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Eight Culture Strains (Detroit) of Human Epithelial-Like Cells.*† (22595)

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Various investigators have reported the development of stable strains of human cells capable of growing on glass surfaces in relatively simple fluid media(1-8). Some strains have been derived from tumor tissue and others from presumably normal tissue. Most have been described as "epithelial" although there are also human fibroblast-like strains in existence. Virologists have given a great deal of attention to one of these human cell strains, the HeLa strain(1), and wide use of this strain has stimulated the development of other human cell strains which might have additional or different useful properties.

A total of 8 epithelial-like strains have now been isolated in these laboratories(9,10). The present report is concerned with histories of the cell strains, procedures used for their isolation and maintenance, and preliminary observations on their morphology and growth behavior.

Histories of the cell strains. Five strains (Detroit-6, -32, -34, -52 and -98) were derived from cultures of sternal bone marrow. Two (Detroit-30A and -56A) were cultured from carcinomatous ascitic fluids and one (Detroit-116P) was obtained from lymphomatous pleural fluid. Table I lists the origin of each cell strain, pertinent data on the

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TABLE I. Histories of Detroit Strains of Epithelial-Like Cells.

Cell strain	Tissue source	Diagnosis of patient's disease	Biopsy material containing malignant cells	Appearance of plaques (days)	Life of strain (mo)*
Detroit- 6	Sternal marrow	Carcinoma of lung	Mass in lung	51	19
" - 32	" "	Carcinomatosis, primary site undetermined	Pleural fluid	65	13
" - 34	" "	Metastatic carcinoma of bone	Tumor of femur	51	13
" - 30A	Ascitic fluid	Carcinoma of breast	Mass in breast, ascitic fluid	24	16
" - 56A	" "	Carcinomatosis, primary site undetermined	Ascitic fluid	55	10
" -116P	Pleural "	Lymphosarcoma	Pleural "	21	8
" - 52	Sternal marrow	Diabetes Mellitus†	None	57	10
" - 98	" "	Refractive error†	"	53	7

* To June 1, 1956. † No malignancies detected.

human donor, the age of the primary culture when epithelial-like cells were first detected, and the subsequent interval (to the present time) during which each strain has been maintained.

Methods of isolation and maintenance. Heparinized aspirated marrow specimens were centrifuged at 4-10°C for 15 minutes at 40 g. The supernatant plasma containing suspended marrow cells was diluted with nutrient solution so that the final mixture contained 125,000 or more cells per ml. Eight ml were placed into each of a series of 3-ounce prescription bottles.‡ The size of the cell inoculum did not appear to be a critical factor between the ranges of 125,000 and 250,000 cells per ml.

Ascitic and pleural fluids were diluted 1:1 in nutritive medium and cultured in 8 ml aliquots without further manipulation. Varying admixture with erythrocytes did not appear to have any deleterious effects. In all instances the bottles were incubated on their flat sides at 36°C.

The nutrient medium used originally was composed of 40% human cord serum, 2% chicken embryo extract, and 58% Hanks' balanced salt solution with phenol red in a final concentration of 0.002% as a pH indicator ($CS_{40}BSS_{58}EE_2$). The nutrient medium in primary cultures was replaced when the pH fell below a range of 6.8 to 7.0. Daily observations were made through the flat sides of the bottles with an ordinary light microscope at 60X or 100X magnification. At intervals, the supernatant fluid containing unattached cells was removed, then centrifuged for 10 minutes at 750 g. Smears of the cell sediments were stained with May-Grünwald-Giemsa for morphologic study. The appearances of such cells in primary marrow cultures have been described in detail(2).

Observations on primary cultures. The marrow cultures from which epithelial-like cells were isolated passed through 3 phases. The first phase was characterized by the presence of specific myeloid cells among which mitotic figures were present. These cells gradually declined in number. The second phase was that of the appearance of monocytoid cells, reticulum cells and macrophages which could be recognized in the culture bottles as large discrete round cells firmly attached to glass. This was followed by the third phase in which spindle-shaped fibroblast-like cells became visible. These phases usually overlapped one another and were variable in time of onset and duration but the sequence of phases was consistent. The carcinomatous ascitic fluids and lymphomatous pleural fluid differed from the marrows in that the fibroblast-like cells appeared within a few days together with the large round cells of monocytoid and macrophage types.

Development of epithelial-like cells. In each instance, the epithelial-like cells were first recognized as isolated plaques or colonies

‡ Owens Oval 3-oz prescription bottles of Duraglas, obtained from McKesson & Robbins, Inc., Detroit.

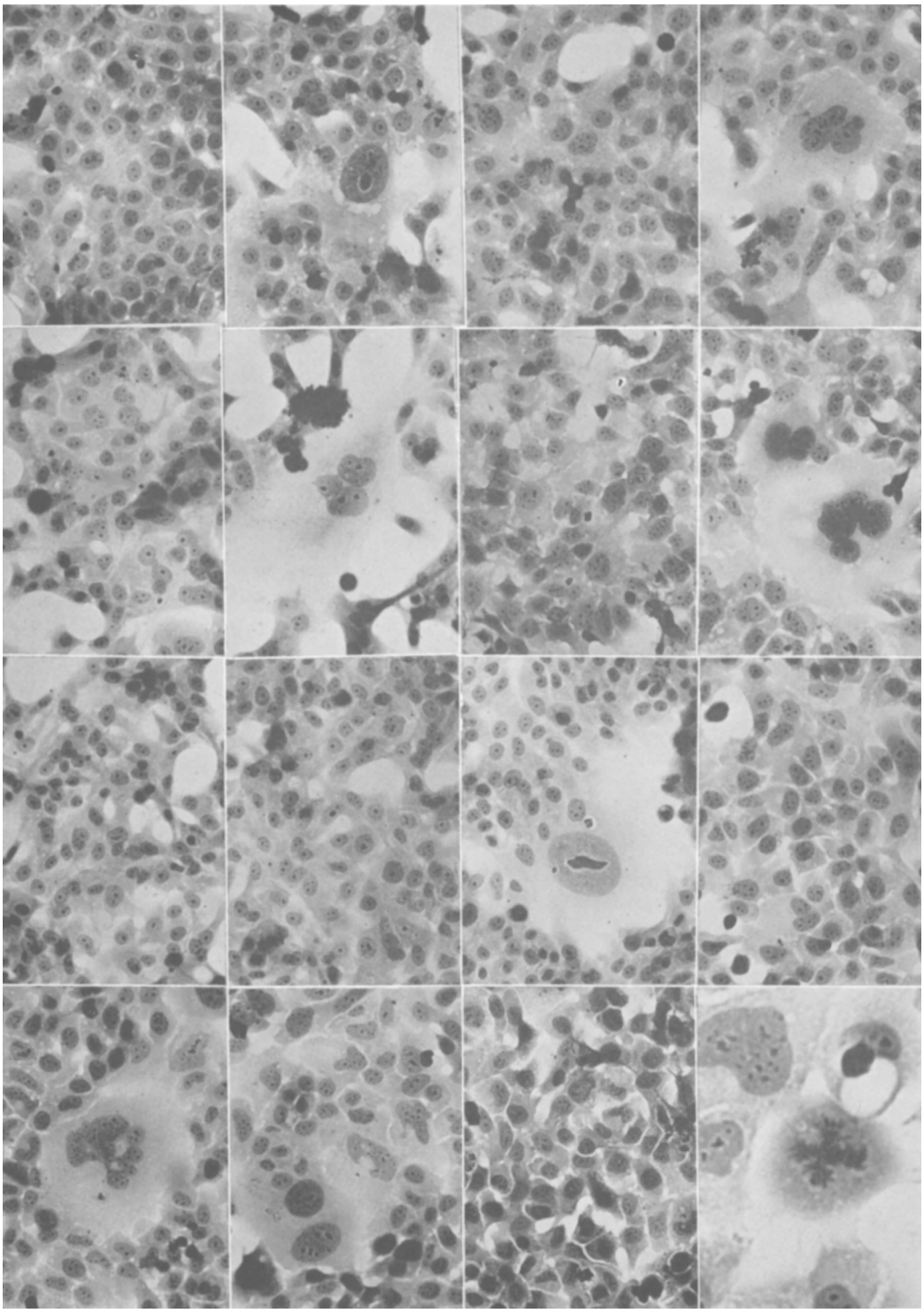

PLATE I. All fields from Bouin-fixed hematoxylin-stained coverslip preparations. Magnifications are 180× except the field in the lower right hand corner, which is 450×. *Top row* (left to right): 1. Detroit-6. 2. Polyploid cell in Detroit-6 culture. 3. Detroit-30A. 4. Polyploid

cell in Detroit-30A culture. *Second row* (left to right): 1. Detroit-32. 2. Polyploid cell in Detroit-32 culture. 3. Detroit-34. 4. Polyploid cells in Detroit-34 culture. *Third row* (left to right): 1. Detroit-52. 2. Detroit-56A. 3. Polyploid cell in Detroit-56A culture. 4. Detroit-98. *Bottom row* (left to right): 1. Polyploid cell in Detroit-98 culture. 2. Polyploid cells in Detroit-98 culture. 3. Detroit-116P. 4. Multipolar mitosis in Detroit-116P culture.

of polygonal cells sometime after the appearance of a fibroblastic phase of growth. Following the development of the isolated plaques, cords and groups of polygonal cells extended from them to form confluent sheets on the glass surface. In several instances, the plaques or portions of the sheets of cells of the primary cultures were removed mechanically for preparing subcultures that continued to grow as sheets of polygonal cells. Subsequently, it was found that frequent subculture after freeing attached cells by trypsin solution resulted in survival of polygonal cells from mixed populations of such cells and spindle-shaped elements. Once the epithelial-like polygonal cells were established in subcultures, there were no reversions to a fibroblast-like form.

Subculture and maintenance of the epithelial-like strains. Originally the cell strains were subcultured and maintained in CS_{40}-EE_2BSS_{58}. In this medium, however, the cultures occasionally were observed to go through irregular periods of poor growth. Following the reports of Eagle(11), all strains have been maintained in Basal Medium (Eagle)¶ plus 20% human cord serum ($BME_{80}CS_{20}$), streptomycin 50 μg/ml and penicillin 50 units/ml. Subculturing is accomplished by replacement of the medium with 5 ml of 0.25% trypsin solution (Difco 1:250) in BME for 10 minutes at 37°C. The trypsin-treated cell suspensions are centrifuged, resuspended in $BME_{80}CS_{20}$ prepared with Hanks' balanced salt solution rather than Earle's as recommended by Eagle(11). The latter modification was introduced in order to maintain the medium at a pH of 7.2 to 7.4 rather than 7.8 to 8.0. This minimizes clumping of cells and results in improved attachment of cells to the surfaces of the culture bottles. Approximately 50,000 cells/ml are placed in the 3-oz prescription bottles in aliquots of 8 ml and incubated at 36°C. Subsequent feedings between transfers or subcultures are made with $BME_{80}CS_{20}$ prepared with Earle's solution. Within 24 hours, sheets of cells are evident. After 3 days confluent sheets cover the flat surfaces of the bottles and after 4 to 5 days, with only one intervening feeding, all the strains yield averages of 400,000 to 500,000 cells/ml.

Preliminary observations on differences and similarities of strains. Plate I illustrates stained cells from sheets of tissue grown on coverslips inserted into Beckman beakers(1) or Leighton tubes.‖ All of the 8 strains grow in the fashion of epithelium, especially as squamous epithelium in the sense these terms are used in the tissue culture literature(12, 13). Furthermore, the 8 Detroit strains cannot be distinguished from each other on the basis of examination of individual cells by ordinary methods of microscopy applied to living or fixed stained preparations. Each of the strains exhibits features found in malignant tissues, namely, anisocytosis, giant nucleoli, polyploidy, abnormal mitoses, and rapid growth even though some of them were derived from tissues of patients free of malignant diseases. On the other hand, there are subtle differences among the various strains. These are evident in the topographic appearances of the sheets of cells that form during proliferation. In Plate II are photomicrographs made from living cultures in the prescription bottles after 3 days of growth in $BME_{90}CS_{10}$. At this stage of growth Detroit-98 and -116P are similar; they appear as confluent monocellular layers. After the same period of incubation, Detroit-52 grows as clumps of rounded cells. Continuous epithelial-like sheets do not appear until the fourth or fifth day of growth. The other cell strains cannot be distinguished by their topographic

¶ Obtained as separate stock concentrates of amino acids, vitamins and glutamine from Microbiological Associates, Inc., Bethesda, Md.

‖ Obtained from Microbiological Associates, Inc, Bethesda, Md.

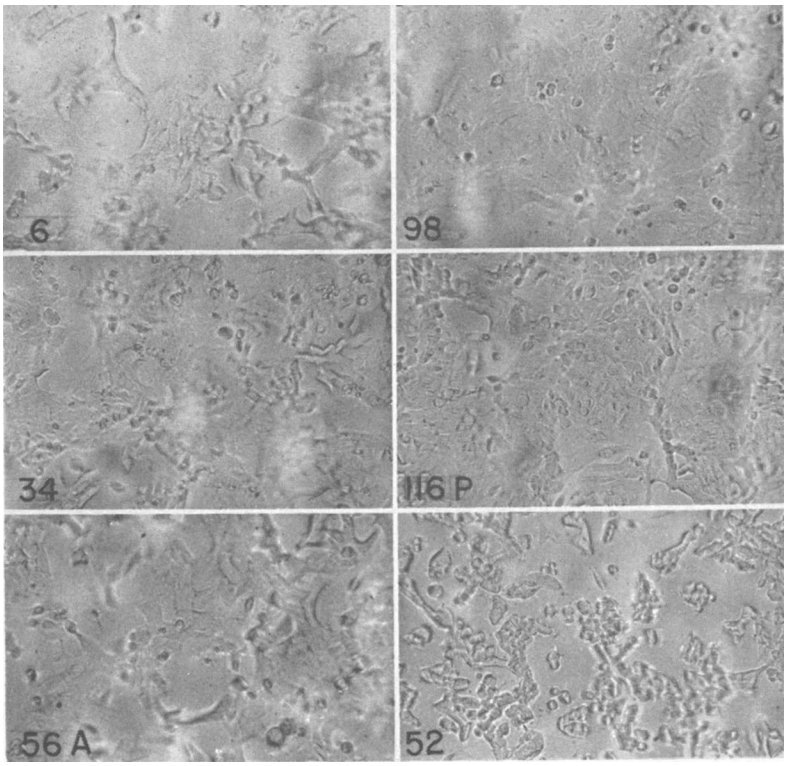

PLATE II. Appearances of unstained living cultures as seen through the flat surfaces of prescription bottles after 3 days of growth. (75×.)

appearances under these conditions (10% serum). If the serum content of the medium is increased to 20%, all strains form confluent monocellular layers earlier.

Discussion. It is emphasized that the designation of the 8 Detroit strains discussed herein as epithelial-like does not imply that they are necessarily true epithelial cells, nor do cytologic phenomena of the types seen in malignant tissues imply that such morphologic features, by themselves, indicate that the cells are cancerous. The cells reported here have growth characteristics that are similar to those of cells derived from epithelial tissues. In the absence of any signs of morphologic differentiation toward the formation of tubules or adenomatous structures these cells must be regarded as examples of "indifferent epithelial-like cells" mentioned by Parker(14). The 8 Detroit strains reported here have derived from tissues free of epithelium as well as from sources known to contain malignant epithelial cells. In each instance, however, the primary material contained a mixture of cells including derivatives of the histiocytic or reticuloendothelial system and the vascular system as well as hemic elements. Although various cell strains developed by others have been referred to as lung cancer cells, skin cells, cervical cancer cells, bone marrow cells, etc., there exists the possibility that the various strains may have developed from a source common to the different tissues used, for example, vascular endothelial cells or histiocytes. Distinctions between morphologically similar strains of cells must be based on more precise morphologic technics and biological activities than have usually been used. An attempt to make use of comparative virological susceptibilities as a means of distinguishing between the various epithelial-like strains will be reported in another communication. It is now apparent that the development of strains of human epithelial-like cells from bone marrow, ascitic and pleural fluids, can be accomplished by

fairly simple methods but the explanation of how and why such cells appear in cultures is more difficult. For example, it was stated by Parker *et al.*(15) that they had observed numerous instances of the development of strains of unidentified cells that multiplied with great rapidity to yield uniform populations of free-living cells. It was implied that such cells were altered in some way during cultivation *in vitro*. The idea is similar to that expressed in discussions of cell modulation(16,17). The environmental conditions that account for the appearances of altered cells or modulation forms are not completely understood. It has been demonstrated by numerous investigators that morphologic and growth characteristics of cells can be modified greatly by deficiencies of essential nutrients. Eagle(11) has recently demonstrated the structural changes resulting from some amino acid, vitamin and salt deficiencies. Morphologic studies have emphasized the need for such information. Leighton *et al.*(6) have described the transformation of normal human fibroblasts into cells that appeared malignant by histologic criteria. It is questionable whether histologic features alone are adequate for determining the malignant potentialities of cells. Some of the morphologic criteria of malignancy are found in Detroit strains that were derived from non-cancerous individuals. The possibility that such strains have become malignant *in vitro* requires proof by methods that have not yet been devised for study of human strains. Puck *et al.*(18) described the isolation of clones of cells from HeLa cultures. The clones represent different phenotypes derived from the original population of cells. The ideas of Parker *et al.*, Leighton *et al.*, and Puck *et al.*, are equally reasonable explanations of the appearance of epithelial-like cells in tissue cultures derived from mixed populations of cells. An unresolved problem relates to the possibility that the stable strains of epithelial-like cells represent the survival of a particular cell type included in the primary cultures.

Summary. The histories of 8 strains of human epithelial-like cells derived from bone marrow, ascitic and pleural fluids have been described. Preliminary observations on their morphology have been reported and the significance of these observations has been discussed.

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