Chromosome Analysis of Five Long-Term Cell Culture Populations Derived from Nonleukemic Human Peripheral Blood (Detroit Strains)*

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The cultivation of adult human cells of normal origin has become a routine procedure during the past several years (4). Among the first stable epithelial-like (Ep-L) cell lines derived from normal human tissues were those established by Chang (1954) (5) from conjunctiva, liver, kidney, and appendix. Although the HeLa strain of cells from cancer tissue had been available before this time, there were objections to the use of cell strains derived from cancers for the cultivation of viruses for vaccine production, or in experiments designed to explain the normal biology of human cells. The development of stable cell lines1 from normal tissue was at first thought to circumvent such objections, but it soon became apparent that cells derived from normal tissues may undergo transformation to new cell types having features suggestive of malignant properties (4). Among them was the ability of the cells to grow as tumors when transplanted into homologous and heterologous host animals (23), and radical alteration in chromosomal complement (4, 12, 18). These observations were consonant with the earlier findings indicating the acquisition of neoplastic properties by cultured mouse cells (24).

Levan (18) reported that the chromosome number of the Chang liver and conjunctival strains was between 72 and 80. Such radical changes in chromosome number have been termed "heteroploid alterations." Hsu and Moorhead (12) reported on the Chang liver and conjunctival lines and also on the McCoy line derived from the synovial fluid of an arthritic patient. All three of these lines from nonmalignant sources displayed a heteroploid condition similar to that described by Levan. Hsu, Pomerat, and Moorhead (14) described the heteroploidy of stable cultures derived from nonmalignant human synovial lining cells (Mayes strain) and, more recently, amnion, skin, and heart (Hsu) (11). Westwood et al. (27) noted the development of heteroploidy in cultivated normal human, monkey, and rabbit tissues.

Additional information is available on the chromosome condition of human epithelial-like stable lines which have been initiated from malignant tissues. Hsu (10) has reported on the HeLa strain, and more recently Hsu and Moorhead (12) on the S-3 HeLa clone, KB strain derived from an epidermoid carcinoma of the mouth, Maben from pleural fluid of a patient with adenocarcinoma of the lung, and Oregon J-96 from the peripheral blood of an individual with monocytic leukemia. All show the heteroploid condition. Berman, Stulberg, and Ruddle (4) reported heteroploidy in five stable Ep-L cell lines of the Detroit strains. These were Detroit -6, -32, -34 from bone marrow, Detroit -80A from carcinomatous ascitic fluid, and Detroit -110P from lymphomatous pleural fluid.

It is noteworthy that cells derived from both malignant and nonmalignant sources were heteroploid in their stable line condition and that the heteroploid condition was fixed between the 65 and 80 chromosome level in most instances heretofore reported. The in vitro transformation of normal cells to the heteroploid condition and the acquisition of neoplastic properties suggest...
a relationship between cancer and heteroploidy. It is conceivable that this possible relationship may be studied by following the fate of normal cells explanted to the tissue culture environment. In this manner, tissue culture systems would serve as a model for the study of the neoplastic process or of cell transformations in general.

To date, nine human, stable Ep-L cell lines of normal origin (above) have been studied, but they were derived from tissues of complex cell content, from different organs, and by various methods of culture. Only a few studies (18) have been performed in which chromosome number and morphology have been studied simultaneously. For these reasons, chromosomal analyses were made of five strains of human Ep-L cells derived from peripheral blood of normal individuals with no past or present history of malignancy and cultivated under uniform conditions.

MATERIALS AND METHODS

Details concerning explanation and cultivation and photomicrographs of cells of the Detroit blood strains are included in a report in press (3).

The cells were cultivated in 8-ounce medicine bottles with 8 ml. of Eagle’s basal medium plus 16 per cent human serum and 100 units/ml of penicillin and 100 μg/ml of streptomycin. Cultures were examined 48 hours following subculture. At this time there were many dividing cells. An inoculum of 100,000 cells/ml (800,000 cells/bottle) was found to give satisfactory results. With smaller inocula, difficulty was encountered in removing the cell sheet with verseae.

Each crop of cells to be examined was divided into two subcrops (A and B). Subcrop A (two to three bottles) was examined without colchicine treatment and was used for the estimation of relative frequencies of the s, 2s, and >2s ploidy levels. Uncolchicinized cells also served as a reference for comparison with colchicinized cells. Each bottle of cells was processed and examined separately. Subcrop A was prepared for study in the following manner: The nutritive supernatant fluid was removed and the cell sheet rinsed with hypotonic versene solution at 86° C. The cells were then incubated for 5-8 minutes with hypotonic versene solution with gentle, intermittent shaking. The hypotonicity of the versene solution aided greatly in spreading the chromosomes, as previously described by Hughes (15) and Hsu and Pomerat (13). Gentle pipetting was used to further separate the cells. Very little cell damage resulted, and chromosomal fragmentation was virtually absent. The cell suspension was then centrifuged at 200 g for 3 minutes. The supernate was decanted and the cell mass mixed with an equal volume of aceto orcein stain. The mixing. The cells were allowed to remain in the stain for 5 minutes. A drop of the cell-stain mixture was placed on a silanized slide, together with a drop of 45 per cent acetic acid. Addition of the acid caused precipitated stain to dissolve and also improved the contrast between chromosomes and cytoplasm. Cells were squashed by means of thumb pressure on an overlying nonsilanized coverslip. Flaming the preparations prior to squashing, as well as tapping the coverslip sharply with a pointed object such as a pencil, improved cell dissociation and spreading. The coverslips were sealed with a paraffin-vaseline mixture, and could be stored as long as a week at 10° C. without deterioration.

The remaining portion of the cell crop (B) (four to five bottles) was made 5.0 × 10^{-4} M for colchicine, by adding 0.1 ml. of 5.0 × 10^{-4} M colchicine solution in Eagle’s basal medium to 10 ml. of nutrient supernate in each tissue culture bottle. Sixteen to 18 hours’ treatment greatly increased the number of mitoses. It was found that a lesser 1:10 dilution did not increase the accumulation of mitoses. Thus, 5 × 10^{-4} M colchicine concentration just exceeded the threshold value of these cells for c-mitosis, as pointed out by Levan for the Chang liver and conjunctival strains (18). The subsequent squash procedure was identical to that given above for subcrop A. Some preparations were made permanent by means of the dry-ice method of Conger and Fairchild (7). The quality of such preparations was unpredictable and inferior to those of wet preparations.

Chromosome counts were made with the aid of an ocular grid and hand counter. Repeated countings of individual metaphase plates indicated that the counting error seldom exceeded ±2 chromosomes when less than 100 chromosomes were counted, but probably exceeded this value when metaphase plates of more than 100 chromosomes were counted. Chromosome morphology was studied by means of photographs and drawings. Drawings were made with the camera lucida. The procedure used was as follows: First the whole metaphase plate was drawn in situ and the chromosomes assigned numbers. In all instances where possible, photographs were obtained of these plates to serve as a check of the accuracy of the drawings. Each chromosome was then drawn individually in the center of the field, using a 20× ocular with the camera lucida. Measurements of chromosomes were made directly from the camera lucida drawings which varied in over-all length from 4.5 to 0.5 cm.

TERMINOLOGY

In the literature the term heteroploidy is used in various ways. Here, it will merely indicate a radical alteration of the chromosome number distinctive from variations of one or several chro-

1 Hypotonic versene solution: 8 parts of solution A: 2 parts of solution B.

2 Solution A: KCl, 0.2 gm.; KH₂PO₄, 0.2 gm.; Na₂HPO₄, 1.15 gm.; and glass-distilled water, 1 liter.

3 Solution B: Solution A plus 80 gm. NaCl. The working solution was made 0.01 per cent for disodium versenate.

4 Gurr’s natural orcein—prepared by dissolving 2 gm. into 100 ml. of boiling 45 per cent acetic acid, cooling, filtering, and storing at room temperature. Source: Starkman Biological Lab., Toronto, Ontario.

mosomes (aneuploidy) or of $4n$, $8n$, multiples of the $2n$ number (polyplody).

In discussing the stable in vitro cell populations, it is convenient to employ the stem-line terminology used by Makino for ascites tumor cell populations (22). The term, stemline ($s$), designates the major numerical component of the cell population as determined by chromosome number. This is generally assumed to act as the prime genitor of all other cell types which may be present. In rat ascites tumor cell strains, it has been shown that at least three stemlines may coexist; they need not be mutually excluding (26).

The double stem-line ($2s$) component consists of the group of cells which have double or nearly double the $s$-chromosome number. At present, it is thought that the $2s$ component is wholly derived from the $s$ but that, under certain conditions, the $2s$ component might itself become the prime genitor of the culture. These concepts suggest that the $2s$ components of different cultures, or the $2s$ component in the history of a single culture, might exhibit varying degrees of autonomy with respect to the $s$ component. These ideas will be discussed in more detail later.6

RESULTS

Morphology of cell strains.—The polygonal Ep-L cells grow on glass as monocellular layers. The nuclei are oval, and in preparations made as dry films stained with May-Grünewald-Giemsa the nuclear chromatin pattern is that of a delicate filigree of small, uniform masses of chromatin separated by very light-staining parachromatin having a total area about equal to that occupied by chromatin. One or more large nucleoli, sometimes one fourth the diameter of the nuclei, are present. The nuclear membranes are very fine, smooth, and distinct. Average nuclear diameters range from 17 to 21 μ. There are numerous variations among cells within a strain and among the cell strains. The most obvious are differences in cell size, shape, and number of nucleoli, frequently of abnormal mitoses, or incidence of multinucleated cells. Clones derived from single cells of various strains exhibit less variation in total cell or nuclear size than is the case for the parent strains. The cytoplasm of the cells is deeply basophilic, homogeneous, or finely vacuolated. In some strains the cytoplasm may contain fine, dustlike azurophilic granulations, although the granulation does not appear to be a constant feature of any of the cell lines derived from normal blood.

Chromosome counts.—Analysis of the five Detroit blood lines (Detroit -B16, -B17, -B78, -B96, and -B110) by chromosome counting in no instance yielded modal values which approximated polyplody or aneuploid values of the normal human chromosome number (Chart 1). The modal values were found to be of a distinctly heteroploid condition.

The modal values of Detroit -B16, -B96, and -B110 were, respectively, 67, 68, and 65 in the $s$-range. When counts were listed in groups of five, the modal values for these lines fell between 63 and 67, or within the 63 group. Photographs of the $s$-condition of these cell lines are given in Figures 1-3. There were no distinguishable differences in the $s$-condition among these lines. The $2s$-component in strains Detroit-B96 and -B110 were twice the value of the $s$-component. Detroit -B16 showed a deviation from the expected pattern, having a $2s$-mode less than double the mode for the $s$-component. An example of the $2s$ chromosome set is given in Figure 4 for Detroit-B16. Detroit-B78 differed distinctly from the other cell lines on the basis of chromosome number, having a mode of 79 at the $s$-level. The -B78 strain could be distinguished easily from other cell lines on the basis of chromosome number. An example of Detroit-B78 cell with the $s$-chromosome complement is shown in Figure 5. Figure 5 and Chart 2 (A, B) are of the same cell. The $2s$-component of this strain was seen to be twice that of the $s$-component on the basis of the few $2s$-cells available for counting. Detroit -B17 was especially interesting, since this cell strain operated primarily at the $2s$-level (Table 1), having a modal value of about 120. It is also seen from Chart 1 that variation in chromosome number within the $2s$-range was great, making the -B17 strain doubly distinctive. Increased variation in this strain may have been due in part to increased counting error. A metaphase cell of Detroit-B17 is shown in Figure 6.

Frequency of stem-line components.—The chromosome counts give evidence for $s$ and $2s$-components in all the blood line populations. However, the relative frequencies of the two components cannot be determined adequately by chromosome counting alone. This is so because it is uncertain to what extent the $s$ or the $2s$-cells are selected for inclusion into the count data. For example, $s$-cells in one preparation may be favored because of their lower chromosome number. However, $2s$-cells sometimes yield more favor-

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6 In the case of the Detroit-B17 cell strain, the stem-line terminology has been somewhat corrupted, since the major component of this cell population, which has a chromosome number centering about 120, is designated $2s$. This has been done so that this cell strain can be compared more easily with the other cell strains from blood.
CHART 1.—Frequency distributions of chromosome numbers of five Detroit cell strains derived from nonleukemic human blood.
CHART 2.—Idiograms of Detroit-B78 (A), -B96 (C), and -B110 (E). Metaphase plates of -B78 (B), -B96 (D), and -B110 (F) cells, from camera lucida drawings.
able chromosome displays on squashing than do the smaller s-cells. In order to circumvent this difficulty, noncolchicinized cultures, otherwise comparable to those cultures which were used for counting, were squashed, and the metaphase figures classified into three categories: s, 2s, and greater than 2s. The last class represented the 4s level, and other very highly polyploid cells. The preparations were studied in a random fashion, and all metaphase plates (with a few exceptions) encountered were classed, even though accurate chromosome counts may not have been possible. The data for these classifications are given in Table 1.

On the basis of this study, it is seen that Detroit -B16, -B96, -B110 were similar, having approximately 25 per cent of their populations in the 2s condition and about 70 per cent in the s range. The -B78 strain had only a very small component in the 2s and greater than 2s range; more than 95 per cent of the cells were in the s class. Strain -B17 had 89 per cent of the cells in the 2s condition, while 6 and 5 per cent of the cells were in the s and greater than 2s condition, respectively.

Photographs of stained preparations of these cell lines growing on coverslips are shown in Figures 7 and 8. These represent typical areas of the cell sheets. In respect to nuclear size, the cells correspond to the frequency of ploidy classes given in Table 1. In Detroit -B110, -B96, and -B16 two sizes of nuclei can be detected. Nuclei of Detroit -B78 and -B17 are uniform in size. The examination of cell sheet preparations also indicates that -B17 has more numerous and larger nucleoli, possibly reflecting its dominant 2s condition.

In the coverslip preparations, it was observed frequently that cells with large nuclei (presumably 2s) were aggregated in groups. Such groupings suggest that the cells are related, having been derived by regular mitoses from one or several 2s-cells. Diplochromosomes indicative of endoreduplication in the sense of Levan and Hauschka (21) were seen in one fragmented cell of Detroit -B110. These are shown in Chart 3 (E). Neither endomitosis nor c-mitosis was commonly observed in noncolchicinized cultures.

Chromosome morphology.—Idiogram analysis of a representative metaphase plate of each cell line is given in Charts 2 and 3. The idiograms were constructed from cells whose chromosomes showed only moderate colchicine-induced contraction. Such cells are considered to be in early c-mitosis. Noncolchicinized metaphase cells possessed relatively longer and thinner chromosomes. Idiogram chromosomes varied in over-all length from 8 μ for M-chromosomes, 5 μ for SM chromosomes, and 3 μ for ST-T chromosomes. The majority of the cells studied showed a greater colchicine effect than depicted as a rule in the figures. Figure 2 shows the more characteristic degree of contraction when colchicine is used at the 5 × 10^-8 M concentration for 15 hours. The more contracted chromosomes are ideal for counting, since overlapping of chromosomes is reduced. On the other hand, morphological studies require the more extended chromosomes of prometaphase or early c-mitosis.

Satellites were seen in the Detroit blood lines but without consistency (Chart 3, C). Satellites are small enough that it is quite possible to miss them, especially when they fold back against their chromosome, or lie against or under adjacent chromosomes. It is possible that squashing may detach satellites, thus giving a falsely low frequency of their occurrence. In his study of Chang liver cells Levan (18) did not report satellite chromosomes. Tjio and Levan (25) indicate the presence of two satellite chromosomes in one idiogram of normal human tissue.

Examples of dicentric chromosomes are given in Chart 3, F. Such chromosomes are presumed to be unstable, producing cells with deviating chromosome numbers (see “Discussion”). The instability of these chromosomes has been pointed out by Levan (20).

Relation of heteroploidy and polyploidy to chromosome morphology.—In his study of cancer cell populations, Levan has observed an alteration in chromosome morphology which became more pronounced in polyploid populations (18, 19). It was of interest to see whether similar morphological alterations had occurred in the Detroit blood lines. To do this, suitable chromosome displays of the metaphase condition were drawn with the aid of the camera lucida under high magnification. The drawings were measured, and, on the basis of median:SM), 5-9.9 (submedian:ST), and greater than 9.9 (terminal:T).

<p>| TABLE 1 |
|-------------------------------|---------|---------|---|---------|</p>
<table>
<thead>
<tr>
<th>Blood line</th>
<th>Total no.</th>
<th>s</th>
<th>2s</th>
<th>&gt;2s</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-16</td>
<td>222</td>
<td>75.7</td>
<td>20.9</td>
<td>3.4</td>
</tr>
<tr>
<td>B-17</td>
<td>356</td>
<td>5.9</td>
<td>89.4</td>
<td>4.7</td>
</tr>
<tr>
<td>B-78</td>
<td>90</td>
<td>97.0</td>
<td>3.0</td>
<td>0</td>
</tr>
<tr>
<td>B-96</td>
<td>145</td>
<td>61.0</td>
<td>36.2</td>
<td>2.8</td>
</tr>
<tr>
<td>B-110</td>
<td>109</td>
<td>70.6</td>
<td>23.9</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Levan (18) has divided human metaphase chromosomes into classes on the basis of arm length ratio. The ranges of the ratios for these groups are 1-1.5 (median:M), 2-4.9 (submedian:SM), 5-9.9 (subterminal:ST), and greater than 9.9 (terminal:T).
Chart 3.—Idiograms of Detroit-B16 (A), -B17 (C), and metaphase plates of -B16 (B) and -B17 (D) cells. Diplochromosomes and dicentric chromosomes of -B110 (F) are illustrated, from camera lucida drawings.
of arm-length ratios, the chromosomes were classed into M, SM, and ST-T groups. The results of these classifications are given in Table 2. The starred data in this table refer to the idiograms which appear in Charts 2 and 3. Only one measurement was made of blood line -B17 because of the difficulty of finding suitable plates in cells having 120 chromosomes. Variations in the number of chromosomes in the different classes within a given strain were observed. Such fluctuations may be due to actual differences or to error which is inherent in a method which is itself arbitrary.

The averaged results of all of the blood lines were quite uniform with the exception of blood line -B96 which had slightly more M chromosomes and fewer SM chromosomes. We do not feel this difference to be significant. The average values for all the blood lines was 58 per cent for the M chromosomes, 32 per cent for the SM chromosomes, and 15 per cent for the ST-T chromosomes.

**DISCUSSION**

**Critique of chromosome analyses.**—The degree of constancy or variation in the chromosome numbers of somatic tissues is one of the major controversies in modern cytology (2). The reason is primarily the difficulty of obtaining adequate chromosome preparations. If squashing is not employed, the probability of chromosome loss or fragmentation is greatly reduced, but the ability to resolve all the chromosomes is impaired. One can be fairly certain that chromosomes have not been lost in squashed cells if their outlines are intact, but there is always the possibility that cytoplasmic fragmentation has occurred at surfaces perpendicular to the line of sight. Such breakages would be almost impossible to detect.

Beatty (1) obtained comparatively slight, symmetrically distributed variations about the 2n-mode in counts on normal mouse corneal epithe-
only infrequently in all Detroit blood lines and were not characteristic of the stem lines. Such abnormal chromosomes would result in cells which differ numerically from the stem-line mode, and if they play an important role in producing numerical variants one might expect the count data to have means lower than the modes. Such a condition was seen in blood lines -B16 and -B96 (Chart 1). Besides dicentric and lagging chromosomes, multipolar spindles may also introduce numerical variants into the population. This process would produce rather extreme deviations from the stem-line mode. However, only a very few radically deviating cells were observed. Strain -B17 showed relatively wide numerical distribution. Although increased counting error may be responsible for much of this variation because of the high chromosome number of these cells, error alone is an inadequate explanation. There is evidence of a bimodal distribution in this cell line, and two stem lines may be operative at the 2s level. The condition in -B17 suggests instability, and it will be of interest to examine this strain at regular intervals.

Further evidence for true chromosomal variation within an s-assemblage comes from the work of Chu and Giles (6). These authors studied chromosome number of clones derived from a wild HeLa population. They found that the clones demonstrated modal values which differed among themselves, and also from the mode of the parent strain. Nevertheless, the modal values of the clones were within the range of variation of the s component of the culture from which they were derived. These observations suggested that variants of the mother culture have been separated by cloning and that such variants established a new modal condition in the cell populations to which they gave rise.

Relation of heteroploidy and polyplody to chromosome morphology.—It is difficult to evaluate comparisons between normal and heteroploid idiograms. This is so because of the lack of uniformity and the paucity of existing data on the normal human karyotype. Normal karyotypes have been examined in short-term tissue cultures of somatic tissues by Hsu (9) and Tjio and Levan (25), and of germ-line cells by Kodani (16, 17). The idiograms of these authors have been classed according to Levan’s method (below), and percentages for M, SM, and ST-T groups are given in Table 2.

At present, uncertainty exists on the actual human chromosome number. Hsu originally reported 48 chromosomes, but has since revised his estimate to 46 (11). Kodani claims the existence of a basic number of 46, but states that one or two supernumerary chromosomes may be present, giving numbers of 46, 47, or 48 in different individuals, but his data apply only to germ-line cells, and his results cannot be extrapolated to somatic tissues. Tjio and Levan report only 46 chromosomes in their material.

Greater uncertainty exists with regard to the frequency of morphological types of chromosomes. The data of Hsu and Kodani are in fairly close agreement, especially with regard to the ST-T group which comprises 4 per cent of the chromosomes in comparison to SM types. Tjio and Levan report that the number of M and SM chromosomes are equal, each representing about 44 per cent of the karyotype while the ST-T group accounts for 18 per cent of the chromosome complement. It is in terms of the ST-T groups that the idiograms of Hsu and Kodani differ from those of Tjio and Levan, the former suggesting one pair of chromosomes, the latter three pairs. It should be emphasized that Tjio and Levan’s figures were given in the form of estimates and that Hsu and Kodani did not class their chromosomes, perhaps indicating their reluctance to employ a method which is admittedly arbitrary. Moreover, it should be realized that these studies were performed on different tissues of different ages, from humans of different genetic relatedness.

Comparison of the normal complement reported by Tjio and Levan with the heteroploid karyotype of the Detroit blood lines showed a small difference in percentage between the M and SM classes. The heteroploid cells had a higher percentage of M chromosomes and a lower percentage of SM chromosomes. The ST-T chromosomes did not differ greatly between the normal and heteroploid cell types. Thus, on the basis of the Tjio and Levan karyotype there is no indication of a radical alteration in the frequency of morphological classes, concomitant with the acquisition of heteroploidy. However, if the data of Hsu and Kodani are used as a criterion of the normal karyotype, it would seem that heteroploid transformation resulted in an increase of ST-T chromosomes. Because of the lack of agreement on the status of the normal karyotype, we are reluctant to speculate on whether morphological changes in the chromosome complement have occurred to any extent in the heteroploid Detroit blood lines.

Significance of 2s cell populations.—It was noted above that, in sheets of the Detroit blood strains, cells with large nuclei (presumably 2s) were aggregated in groups. Inasmuch as such groupings suggest that the cells are related, this would indicate a capacity for division among 2s cells.
Furthermore, the autonomy of the 2s cells is indicated by their vigorous appearance and their relatively high mitotic index. In opposition to the notion of self-generation, the presence of the 2s cells in the blood strains can be explained on the basis of generation from the s component by means of endoreduplication, endomitosis, or c-mitosis. The presence of the 2s component may depend on both generation from the s component and its own mitotic capacity. The capacity of the 2s component to reproduce itself is also suggested by the chromosome count data from the Detroit-B16 strain. In this strain the modal value for the 2s component is definitely lower than expected on the basis of a doubling of the s component. This suggests the autonomy of the 2s cell population.

Mutation as a mechanism for cell transformation in vitro?—While the present observations were restricted to terminal events in heteroploid transformation, it is worth while to see how the heteroploid state of the Detroit blood strains affect hypotheses which have been advanced to explain chromosome changes in autonomous cell systems. Levan (19) has emphasized the possible role of mutation in the evolution of a variety of murine cancers. His term, mutation, included a variety of events ranging from changes at a genic level to chromosomal rearrangements. It is thought that a number of such mutations must occur before cells can increase their autonomy; a number of mutational events are required for a cell to acquire the ability to grow independently. The idea implies that additional mutations may be necessary for the adaptation of a cancer cell population to a new form of existence such as the ascites form or growth in vitro. The work of the Kleins (cited by Levan, Exper. Cell Research, 11:613, 1936) supports this thesis, since, in the adaptation of solid tumors to the ascites form, a series of stepwise adaptations frequently occurs; this is suggestive of polygenic alterations. While this mechanism may explain the progression of cancer cell populations from one form to another, it does not follow that the primary induction of neoplasia can be explained similarly.

Levan, like Winge (28), attaches significance to polyploids in the possible accumulation of mutations. Polyploids are considered important in such a process because of their supposed ability to accumulate genic alterations without a loss in vitality. In considering the properties of polyploids, particularly in plants, Levan says: "In an autotetraploid, each chromosome is buffered by the presence of three other chromosomes of the same kind, so a genetic or gross chromosomal change that would be deleterious to the diploid may survive in the tetraploid, because it is compensated for in the rest of the genotype" (19).

The importance of the tetraploid in the adaptation of cell populations to various environmental conditions is stated in an additional quotation from Levan: "The evolutionary significance of tetraploidy in tumors is that it endows the stem line with the capacity of accumulating in the population a much more diverse supply of genotypes than is possible on the diploid level. This diversity forms the basis for further evolutionary developments. The acquirement of a polyploid stem line is therefore an important milestone for tumors on their way towards progression" (19).

However, many mouse and rat tumors have stem lines which are near diploid. Thus, it is true that autonomy need not involve the development of 4n or near 4n modes. In an isologous environment cell populations may not require extensive reshaping of their genotypes to adjust to their modified mode of existence. Nevertheless, it is known that cell lines can more easily adjust to a homologous environment if they are polyploid (8).

The results of chromosome analysis of the Detroit blood lines seems to fit well with the Levan notion of mutation accumulation in tetraploids. Four of the cell lines, namely, -B16, -B78, -B96 and -B110 had s modal values which were clearly hypotetraploid. The fact that these transformed populations, as well as many comparable stable lines studied in other laboratories, show similar levels of heteroploidy is suggestive of a common mechanism in the genesis of stable cell populations. It can be suggested that these lines have arisen from tetraploids which have accumulated by chance the right combination of mutations which permit these cells to exploit the tissue culture environment. The shift in chromosome number from the tetraploid to the hypotetraploid or heteroploid level can be explained simply. The loci which confer fitness to the in vitro adapted cell may be present on only a portion of the chromosomes of the tetraploid complement. Physiologically superfluous chromosomes could be lost by the various mitotic accidents, such as dicentric formation and lagging, which are known to occur in tissue culture cells. It is conceivable that the loss of unnecessary chromosomes would, in fact, enhance cell vitality. A reduction from the tetraploid level to a hypotetraploid condition actually has been observed in the rat MTK-IV ascites tumor (22). Makino (22) described this tumor as having a slightly hypotetraploid number of 75 when it was first examined. Recent samples
gave a modal value of 60 chromosomes. Makino states: "The cell population as a whole may have gradually shifted toward triploidy from the hypotetraploid start. From this fact it is likely that the present tumor may have originated from tetraploid predecessors, and therefore that hypotriploid cells now forming a stem line are not simple euploid multiples of the basic complex."

The existence of hypertetraploid cell populations such as -B17 and the 7-month Mayes strain (14) would at first appear to counter the notion of the development of heteroploid populations from tetraploid cells. Yet, such hypertetraploid lines, which are the exception rather than the rule, might represent a secondary event in the history of a stable cell line. This would involve a shift in the functional stem line from the s to the 2s level. The fact that a shift to the 2s level would represent an additional improbable event in a series of improbable events would account for the rarity of its appearance. One might expect such events to occur relatively late in the history of a cell line, assuming that environmental conditions remain relatively uniform. While we do not have sufficient data to validate this point, it is interesting that -B17 is the second oldest strain in the blood line series. Similar transformations have been reported for certain rat and mouse ascites systems. Makino (22) reported that a subline of the Hirosaki rat sarcoma has undergone conversion to a hypotetraploid condition. Since the original s mode of the Hirosaki strain was at the diploid level, this would correspond to a shift in the s mode of blood line -B17 cells to the 2s mode. Similar conversion has been observed in substrains of the mouse TA3 mammary adenocarcinoma which have been cultivated in different laboratories in the ascites form (19). The most likely explanation for the development of these hypotetraploid mouse strains is that they also arose from the 2s component. A hypotetraploid condition would result by the loss of superfluous chromosomes. This kind of transformation may actually be taking place in blood line -B17. In this strain, there is already some indication of reduction from the expected 2s level.

It may be speculated that stable heteroploid cell lines arise in the following fashion: Predominantly diploid cells of the normal karyotype are explanted into the tissue culture environment. This environment will not support the growth of these cells indefinitely unless they adapt genetically. Genetic adaptation occurs by means of the accumulation of certain essential, randomly occurring mutations. Such mutations accumulate more readily in polyploids. Tetraploids themselves might tend to accumulate in vitro due to impairment of normal cell (2n) division, the tissue culture environments perhaps stimulating endoreduplication, endomitosis, c-mitosis, etc. Tetraploids might also be better equipped to survive in vitro owing to a more favorable surface volume ratio and a quadruple set of chromosomes. Adapted tetraploids may then undergo a second change involving the loss of superfluous chromosomes, resulting in the fully adapted heteroploid cell. The cells better equipped to deal with the conditions of culture possess an adaptive advantage. Selection would operate, speeding the process of transformation to a stable type population.

At present the notion that mutational changes afford a mechanism for transformation to a tetraploid condition is theoretical. Alternate theories are also available, such as the selection in vitro of heteroploid cells already present in explanted tissues. It is felt that various possibilities can be proved or disproved by means of cloning experiments. Such work is now in progress.

**SUMMARY**

1. Chromosome counts of five stable epithelial-like cell strains derived from normal human peripheral blood all showed pronounced heteroploidy. The stem-line number in blood lines -B16, -B17, -B96, and -B110 was 65. Blood line -B17 had its s-mode at 80. The majority of cells in strain -B17 existed in the 2s range, having a mode of 120. The 120 mode in strain -B17 may actually represent the functional stem line of this strain.

2. Determination of the relative number of cells in the s and 2s condition showed that in the strains -B16, -B96, and -B110 approximately 75 per cent of the cells were in the s condition.
Fig. 7.—Detroit-B17 coverslip preparation. Note the relatively large nuclei of cells of this strain, as compared with those of the Detroit-B78 cells shown in Figure 8. Bouin fixation, H. & E. stain.

Fig. 8.—Detroit-B78 coverslip preparation. Bouin fixation, H. & E. stain.
and approximately 25 per cent in the 2s condition. Strain -B78 had 97 per cent in the s condition and 2 per cent in the 2s condition, while strain -B17 had 6 per cent in the s condition and 90 per cent in the 2s condition. These relationships correlated with observations of nuclear size in cell sheet preparations.

3. Indirect evidence was given for true variation in chromosome number within the s component and autonomy of the 2s component with respect to the s component.

4. Studies on chromosome morphology indicated no significant difference between strains on the basis of morphological types as determined by arm length ratios. Distributions of morphological types in accumulated data of all the cell strains showed 53 per cent medial chromosomes, 32 per cent submedial chromosomes, and 17 per cent subterminal and terminal chromosomes. It was uncertain that morphological differences existed between the Detroit blood lines and chromosomes of the normal human karyotype.

5. The significance of the findings with respect to current hypotheses concerning the development of stable heteroploid in vitro cell strains from human sources was discussed. It is speculated that heteroploid cells may develop from tetraploid cells by a mechanism of mutation and selection.

REFERENCES

Chromosome Analysis of Five Long-Term Cell Culture Populations Derived from Nonleukemic Human Peripheral Blood (Detroit Strains)

Frank H. Ruddle, Lawrence Berman, Cyril S. Stulberg, et al.


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