that meaningful between-lava comparisons of the Th/U ratio should be supplemented by examination of polished as well as thin sections. A practice of collecting at least two samples from each lava, with one from the center of the lava, is also encouraged in order to test for gross variations that may originate during initial cooling (*21*).

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21. We suspect that when consistent between-lava values for Th/U ratios are observed, it is not impossible that a sampling bias may be in evidence. For example, J. M. Rosholt, M. Tatsumoto, R. J. Knight [*Program of Annual Meeting* (Geol. Soc. Amer., 1966), p. 181] report very constant Th/U ratios from five historic and one Pleistocene flow in Hawaii. If samples were taken from the cooled surface of each lava, then the specimens could be expected to have virtually identical cooling histories. This does not mean that such samples are not "representative" of the lavas, although we might add that the term "representative" is in need of very careful scrutiny, when applied to extrusives. Our point is that, if subsequent erosion exposes the interiors of the lavas, between the upper and lower cooling faces, then resampling may yield different Th/U values.

9 November 1966

Contact Inhibition in Colony Formation

Abstract. *Contact inhibition of replication of the established mammalian cell line 3T3 was examined during growth of the colony and compared with that of the Chinese-hamster cell line CHL-1. The growth curves of cells in the colonies conformed to the predicted exponential and linear rates for CHL-1 and 3T3 respectively. Autoradiographs of colonies in which DNA was labeled with tritiated thymidine showed that in 3T3 colonies, only peripheral cells were labeled, while CHL-1 colonies were labeled throughout.*

Simple methods for growth of macroscopic colonies from mammalian cells in vitro have made possible quantitative studies of cellular reproduction, genetics, and interactions with viruses (*1*). With these microbiological methods, the growth of mammalian cells in a particular medium can be expressed in terms of the plating efficiency (that is, the number of cells in a population capable of initiating self-sustaining multiplication) and of the generation time in the exponential reproductive phase. Contact inhibition of replication, however, is a property of some mammalian cells cultivated in vitro; this property interferes with unlimited proliferation of all of the cells in the colony. As a result, the differences between colonies of cells with and without contact inhibition can be used to identify and analyze the property.

To examine the effects of contact inhibition in colony growth, we have compared the 3T3 cell-strain isolated by Todaro and Green (*2*) with the Chinese-hamster cell strain CHL-1 isolated by Puck (*3*). The media and methods of cultivation and plating have been described (*2, 4*). The 3T3 cells formed less distinct colonies and consistently had a lower plating efficiency and growth rate than the CHL-1 cells (Fig. 1, a and b). The average plating efficiency of 3T3 [with 50 cells as the minimum colony size (5)] in ten separate experiments was 29 percent, whereas that of CHL-1 was seldom less than 70 percent. The generation time estimated from a cell count of the col-

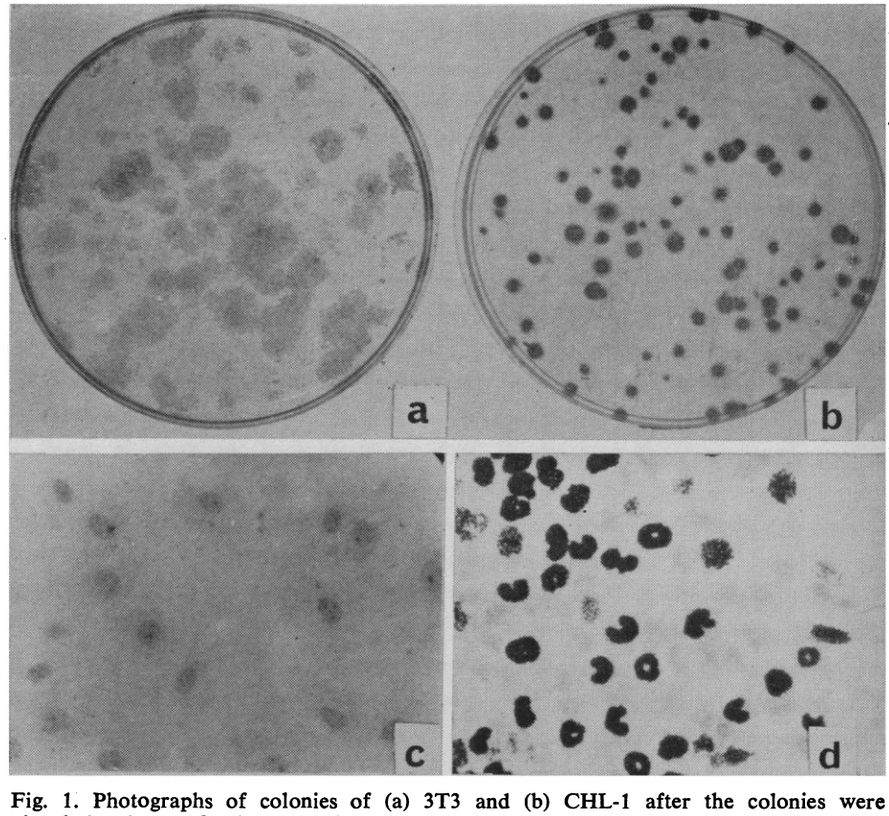

Fig. 1. Photographs of colonies of (a) 3T3 and (b) CHL-1 after the colonies were plated, incubated, fixed, and stained (actual size). (c and d) Photomicrographs of the center of colonies in which DNA was labeled with H^3-thymidine and of which autoradiographs were made (c) 3T3 and (d) CHL-1 (× 375).

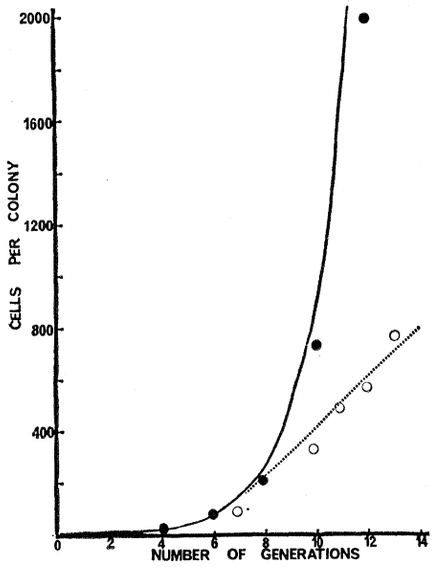

Fig. 2. Growth rates of CHL-1 (solid circles) and 3T3 (open circles). The solid line represents the theoretical exponential curve; the broken line represents the theoretical linear growth-rate starting at about 60 cells per colony.

onies of 3T3 was about 24 hours, whereas that of CHL-1 was about 12 hours.

To determine if the difference in the numbers of cells in the colonies of CHL-1 and 3T3 reflected the effects of contact inhibition, we established

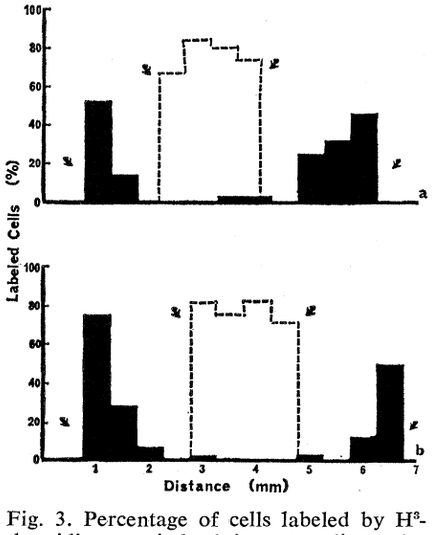

Fig. 3. Percentage of cells labeled by H^3-thymidine, as judged in autoradiographs, as a function of the distance (a) horizontally through a colony and (b) vertically through the same colony. The total cells and labeled cells were counted in adjacent squares (0.5 mm by 0.5 mm). The solid areas represent values for 3T3; under the broken line are the values for CHL-1. Arrows indicate the edges of the colony.

growth curves of the two cell types. With contact inhibition of replication, one would expect that, after the colonies reached a size in which the central cells could not escape contact with their neighbors, the growth rate would be a linear expression of the number of peripheral cells; without contact inhibition in the colonial population, the growth rate would continue exponentially. The results of the cell counts of colonies as a function of the number of generations are presented in Fig. 2. The growth rate of 3T3 colonies became approximately linear; that of the CHL-1 colonies, became approximately exponential. The expression of contact inhibition within the colony was detected from the 60-cell stage. This agrees with the fact that, from the sixth generation on, growth is linear rather than exponential.

To test whether only the cells on the circumference of the colonies were multiplying, we measured DNA synthesis by radioactive labeling and autoradiography. The colonies were grown on glass slides in petri dishes until they reached the desired size. The medium was then changed to Saline F (*4*) with fetal bovine serum and H^3-thymidine; the medium was 2 percent fetal bovine serum and had a final specific activity of 1 μc/ml. The incubation was continued for one generation time, and the radioactive solution was removed. The cells were washed twice with Saline F and fixed with 10 percent formaldehyde. The slides were then rinsed in distilled water, immersed for 1 hour in cold, 5 percent trichloroacetic acid, rinsed in distilled water, and air dried. For autoradiography, the slides were dipped in Kodak NTB-2 liquid photographic emulsion and exposed for 5 to 8 days at 4°C before being developed (*6*).

The autoradiographs of the center of a 3T3 colony had no grains, whereas the CHL-1 cells were labeled throughout (Fig. 1, c and d). The edges of the 3T3 colonies, however, did have labeled nuclei. We determined the distribution of the percentage of labeled cells by scanning the colony in two directions (Fig. 3). Whereas 60 to 80 percent of the peripheral 3T3 cells had labeled nuclei, less than 5 percent of those in the center of the colony were labeled.

Results obtained in analysis of clonal isolates by this method were the same as those obtained with the original 3T3 strain. Analysis of the percentage of cells having the property of contact inhibition in a primary culture is possible with this technique.

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Plasma Membrane: Substructural Changes Correlated with Electrical Resistance and Pinocytosis

Abstract. *Inducers of pinocytosis in amoeba cause as much as a 50-fold decrease in the electrical resistance of the plasma membrane prior to the formation of the typical tunnels and vacuoles. In this state the thickness of the electron-transparent core or lamella of the unit membrane is at least twice as thick as that of the control. The changes in structure and resistance as well as the induction of pinocytosis are dependent on the initial external concentration of calcium. These changes are rapidly reversed when the concentration of calcium in the external medium is increased.*

Cultures of the amoeba *Chaos chaos* L. (*Pelomyxa carolinensis*) fed washed paramecia were grown in a fluid containing 1.0 mmole of $CaCl_2$, 2.0 mmole of NaCl, and 0.4 mmole of KH_2PO_4-Na_2HPO_4 per liter of ion-free water with a *p*H of 6.9. The test solutions, described by notation of the Ca^{2+} concentration only, were identical except that the concentration of calcium chloride was varied. Solutions were made with ion-free water (resin supplied by Continental Water Conditioning Corp.) and kept in disposable