other than sedimentation.

Other plant cell organelles have been considered with respect to possible roles in geotropism. These include mitochondria (14) and the Golgi apparatus (15-18). The dictyosomes of the Golgi apparatus, despite their generally accepted relationship to internal membranes, appear to be positioned in a manner strongly related to the gravity vector (16,17). Whether they are serving as statolith or responding to metabolic gradients is unknown, but one might consider the following metabolic interrelationships as a testable alternative to the statolith theory: 1) Cell wall compression produces a membrane response. 2) This response consumes auxin. 3) Auxin is transported down its gradient. 4) Cell wall synthesis is stimulated. 5) New wall synthesis depletes Golgi products. 6) The cell produces more active dictyosomes. 7) Golgi forms in direction of the secretion (as in animal systems).

ORGANELLES IN MAMMALIAN CELLS

Animal cells differ explicitly from plant cells in their lack of a need to synthesize a cell wall in a particular direction. If plant cells need to respond to gravity for this purpose only, then one would not expect the intracellular activities of animal cells to be very responsive to gravity. An analysis of the constituents of the mammalian cell should indicate whether or not there exist any organelles that can sediment under the influence of gravity. Biophysical research in the past decade has added considerably

to our knowledge of the structural and hydrodynamic properties of chromosomes, plasma membranes, nuclear membranes, cytoplasm, nucleoplasm, chromatin, nucleolus and membranous organelles. Using recent measurements, an attempt is made here to estimate the effects of the gravitational field upon the position and motion of the cells' densest structures.

The Nucleolus

Earlier theoretical work indicated that the nucleolus might be a sufficiently large dense structure to be influenced by gravity (19). This would certainly be the case if the nucleolus could be considered as a solid object suspended in a viscous liquid medium. However, this is not the case. Our current understanding of the nucleolus (see Fig. 1) is that its role is the synthesis of ribosomal RNA and the assembly of ribosomes (20,21). Although it is truly a densely packed structure, it is not isolated from the surrounding nucleoplasm as a solitary hydrodynamic unit. Instead it has threads of chromatin running through it--presumably the chromatin which contains ribosomal DNA genes (21). The nucleolus is therefore suspended in the nucleus by a number of threads, and its motion is therefore constrained by the motion of the chromatin with which it is associated. Hence, as shown in the electron micrographs of Fig. 1, there is little or no evidence for the sedimentation of nucleoli to the bottoms of nuclei in cultured human cells. On the average, the nucleolus is just about as close to the top of the nuclear membrane as it is to the bottom. It is to be learned from this discussion that fibrous materials in the cell can greatly influence the response of its organelles to gravity.

The Cell Nucleus

Now let us consider the nucleus as a whole. Recent studies have indicated that the cell cytoplasm can be considered as a network of microfilaments and microtubules (23). The increasing rate at which contractile proteins are being discovered in so-called non-contractile cells is so alarming that we wonder why they were not previously found. Two classes of structure are of interest to our discussion. The main protein of microtubules is tubulin (24). The tubulin exists in sub-units of microtubules. The sub-units are assembled into tubules for such purposes as the guiding of chromosomes at mitosis, the strength and movement of cilia, and for axoplasmic flow in nerve axons. The assembly of these sub-units into tubules is inhibited by colchicine and similar vinca alkaloids. Microfilaments, on the other hand, appear to consist of a mixture of actin, myosin, and other contractile muscle proteins (25). Microfilaments have been considered essential for the normal migratory behavior of cultured fibroblasts (26). Cytochalasin B interferes with the normal action of microfilaments (27). Figure 2 indicates the presence of both actin and myosin in the microfilaments of cultured fibroblasts and shows that these microfilaments envelope the cell nucleus.

It appears that the nucleus is positioned in the cytoplasm under constraints imposed by microfilaments and/or microtubules. If cultured cells attached to coverslips are centrifuged at moderate speed, one finds that cells remain intact without significant displacement of their nuclei. If, on the other hand, one treats cultured cells attached to coverslips with cytochalasin B and then subjects the attached cells to a centrifugal field, it is found that the centrifugal acceleration is then adequate to enucleate the cells (28). If one were to assume that the nucleus is a hydrodynamic unit approximated as a sphere 12 microns in diameter with density 1.14 suspended in a fluid with viscosity 17 centipoise and density 1.03, then one would anticipate a sedimentation velocity of the cell nucleus equal to about 20 micrometers per hour. Clearly, all nuclei would sediment to the bottoms of their cells in a few minutes. That this is not the case is observable in mammalian tissue sections in which the nuclei are always central and in vertical sections of cultured cells (Fig. 1), where the nuclei are also rather centrally positioned. Evidently, microfilaments or other cellular structures deny the cell nucleus any motion induced by gravity.

The effect of gravity on nuclear shape is now considered. It has been noted that isolated cell nuclei are more susceptible to deforming forces than are nuclei within

cells. Evidently the deformibility of cell nuclei is also influenced by cytoplasmic materials. If the nucleus were to be pictured as a colloidal sol inside a deformable bag, one would expect nuclei to be broader at the bottom than at the top where up and down are defined by the gravitational vector. If cells from sectioned tissue ever indicated such an anisotropic feature it was never reported. Upon examining human cells in culture such as in Fig. 1, in which the gravity vector is clearly defined, one might seek a gravitational affect in the form of nuclei which are broader in their lower halves than in their upper halves and in which the top radius of curvature is much less than the lower radius of curvature of the nucleus. Indeed, one finds evidence for this occurring in a significant proportion of cells examined. It should be cautioned, however, that such anisotropic nuclear shape might have nothing to do with the gravitational field because the nucleus may assume this shape simply because the cell which surrounds it is broader at the bottom as a consequence of being attached and spread at its bottom surface and not at its top surface. There is, therefore, no concrete evidence that gravity influences nuclear position or nuclear shape.

Chromosomes

Finally, let us consider the possibility of a gravitational effect on chromosomes at mitosis. Ever since the discovery of chromosomes, scientists have been fascinated by their movements during cell division. Their kinematics and mechanics have been considered in detailed physical theories of the mitotic process. As we have done with the other organelles, let us consider the sedimentation velocity of a free chromosome suspended in the fluid matrix of the mitotic cell. The general equation of motion for a sedimenting particle is

$$m \frac{d^2x}{dt^2} = F_{grav} - F_{buoy} - F_{drag}$$

or

$$o = mg - V\rho_o g - fv \qquad (1)$$

where the friction factor, f, for a long prolate ellipsoid is estimated as

$$f = 9\pi \sqrt[3]{3V/4\pi} \, . \qquad (2)$$

Substituting for f and solving equation (1) for the terminal velocity we find that

$$v = \frac{Vg\,(\rho-\rho_o)}{9\,\pi \sqrt[3]{3V/4\pi}} \qquad (3)$$

in which all of the values in equation (3) are known. These values and their sources are tabulated in Table I.

We may also use the equation of motion (equation 1) as a force balance equation. By using the boundary conditions that velocity and acceleration equal 0, we may determine the difference between the gravitational and buoyant force and thereby estimate the force required to prevent the chromosome from sedimentating in the cytoplasm. The constants needed for this calculation are given in Table I and Figure 3. The calculations applied to a "typical" mammalian chromosome and indicate that if the chromosome were suspended in a free solution with cytoplasmic density and viscosity it would sediment with $v \simeq 2 \times 10^{-7}$ cm/sec. Assuming v = o in equation (1) leads to a balancing force of about 10^{-8} dyne, or less than that of the covalent bonds which exist within the cross section of a spindle fibre.

One may now question whether or not the mitotic spindle can exert the force required to prevent chromosome sedimentation in the cytoplasm. A set of experiments was done in the following way: cultured Chinese hamster M3-1 cells (34) and cultured human kidney T-1 cells (35) were allowed to attach and grow on the surface of plastic tissue culture bottles (Falcon #3012) for 24 hours in the horizontal position, after which half of the sample bottles were filled with medium and oriented vertically. After 18-20 more hours of incubation at 37°C, the cultures were rinsed with Hanks' balanced salts solution and fixed without changing their orientation. They were stained in the horizontal position with Harris' hematoxylin and mordanted tap water. The angle Θ subtended by the direction of the spindle and a vertical line (Fig. 4) was estimated within 30° intervals microscopically, and the number of dividing cells lying in each 30- degree interval was determined (Table II). The following results are to be expected: 1) If mitosis is oriented by the growth surface only, there will be an equal proportion of cells in each 30-degree interval in both vertical and horizontal cultures, and a preference for chromosome motion parallel to the growth surface. 2) If mitosis is oriented by gravity alone, there will be a preferred orientation around $\Theta=90^{\circ}$ (interval 3) in the vertical culture. 3) If both gravity and the growth surface act to orient mitosis, there will be a preferred orientation around $\Theta=90^{\circ}$ and a preference for chromosome motion parallel to the growth surface in the vertical cultures.

Cells were assigned to groups 1 through 6 according to the value of Θ. An isotropic culture should have roughly equal numbers of dividing cells in each group. The existence of anisotropy should be indicated by an excess of dividing cells in one or two of the angular intervals. In horizontal flasks isotropic distributions were generally found. Nevertheless, the proportion of mitoses oriented at each angle in horizontal cultures was used as a baseline against which to compare the proportion at the same angle in vertical cultures, and to determine the effect of growth on the vertical surface, the following vertical-to-horizontal ratio was defined:

$$\frac{V}{H} = \frac{\text{proportion of cells in } \Theta \text{ interval, vertical}}{\text{proportion of cells in } \Theta \text{ interval, horizontal}}, \qquad (4)$$

then histograms were prepared of V/H vs. Θ interval.

An example of such a histogram is given in Figure 5, which suggests that the proportion of mitoses oriented at each angle did not differ significantly between horizontal and vertical cultures in this experiment.

Chinese hamster M3-1 cells grow into colonies with a large axial ratio. If the plane of division occurs with greater frequency at a particular position for cells grown on a vertical surface, then the long axis of the resultant colonies should be preferentially oriented. The angle subtended by the long axis of the colonies and the long axis of the bottle was estimated for vertically- and horizontally- grown cultures and the corresponding V/H ratio determined for each angle in Figure 6.

In order to avert the ambiguities associated with counting small numbers of dividing cells (about 300 cells per dish were measured), experiments were designed so that the direction of division could be determined for a large number of cells plated at relatively low density. Human kidney T1 cells were plated and the vertical bottles were oriented as soon as the cells were firmly attached; thus, the first division occurred after the bottles had been oriented. The oriented bottles were then incubated for exactly one generation time (about 24 hours) and stained. The plane of division was determined for 1,000 cells in two experiments. The V/H ratio presumably has the same meaning as in experiments in which only dividing cells were measured, as the angles were determined only for colonies containing two cells. The distribution of the V/H ratio is given in Figure 7.

If there is any effect of vertical incubation upon orientation of cell division, it is probably small and difficult to reproduce.

Cultured Human Cells in Weightlessness

The above conclusions concerning a lack of obvious effect of the gravity vector on the orientation of mammalian cell division is borne out in the studies of Montgomery (36), in which cultured human WI-38 fibroblasts were grown during the 59-day mission of Skylab. The population doubling time in flight, 22.3 ± 3.1 hr did not differ significantly from that at 1g, 20.4 ± 4.8. The speed of cell migration on the culture vessel surface was the same, and no ultrastructural or karyotypic differences could be observed. Cells that had rounded for mitosis did not even require the gravitational force to reattach to the surface upon which they were growing.

Experiments in the laboratory and in space indicate that the cell division process in cultured mammalian cells is rather sensitive to the influence of gravity.

DISCUSSION

Some of these concepts lead to interesting questions concerning the role of gravity in organic or chemical evolution. For example, one might ask would the ideal shape of an organism in the absence of gravity always be a sphere? In other words, would an organism evolving in space be spherical rather than shapely as organisms evolved on earth in the presence of gravity? At the subcellular or organelle level even more serious questions persist: Do particles that sediment in plant cytoplasm really behave as geotropic sensors? If they do, how do they inform the cell what to do? Does gravitational stress lead to an intracellular contractile response? Many of these considerations overlook the existence of internal cellular membranes which, in eukaryotic cells, exist in great abundance.

Perhaps the sedimentation of particles in cells has been considered too simplicistally and one needs to include considerations of such phenomena as the Dorn effect in which an electric field results when a particle sediments. Such fields can be as great as 20 millivolts.

Also, droplet sedimentation should probably be given more serious consideration as it is a phenomenon related to larger hydrodynamic units whose density depends on particle concentration.

Other questions of biological interest include, Why are plant tumors not geotropic? Do plant tumor cells disregard gravity? Is something missing in their differentiated structure? Also, simple plants such as the mold, Phycomyces, respond to gravity without possessing any apparent sedimenting cytoplasmic particles.

Research on earth and in space has not yet led to concrete evidence that sedimenting intracellular particles play a role in determining the relationship between cellular activities and the gravity vector.

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TABLE I. HYDRODYNAMIC VALUES FOR A METAPHASE CHROMOSOME (SEE FIGURE 3) USED FOR APPLICATION TO EQUATION (3). CHROMOSOMES HAVE BEEN EXAMINED HYDRODYNAMICALLY IN ISOLATION (31,32), AND CYTOPLASMIC VISCOSITY HAS BEEN STUDIED BY PARAMAGNETIC RESONANCE (33).

$$V = 2\pi r^2 \ell = 25 \times 10^{-12} \text{ cm}^3$$

$$g = 980 \text{ cm/sec}^2$$

$$\rho - \rho_o = 1.35 - 1.04 = 0.31 \text{ g/cm}^3$$

$$\sqrt[3]{3V/4\pi} = 2.1 \times 10^{-4} \text{ cm}$$

$$\eta = 5 \pm 2 \text{ dyn-sec/cm}^2$$

$$v \simeq 2 \times 10^{-7} \text{ cm/sec}$$

TABLE II. ANGULAR INTERVALS (SEE FIGURE 4) USED TO CLASSIFY ORIENTATION OF MITOTIC CELLS AND COLONIES ON HORIZONTAL AND VERTICAL CULTURE FLASKS.

CLASS	RANGE OF θ (DEGREES)
1	135 - 165
2	105 - 135
3	75 - 105
4	45 - 75
5	15 - 45
6	-15 - 15

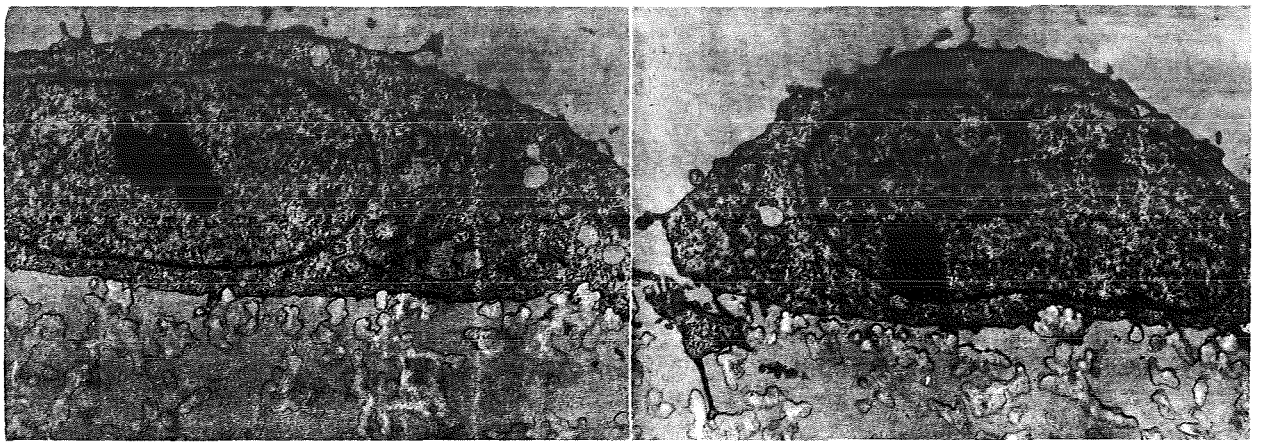

Figure 1.- Electron micrographs of vertical sections of cultured human liver cells grown on horizontal Millipore filters. The location of nucleoli is variable, and the nuclei tend to be broader at the base. (Micrographs courtesy of Helge Dalen (ref. 29).)

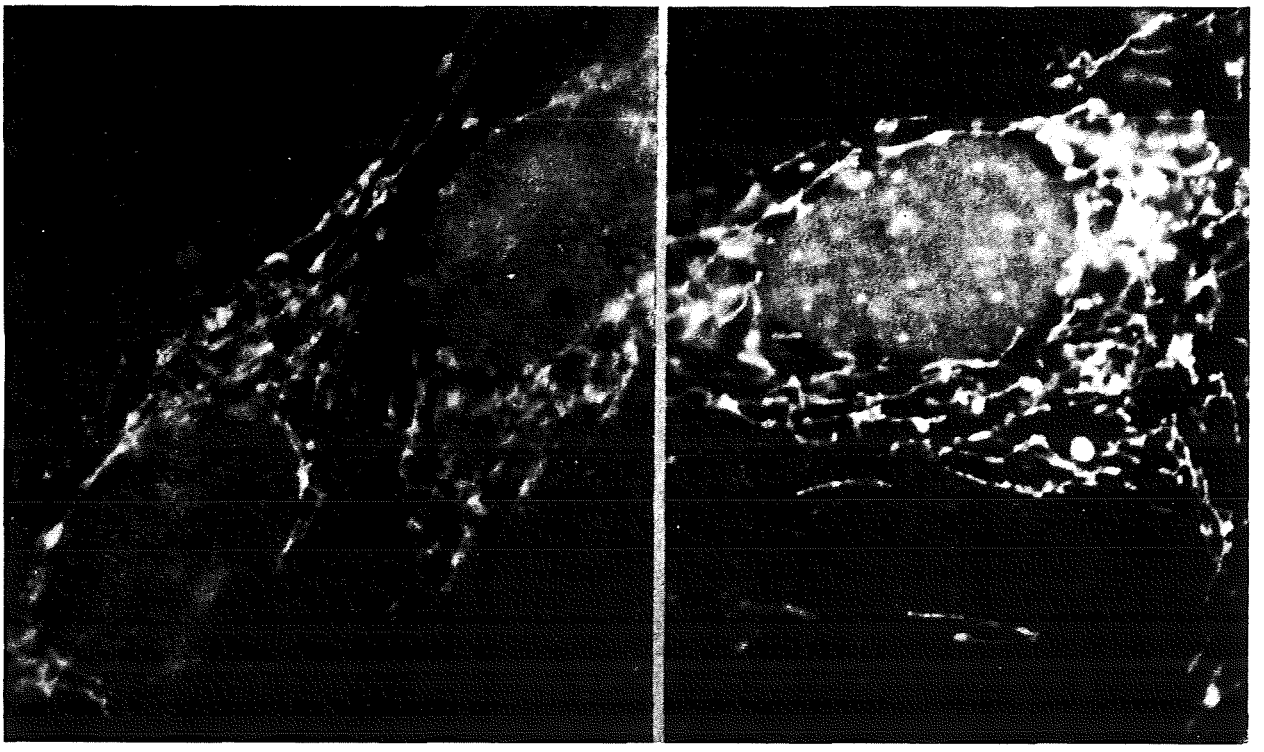

Figure 2.- Fluorescence micrographs of cultured human embryonic lung cells fixed in acetone, extracted with glycerol, and "stained" with fluorescent antibody against heavy meromyosin to show presence of myosin (left) and "stained" with heavy meromyosin in addition to the same fluorescent antibody to show presence of actin in filaments (right). (Micrographs courtesy of Alex L. Miller (ref. 30).)

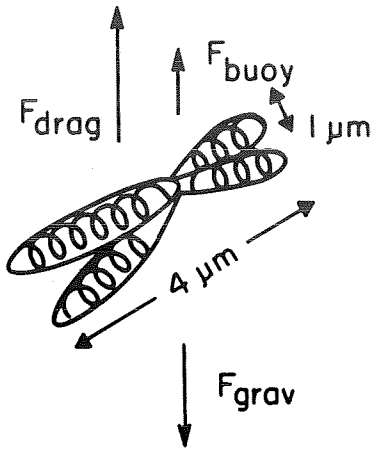

Figure 3.- Assumed properties of a metaphase chromosome suspended in cytoplasm. (See table I.)

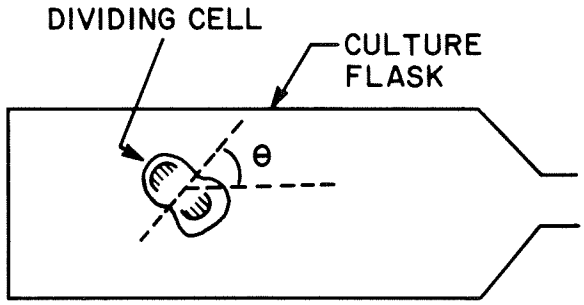

Figure 4.- Illustration of analysis of orientation of mitosis in horizontal and vertical cell culture flasks. The diatram defines the mitosis orientation angle θ.

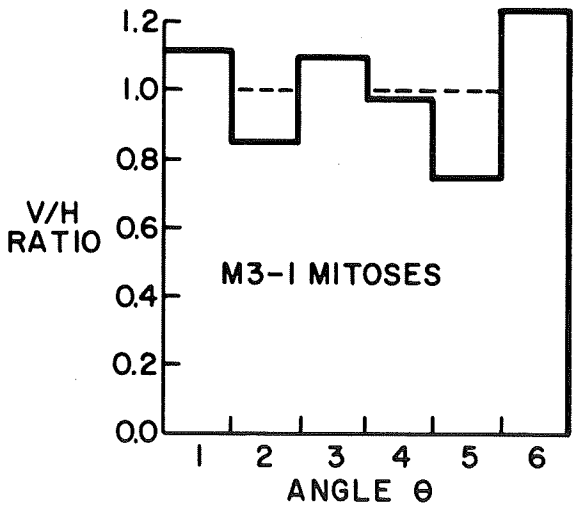

Figure 5.- Histogram showing the ratios of mitoses in vertical to those in horizontal culture flasks at each interval of the mitosis orientation angle θ, defined in figure 4 and in table II.

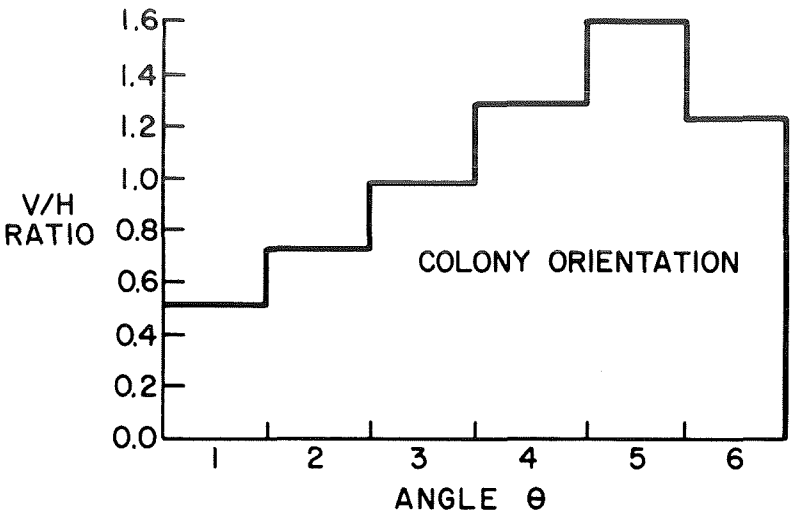

Figure 6.- Histogram showing the ratios of M3-1 cell colonies in vertical to those in horizontal culture flasks oriented with their long axes in each interval of the colony orientation angle θ, defined in figure 4 and in table II.

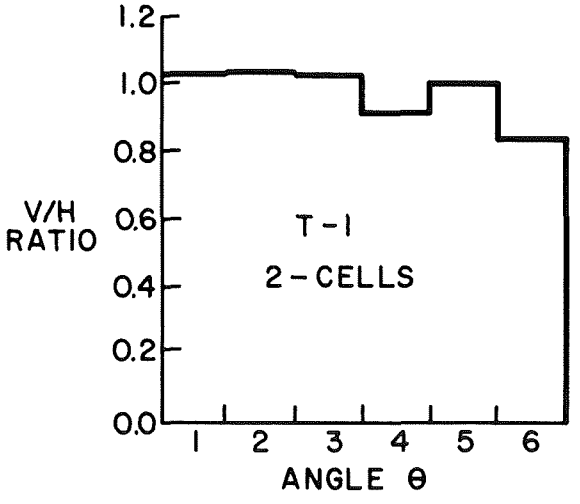

Figure 7.- Histogram showing the ratios of T-1 (two-cell) colonies in vertical to those in horizontal culture flasks having their plane of division in each interval of the division plane angle θ, defined in figure 4 and in table II.