# CLONAL GROWTH IN VITRO OF EPITHELIAL CELLS FROM NORMAL HUMAN TISSUES*

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PLATES 48 AND 49

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In previous communications (1, 2), we have described the growth *in vitro* of single HeLa cells isolated from a human cervical carcinoma (3), under conditions such that each cell produces a well defined macroscopic colony within a period of 9 days or less. The present paper describes the application of this plating technique to a variety of epithelial cells originating from normal human tissues, their establishment as clonal cell lines, and some features of their colonial development. These cells will be compared with S3, the clonal HeLa cell which has been adopted in this laboratory as a standard of reference because of its consistently reproduceable behavior over a period of 2 years.

## *Methods and Materials*

The cell strains here described were epithelial in type and had been previously isolated from normal human conjunctiva, liver, kidney, and appendix by Dr. R. S. Chang, (4) who kindly supplied us with specimens of each. These cells had been grown for approximately 40 to 70 serial subcultures (using macroscopic inocula) in Chang's medium, consisting of human serum (20 per cent), chick embryo extract (5 per cent), and balanced salt solution (75 per cent) (4). In addition, we have used our complete growth medium previously described (2) with some slight modifications. Table I summarizes the composition of all the solutions used in the present study.

Glassware is washed by the procedures which have become standard in tissue culture. Incubation is carried out at 37°C. in tight boxes, continuously flushed with a water-saturated mixture of 5 per cent $CO_2$ in air, at a rate equivalent to about 1 complete change per hour. Commercial liquid $CO_2$ is purchased in 20 lb. standard (black top), top delivery tanks and successively passed through: (*a*) a coarse alumina filter[1] to trap oil, (*b*) a two-stage, automatic pressure regulator gauge,[2] and (*c*) a gas flow meter[3] leading to one branch of a Y-tube. Air from the laboratory supply line, reduced to 3 pounds per square inch through a

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‡ Contribution No. 59.

[1] The filter, fabricated in our machine shop, is charged with alumina mesh 4–8, grade F1, obtained from Aluminum Co. of America, New Kensington, Pennsylvania.

[2] Matheson Co., Joliet, Illinois (Matheson No. 8).

[3] Hoke, Inc., Englewood, New Jersey (bantam flow gauge No. 994).

TABLE I

*Composition of Various Solutions Employed in This Study*

| | |
|---|---|
| (*a*) *Saline A:*— | |
| | *gm./liter* |
| Phenol red | 0.02 |
| NaCl | 8.00 |
| KCl | 0.40 |
| Glucose | 1.00 |
| $NaHCO_3$ | 0.35 |
| (*b*) *Trypsin:*— | |
| 0.5 gm. of trypsin (1-300)* to 1 liter of saline A | |
| (*c*) *Hanks's Saline:*— | |
| | *gm./liter* |
| Phenol red | 0.02 |
| NaCl | 8.00 |
| KCl | 0.40 |
| $CaCl_2 \cdot 2H_2O$ | 0.18 |
| $MgSO_4 \cdot 7H_2O$ | 0.20 |
| $Na_2HPO_4 \cdot 7H_2O$ | 0.09 |
| $KH_2PO_4$ | 0.06 |
| Glucose | 1.00 |
| $NaHCO_3$ | 0.35 |
| (*d*) *Nutrient Solution (Based Largely on a Composition Originally Developed by Charity Waymouth (6))*:— | |
| | *gm./liter* |
| L-Arginine | 0.0375 |
| L-Aspartic acid | 0.0300 |
| L-Cystine | 0.0075 |
| L-Glutamic acid | 0.0750 |
| Glycine | 0.100 |
| L-Histidine | 0.0375 |
| DL-Isoleucine | 0.0250 |
| L-Leucine | 0.0250 |
| L-Lysine | 0.0800 |
| L-Methionine | 0.0250 |
| β-Phenyl-L-alanine | 0.0250 |
| L-Proline | 0.0250 |
| L-Threonine | 0.0375 |
| L-Tryptophan | 0.0200 |
| L-Tyrosine | 0.0400 |
| DL-Valine | 0.0500 |
| Biotin | 0.00010 |
| Calcium pantothenate | 0.0030 |
| Choline | 0.0030 |
| Folic acid | 0.00010 |
| Niacinamide | 0.0030 |
| Pyridoxine | 0.00050 |

TABLE I—*Continued*

| (*d*) *Nutrient Solution (Based Largely on a Composition Originally Developed by Charity Waymouth (6))*:—Continued | *gm./liter* |
|---|---|
| Riboflavin | 0.00050 |
| Thiamin | 0.0050 |
| Glutamine | 0.0500 |
| Hypoxanthine | 0.0250 |
| Glucose | 4.80 |
| Phenol red | 0.0125 |
| NaCl | 7.00 |
| KCl | 0.20 |
| $CaCl_2 \cdot 2H_2O$ | 0.14 |
| $MgCl_2 \cdot 6H_2O$ | 0.10 |
| $Na_2HPO_4$ | 0.23 |
| $KH_2PO_4$ | 0.10 |
| $NaHCO_3$ | 2.24 |
| Penicillin | 0.250 |
| Streptomycin | 0.250 |
| (*e*) *Complete Growth Medium*:— | |
| Nutrient solution | 40 per cent |
| Mammalian serum (2 parts pooled human to 1 part horse unless otherwise specified) | 30 per cent |
| Hank's saline | 30 per cent |
| (*f*) *Other Growth Media*:— | |
| Nutrient solution | 40 per cent |
| Mammalian serum | As specified |
| Hanks's saline | To 100 per cent |

* Nutritional Biochemicals Corp., Cleveland.

reducing valve[4] is delivered through a gas flow meter[5] into the other branch of the Y-tube at a rate sufficient to make the final mixture 5 per cent in $CO_2$. The mixture is passed through a sintered glass filter[6] submerged in water at 37°C., and finally delivered into a perforated metal tube running vertically the height of the incubator. Water-filled pans inside the incubator help maintain the needed high relative humidity.

The trypsinization procedure previously described (2) has been modified somewhat, as follows: Growth medium is removed from the bottle containing the cell monolayer to be harvested, which is then washed *in situ* with saline solution A (Table I), by gently rocking the solution (1 to 2 mm. deep) over the cell layer for a few seconds at room temperature. This solution is removed and replaced with an equal volume of the 0.05 per cent trypsin solution (Table I). After 10 to 15 minutes' incubation at 37°C. with occasional gentle agitation, the tryptic action is ended by dilution with an equal volume of complete growth medium at room temperature. The suspension is pipetted for the minimal period of time needed to disperse cell clumps, and aliquots are plated as has been described (2).

[4] Matheson Co., No. 70 reducing valve.

[5] Hoke, Inc., (delivery range: 0.05 to 0.7 liters per minute of $O_2$).

[6] Corning Co., Corning, N. Y. (gas-dispersion fritted disc, coarse porosity, No. 39530).

*Fixing and Staining Procedure.*—Plates containing colonies to be fixed and stained are gently washed several times with 0.15 M NaCl to remove all extraneous protein. Colonies are fixed by flooding the plates with Bouin's fixing solution (5) to a depth of 1 or 2 mm. for 5 minutes. The fixative is then poured off, the plates are rinsed once or twice with alcohol, and a suitable stain applied. Giemsa or hematoxylin are especially satisfactory for direct or photographic visualization.

Vital staining is often useful to identify colonies suitable for isolation and for examination of morphological development. Sterile neutral red is added directly to the nutrient fluid medium in a final concentration of 1:13,000. After incubation at 37° for 5 to 10 minutes, the colonies may be visualized.

In the experiments which follow, the uncertainty of all numerical measurements is indicated by the notation ($\pm\chi$) in which $\chi$ is the standard deviation.

*Definitions.*—The terms, *clone*, *plating efficiency*, and *parental strain*, are used as previously defined (2, 7). The term, *normal*, when used to describe cells, refers only to their origin from apparently normal human tissue and does not imply any knowledge of their latent potentialities.

## EXPERIMENTAL RESULTS

*Growth of Clones from Human Cells of Normal Tissue: Plating Efficiency, Growth Rate, and Frequency of Mitosis.*—Single cells plated from these various tissues by the techniques described earlier in connection with the HeLa cell, readily multiplied to give macroscopic colonies. A typical plate illustrating the colonial growth from conjunctiva cells is shown in Fig. 1. The parental populations yielded plating efficiencies in complete growth medium lying between 50 and 100 per cent. It is noteworthy that for these cells no "feeder" layer (2) was required to obtain good yields of colonies from isolated cells, in the media here employed.

Single colonies were picked by means of the steel cylinder technique (2) subcultured, and developed into new clonal stocks, from each of the four normal cell strains described. In order to insure genetic purity, each strain is passed as routine through two successive single cell isolations, before it is accepted as constituting a clonal population. Further studies were then carried out on these clonal stocks (designated C1, 2; L1, 2; A1, 2; and K1, 2 for clones isolated from conjunctiva, liver, appendix, and kidney, respectively) rather than the more heterogeneous parental populations.

The growth rates and plating efficiencies of these normal cells were measured in media in which the cell had originally been maintained in continuous subculture, as well as in our own growth medium, made up with a variety of mammalian sera. Table II presents a comparison of plating efficiencies of the clonal cells so isolated. All the cells lines shown have exhibited relatively constant plating efficiencies throughout a period of more than 8 months. While most of the differences in plating efficiencies shown are probably not significant, some, like that of Appendix A1 in 10 per cent porcine serum, suggest definite differences in nutritional requirements. Further studies are in progress to determine the frequency and range of variation of genetically controlled

nutritional requirements of different mutant cells isolated from a given tissue; and whether specific nutritional properties exist which are characteristic of the tissue of origin of each cell type (7).

Techniques for simple and accurate determination of the growth curve and division time of HeLa cells have been described in detail (2). Similar determinations have been carried out with most of the normal cell clones. The growth curve of conjunctiva-C1 cells in complete growth medium, as presented in Text-fig. 1, is representative of the cells studied. These conjunctiva C1 cells display a generation time of 23 hours. Similar growth curves, corresponding to generation times of 20, 23, and 22 hours, have been obtained in this medium

TABLE II

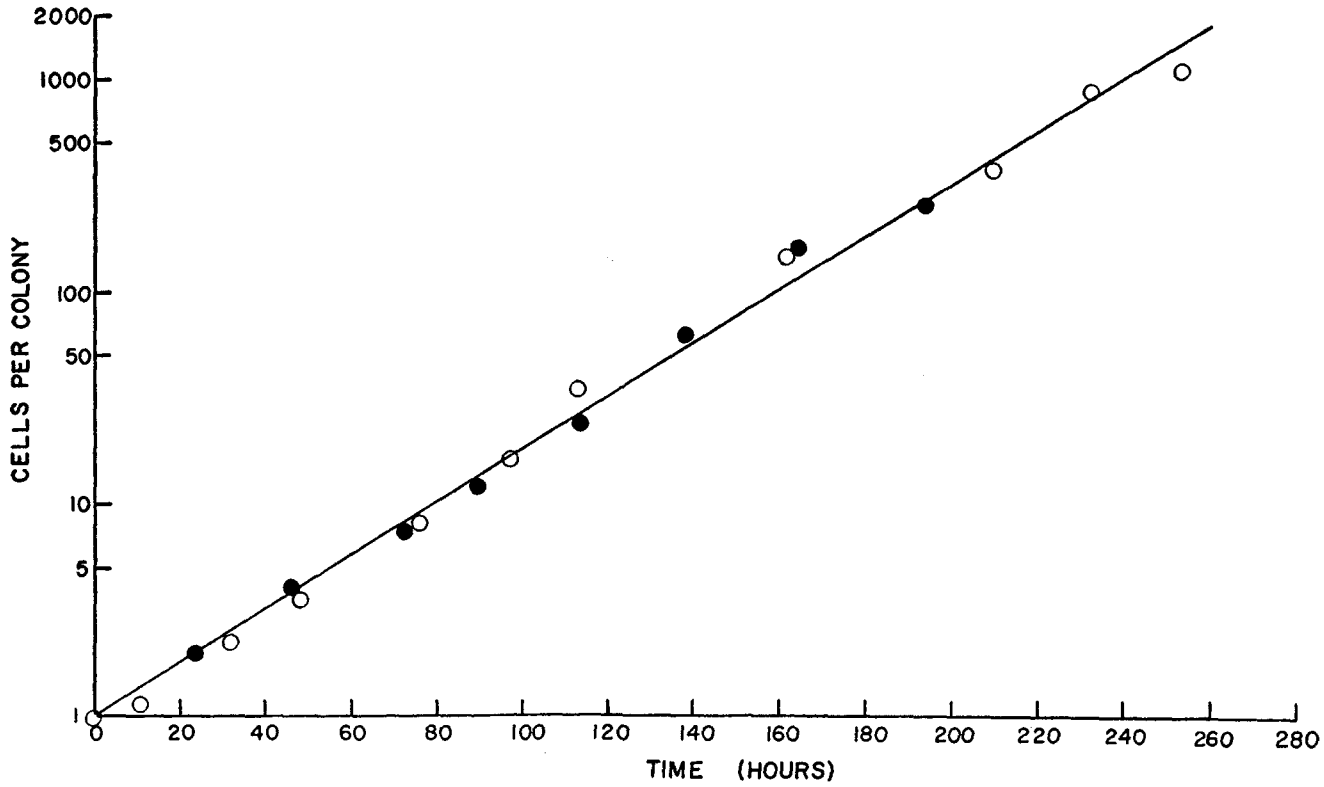
*Measurements of the Plating Efficiency of Cells from Normal Tissues Plated as Single Cells in Complete Growth Medium, Chang's Medium, and Nutrient Solution plus 10 Per Cent Porcine Serum*

The HeLa S3 cell yields essentially complete efficiency (80 to 100 per cent) in all three media.

| Cell | Plating efficiency (per cent) | | |
|---|---|---|---|
| | Complete growth medium | Chang's medium | Nutrient solution plus 10 per cent porcine serum |
| | *per cent* | *per cent* | *per cent* |
| Conjunctiva-C1 | 82 | 81 | 37 |
| Liver-L1 | 51 | 83 | 41 |
| Liver-L2 | 93 | 70 | 72 |
| Kidney-K1 | — | 67 | 70 |
| Kidney-K2 | — | 46 | 71 |
| Appendix-A1 | — | 57 | 10 |

for Liver-L1, Kidney-K2, and Appendix-A1 cells, respectively. These values are not significantly different from that of the HeLa of carcinomatous origin (2).

The duration of mitosis in the HeLa S3 cell under the conditions here employed was determined by continuous observation of individual cells plated in the standard manner, and maintained on the stage of a phase contrast microscope in a thermostated, humidified box with an atmosphere of 5 per cent $CO_2$. A series of such measurements yielded a value of 49 ± 9 minutes as the mitotic time in complete growth medium at 37°C. Mitotic frequencies measured by scanning large numbers of S3 colonies arising from single cells and containing hundreds or thousands of individuals, were found to be 0.038 ± 0.006 under these conditions. If the occurrence of mitosis is distributed randomly in such




TEXT-FIG. 1. Typical growth curve exhibited by single cells of the conjunctiva C1 clone. The solid and hollow circles respectively represent two different experiments carried out some months apart. While the present curve has been drawn as a straight line, a lag period of approximately 10 hours is commonly observed in such experiments, and is suggested by the initial points, but is difficult to quantitate because these points have the largest uncertainty. Each point on the curve represents the mean of cell counts from about 10 or more colonies. Single cells from clonal stocks of liver, appendix and kidney showed similar growth curves in the same medium.

populations, the generation time can be calculated as the ratio of the duration of mitosis to the mitotic frequency. The value so obtained in 19 hours, which is in excellent agreement with the experimentally determined value of 20 hours for the generation time of the HeLa cell (2). This agreement permits the conclusion that in the colonies developing under the present conditions, mitosis occurs randomly rather than synchronously among the cell population.

Determination of the mitotic frequency for normal tissue cells yielded values comparable to that of the HeLa cell: $0.04 \pm 0.025$ and $0.03 \pm 0.025$ for the Conjunctiva C1 and Liver L2, respectively. In each case, about 20 colonies, each containing approximately 1000 cells, were scanned. Presumably, therefore, the fraction of the generation time occupied by the mitotic process is the same in these cells as in the HeLa cell, in complete growth medium.

*Cellular and Colonial Morphology.*—In all of the media used in this study, the colonial and cellular morphologies of all the cell types derived from the four different normal tissues here studied were so similar as to be indistinguishable from each other.

In our previous study it was shown that the HeLa cell can assume two distinct forms, depending on whether human serum is present or absent in the growth medium. In the presence of 20 per cent human serum, the cells assume highly stretched shapes with large cross-sectional areas, and migrate extensively; whereas, if horse, calf, or porcine serum is employed, the developing colonies consist of tightly packed, non-migrating, columnar cells, with small, cross-sectional areas (2). All the epithelial cells from normal tissues resembled the HeLa cell in displaying this differential growth in the presence or absence of human serum, respectively. However, the magnitude of the response was considerably less than that achieved by HeLa cells under the same conditions. A typical example is shown in the photographs of Fig. 2.

A clear cut difference in morphology between these cells as a group and that of the carcinomatous HeLa when grown under identical conditions, was also noted: The normal cells occupy a larger cross-sectional area when stretched on glass than does the HeLa cell. This difference was exhibited in all of the growth media employed. In quantitation of these relationships, detailed measurement of free cell volumes and of the cross-sectional areas of the stretched cells attached to glass were carried out for the conjunctiva and liver clonal cells, as representative of the group of normal cells and compared with HeLa S3 grown under identical conditions. The results of Table III indicate the increased cross-sectional area of the conjunctiva, C1 clone, representing the epithelial cells from normal tissues, as opposed to the HeLa. They also show that, despite large differences in the cross-sectional areas of the glass-attached cells, the total cell volume remains constant, and is practically identical for normal cells and for the HeLa S3. The nuclear area of both cell types shown in Table III was also the same, averaging 300 $\mu^2$.

The cells from the normal tissue resemble those of HeLa (2) in that the cross-sectional area exposed by cells in the center of a colony tends to become smaller as the colonies exceed 1000 to 2000 cells in an area of about 2 to 4 mm.$^2$ presumably because of interactions (*i.e.* physical pressures and chemical exchanges) exerted by cells on their close neighbors (2).

*Morphologic Abnormalities: Multinucleated and Giant Cells.*—Abnormal cell morphology is always of interest and the phenomena of multinuclearity and giant formation are particularly so since, under some conditions at least (8), they indicate that nuclear reproduction may be occurring either more or less rapidly, respectively, than that of the cytoplasmic constituents. A high degree of multinuclearity could also decrease the usefulness of cells cultivated by this method, for genetic and radiation studies.

The frequency of multinuclearity was measured and found to be low in all colonies examined, but particularly low in the larger colonies. The number of

TABLE III

Demonstration that in complete growth medium, the cells from normal tissues have the same volume as the carcinomatous HeLa, but have much larger cross-sectional areas, indicating a higher degree of spreading on the glass surface. A clonal conjunctiva cell is used as representative of the group of epithelial cells originating from normal tissues. Both it and the HeLa cell were grown under identical conditions, utilizing the same batch of complete growth medium, to eliminate any differences due to changes in composition of serum components arising from different donors.

| Clonal cell | Cell volume | Cell surface area on glass |
|---|---|---|
| S3 | $(2.5 \pm 0.45) \times 10^3 \mu^3$ | $850 \pm 375\ \mu^2$ |
| C1 | $(2.1 \pm 0.6) \times 10^3 \mu^3$ | $2010 \pm 600\ \mu^2$ |

multinuclear cells per colony tends to remain constant as the colony size increases, as shown in Table IV for the conjunctiva cell, so that for colonies of 1000 or more cells, the frequency of these forms ranges around 0.1 to 0.3 per cent (Table IV). Essentially similar results were found for colonies of S3, C1, and L1 cells. Whether these abnormalities tend to form mainly during the early period of a colony's development, or whether a steady state is reached in which they disappear as rapidly as they form, remains to be determined.

A series of counts was carried out, analyzing degrees of multinuclearity. The data are presented in Table V. This distribution indicates that the HeLa cell exhibits no markedly different distribution of the various degrees of multinuclearity than obtains with cells from non-cancerous epithelium. The data also permit certain conclusions about the genesis of multinuclearity in such cells. (See Discussion.) Mitoses have been observed involving either one or both nuclei of a binucleate cell.

Giant cells are occasionally observed in these colonies, often achieving diameters 5 to 10 times that of normal cells (Fig. 3). These giants presumably

represent individuals in which cytoplasmic growth has outstripped nuclear division. We have found no morphological difference between these rare giants arising spontaneously and the ones produced in large numbers by high energy irradiation (9). The distribution of spontaneously occurring giants which was

TABLE IV

*The Frequency of Multinucleated Cells in Conjunctiva C1 Colonies of Various Sizes Grown in Complete Growth Medium. An Almost Identical Distribution Was Observed for the Liver L2 Cell*

| Colony Size | Multinucleates | |
|---|---|---|
| (No. of cells) | No. | Frequency |
| | | *per cent* |
| 452 | 4 | 0.88 |
| 490 | 4 | 0.82 |
| 959 | 3 | 0.31 |
| 1130 | 2 | 0.18 |
| 1660 | 5 | 0.30 |
| 1970 | 3 | 0.15 |
| 2470 | 6 | 0.24 |
| 3120 | 5 | 0.16 |
| 3310 | 4 | 0.12 |
| 6200 | 4 | 0.06 |

TABLE V

*Distribution of Multinuclear Forms among the Colonies Developed from Clonal Cells of a Carcinomatous Strain (HeLa S3) and a Normal Epithelial Strain (Conjunctiva, C1) Grown in Complete Growth Medium*

| Clonal cell type | Total No. of cells examined | Binucleates | | Trinucleates | | Tetranucleates | |
|---|---|---|---|---|---|---|---|
| | | No. | Frequency | No. | Frequency | No. | Frequency |
| | | | *per cent* | | *per cent* | | *per cent* |
| S3 | 21,500 | 121 | 0.56 | 15 | 0.07 | 2 | 0.009 |
| C1 | 14,200 | 83 | 0.58 | 6 | 0.04 | 0 | — |

found to be similar in the colonies of HeLa S3 carcinoma and those of the normal epithelial cell here described, had the following characteristics:

(*a*) In colonies of less than 2000 cells, the frequency of giants is much smaller than that of multinucleated forms, and in fact, is too small to be accurately counted. Thus, in a series of 20 colonies of conjunctiva C1 and 15 colonies of Liver L2, with cells per colony ranging from 400 to 6000, not a single giant was found.

(*b*) In marked contrast to the case of multinucleate forms, giant cells tend to increase in frequency as the colonies age, presumably reflecting exhaustion of

nutrients or accumulation of toxic substances. For example, in a carefully followed series of plates seeded with single liver cells, no giants were found in a series of fifteen colonies which had been incubated for 9 days, after which they were fixed and stained and were found to have reached a size of 1000 to 2000 cells. A similar set of plates incubated for an additional 3 days during which the cell number had increased to 2000 to 4500, contained on the average 5 giants per colony. The development of giant cells following a toxic change in the nutrient medium has been observed often in tissue culture.

(*c*) Occasionally giant cells may appear very early in the course of colony development from single cells, and in this case the per cent of giants remains fairly constant throughout the logarithmic period of growth. It is our impression that this phenomenon reflects some inadequacy in the medium.

## DISCUSSION

The foregoing data indicate that cells with epithelial morphology from a variety of normal tissues show no significant difference from the carcinomatous HeLa S3 strain in: the ability of their isolated single cells to form colonies, in the maximum growth rate achieved by such developing colonies, or in the frequency of multinucleated or giant cells they contain.

It is noteworthy that the parental populations of all the epithelial cell strains described exhibited a plating efficiency close to 100 per cent, indicating that in these cell lines, as in the HeLa, the ability to reproduce indefinitely is shared by virtually every member of the population. Techniques are now under study to permit determination of growth potential of cells isolated directly from tissues with minimal or no previous passage in tissue culture.

The cells obtained from the normal tissues also resemble the HeLa in their ability to increase their stretched area on glass when grown in human rather than bovine or porcine serum. The only consistent difference observed in the present experiments between the carcinomatous cell and those derived from normal epithelium lies in the fact that the latter, while possessing the same total volume as the former, exhibit a larger cross-sectional area, when stretched on glass. While this surface area change might conceivably reflect a difference related to invasiveness in these cell strains, such conclusions must be approached with great caution, not only because of the small numbers of cell types here studied, but because the presence or absence of invasiveness cannot be determined solely on the basis of the state of the tissue from which the cell strain originated. The significant number of instances in which cells from normal tissues have been observed to acquire cancerous propensities after growth in tissue culture (10–12) makes necessary much further study before reliable conclusions may be reached.

The distribution of multinuclear cells is low and similar in colonies developing by the plating procedure here described from the clones obtained from

carcinomatous or from normal epithelial tissues. This observation is of interest, since it indicates that neither cell possesses an intrinsic tendency to manifest nuclear aberrations in any marked degree, at least when grown under the favorable conditions which are established in the standard plating procedure.

Of further interest is the fact that the particular distribution of multinucleated cells described in Table V cannot arise solely from a process in which the mechanism governing the appearance of each additional nucleus in any cell is a random event with a constant probability. For example, in that case, one would expect the frequency of trinucleate cells to be the square of that for binucleates; *i.e.*, 0.0031 per cent instead of the observed values for C1 and S3 which are ten and twenty times greater, respectively (Table V). Similarly, the finding even of two tetranucleate S3 cells observed in this series represents a frequency 500 times greater than that demanded by this theory.

The observed distribution could be accounted for if one postulated that a multinucleate cell cannot arise unless some predisposing condition is first established within the cell. (Presumably this might be some degree of inhibition of cytoplasmic multiplication.) Once this condition arises, then the development of an additional nucleus in the cells so affected is a random event with a constant probability. This picture quantitatively predicts the observed distribution of the different multinucleates as follows:—

Let the frequency of occurrence of the condition predisposing to multinuclearity be $p$. Let the probability of development of an additional nucleus in such a cell be $n$. Then the frequency of binucleate cells is $pn$; of trinucleates, $pn^2$, and of tetranucleates, $pn^3$.

From the data of Table V, we can set up the following simultaneous equations for the S3 cell:—

$$pn = 0.56 \text{ per cent} \quad (1)$$

$$pn^2 = 0.07 \text{ per cent} \quad (2)$$

$$pn^3 = 0.009 \text{ per cent} \quad (3)$$

Solution for $n$ from Equations (1) and (2) yields a value of 0.125, while from Equations (2) and (3) the value of 0.13 is obtained, which is an excellent agreement. Similarly, the values for $p$ obtained by substituting $n = 0.127$ in these three equations, are 4.4 per cent, 4.35 per cent, and 4.4 per cent, respectively. This series of unexpectedly good agreements with the demands of the theory would appear to justify entertainment of the proposed picture as a working hypothesis. It is evident from Table V that, within the limits of uncertainty of the data for C1, this cell does not differ significantly from S3 with respect to the parameters determining multinuclearity. Analysis of the frequency of the various types of multinuclear cells under these conditions may provide an important tool for study of the dynamics of interaction of the nucleus and cytoplasm.

The ability of single cells from a variety of human tissues to be grown into colonies by the simple plating procedure described here opens the possibility for extending the quantitative types of metabolic and genetic investigations on growth rate, mutant isolation, high energy irradiation, and other kinds of

studies like those now current with the HeLa strain (7, 9), to cells from these other tissues. Studies are now in progress comparing the response to x-rays, hormones, and nutritional stresses of clonal cells from normal epithelial tissues with that of the HeLa S3. Experiments which will be described later have demonstrated that human fibroblasts can also be grown from single cells by these procedures.

SUMMARY

Tissue culture strains of cells from four different normal human tissues—liver, conjunctiva, kidney, and appendix—have been grown by the plating procedure previously developed for the HeLa strain of cervical carcinoma cells. This technique results in colony formation from isolated single cells, in a manner completely analogous to the plating of bacteria in semisolid nutrient media.

Clonal cell strains have been isolated from each cell type. All behaved exactly alike in all properties studied except that some differences in plating efficiency were displayed in some of the growth media employed.

The cells from normal human tissues resembled the HeLa S3 carcinomatous cell in the following properties:— (*a*) Single cells displayed a plating efficiency close to 100 per cent in an appropriate medium. (*b*) They all grew as an epithelial sheet on glass, the cells being closely packed and polygonal in shape. (*c*) They had mean generation times of 20 to 23 hours in the nutrient media employed. (*d*) The mitotic frequency was constant, and therefore the duration of mitosis was the same for all the strains studied. (*e*) The incidence of multinuclearity and giant formation was very low and similar in both types of cells. (*f*) Both classes of cells had the same total volume, and the same nuclear cross-sectional area. (*g*) Both also showed a tendency to spread more in the presence of human serum (concentration of 20 per cent or more) than in porcine serum. However, this differential morphological response was much more marked in the HeLa cell than in those from normal tissues.

The only difference noted in the behavior of these two groups of cells lay in the tendency of the cells from normal tissues always to exhibit a greater cross-sectional area when spread on glass than the HeLa cell in the same medium.

The frequency of occurrence of different types of multinuclearity in the HeLa cell and cells from normal tissues has been measured. The data suggest that multinuclearity depends on two factors: a necessary, predisposing state in the cell, and a random, independent event causing the appearance of an additional nucleus in such a prepared cell.

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## EXPLANATION OF PLATES

### PLATE 48

FIG. 1. Typical plate inoculated with 150 single conjunctival cells, and grown in complete growth medium at 37.5°C. for 12.5 days in an atmosphere of 5 per cent $CO_2$ + 95 per cent air. The average plating efficiency on a series of 3 such plates was 78 per cent, and the average number of cells per colony was 2500. Actual size.

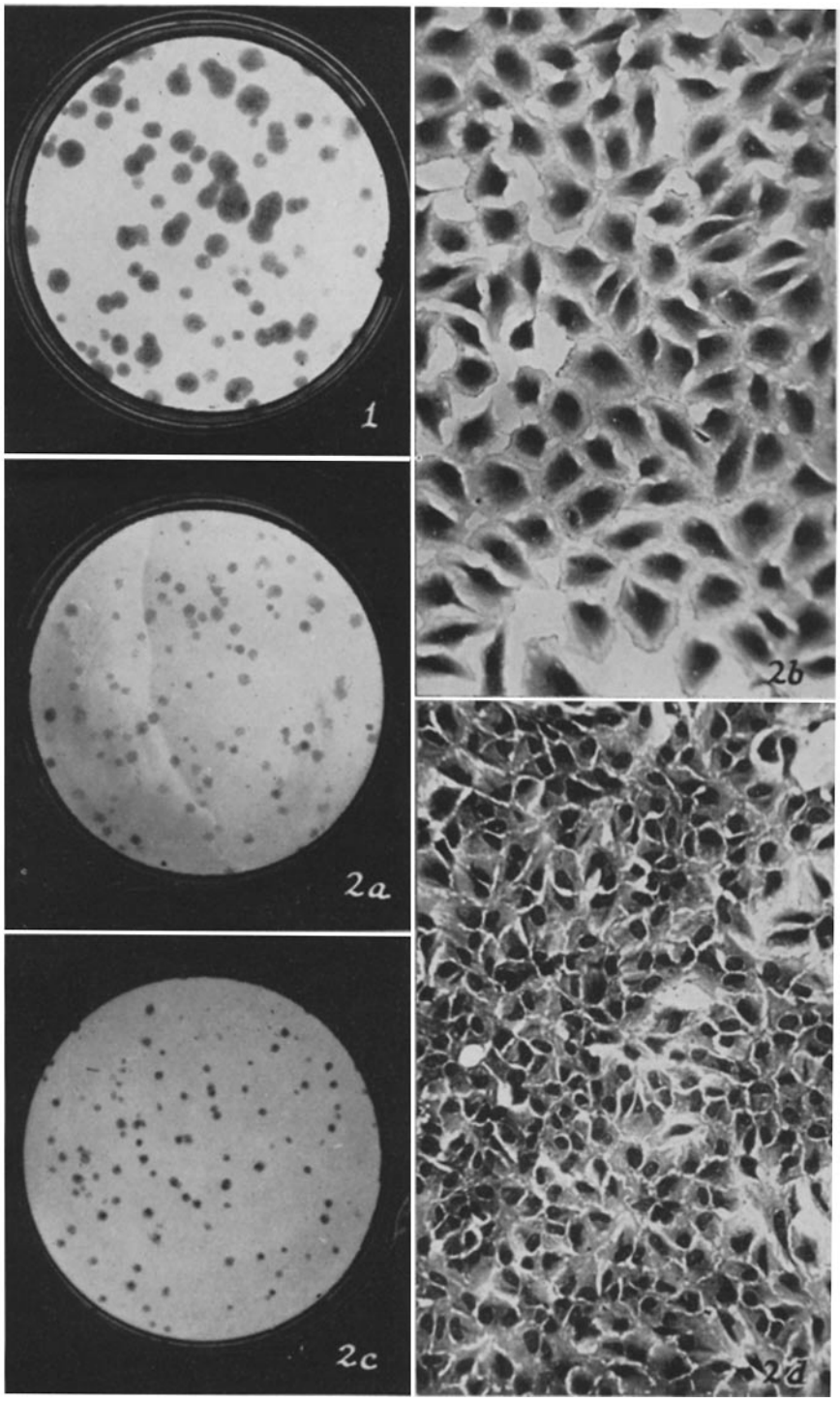
FIG. 2. Demonstration that epithelial-type cells from normal human tissues resemble the HeLa in assuming a smaller cross-sectional area when grown in 10 per cent porcine rather than 20 per cent human serum. Since all four of the normal cell types are indistinguishable in this respect, the liver L2 cell is shown as representative of the group.

FIG. 2a. Plate demonstrating colonies developed from single liver cells grown in standard nutrients plus 20 per cent human serum. Normal size.

FIG. 2b. Typical colony from plate 2a. ×115.

FIG. 2c. Plate identical with that of 2a, but containing 10 per cent porcine instead of 20 per cent human serum. Normal size.

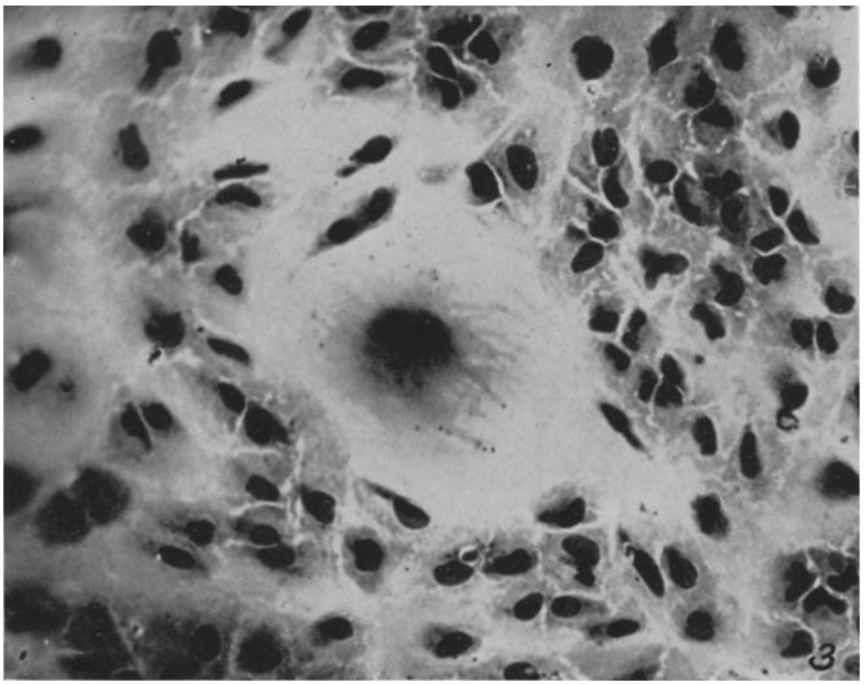
FIG. 2d. Typical colony from plate 2c. ×115. While these cells are clearly smaller than those of 2b, the difference is not as great as that exhibited by the HeLa cell under these conditions (2).




(Marcus *et al.*: Clonal growth of epithelial cells)

PLATE 49

FIG. 3. Spontaneously occurring giant cell in a colony of Conjunctiva C1, grown from a single cell plating in complete growth medium. ×200.



(Marcus *et al.*: Clonal growth of epithelial cells)