# AN UNUSUAL BEHAVIOR OF STRAIN HeLa CELLS IN TISSUE CULTURE

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The differentiation attained by emigrating embryo chick cells cultivated under strips and sheets of dialysis cellphane (1–4) in multipurpose culture chambers (5) prompted us to make a similar study of malignant tissues and strains. One of the more provocative results obtained with HeLa cells (6, 7) is contained in this note.

The environmental conditions resulting when sheets of cellophane are laid over embryo chick tissues on coverslips of the multipurpose culture chambers have been found to constrain the cells in such a way that dedifferentiation does not occur even after prolonged cultivation periods (8, 9). Some explants have been maintained in this condition for 2 years, and the cells did not display morphologic features characteristic of malignant cells. These observations suggested that an environment was produced by this chamber construction that provided physiological conditions resembling more closely those found in the normal host than those produced by less confining chamber techniques in which proliferation continues unrestrainedly. It was postulated, therefore, that if cells which had already undergone malignant transformations (a malignant strain) were placed in dialysate environments, a similar constraining effect might force a degree of differentiation to occur, if such a phenomenon were at all possible.

When cellophane sheets are laid over clumps of strain HeLa cells on the coverslips of multipurpose culture chambers, the resulting compression markedly flattens the cells against the coverslips (7), and this often produces cell membrane dehiscences. Conversely, depending upon local compression factors, cellular masses can be maintained in the above condition for long periods of time without, in the majority of cells, rupturing membranes. A variation of this technique involves the use of two sheets of dialysis cellophane against the coverslip wall of a chamber. In this way, the cells are explanted between the two cellophane sheets. These provide "soft" surfaces that may more closely resemble the normal physiological environment and, generally, do not produce severe cellular rupturing.

HeLa cells were established in this novel way in December 1960, and one of the chamber cultures with two cellular clumps (Fig. 1) contained the cells of interest in this report. By macroscopic observation these cell clumps appeared essentially unchanging over a 6-month period. Microscopic observation, however, revealed that the cellular patterns were continually changing. Other cellular explants of this size, cultivated directly on the coverslips without the cellophane, completely covered the glass walls of the chamber in a few weeks and had to be discarded. The cultures of this report were scanned at weekly intervals, and after 3 months an area was observed (Fig. 2) in which there were unusual clusters of cells. A closer inspection of these revealed an orderly arrangement of cells which had the appearance of a histological section of columnar epithelium (Fig. 6). This and an adjacent area were followed for the next 3 weeks, and it was observed that the cells in their orderly array were gradually dispersed (Figs. 3 to 5). As this was taking place, however, an adjacent area was undergoing a similar formation. This second area did not develop so completely as the original one.

Mitoses were not evident in these masses on direct observation; however, a time-lapse sequence of 5 days' duration revealed two mitotic divisions occurring in the chains of cells.

Many of these cells had phase dark circular juxtanuclear formations which, in all probability, represented the Golgi complex (4, 10). The underlying and overlying dialysis cellophanes limited the resolution of high power phase contrast microscopy, so that the ultimate magnification was obtained with a × 43 objective.

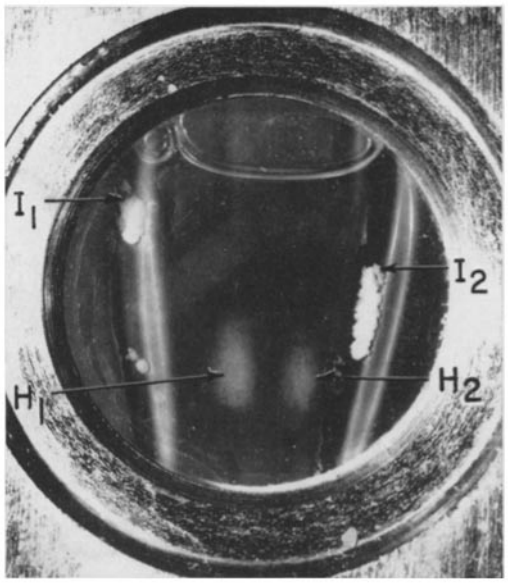


FIGURE 1

A macroscopic view of the multipurpose culture chamber with two HeLa explants ($H_1$ and $H_2$) lying between two sheets of dialysis cellophane. The cellophane sheets were adjacent to the top coverslip. The cellular mass had been in cultivation 158 days at this time. The white longitudinal masses ($I_1$ and $I_2$) were accumulations of cellular debris which progressively increased in size and density. × 2.

## DISCUSSION

The normal appearance of the HeLa cells, a tissue culture strain derived from a human carcinoma of the cervix 10 years ago (6), shown in this report offers a challenge to our concepts of the malignant transformation. Of course, it would be presumptuous to say that a redifferentiation of malignant cells occurred, since we do not even know for certain what is involved in such a phenomenon. The fact is apparent, however, that some cells of a malignant strain appeared similar to normal cells in that they achieved an organization characteristic of tissue formations. These malignant cells were simply introduced into a physiological environment previously shown to be useful for the cultivation and maintenance of normal differentiated (or semidifferentiated) cells, and after 3 months were found to show forms which were no longer typical of malignant elements. This finding is reported simply to mark the observed phenomenon.

A more intensive study is now under way which will attempt to duplicate this finding, as well as to determine the effect on other malignant strains of these peculiarly surfaced, complete dialysate environments.

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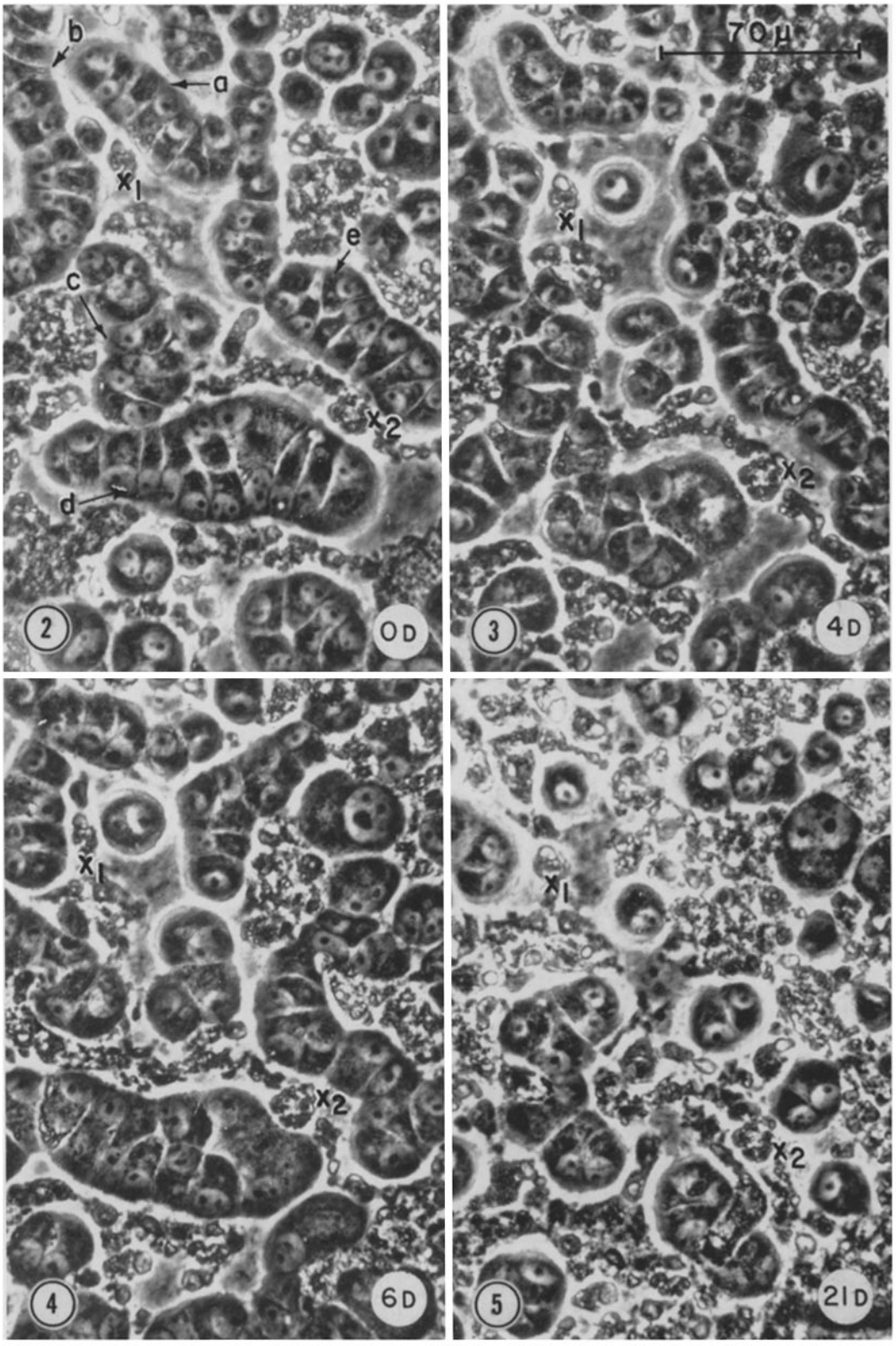
FIGURES 2 THROUGH 5

These are phase contrast photographs of the special clusters of HeLa cells. They were taken on 4 different days *oD* being the 1st day, *4D* the 4th day, *6D* the 6th day, and *21D* the 21st day. The main cellular masses *a*, *b*, *c*, *d*, and *e* and two identifying fragments of debris, $x_1$ and $x_2$, are shown. The rearranging of these masses is indicated by this 21-day series. Cellular masses *a* and *b* are shown in greater detail in Fig. 6. A magnification line for Figs. 2 through 5 is in Fig. 3. × 470.


b
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e
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x2
d
2
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70 µ
x1
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3
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x1
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6D
x1
x2
5
21D

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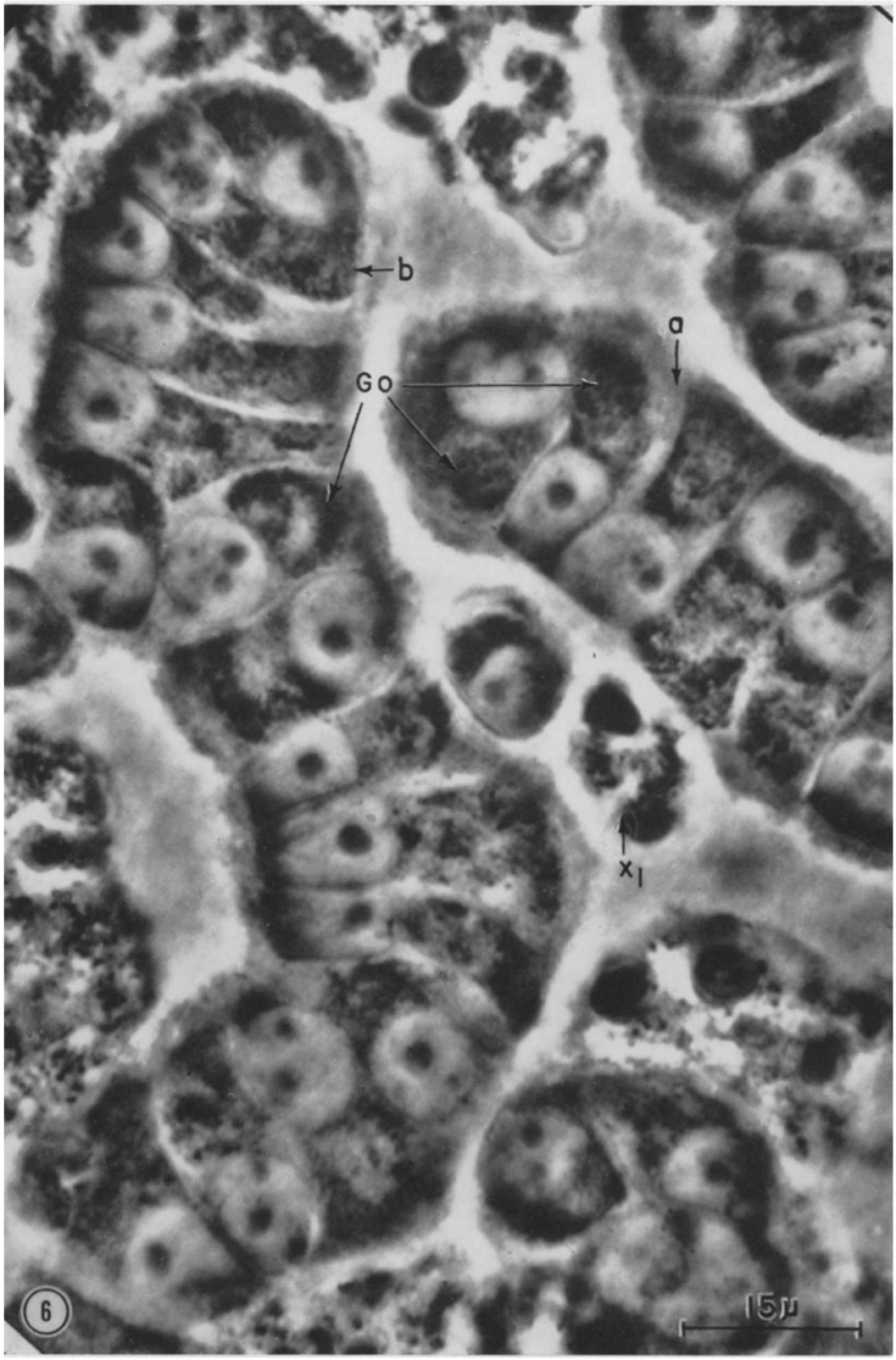
FIGURE 6

This is a phase contrast view of cellular masses *a* and *b*, as recorded on the first day of observation (*OD*, Fig. 2). The round nuclei in a polar position make these masses appear as a columnar epithelium. The Golgi complexes (*Go*) of three cells are shown, and the localizing debris ($X_1$) is indicated. × 1800.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