A STUDY OF THE CELL GROWTH OF A SINGLE-LAYER CULTURE OF CaVe STRAIN ON A NUTRIENT MEDIUM WITH BOVINE AND RABBIT SERA

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Several authors (11, 12, 14, 17, 19, 20) have found a change in the character of the growth single-layer cultures of human cells in media with animal sera. However, other changes in the properties of these cultures and their quantitative nature have been insufficiently studied (6, 7, 8, 19). Meanwhile, such investigations have great significance in the elaboration of the genetics of somatic cells (2, 4, 10, 15, 17, 18) and for understanding the problems of cell adaptation to existence in vitro (1, 5, 6, 8, 9, 14-17, 19, 20).

The purpose of the present work was a study of the character of the growth of a single-layer culture of cells of the CaVe strain and of the clone line CaVe K-17 obtained from this strain, cultivated on synthetic nutrient medium No. 199 with bovine or rabbit sera.

EXPERIMENTAL METHODS

The investigation was carried out on a single-layer culture of human cells of strain CaVe obtained from human cancerous stomach tissue (3, 4). Besides the parent culture* a clone line of strain CaVe K-17, obtained in our laboratory by the U. Min' method (10, 11) was used. 200,000 cells of both culture lines were inoculated into Carrell flasks in two variants of the experiment: 1) with 10% bovine serum (CaVe b); 2) with 10% rabbit serum (CaVe r). All four CaVe culture lines were examined quantitatively for seven days by the method described earlier (6). In each experiment no fewer than 7 Carrell flasks were used; calculation of the living and dead cells and also measurement of the pH of the medium were made daily at the same time of day. Each experiment was repeated 3-4 times; as a rule, parallel growth occurred in both kinds of sera. Strain CaVe was first cultured for about six months in media with bovine serum, while strain CaVe K-17 was used in the experiments from the first transfers from the time it was obtained. Besides calculation of the coefficient of proliferation (or growth potential), determined as the ratio of the number of cells growing to the number inoculated by the standard method (1), the total number of cells was calculated and the number of living and dead cells compared in each line.

EXPERIMENTAL RESULTS

The usual epithelial-like type of growth is observed during growth of the cells in medium with bovine serum (Fig. 1, a). Cell colonies of a different shope with smooth or undulating edges are found. The colonies consist of polygonal and, more infrequently, round cells in which nuclei are clearly seen with one or more nucleoli, in certain places polymorphism of the cells and nuclei is encountered. According to the degree of growth, certain colonies begin to run together, and the single off-shoot cells noted in the first days along the edge of the colonies disappear.

Another type of growth is observed during growth of strain CaVe on medium with rabbit serum (Fig. 1, b); the cells grow in the form of a peculiar flocculent diffuse net with small compact areas, usually with rough edges. A large number of off-shoot cells is noted; polymorphism of the cells is even sharper than during growth on medium with bovine serum. Cells with two nuclei and cells with large and giant nuclei are found, and also cells with conglomerate nuclei.

*Obtained from the Institute of Experimental and Clinical Oncology, AMN SSSR in 1961.

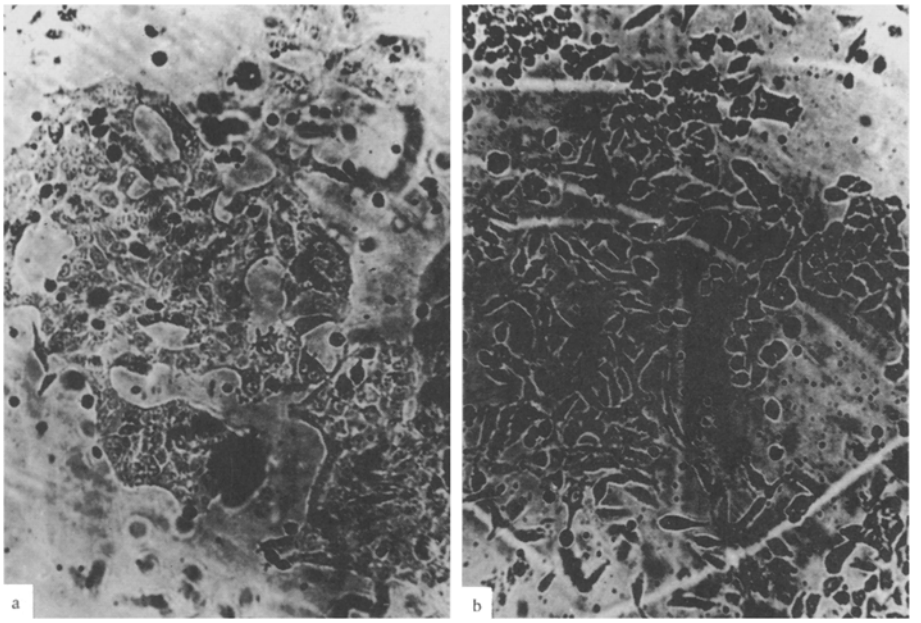

Fig. 1. Viable culture of strain CaVe growing on medium No. 199 (seventh day of observation). a) 10% bovine serum added; b) 10% rabbit serum. Magnification 90 x .

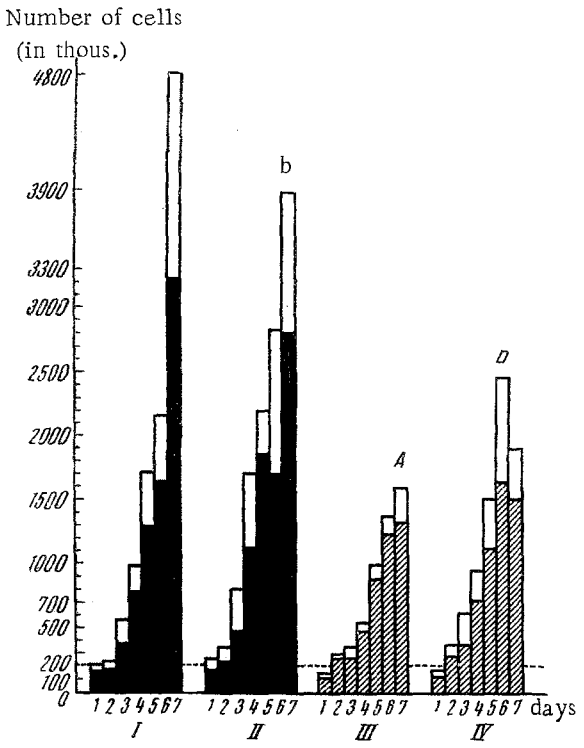

Fig. 2. Dynamics of cell growth of strains CaVe (A) and CaVe K-17 (B) during seven days of culturing. I, II) medium with bovine serum; III, IV) medium with rabbit serum. White areas of columns—percent of dead cells.

Clone lines of strain CaVe K-17 do not have such sharp morphological variations during growth on media with rabbit or bovine sera. In both sera the growth is epithelial-like; on rabbit serum the layers around the edge are somewhat broken up, the cells are more polymorphic, giant cells with nuclei with irregular shapes or with aggregate nuclei are found, but a tendency toward growth in layers or fused layers is quite well defined.

Daily measurement of pH for a week showed that in all culture lines it changed identically, from 8.2-8.0 on the first day to 7.2-7.0 on the seventh day.

Calculation of the number of living and dead cells in the four culture lines showed definite differences. A high multiplication rate of the parent CaVe culture on medium with bovine serum was found, while in medium with rabbit serum the number of cells on each of the 7 days of observation was less (Fig. 2).

Similar differences were observed in clone lines of strain CaVe K-17 (see Fig. 2) growing on medium with bovine and rabbit sera, but in this case there were fewer differences between them. At the same time, strain CaVe K-17, in comparison with the parent culture CaVe, had somewhat less intensive growth on medium with bovine serum and somewhat more on medium with rabbit serum.

Differences found during calculation of the total number of cells confirm the data from the calculation of the coefficient of proliferation, lasting more than a year from transfer to transfer.

It should be noted that CaVe cells growing on medium with rabbit serum were removed from the glass with Versene more quickly (5-8 min.) than cells cultured on medium with bovine serum (15-20 min.).

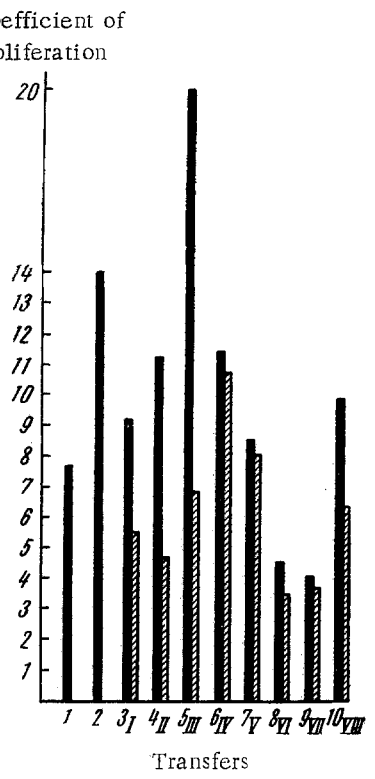

Fig. 3. Comparison of the coefficients of proliferation of CaVe K-17 in ten successive transfers in medium with bovine serum (black columns) and in 8 successive transfers in medium with rabbit serum (crosshatched columns). Calculation made on the 7th day.

A study of the coefficient of proliferation of cells of the clone line CaVe K-17 on the seventh day during cultivation in medium with bovine serum (1-10 transfers) and in medium with rabbit serum (1-8 transfers) was of great interest. As seen from Fig. 3, the coefficient of proliferation in both variants of the experiment varied from transfer to transfer, but in medium with rabbit serum it always was somewhat lower than in medium with bovine serum, which is confirmed by the higher results of the daily calculations (see Fig. 2).

Thus, noticeable differences were found in the character of growth of the parent culture of strain CaVe and clone lines CaVe K-17 during their cultivation in medium with bovine or rabbit serum. In the first case epithelial-like growth with very intensive proliferation of the cells is observed, while during growth in medium with rabbit serum the character of the growth was unusual and to some degree resembled the growth of fibroblasts, with a noticeable reduction in the coefficient of proliferation. This difference is retained during daily observations and in a series of successive transfers. In regard to the clone line CaVe K-17, during cultivation in media with both heterological sera, there were similar but less clearly expressed differences.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.