Therefore

$$\phi = v_0(-bp + 2c\theta)(rb/2c)$$

By substituting $2c\theta$ from Eq. 7,

$$\phi = [(v_0b^2p)/2c](r^2 - r) \qquad (8)$$

In Eq. 8, ϕ has the minimum value when the parameter r has the value ½. The temperature profile with the greatest stability has the equation

$$\theta = (b/4c)p$$

The rate of decrease of temperature with pressure, $b/4c$, is about 0.010°C/bar. This is one-half of the decrease of temperature of maximum density with pressure; corollary III is therefore proved.

It has been reported (*1*) that among the temperature profiles reported by Strøm (*2*), those that cross the line of maximum density tend to change sign of slope where they cross the line. No reported temperature profile coincides with the computed line of maximum density. Finally, Strøm's "envelope," which tends to parallel all his reported profiles, has a slope of about 0.011°C/bar, which is one-half the slope of the line of maximum density and, as we have shown, is the line of greatest stability; thus all three of the proposed corollaries are consistent with Strøm's data.

My conclusions are also supported by Johnson's observations on Great Bear Lake (*3*). He noted that the temperature profile of 26 July 1963 reversed its sign of slope at about the depth at which it crossed the computed line of maximum density. The observed decrease in temperature with depth in Great Bear Lake was about 0.011°C/bar, which is about the temperature profile with the greatest stability according to corollary III. I conclude that data reported by Strøm and Johnson are in agreement with the line of maximum density previously computed by me at 0.021°C/bar (*1*).

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References

1. H. Eklund, *Science* **142**, 1457 (1963).
2. K. M. Strøm, *Geofys. Publikasjoner Norske Videnskaps-Akad. Oslo*, **16**, No. 8 (1945).
3. L. Johnson, *Science* **144**, 1336 (1964).

16 October 1964

Cytopathic Effect of Rubella Virus in a Rabbit-Cornea Cell Line

Abstract. *A rabbit-cornea cell line has been found susceptible to three strains of RK-13–adapted rubella virus. The lesions are very pronounced and appear within a week.*

Weller and Neva first described cytopathic effects of rubella virus in continuously cultured primary human amnion cells (*1*). The changes were, however, slow to appear and difficult to detect. McCarthy *et al.* overcame this difficulty; they used a rabbit-kidney cell line (RK-13) which was sensitive to rubella virus and which showed upon infection very characteristic microfoci formation (*2*). The search for microfoci in inoculated cultures is, however, occasionally difficult and laborious. Cytopathic effect of rubella virus involving the entire cell sheet was reported by Günalp in a line of green-monkey kidney cells (GMK, AH-1) where changes appear in about 2 weeks unless the virus has been previously adapted to the cell line (*3*).

I now report the cytopathic effect of rubella virus in a rabbit-cornea cell line established some years ago at this institute by M. Volkert.

The following virus strains were used in the experiment: "Judith" supplied by A. Svedmyr, Stockholm; "RV" supplied by J. L. Sever, Bethesda, Maryland; and "Orta" supplied by J. L. Melnick, Houston, Texas. The three virus strains were adapted to RK-13 cells in this laboratory. For comparison, the virus strains were titrated simultaneously in the rabbit-cornea cell line and in RK-13 cells originally supplied by G. M. Schiff, Bethesda. The growth medium for the rabbit-cornea cells was Earle's solution supplemented with 10 percent of calf serum, and containing (per liter) lactalbumin hydrolyzate, 1.7 g; yeast extract "Difco," 0.57 g; sodium bicarbonate, 0.84 g; streptomycin sulfate, 0.05 g; and penicillin, 200,000 international units. The RK-13 cells were grown in Medium 199 supplemented with 5 percent calf serum and containing (per liter) sodium bicarbonate, 1.12 g; streptomycin sulfate, 0.05 g; and penicillin, 200,000 international units. Both cell lines were grown at 37°C. The maintenance medium for both cell lines was Medium 199 with sodium bicarbonate (2.24 g/liter); penicillin, streptomycin, and 1 percent of inactivated calf serum. After the medium was changed the tubes were inoculated with rubella virus and rotated at 34°C.

When uninoculated, the rabbit-cornea cell line (Fig. 1) forms a monolayer of densely packed oblongated cells with a fibroblast-like appearance.

In cultures inoculated with rubella virus (Fig. 2) the cytopathic effect usually is discernible 4 days after inoculation. On the 7th day an extensive degeneration of the cell sheet is seen which on the 8th to 10th day reaches total degeneration with the cells falling off the glass.

In cells removed by the collodion-membrane method (*4*) and stained by hematoxylin and eosin the first manifestations of the cytopathic effect is vacuolation of the cytoplasm at the poles of the cell. Next a rounding of the nucleus from the normal oval to a circular shape is seen after a retraction of the cytoplasm around the altered nucleus. Further, the nucleus becomes more dense, and the cytoplasm develops an "ink-spot" appearance with pear-shaped drops radiating from a

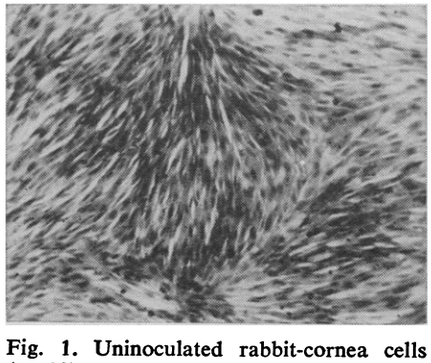

Fig. 1. Uninoculated rabbit-cornea cells (× 83).

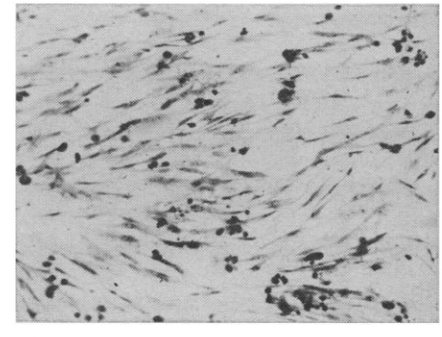

Fig. 2. Rabbit-cornea cells inoculated with 100 $TCID_{50}$ rubella virus, strain Judith; 7th day after inoculation (× 83).

Table 1. Titers ($\log_{10}$ $TCID_{50}$*/0.2 ml) of RK-13–adapted rubella virus strains in rabbit-cornea cells.

Virus strain	Cultured cells	
	RK-13	Rabbit cornea
Judith	3.7	3.2
RV	3.3	3.1
Orta	2.5	2.3

* Tissue culture infective dose, 50 percent effective.

larger circular drop, inside which remainders of the nucleus may be seen.

The cytopathic effects in rabbit-cornea cells of the three different strains of RK-13–adapted rubella virus were identical, and titrations simultaneously performed in the two cell lines gave for each of the three rubella virus strains titers of the same level (Table 1).

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References and Notes

1. T. H. Weller and F. A. Neva, *Proc. Soc. Exp. Biol. Med.* **111**, 215 (1962); *Federation Proc.* **22**, 208 (1963).
2. K. McCarthy, C. H. Taylor-Robinson, S. E. Pillinger, *Lancet* **1963-I**, 593 (1963); C. H. Taylor-Robinson, K. McCarthy, S. G. Grylls, E. M. O'Ryan, *ibid.* **1964-I**, 1364 (1964).
3. A. Günalp, *Proc. Soc. Exp. Biol. Med.* **118**, 85 (1965).
4. J. F. Enders and T. C. Peebles, *ibid.* **86**, 277 (1954).
5. This study was part of work undertaken while the author held a scholarship grant from the Royal Veterinary and Agricultural College, Copenhagen.

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Growth of Mouse Mammary Glands in vivo after Monolayer Culture

Abstract. *Mouse mammary tissue grown in primary cell culture did not display specialized characteristics of the parent tissue. When implanted into gland-free mammary fat pads, not only did some of these cultured cells grow and produce normal mammary outgrowths, but some of the outgrowths formed glands which displayed a variety of culture-induced abnormalities.*

The establishment of monolayer cultures from differentiated tissues necessitates a number of changes, notably the immediate loss of tissue-specific structure and the subsequent loss or reduction of identifiable functional activity (*1*). Although Moscona (*2*) has shown that the dissociation of tissues into their component cells does not in itself preclude the reestablishment of histotypic patterns, the ability to form structured aggregates is mainly limited to embryonic cells (*3*). Furthermore, the process of cell culture produces a decline in the ability of dissociated embryonic cartilage cells to reaggregate (*4*).

Functional changes also may occur rapidly. For example, in cultures of embryonic cartilage a progressive reduction in the formation of extracellular cartilaginous matrix occurs until none can be observed after about 10 days (*4, 5*). Bovine mammary tissue has been placed in cell culture by Ebner *et al.* (*6*), who studied the fate of certain mammary-specific biochemical activities. These activities were observed to decline within a few days, but in a nonparallel manner.

In other cases certain characteristics of the differentiated state may reappear after long periods of time in response to changes in the cellular environment. Chick iris epithelium grown as a colorless sheet of cells for a period of a year or more can form pigment after a change in medium (*7*). More recently, Priest and Priest (*8*) have reported that rat fibroblasts grown in culture for 15 transfers without the production of collagen showed biochemical and morphological evidence of collagen formation when placed in diffusion chambers implanted into the rat peritoneal cavity.

These results point up the classic difficulty of determining whether culture-induced changes represent a process of selection, an actual change in differentiative potential, or merely a reversible alteration that is dictated by limitations of the culture situation—alternatives that have been reviewed and discussed by Harris (*9*). One of the principal difficulties in these investigations is the shortage of suitable phenotypic markers. The failure of cells to produce a given structure or display a certain function when studied only in vitro could mean only that the conditions of culture do not favor these activities. The ideal situation in which to test the state of differentiation of cells is that found in vivo—the return of the cultured cells to their site of origin in an isologous animal. Retransplantation of cultured cells into isologous hosts can be employed in the case of neoplastic cells, which may be easily distinguished from host tissues after a period of growth. Cultured normal cells, which are likely to be confused with host material, must be implanted in unusual sites such as the anterior chamber of the eye, unrelated organs, or diffusion chambers—locations which may not allow full expression of differentiative potential.

Some of these difficulties can be avoided by transplanting into the gland-free mammary fat pads of isologous mice according to the techniques of DeOme and Faulkin (*10*). Fragments of mammary gland transplanted into these gland-free fat pads grow and develop into normal mammary structures. At necropsy the glands may be removed, fixed, and stained with hematoxylin; this results in an optically clear structure in which even subtle characteristics of the parenchymal morphology are clearly visible and in which there is virtually no possibility of confusing host with transplanted tissues.

This report concerns experiments in which the transplantation of cultured cells into gland-free fat pads was used in order to measure the effect of primary monolayer culture on the ability of mammary cells to reconstruct normal, functional mammary tissues.

Cell cultures were established by a method based on that of Lasfargues (*11*). The mammary glands were removed from young adult mice of the C57BL/Crgl or BALB/cCrgl strains that were from 14 to 17 days into their first pregnancy. These glands, which showed good lobuloalveolar development but lacked obvious secretion, were finely minced, placed in Hanks solution containing 0.05 to 0.1 percent collagenase, and stirred at room temperature for 90 minutes or until digestion was nearly complete. After pelleting, the cell suspension was pipetted repeatedly until microscopic examination showed only single cells and small clumps containing about one or two dozen cells. The cell suspension was washed, diluted with growth medium in the ratio of 0.08 ml of packed cells per 100 ml of medium, and grown in prescription bottles at 37°C. The nutrient was changed on alternate days and consisted of Medium 199 supplemented with 10 percent newborn or fetal calf serum, insulin (10 μg/ml), penicillin (50 units/ml), and streptomycin (50 units/ml).

During the first week of culture, cells of typical epithelial morphology proliferated until a nearly continuous sheet was formed (Fig. 1*A*). Between the 5th and 7th days fibroblasts became prominent in the spaces not occupied by epithelial cells, but the two cell types were not seen to intermix even in