human cell culture. morphology of the detroit strains

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the isolation of increasing numbers of human cell strains has stirred up old problems and created new ones. for example, continuously maintained transferable cells lose their resemblance to those of the original explanted tissues, and there are many similarities among cultured cells derived from different sources. furthermore, in vitro the cells may acquire features pathologists rely on for recognition of malignancy. this occurs in cells derived from noncancerous as well as cancerous sources.

during the past few years we have studied thirteen strains of human cells developed in our laboratories from bone marrow (detroit-6, -32, -34, -52, -98, -143), carcinomatous ascitic fluid (detroit-30a, -56a), lymphomatous pleural fluid (detroit-166p), nonleukemic peripheral blood (detroit-173b, -b16, -b17), and a hamartoma of liver (detroit-501). others have had similar results with various normal and malignant tissues (1, 29, 37, 39, 41, 46). these strains have been maintained in continuous culture by repeated transfers for 8-26 months. their use for virologic studies has been reported (6, 42, 44, 45). this paper deals mainly with morphology as observed by bright-field microscopy.

materials and methods

for maintenance of strains we use eagle’s basal medium (11), to which serum of human or animal origin is added. suspensions of cells from marrow, blood, ascitic or pleural fluid, or one prepared by mincing and trypsin-treatment of solid tissues, are placed in ordinary prescription bottles lying on their flat sides. cells settle on the glass and proliferate. morphologic studies of fixed and stained preparations at high magnifications are made on films prepared on removable coverslips placed in 5-ml beckman beakers or leighton tubes. the details of isolation, maintenance, and transfer of cells were given in previous papers (5, 6, 45). additional special technics will be described in connection with related observations.

observations

there are subtle differences among the ep-l detroit strains, but their significance is not clear. morphologic findings exhibited in common will be emphasized. variances shown to have functional meaning will be discussed in another publication.

morphologic phases in the cultures.—four phases can be observed: (a) unchanged cells of the original explant, (b) large round cells, (c) fibroblast-like cells, and (d) epithelial-like cells. the times of appearance and duration of phases are variable (table 1).

in the first phase of bone marrow cultures leukocytes and their precursors attach to glass. mitotic activity continues for a while, but the number of these cells declines and ultimately they disappear. this is followed by the second phase, in which...
large round histiocytoid and monocytoid cells increase in number. The third phase begins with the appearance of spindle-shaped and stellate cells which proliferate to cover the entire culture areas in palisades and networks. In cultures from which Ep-L strains develop, a fourth phase begins with isolated foci of polygonal cells. Thereafter, colonies of these cells increase in area and coalesce, while the others decrease in number and finally disappear. Repeated transfer results in the isolation of strains of polygonal cells which maintain their appearance without reversion to a Fb-L state.

In cultures of lymphomatous pleural and carcinomatous ascitic fluids the second phase appeared ample, in a culture of Fb-L type maintained for months we observed a change to an Ep-L condition. During the period of transition when the cultures contained Fb-L sheets and foci of Ep-L cells the smears contained both types. Figure 9 shows a smear containing a group of typical Fb-L cells, among which were two Ep-L cells appearing almost black in the photomicrograph because of the intense staining of their cytoplasm. With MGG staining we found that single-strength Giemsa was adequate for Ep-L cells, whereas Fb-L cells required triple-strength Giemsa.

The Ep-L cells had homogeneous, reticular, or finely vacuolated cytoplasm, although on occasional transfers distinct larger vacuoles were present, or was skipped, to be followed quickly by the third and fourth phases. These events were illustrated in previous publications (5, 6).

In several nonleukemic peripheral blood cultures the second phase was followed by the fourth without an intervening fibroblast-like phase.

Growth rates of established lines are shown in Table 2.

Morphology of trypsin-treated cells.—When cells have covered the culture areas they are harvested by applying trypsin solution by methods previously described (6). The resulting cell suspensions are divided and transplanted. Smears from thick suspensions are air-dried and stained with May-Grunwald-Giemsa (MGG). Figures 1–9 show specimens from various strains.

The isolated cells of Ep-L lines had round or oval nuclei and deeply basophilic cytoplasm with sharply defined edges, although small excrescences might be present (Figs. 1 and 2). When these were numerous, there were detached fragments of cytoplasm adjacent to the cells (Figs. 3 and 5). On the other hand, cells from Fb-L cultures treated in the same way had hyaline ectoplasmic borders which could be seen best when the cells were in apposition (Fig. 8).

The tinctorial properties of cells which grow as Ep-L strains were different from those which grow as Fb-L strains. The degree of cytoplasmic basophilia was much greater in the former. For instance, in a culture of Fb-L type maintained for months we observed a change to an Ep-L condition. During the period of transition when the cultures contained Fb-L sheets and foci of Ep-L cells the smears contained both types. Figure 9 shows a smear containing a group of typical Fb-L cells, among which were two Ep-L cells appearing almost black in the photomicrograph because of the intense staining of their cytoplasm. With MGG staining we found that single-strength Giemsa was adequate for Ep-L cells, whereas Fb-L cells required triple-strength Giemsa.

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**Table 1**

AGE OF PRIMARY CULTURES WHEN VARIOUS PHASES APPEARED DURING THE DEVELOPMENT OF THE DETROIT Ep-L CELL STRAINS

<table>
<thead>
<tr>
<th>Source</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow of cancer patients</td>
<td>Det-6</td>
<td>Det-32</td>
<td>Det-34</td>
</tr>
<tr>
<td>Bone marrow of cancer-free patients</td>
<td>Det-28</td>
<td>Det-54</td>
<td></td>
</tr>
<tr>
<td>Bone marrow of cancer patients</td>
<td>Det-28</td>
<td>Det-54</td>
<td></td>
</tr>
<tr>
<td>Carcinomatous ascitic fluids</td>
<td>Det-30</td>
<td>Det-34</td>
<td></td>
</tr>
<tr>
<td>Lymphoma pleural fluid</td>
<td>Det-50</td>
<td>Det-56</td>
<td></td>
</tr>
<tr>
<td>Nonleukemic peripheral blood</td>
<td>Det-11</td>
<td>Det-11</td>
<td>Det-12</td>
</tr>
</tbody>
</table>

**Table 2**

GROWTH RATES OF DETROIT Ep-L STRAINS BASED ON CELL COUNTS OF TRYPSIN-TREATED SUSPENSIONS

<table>
<thead>
<tr>
<th>Source</th>
<th>Initial Cell Count (X 10⁶)</th>
<th>After 4-5 days Cell Count (X 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow, cancer patients (Detroit-6, 32, 34)</td>
<td>2.8-4.4</td>
<td>7.6-61.7</td>
</tr>
<tr>
<td>Bone marrow, cancer-free patients (Detroit-32, 34)</td>
<td>2.8-4.6</td>
<td>9.6-46.0</td>
</tr>
<tr>
<td>Carcinomatous ascitic fluids (Detroit-30A, 56A)</td>
<td>2.7-4.6</td>
<td>15.5-54.0</td>
</tr>
<tr>
<td>Lymphomatous pleural fluid (Detroit-116P)</td>
<td>3.0-4.7</td>
<td>22.4-46.7</td>
</tr>
<tr>
<td>Nonleukemic peripheral blood (Detroit-172B)</td>
<td>3.0-4.5</td>
<td>8.5-42.5</td>
</tr>
</tbody>
</table>

* Total cell count for 3-ounce bottle.

The nuclear chromatin pattern in all strains is a delicate filigree of very small, uniformly distributed masses. The areas occupied by chromatin are generally greater than those occupied by parachromatin. On the whole, the nuclear patterns re-

4 One drop of Giemsa stock solution in 1 ml of water buffered to pH 6.8.
semble those of reticulum cells of hematopoietic tissues (8) and many forms of metastatic tumor cells seen in MGG-stained dry films of human bone marrow (48).

Distinct nucleoli are present in all strains. They are usually multiple, but different strains have varying percentages of cells with single large nucleoli. The 30A strain from carcinomatous ascitic fluid of a patient with cancer of the breast had a marked predominance (90 per cent) of cells with single, large, round nucleoli having diameters over one-fourth that of the nuclei. In this respect they resemble HeLa cells (Gey et al.) (16) very closely.

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Morphology of cell sheets.—The similarity of various Detroit Ep-L strains was due to their characteristic formation of monolayered layers of polygonal cells (Fig. 12). Unlike cultures of monocytes or macrophages, these were not discrete cells lying close to one another but were cells intimately in contact over broad surfaces with connecting filaments of cytoplasm resembling intercellular bridges, although without the regular structure seen in squamous epithelium. If cultures were allowed to proliferate without transplantation after cells had covered the available areas of the glass, the sheets became thicker and stratified.

Fb-L cells had an entirely different appearance (Fig. 13). They formed palisades and networks of spindle-shaped and stellate elements which became multilayered rapidly if the cultures were not divided and transplanted. Under different conditions the Fb-L cells changed considerably in length and width. In freshly transplanted cultures of low cell density, the cells were shorter, broader, and irregularly stellate. Cultures of high density contained cells of long bipolar form.

Morphology of cell colonies.—The colonial behavior of cultures of epithelium is usually different from that of fibroblastic connective tissue. When Fb-L cultures changed to Ep-L cultures in bottles, the colonial behavior of cell clusters changed also.

Morphology of sponge matrix cultures.—Three-dimensional growths of tissue were prepared by placing suspensions of cells in pieces of cellulose sponge. Portions of sponge 8 × 5 × 1 mm were washed and sterilized according to the method of Leighton (28). A bottle culture was subjected to trypsin-treatment, and the free cells were sedimented by centrifugation, washed in balanced salt solution, and resuspended in 0.5 ml. of medium. The concentrated suspension containing approximately 2 to 3 million cells was placed in an embryological staining dish to which six pieces of sponge were added. After the sponges had soaked up the cell suspension, two pieces were placed in each of three 16 × 150 mm. roller tubes containing 2 ml. of medium. In a few days some of the interstices of the sponges were filled with tissue. At intervals sponges were removed for sectioning. The Ep-L cells produced masses large enough to be studied conveniently after 9–10 days of incubation, but Fb-L cells only occasionally formed masses of tissue of grossly visible size even after 40 days.

The different strains grew as tissue resembling undifferentiated epithelium (Figs. 14 and 15). This was also characteristic of sponge matrix cultures of HeLa cells prepared from suspensions. In some of our strains a distinct layer of cuboidal cells formed as a limiting membrane on the surfaces in contact with medium (Fig. 14). As the masses of Ep-L cell tissue became larger, there usually was necrosis of the central parts, although mitotic activity and vigorous growth persisted in the peripheral portions even after 43 days. Necrosis of the central parts produced pseudocystic structures. This occurred at different ages of the cultures of various strains.

Morphology of cells transplanted to chorioallantoic membranes of chicken embryos.—The main purpose of these experiments was to investigate the possibilities of studying morphogenetic or histogenetic potentialities of transplanted Ep-L cells in embryonic hosts because of the properties of mesenchymal frameworks or fibroblasts in tissue culture of enhancing morphogenesis of epithelial cells (88). Ten-day-old embryos were given inoculations of washed trypsin-treated cells placed directly on the chorioallantoic membranes which had been dropped by the artificial air sac method of Burnet (7). The inocula contained 100,000–500,000 cells suspended in 0.2–0.3 ml. of maintenance medium (6) or basal medium. The eggs were not rotated.

Additional photomicrographs of sheets of Detroit cells have been published elsewhere (5, 6).
The involved areas of the membranes were removed and fixed in Bouin's fluid. The cell lines studied were Detroit-6, -32, and -34 from the bone marrow of cancer patients and Detroit-98 from the bone marrow of a cancer-free individual. Figure 11 shows the proliferating Ep-L cells of the Detroit-92 strain. There was formation of cylinders of cells supported on definite basement membranes (Fig. 16). Figure 17 shows the loose and irregular foci and small cords of cells of an implanted strain derived from bone marrow of a cancer-free individual. An intense desmoplastic reaction resulted from an attempted passage of a portion of the involved chick tissue to the chorioallantoic membrane of a second embryo in which we observed a nodule composed mostly of the host's fibrous tissue enclosing occasional isolated cells of the initial lesion, indicating that they were able to penetrate the new host. At present no significance can be attached to differences in reactions caused by cells from cancer and noncancer sources, because only a few experiments have been carried out. These preliminary observations are reported to indicate the possibilities of this method for studying morphogenic properties of cells which produce only undifferentiated cell masses or sheets in vitro.

Variations in morphology of cells.—Although within each Ep-L cell line there are numerous aberrations of morphology, the strains are alike in displaying prominently the types of abnormalities commonly associated with cancer. Among these attributes are giant cells, great anisocytosis, variations of shape and staining properties, multinucleated cells, micronuclei, giant nuclei, multi-formed nuclei and nucleoli, giant nucleoli, atypical multipolar mitoses, contracted or superfragmented chromosomes, and halo-like areas around nucleoli, all of which have been noted in human cancers, human pleural and ascitic malignant effusions, and in tissue cultures of human cells derived from cancerous tissue as well as in tissue cultures which were considered to have undergone malignant transformations (18, 21, 22, 27, 38). We found no evidence that these phenomena are less frequent in Detroit Ep-L lines derived from noncancerous sources as compared with sources containing cancer cells. The incidence of multinucleated cells in cultures derived from nonleukemic peripheral blood was 94 per 1000, while in cells of cultures from carcinomatous ascitic fluid it was 47 per 1000. In peripheral blood strains the incidence of cells with more than three nuclei was 23 per 1000, but it was only 5 per 1000 in cancerous ascites strains.

Various types of abnormalities and alterations of cell morphology are illustrated in Figures 18 and 19. The greater pleomorphism of cultures from nonleukemic blood can be appreciated by comparing smears of trypsin-treated cells (Figs. 1 and 7).

Chromosome counts of cells from 2-day-old cultures were made on squash preparations according to the hypotonic methods of Hughes (24) and Hsu and Pomerat (28), with certain modifications. Cell sheets were washed with isotonic phosphate buffer and then treated for 15–20 minutes with hypotonic phosphate buffer7 with 0.02 per cent disodium versenate. During the period of treatment the culture flasks were gently agitated. The partially dispersed sheets were squirted through a 10-ml. pipette. The cell suspension was centrifuged and the supernate decanted. This left a packed mass of cells which was mixed with twice its volume of aceto-orcein stain.8 The cells remained in the stain for 5 minutes. A drop of the mixture was placed on siliconized slides and squashed by means of thumb pressure on an overlying nonsiliconized coverslip. Repeated counting of chromosomes of metaphase cells of the 65–75 condition showed that the error was no more than two chromosomes, and was generally less. The counts for strains Detroit-6, -30A, -92, and -34, and 116P indicated extensive heteroploidy. Among the 240 cells studied there was none with the normal diploid number, 46–48, of human somatic cells (47). In lines from bone marrow of patients with cancer (not shown to have cancer in the marrow itself) the modal values were between 68 and 70. In a strain from lymphomatous pleural fluid containing numerous lymphoblasts the modal value was 80–85. All strains gave indication of a small percentage of the cell population existing in conditions representing multiples of these modal values. Small numbers of cells had extremely high chromosome numbers, with a few counts as high as 500 chromosomes. The frequency of cells with such extremely high counts is under-represented in the histograms (Chart 1) because of the difficulty of counting accurately in cells with very high chromosome numbers. Figure 10 shows a cell in a smear made from a suspension of trypsin-treated cells which were maintained for a short time in hypotonic medium. The chromosome number is probably in the vicinity of 260, which represents 3–4 times the modal values observed in the Detroit lines.

Preliminary study of karyotypes has shown an increased incidence of chromosomes having a sub-
terminal centromere when they are analyzed by the method of Levan (32).

Transformation of Fb-L cell strains to Ep-L cell strains.—As previously noted, the development of polygonal cell sheets began as isolated foci of such cells in otherwise Fb-L cell cultures. The foci enlarged and replaced the spindle-shaped and stellate cells. Sometimes this occurred in cultures which seemed to be on the verge of dying out, as indicated by the disappearance of Fb-L cells both by degeneration and detachment of cells from preparations with cultures on removable coverslips provided suitable material. Since the Ep-L strains had already been established as such, we have studied a strain of Fb-L cells from human embryos (MAF) which was undergoing a similar alteration in our laboratory.

Originally, the MAF cultures were composed entirely of spindle-shaped and stellate cells. In scattered areas the cytoplasm of these cells became more deeply basophilic, as indicated by the increased intensity of the blue color in MGG-stained glass. These events were characteristic of cultures derived from bone marrow, carcinomatous ascitic and lymphomatous pleural fluids, and a hamartoma of liver; but in the case of cultures originating from nonleukemic peripheral blood the polygonal cell foci emerged among the phase 2 cells, although occasional Fb-L cells of short life span were seen in some specimens.

At first we were able to make these observations only on living cultures or in smears of trypsin-treated cells, but once the sequence from phase 2 and Fb-L cells to Ep-L cells was found to be quite regular we sought means of making serial observations on permanent fixed preparations which could be examined in detail. The Leighton tube

CHART 1.—Frequency distributions of chromosome numbers of five Detroit Ep-L strains

preparations, which may be a sign of an increased concentration of ribonucleoprotein (9, 19, 25). While this took place the affected cells became broader and shorter and their nuclei became larger and rounder, and usually the nucleoli also became more brightly stained and distinct (Fig. 20). There were numerous examples of such altered cells with deeply basophilic cytoplasm still attached by extensions of cytoplasm to adjacent spindle-shaped and otherwise typical Fb-L cells. As the focus of change enlarged, more and more cells in the region were similarly altered; those near the center of the focus became more squat until they formed closely approximated polygonal

* From Microbiological Associates, Bethesda, Md.
cells existing in small cords and sheets. Some of the polygonal cells divided by mitosis; cords of increasing length and width formed, and at the same time more foci of change appeared. When the cultures of mixed cell population were divided and transplanted after treatment with trypsin, the new cultures started out with increased numbers of polygonal cell foci, and, as more transfers were made, the numbers of polygonal cells increased at the expense of the others. After several transfers the new cultures started out as sheets of polygonal cells, and thereafter spindle-shaped cells with their relatively pale-staining cytoplasm no longer appeared. This alteration of cellular morphology was reflected functionally in several ways: increased rate of proliferation in bottle cultures; ability of the altered cells to grow readily in sponge matrix cultures; change in the growth pattern of plasma clot cultures.

**DISCUSSION**

Our experience with cultures of suspensions of human cells shows that identification of tissue culture cells merely on the basis of the tissue of origin is misleading. Various reported human cell lines were derived from mixed cell populations. However, there are many similarities among those Ep-L cells which can be maintained in vitro. The uniformity of end results, even though the primary sources are as widely divergent as ascitic cancer cells and normal leukocytes, is becoming generally recognized. Marcus et al. (35) noted that cells with epithelial morphology from a variety of normal tissues show no significant differences from the carcinomatous HeLa SS strain in ability of their single isolated cells to form colonies or in the frequency of multinucleated or giant cells. Leigh-ton et al. (cited by Moore et al., Science, 124:129, 1956) found that Chang's conjunctiva and Henle's intestine 407, both derived from normal individuals, are indistinguishable cytologically from what are considered to be tumor cell lines. Likewise, when various Detroit Ep-L cells are examined by the Papanicolaou technic they are indistinguishable. Fennell (cited by Moore et al., Science, 124:129, 1956) found this to be the case when "normal" Chang liver cell and "tumor" cell lines were studied in this way. Even if a strain were developed from a single cell of cancer tissue, there would be no way of knowing that the one selected was originally malignant, unless it can be assumed that every cell in a cancer is so.

Attempts to distinguish between Ep-L cells on the basis of their morphogenetic potentials by observing their behavior in clot and sponge matrix cultures or by transplantation experiments have yielded some interesting but as yet unexplainable results. Polygonal cells grew in plasma clots and sponges as masses resembling undifferentiated epithelium, but when the cells were transplanted to chick embryos some morphogenic properties were revealed. Moore et al. (38) observed this to a small extent in human volunteers given inoculations of human tissue culture cells, and more recently we have been informed of successful transplantation of human tissue culture cells in unconditioned hamsters by Foley and Handler, who observed morphogenic potentialities in subsequent passages. In the chorioallantoic membranes, cells from bone marrow cultures of a cancer-free individual formed cylinders and cell groups quite like those of malignant epithelial tumor metastases in spite of the fact that cells with such morphogenic potentialities are unknown in human bone marrow.12

The cells of Ep-L lines grow at rapid rates, as might be seen in some hyperplastic or neoplastic conditions. The morphologic difference between hyperplasia and neoplasia in vivo is a small one compared with the differences between the original cells of the explanted materials and those maintained in vitro, when such are sought by the ordinary microscopical methods we have used. There are instances in human pathology in which normal-looking cells comprise tumors with the clinical aspects of cancer. Some of the malignant lymphomas can be cited as examples (3). On the other hand, in various inflammatory and reactive conditions unusual cells with abnormal features are often present. The gradation between reactive hyperplasia and neoplastic growth is continuous, at least as observed by pathologists familiar with human tissues (18).

Human cells undergo profound alterations in tissue cultures. This can be appreciated by comparing normal leukocytes with the cells of the Detroit-173B strain derived from peripheral blood. The tissue culture cells do not resemble any known elements of normal blood or tissues. Thus, the conditions imposed on cells in culture with the methods we have described appear to expose morphologic potentialities not realized in vivo. A question to be answered is: What factors operate in vivo to suppress or control the expression of

11 G. E. Foley and A. H. Handler, personal communication.

12 The inclusion of cells from normal skin or its appendages cannot be ruled out when material is obtained by aspiration from bone marrow, body cavities, or blood vessels, but in such an event the explanation of the cellular changes taking place in cultures is equally obscure.
morphologic potentialities which can be observed
in vitro?

A part of the importance of determining
whether or not human cells acquire malignant
properties in tissue cultures is the formidable prob-
lem of understanding the factors leading to trans-
formations of any kind taking place in vitro. In the
development of Ep-L cell strains there is often a
change from the Fb-L to the Ep-L cell form. This
has been observed and reported as a typical
sequence by us and others (36). Our observations
indicate that the change takes place in individual
cells, rather than as the survival of a preformed
cell type. The former concept is expressed in the
morphologic phenomena we have described, but it
acquires greater validity from the observations of
Parker et al. (40), who showed that the altered
cells developed in clones derived from single cells,
thus indicating that the altered cells need not
come from persisting cells of a mixed population
and are not due to selective advantage of a par-
ticular cell type.

Because of the appearance of phenomena usual-
ly associated with cancer (36) even in cells origi-
nating from normal or noncancerous sources,
much attention is being directed to the possibility
that human cells maintained in vitro have become
malignant. It has been shown that this can occur
in other types of mammalian cells (12, 17, 18). In
the Detroit Ep-L strains heteroploidy was exten-
sive, and pleomorphism was prominent in all cell
lines. Levan (31), using the observed heteroploidy
condition in various lines of serially cultured cells
as a basis, postulated that at first there may arise
a high incidence of polyploidy in the population
which could be the result of numerous environ-
mental factors. Cells in the polyploid state, be-
cause of this “buffered condition,” are able to un-
dergo gene mutation and chromosomal rearrange-
ments with a greater change of continued and per-
haps enhanced viability. These changes presuma-
bly alter the properties of the cell, and those cells
having characteristics more suitable for existence
in the tissue culture environment will tend to be
selected. Chromosomes or parts of chromosomes
which do not contribute to a genome adapted to
the tissue culture environment may be lost by
various mechanisms, thus giving rise to a chromo-
somal complement radically different from the normal
karyotype. We believe that the heteroploid condi-
tion in five lines of Detroit cells tends to support
this notion. However, in the case of human tissues
and cells, at least, one must be cautious in apply-
such information. For example, in reversible
and nonmalignant bone marrow lesions one may
find numerous examples of bizarre erythroblasts
whose nuclear aberrations are consistent with
extensive polyploidy and heteroploidy. In some of
the hematological literature these features were
considered as probably indicative of malignant
disease involving the erythropoietic tissue (erythro-
blastoma) (34), but in a study of cellular giantism
in human erythropoiesis Berman (4) showed that
such changes may occur as reversible manifesta-
tions of nonmalignant disorders of the hematopo-
estic system. Similar abnormal findings were de-
scribed by Beritic and Vandekar (2) in patients with
plumism. In spite of their deceptive appearances
we know from experience that these changes do not
constitute malignant disease. It is interesting that
much of the research on these points is based on
studies of cells derived from malignant tumors as
compared with cells from normal tissues; these
may represent the extremes of a continuous spec-
trum, between which are cells from a large variety
of noncancerous reactive, inflammatory, or hyper-
plastic conditions. Cells from noncancerous patho-
logical tissues may be the proper objects for con-
trast studies.

On the other hand, increasing degrees of chro-
mosomal aberrations, especially heteroploidy,
seem to go hand in hand with increasing degrees of
transplantability in the ascitic tumor lines
studied by Hauschka et al. (20). At present the
best experimental method for determining malig-
nancy is to observe what happens to an animal re-
ceiving transplants of cells. If we are right in con-
sidering transplantability, particularly to uncondi-
tioned hosts, as an indicator of malignancy, the
association of heteroploidy and transplantability
as concomitant or causally related phenomena
becomes very important.

With this in mind we might now reconsider the
usefulness of long-term continuous culture of hu-
man cells. It seems likely that the so-called stable
strains undergo continuous changes. The use of
human cell lines in virological research may com-
pound an already difficult problem, because both
virus and substrate may be undergoing changes.
A more desirable substrate would be provided by
short-term fresh strains of human cells derived
from easily renewable sources of adult tissue, in
the manner exploited by virologists who use fresh
monkey kidney cells. However, this problem con-
fronting the virologist because of the changing
character of long-term tissue cultures of human
cells can be turned to advantage in the study of
neoplasia. The difficulty of investigating the ori-
gins of cancer in human tissues arises from the
lack of suitable material from the living patient.
In tissue cultures maintained for long periods,
periodic reappraisals of morphologic and bio-

chemical changes might be correlated with alterations of biologic behavior, when methods are developed for titrating "malignancy" by means of serial determinations of transplantability in suitable animals hosts.

**SUMMARY**

The similarities of various Ep-L cell strains derived from cancerous and noncancerous human sources have been described. Variations in cell morphology, polyploidy, and heteroploidy have been found to exist in similar degree in all strains. The morphogenesis of Ep-L cells from Fb-L cells was pointed out, and attempts to identify cell types by various methods were described. The significance of the findings was discussed.

**REFERENCES**


Fig. 7.—Smear, trypsin-treated cells, Detroit-173B strain. MGG stain. ×900.

Fig. 8.—Smear, trypsin-treated cells, Fb-L cell strain derived from human embryo (MAF). MGG stain. ×900.

Fig. 9.—Smear, trypsin-treated cells, MAF strain. The field includes two very dark-staining cells of Ep-L type from a culture of Fb-L type containing foci of polygonal cells. MGG stain. ×900.

Fig. 10.—Heteroploidy in a smear of trypsin-treated Ep-L cells maintained in hypotonic medium. MGG stain. ×975.

Fig. 11.—Section of chorioallantoic membrane produced by Detroit-8t cells. Bouin fixation, iron-hematoxylin stain. ×42.
Fig. 12.—Sheet of Detroit-6 cells. Bouin fixation, hematoxylin stain. X480.

Fig. 13.—Sheet of Detroit-148 cells, culture derived from human bone marrow. MGG stain. X480.

Fig. 14.—Section of 9-day sponge matrix culture of Detroit-6 cells. Zenker fixation, hematoxylin and eosin stain. X480.
FIG. 15.—Section of 9-day sponge matrix culture of Detroit-173B cells. Note the relatively great pleomorphism in this material derived from nonleukemic peripheral blood. Zenker fixation, hematoxylin and eosin stain. X480.

FIG. 16.—Section of lesion in chorioallantoic membrane produced by Detroit-88 cells. Bouin fixation, iron-hematoxylin stain. X480.

FIG. 17.—Section of lesion in chorioallantoic membrane produced by Detroit-98 cells derived from bone marrow of a cancer-free individual. Bouin fixation, iron-hematoxylin stain. X480.
Fig. 18.—Sheet of Detroit-6 cells. Bouin fixation, hematoxylin stain. ×480.

Fig. 19.—Sheet of Detroit-56A cells. Bouin fixation, hematoxylin stain. ×480.

Fig. 20.—Focus of change from Fb-L to Ep-L condition in MAF culture. The polygonal cells in the center of the field have more deeply staining cytoplasm than is seen in the surrounding Fb-L cells. MGG stain. ×230.
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