The Chromosomes of Primary Human Amnion Cells and FL Cells*

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SUMMARY

Chromosomal counts and analysis of karyotypes of human amnion cells in primary culture and of cells of the FL line of "transformed" amnion cells are reported. Primary human amnion cultures were made up of cells with exact diploid number of chromosomes, at least during the first 3 weeks *in vitro*, and of a few exact tetraploids. The sex, as determined from the chromosomes, agreed with the phenotypic sex of all studied cases (24 infants). FL amnion cells were heteroploid in the hypertriploid region. Seventy-eight per cent of the FL cells had chromosome numbers between 72 and 76, 2 per cent had numbers close to the double of this range. The human origin of the FL line is still evident from karyotype analysis. A single submetacentric, very large, marked chromosome was characteristic for all examined FL cells; other abnormal chromosomes were less consistently observed. Major changes in chromosome numbers have not occurred within the last 5 years of continuous cultivation. The results are further evidence for the justification of using amnion cells in primary culture as nonmalignant cells in model experiments *in vitro* and FL cells as representatives of malignant cells.

The human amniotic membrane at term has proved to be an excellent source of epithelial cells for tissue culture (21) and virus research (10, 12). Cultivated amnion cells as primary culture cells and in established ("transformed") cell lines differ in many respects. The primary cells usually have a limited growth capability *in vitro*. They may show considerable mitotic activity in the first 2–4 weeks in culture (we have observed a mitotic index as high as 1.8 per cent). Although primary cultures may maintain morphologically intact cells for several months, the proliferative potential declines after the first weeks, and subcultures become increasingly difficult to establish. This failure to multiply continuously may be due to a genetic limitation of growth, although suboptimal *in vitro* conditions cannot be excluded. Cells of established lines seem to have unlimited growth potential *in vitro;* they are, however, clearly altered in many respects. The increased mitotic index (sometimes 7–8 per cent), irregularities of mitotic figures, hyperchromatic nuclei with increase in number of nucleoli, and the relatively scant cytoplasm and their tumor-forming capacity in cancer patients and conditioned animals (9, 13) make them "cells with malignant characteristics." The malignant features of "transformed" amnion cells in contrast to the nonmalignant characteristics of primary culture amnion cells make possible model experiments *in vitro* which may add information on the differences between malignant and nonmalignant cells and enable studies of their interactions when grown together in tissue culture (7). The fact that both cell types are derived from the amniotic epithelium may be pertinent for such studies.

The present paper presents data from a study of the chromosomes of primary human amnion cells and "transformed" FL human amnion cells (11) as propagated for a large series of experiments in progress.

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MATERIALS AND METHODS

The primary human amnion cells were prepared as follows: A suspension of amnion cells was ob-

tained by trypsinization of amnion membranes obtained from human placentas at term. The medium (Bodian's) consisted of two parts of pooled human serum and eight parts of Hanks solution to which had been added: lactalbumin hydrolysate, 0.5 per cent; glutamine, 2.5 mg. per cent; valine, 0.5 mg. per cent; and glucose, 0.2 per cent. Penicillin (100 units/ml) and streptomycin (0.1 mg/ml) were included. Cells were seeded at a concentration of 200,000 cells/ml in plastic Petri dishes (35 mm. in diameter) containing sterile glass coverslips 22 × 22 mm. The medium was changed every

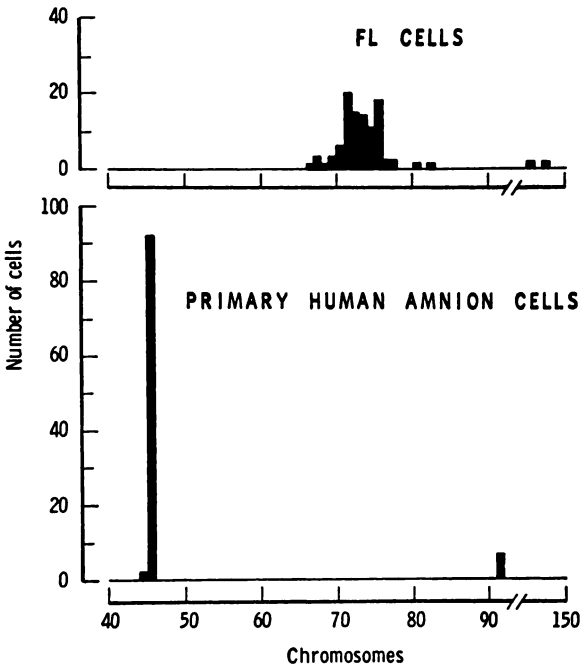

CHART 1.—Comparison of the distribution of chromosome numbers in primary human amnion cells and FL cells. Abscissa: chromosome number per cell. Ordinate: number of cells.

2–3 days. Proper pH was maintained by enclosing several Petri dishes in airtight plastic boxes or by incubation in a 5 per cent CO_2 atmosphere.

Stock cultures of FL cells (not cloned) were carried in LY-medium (10) with 20 per cent human serum. For chromosomal analysis the cells were seeded in Bodian's medium with 20 per cent human serum as described for primary cultures, since pH control of this medium was easier under the experimental conditions. For these fast-growing cells a seeding of 10,000–20,000 cells per ml. in 1–1.5 ml. medium per plastic Petri dish was suitable.

To obtain maximal mitotic activity chromosome preparations were made on days following the change of fluid of the cultures. The age of the primary cell cultures varied from 6 to 20 days; the FL cultures were usually 3–5 days old when analyzed. To facilitate chromosome analysis the cultures were pretreated for 1–2 hours with colchicine, 2.5 μg/ml, added directly to the culture medium without removal of the cells from the incubator. Subsequently, the medium was replaced with hypotonic solution for 30 minutes at room temperature. Depending on the density of the cells, 0.5–0.9 per cent solution of sodium citrate was used. The cells were fixed in absolute ethanol, 3 parts, and glacial acetic acid, 1 part, which was added very slowly to the hypotonic solution drop by drop, and the coverslip was then immersed in full-strength fixative for 10 minutes. The next step was quick-drying of the preparation in the air, and then the cells were stained with 2 per cent natural orcein in 60 per cent acetic acid. Either the chromosomes were examined in temporary preparation, where the coverslip was inverted over a drop of aceto-orcein on a slide and the edges sealed with Krönig's cement, or sometimes the preparations were dehydrated in alcohol after staining and mounted in Euparal. Temporary preparations were sometimes made permanent by the method of Conger and Fairchild (3). For counting of the chromosomes the preparation was scanned under low-power microscopy for suitable spreads, which then were examined with a 100× oil immersion objective and traced on paper with the aid of a drawing apparatus (Treffenberg type) mounted on the microscope. Microphotographs were taken on a Zeiss photomicroscope with a 35-mm. camera. Depending on the staining intensity, bright-field or phase-contrast microscopy was used. Pictures of karyotypes were made by cutting the individual chromosomes out from an enlarged print and gluing them in the desired arrangement on white paper.

RESULTS

Primary Amnion Cells

The chromosome number of human amnion cells in primary culture was determined from exact counts of 100 well spread metaphase plates in cultures from eight different amniotic membranes (Chart 1). The frequency of tetraploid cells in the populations may not necessarily be correct, since the higher number of chromosomes in such cells may make them less suited for exact counting than cells in the diploid range. Their actual frequency was determined by counting roughly all metaphase plates encountered in the preparation and by determining which cells were in the diploid or tetraploid range. In different primary amnion cultures the incidence of tetraploids varied from 5 to 15 per cent. Endo-reduplication was frequently encountered. One preparation showed even 75 per cent of

the tetraploids in endo-reduplication. Our results show, therefore, that primary human amnion cultures are made up of cells with exact diploid number of chromosomes in the great majority of cases, at least during the first 3 weeks *in vitro*, and of a few exact tetraploids.

The sex of cells in amnion cultures from 24 individual placentas was determined from the chromosomes. After checking the total number of chromosomes the number of small acrocentrics was determined. The small acrocentric Y-chromosome usually could be distinguished from the other small acrocentrics (chromosomes 21 and 22) by its slightly greater size and the difference in morphology. The cells from thirteen amniotic membranes were found to be of female, from eleven membranes of male sex. In each case the chromosomal sex as determined in the tissue culture preparation agreed with the phenotypic sex of the infant as reported by the hospital. In a few cases the total karyotype was determined and was always found to be consistent with the normal human idiogram as described by the Denver Committee (15) (Figs. 1, 2).

FL Amnion Cells

The examination of the chromosomes of FL cells revealed a very different picture. The cells were heteroploid. No diploid or near diploid counts were obtained, and exact tetraploid values were absent. The number of chromosomes was mainly in the hypertriploid region.

The results of chromosome counts of 100 FL cells are shown in Chart 1. Thus, 78 per cent of the cells had chromosome numbers between 72 and 76, which number represents the stem-cell region. Only 2 per cent of the cells had close to the double of this range representing the 2*s*-region. Attempts were made to classify and compare the FL chromosomes with those of the normal human idiogram. The general picture (Figs. 3, 4) indicated the human origin of this cell line, with metacentric and submetacentric chromosomes occurring much more frequently than teleocentrics. Detailed karyotype analysis was made on ten cells. Individual chromosomes that differed from any normal human chromosomes could be found, whereas others closely resembled normal chromosomes. Variations from cell to cell, both in total chromosomal number and relative frequency within the morphological groups, proved a further obstacle for such attempts.

A constant feature of the FL cells, however, was a single submetacentric marker chromosome (designated *M* in Fig. 4). It was definitely the largest chromosome and has no counterpart in normal human cells. A minute chromosome was frequently but not invariably observed. An attempt to arrange the chromosomes of an FL cell with Patau's classification (20) as a model is shown in Figure 4. Obviously, some of the chromosomes do not fit into the normal human picture, and the assignment to a group or in the groups is for some of them open to question.

The number of chromosomes in each group varied from cell to cell, except for the large acrocentrics of group D whose number remained relatively constant, nine or ten per cell.

DISCUSSION

Thus, in our cell preparations the chromosomal complement of human amnion cells in primary culture has not showed any aberrations from the normal picture. The occurrence of cells with the exact tetraploid number is not abnormal, since such cells are present in normal tissues—e.g., the liver. Tetraploid and even octaploid nuclei in the human amnion epithelium *in vivo* have been reported by Klinger and Schwarzacher (16).

Inconstancy of chromosomal number of cells of mammalian embryonic membranes was indicated by early work as reviewed by Beatty (1), but chromosomal analysis at that time was inexact. Reports of early *in vitro* passages of human amnion cells have indicated a variation of chromosome number. Levan (17) reported variation extremes of 44–48, 65–75, 92, but he suggested that "transformation" was taking place. Nakanishi *et al.* (19) obtained a range of 41–52 chromosomes with the most frequently observed values at 46 (25 per cent) and 48 (21 per cent) in primary culture of a human amnion fixed on the 25th day of *in vitro* life. For three analyzable metaphases in fresh amnion epithelium not cultivated, *in vitro* values of 42, 48, and 55 were obtained; cultures from this amnion were changed on the 35th day *in vitro* into a population of "transformed" cells, with chromosome numbers ranging from 60 to 77, and then assumed the capacity to grow continuously.

In this chromosomal study no evidence of cell "transformation" has been observed in the primary cell cultures. Thus, it appears that our *in vitro* conditions have been more favorable for the maintenance of normal chromosomes. The limited time of cultivation (3 weeks) during which our cells have been observed may also have been of importance in this respect (6).

We suggest that amnion cells in primary culture could be used for examination when chromosomal studies are indicated on the newborn either by family history or from clinical examination (Mongolism or other congenital malformations). Our

results have indicated that the chromosomal picture observed in the amnion cultures is the same as that of the infant. For cases of mosaicism, of course, no individual tissue would be sufficient for complete chromosomal analysis.

Although some information about X-chromosomes may be ascertained from sex chromatin observations in cultured amnion cells (18), chromosomal analysis, including both X- and Y-chromosomes, yields more complete information about possible sex abnormalities.

Our analysis of the chromosomes in "transformed" FL amnion is in general agreement with previous reports on "transformed" cells of human origin. For most such lines the chromosome numbers were in the region of 60–85 regardless of the tissue of origin. Several transformed human amnion lines have been examined. Nakanishi *et al.* (19) found 69–78 chromosomes per cell in the 84th subculture of Fernandes' line (5); similar counts were obtained by Defendi *et al.* (4) for this line. Hayflick (14) in the WISH-line observed counts in the low seventies, with metacentrics predominating. Clausen and Syverton reported an average of only 64 chromosomes for the HAT-1 line (2). The chromosomes of FL cells were first studied in 1957 by Levan (17); the most frequent number was 76, with distribution between 72–82 or 70–88 in two sets of preparations. A similar range (69–75) was found in 1961, and many departures from the normal human karyotype—not only in number but also in chromosomal varieties—were observed (8).

Although for certain of the FL cell chromosomes the placement in Patau's classification is uncertain, such attempts may be of importance in respect to future comparison with other transformed (or actually malignant) cells and for a possible explanation, at the chromosomal level, of the mechanism of transformation.

The FL cells in the period of examination had been cultured continuously for 6 years. Major changes in chromosomal numbers have not occurred at least during the last 5 years of continuous culture. For a more thorough understanding of the transformation of human cultured cells a complete karyotype analysis of the cells around the actual time of transformation appears pertinent.

The results presented in this paper have further justified the use of amnion cells in primary culture, when propagated under the described conditions, as nonmalignant cells in model experiments *in vitro* and FL cells as their malignant counterpart.

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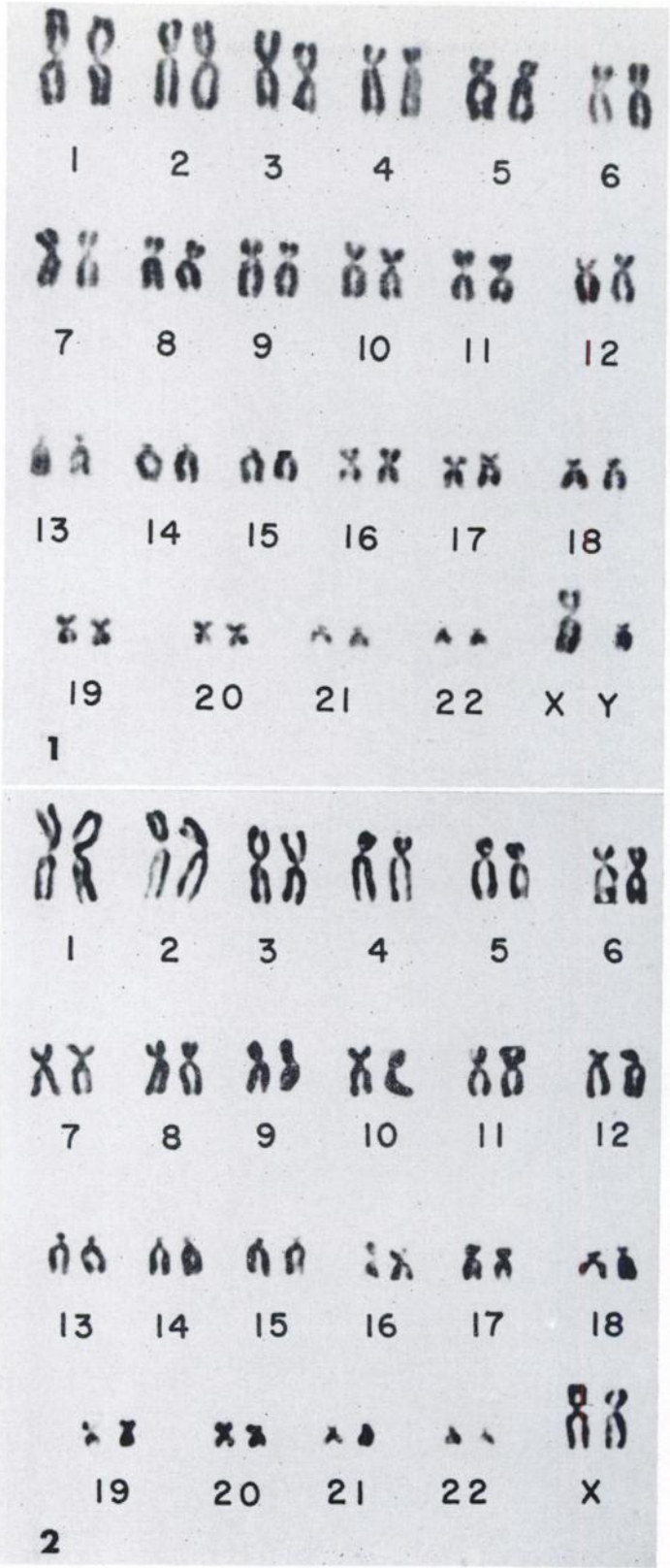
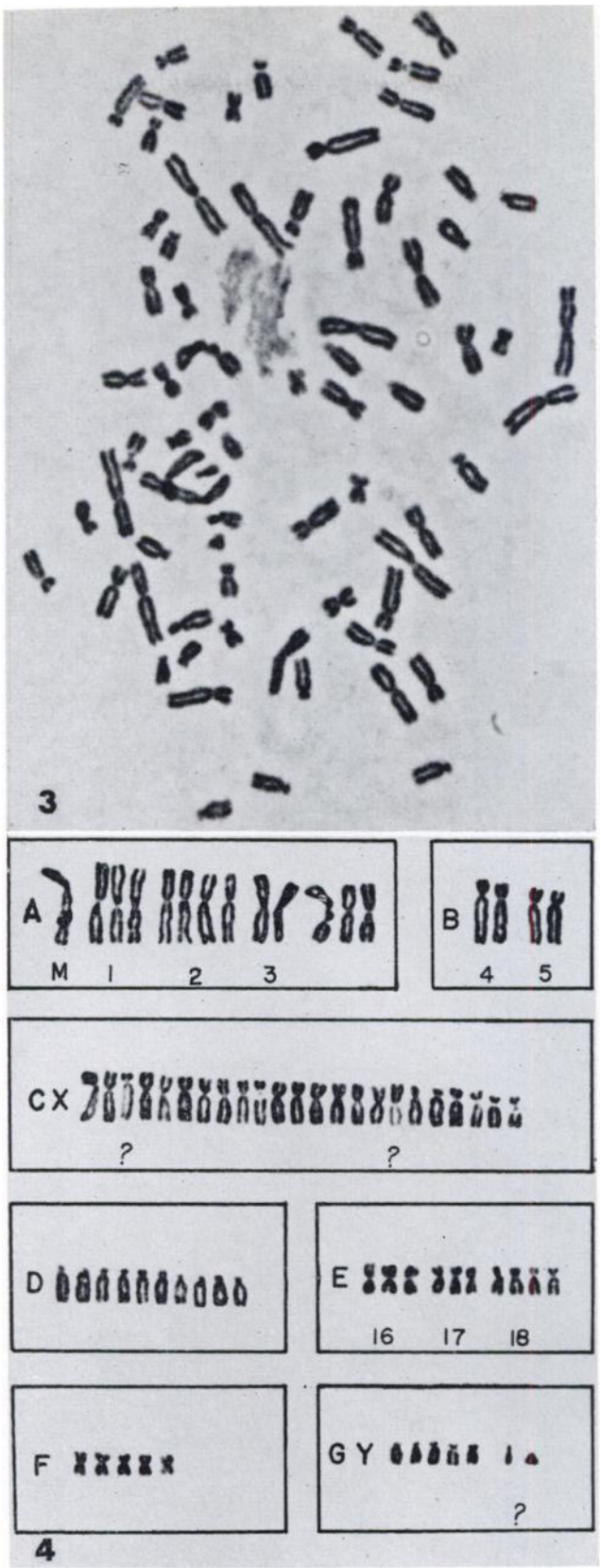


FIG. 1.—Karyotype analysis of a metaphase from a male human amnion culture. The chromosomes are numbered according to the Denver classification (15). Mag. ×1656.

FIG. 2.—Karyotype analysis of a metaphase from a female amnion. Mag. ×1795.

FIG. 3.—Metaphase with 72 chromosomes from an FL cell culture. Mag. ×1450.

FIG. 4.—Karyotype analysis of the metaphase shown in Figure 3, with Patau's classification (20) as a model. Some of the normal-appearing chromosomes are given numbers according to the Denver classification (15). The large marker chromosome is indicated by *M*. Some other obviously "abnormal" chromosomes are indicated by question marks. Mag. ×1186.



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