Technical Papers

Nucleation and Growth of Cell Colonies

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In order to apply mathematical methods to the description of a natural process, it is necessary to invent a model of some sort that operates as the natural process in question is presumed to operate, and that can be handled mathematically. Frequently the model is oversimplified, either because the process is not well understood or because it is very complicated.

In preliminary studies, oversimplification often leads to greater clarity of the basic assumptions in the analysis, and to a more direct check of their validity. If a simple model stands up semiquantitatively under the test of experiment, it may be modified and improved from time to time as desired and as the experimental evidence warrants. If, on the other hand, it is in qualitative disagreement with experiment, it can be abandoned right away without excess wasted effort.

The growth of a cell colony is a natural process that is neither simple nor well understood. At present, therefore, no more than the most modest of models can be proposed to represent it. Such a model is suggested in the following paragraphs. Many factors that influence cell colony growth are assumed to be of secondary importance, and they have been ignored in the interest of simplicity. Nevertheless, the oversimplified model operates in semiquantitative agreement with experiment, and it is hoped that it may further our understanding of cell colony growth.

Sanford, Earle, and Likely (*6*) have drawn attention to the problem of growing single isolated tissue cells *in vitro*. Their work and that of others suggests that the relationships between the size of a tissue explant and its subsequent growth characteristics are similar to those observed for growth in many physical systems.

For example, corresponding to a given geometry, cell type, and culture medium, there is a critical size for cell colonies such that larger colonies live and grow while smaller colonies die out (*4–6*). These observations are consistent with the idea that cells produce one or more water-soluble substances, f, that they require for growth and reproduction. Whenever the f concentration drops below a minimum value C^* and remains below C^* until a cell consumes its stored precursors of f, the cell dies.

Consider a single cell transplanted to a large volume of culture medium that contains no f. The f that the cell produces diffuses away into the surrounding solution, and the cell dies before the local concentration rises above C^*. However, a sufficiently large and compact group of cells introduced into a large volume of medium will continue to grow, for loss of f can occur only at the boundary of the group. By increasing the size of a colony of constant density, the boundary area can be made as small as desired relative to the volume occupied by the group, and the loss of f per cell thereby can be decreased indefinitely. As long as the density of the cells in the colony is sufficiently great that each cell can raise the concentration of f above C^* in its share of the volume, a critical size group will exist such that members of larger groups will grow and reproduce, while members of smaller groups will die.[1]

Similarly, there is a critical size for the fire in a coal-fired furnace, and the phenomenon of combustion in such a furnace provides a physical analogy to the growth of cell colonies. A colony of one or two burning coals in a furnace of unburned coal fails to reproduce, and dies (i.e., goes out), while a very large colony (several shovelfuls of burning coals) grows and fills the furnace. In this analogy, a lump of burning coal represents a living cell, unignited coal represents culture medium, and diffusion of heat represents diffusion of the substance f that is necessary for cell growth and multiplication. Unless the concentration of heat (i.e., the temperature) is high enough, combustion ceases. If the f concentration is too low, cell growth ceases.

Phenomena of change or transformation in physical systems are very frequently described by the concepts of nucleation and growth (*2, 7*). Whenever a region with properties distinct from those of the parent material tends to grow if it exceeds a certain critical size and to disappear if it is smaller than critical size, large growing regions that appear spontaneously and persist are said to have formed by a process of nucleation, and to enlarge subsequently by a process of growth.

In this terminology, the spontaneous appearance of cancer may be a phenomenon of nucleation and growth. Suppose that cancer cells appear from time to time here and there in a tissue, by random mutation or other means. There are then two alternatives regarding their growth. A single cancer cell isolated in normal tissue either can lead to cancer, in which case the nucleation idea collapses into the trivial situation where a single individual is a critical-size colony, or for some reason it cannot lead to cancer. The suggestion is made that single cancer cells isolated in normal tissue find themselves in a somewhat foreign environment, as do the cells of tissue explants in culture media, and that such cells are subcritical for malignant growth. Only when a sufficiently large and closely bunched group of cancer cells is formed is a supercritical colony produced and cancer nucleated. The cancer then can grow.

It is not necessary for the success of the nucleation viewpoint that an isolated cancer cell die, as does an

[1] It should be pointed out that the converse of this hypothesis at present is equally tenable (*6*). A substance f, toxic to cells, may be present in the culture medium. Then only large groups of cells can destroy enough f to bring the concentration to a tolerable level.

isolated cell in a large bath of culture medium. On the contrary, evidence to be described in a later paper suggests that isolated cancer cells may continue to survive, leading relatively normal lives as dictated by the unchanged chemistry of their normal neighbors, until a supercritical colony is formed wherein the altered chemistry of cancer cells no longer is diluted to ineffectiveness.

The idea that there is a critical-size cell colony for the nucleation of cancer is not foreign to current thought. Karnofsky (*3*) has suggested that there is a critical size for the growth of metastases, and it is known that small tumor transplants are less likely to survive than larger ones. It now is proposed that a critical-size colony also is required for the spontaneous appearance of cancer in a previously cancer-free individual, and a tentative model for the process, based upon the nucleation concept, is suggested.

The experiments of Sanford, Earle, and Likely (*6*), dealing with the growth of subcritical and supercritical cell colonies and with the influence of container geometry upon the growth characteristics, can be analyzed quantitatively by making use of the nucleation concept. The following assumptions are made:

(1) All cells in a colony are identical.

(2) A cell produces substance f at constant rate.

(3) If, after a day or two, the concentration of f at a cell remains below a critical value C^*, the cell dies.

Let the diffusion coefficient for diffusion of f in water be D. The steady state concentration of f due to a spherical source of strength Q in an infinite bath initially of zero f concentration is (*1*)

$$C = Q/4\pi Dr \tag{1}$$

where C is the concentration of f in units/cm^3, Q is the rate of production of f at the source in units/sec, D is the diffusion coefficient in cm^2/sec, and r is the distance in cm from the center of the source. The expression is valid for $r \geqq r_1$ where r_1 is the radius of the source.

Now consider a capillary of length L, radius r_0, having a source of strength Q at its center, the capillary being immersed in an infinite bath. The f concentration at the ends of the capillary is approximately zero. The rate of transfer of f along the axis of the capillary is

$$P = -D\ dC/dx \tag{2}$$

units/cm^2 sec. At steady state, the concentration gradient is linear from the center of the capillary to each end,

$$P = Q/2\pi r_0^2,$$

and

$$dC/dx = -C_0/(L/2)$$

where C_0 is the concentration at the center of the capillary. Hence

$$C_0 = QL/4\pi Dr_0^2. \tag{3}$$

Let n_0^* be the number of cells in a colony of critical size at the center of a capillary. Then

$$C^* = n_0^*\ qL/4\pi Dr_0^2, \tag{4}$$

where q is the rate of production of f per cell. For a critical-size colony in an infinite bath, equation 1 requires

$$C^* = n_1^*\ q/4\pi Dr_1 \tag{5}$$

where n_1^* is the critical number of cells and r_1 is the radius of the colony. The critical size is defined to be that for which the outermost cells are just at the borderline of survival.[2]

Combining equations 4 and 5, the relationship between the critical number of cells for a colony in a large bath and for a colony in a capillary is

$$n_1^* = n_0^*\ (r_1 L/r_0^2). \tag{6}$$

According to Sanford, Earle, and Likely (*6*), the value of n_0^* lies between 1 and 4 for strain "L(6)" cells grown in "normal" culture medium. They give capillary dimensions as

$$r_0 = 0.01 \text{ cm}$$
$$L = 0.45 \text{ cm}.$$

Taking the geometrical mean

$$n_0^* \approx \sqrt{1 \cdot 4} = 2$$

the calculated value of n_1^* is

$$n_1^* = 90\ r_1/r_0. \tag{7}$$

Taking the radius of the volume of culture medium per cell in the interior of a cell colony to be

$$r_c \approx 0.8 r_0 = 0.008 \text{ cm}$$

as determined from healthy cell colonies growing in capillaries (Fig. 7 of reference *6*), the number of cells in a colony of radius r_1 is

$$n_1^* = (r_1/r_c)^3. \tag{8}$$

Combining equations 7 and 8,

$$(r_1/r_c)^2 = 90\ r_c/r_0 = 72$$

and

$$n_1^* = 610 \text{ cells}. \tag{9}$$

According to these calculations, therefore, cell colonies containing less than about 600 individuals (as closely packed as those in a colony growing in a capillary) will fail to grow and will die when placed in large flasks of "normal" culture medium. Only colonies containing more than 600 cells will grow and persist. Although the value $n_1^* \approx 600$ cells is valid only for the particular culture medium, temperature, cell strain, and colony density under consideration, the relative values of n_1^* and n_0^*, as given by equation 6, depend only upon colony density.

The critical size of "L(6)" explants grown in large volumes of medium has not been determined experimentally. However, analysis of Figs. 11 and 12 of reference *6* shows that groups of about 300 cells of the assumed closeness of packing are subcritical, whereas it is stated that larger groups of cells are supercritical. The published data indicate only that a critical number of cells does exist, and that this number exceeds 300.

[2] Colonies slightly smaller than the critical size so defined may persist if the mitotic rate of healthy cells is sufficiently great to replace dying cells at the periphery of the colony. Assuming that the mitotic rate is small, the effect is not important.

The purpose of this analysis has been twofold: first, to suggest that the concepts of nucleation and growth, developed for physical systems, apply to the growth of cell colonies, and second, to show that elementary diffusion equations can be employed to calculate the influence of container geometry upon the critical size of tissue explants. It is further suggested that the spontaneous appearance of cancer may be a process of nucleation in that single cancer cells in normal tissue may be subcritical with respect to malignant growth.

References

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The Action Potentials Obtained from Venus's-Flytrap

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Venus's-flytrap, *Dionaea muscipula*, is included in a small group of plants designated by botanists as carnivorous because of its reputed ability to digest insects and small bits of living matter caught in the trap structure from which the plant takes its popular name. It is found growing in the wild state only in the eastern part of the Carolinas of the United States.

In 1769, William Young of Philadelphia brought a live specimen to England. Bourdon-Sanderson (*2, 5*) as early as 1873 discovered the electromotive properties of the leaf of *Dionaea* in the excited and unexcited states, using specimens from the laboratory of the Royal Gardens at Kew. Bose (*1*) criticized these earlier results and Bourdon-Sanderson's later results (*3, 4*), published in 1882 and 1888, as being more or less contradictory.

According to Bourdon-Sanderson one should expect, as a result of stimulating the inner surface of *Dionaea* in its prime, to observe a short-lived positive electrical increase, followed by a larger negative rise and fall in potential, i.e., a diphasic action potential. This indicated that the inner surface of the leaf trap first became positive and then negative as the excitatory process passed under the nonpolarizable electrodes placed symmetrically on the two outer surfaces of the trap. When the inner surface was excited, mechanically or electrically, it was found that the electrical disturbance was greater in the neighborhood of the seat of excitation than at a distance from it. The electrical transient lasted about 1 sec and its propagation speed, at 38° C, was about 20 cm/sec. The mean maximum negative potential variations as the result of a strong stimulus was about 20 mv.

The deflections were originally measured with a 5,244-ohm Thomson galvanometer, a 10,000-ohm resistance in series, and nonpolarizable electrodes placed across the leaf. The sensitivity of the instrument was 350 scale divisions for 0.001 Daniell, i.e., 3×10^{-6} v per division. Later measurements of the maximum magnitude of the fluctuations were made with a Lippmann capillary electrometer.

To get a better understanding of the nature of these electrical transients, it is important not only to know that they are local electrical negative potential changes, but also the precise shape of the after potentials, negative and positive, and their temporal variations under controlled conditions. In order to avoid the necessary corrections for the individual characteristics of the electrical deflecting instruments involved in following such rapid fluctuations in potential, one resorts in modern practice to the use of a cathode ray oscillograph.

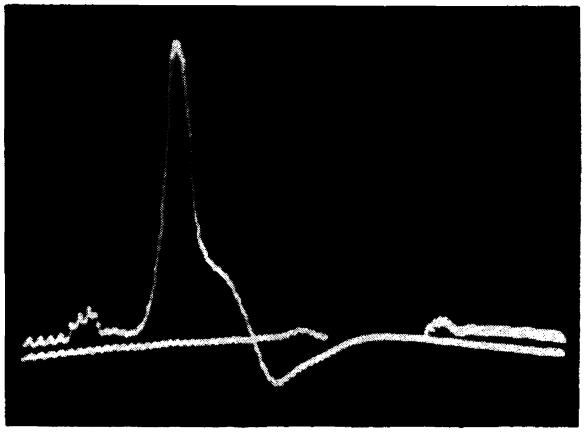

FIG. 1. Oscillograph record of an action potential of Venus's-flytrap, *Dionaea muscipula*. Electrodes taken off under side of trap. Bending of trigger hair used as stimulus. Negative potential up, time scale logarithmic, 60-cycle frequency; stimulating artifact, negative spike potential, negative after potential followed by positive after potential; maximum 0.13 v above resting potential, 24° C.

In neural action potential measurements, the initial negative variation or spike lasts about 2 msec, reaching its crest in about 0.5 msec. In *Dionaea* it was found that the negative variation existed for several tenths of a second. Such a pulse can be highly distorted if amplified by an ordinary resistance-coupled amplifier, such as can be used to amplify the neural spike, unless the coupling circuits have been designed to possess unusually high time constants.

With this in mind, the following instrumentation was devised. A Dumont 208B cathode ray oscillograph was used. The transients on the 5-in. fluorescent screen were compressed to 2 in. and photographed with a Monitor camera equipped with a f/4.5 lens. Lowest frequency X-axis was 2 cycles per sec. Lowest frequency Y-axis was 4 cycles per sec with a 5% distortion at 2 cycles per sec, square wave input.

Since the Y-axis amplifier of the instrument is designed so that the final two stages are directly coupled, and