THE STUDY OF DIFFERENT METHODS OF CELL DIVISION IN TWO TRANSPLANTABLE LINES OF NORMAL AND MALIGNANT HUMAN CELLS

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The development of a new method of cultivation of cells in synthetic liquid nutrient media (monolayer cultures) has provided new opportunities for the study of methods of cell multiplication and their dependence on the nature of the cells and the external environmental conditions. References in the non-Soviet literature deal mainly with mitosis [6-11, 12], for the significance of amitosis remains for discussion.

The work of S. Ya. Zalkind and co-workers [2, 5] demonstrated the dynamics of the mitotic activity of certain transplantable cell lines in relation to the periods of cultivation. The importance of amitosis in the multiplication of individual lines of cells also was stressed in these investigations. Other authors have reported the presence of amitosis [1, 3]. The role of amitosis has also been emphasized in symplast formation under the influence of the action of certain viruses on the cell [6]. A. T. Kravchenko and co-workers [4], when reporting that motion pictures of HeLa cells frequently reveal binuclear and multinuclear cells and that during cultivation in fibrinolytic serum giant cells appear and the mitotic activity is depressed, stated that they were unable to observe amitoses. These workers reached no final conclusion regarding the way in which binuclear and multinuclear cells arise, and the significance of the numerous "invaginations of the nuclear membrane within the nucleus," bean-shaped or scalloped in appearance, which they described.

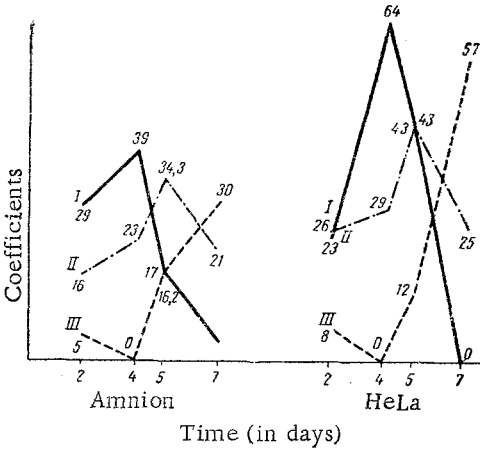

Fig. 1. Dynamics of coefficients of mitosis (I), amitosis (II), and gemmation (III) in lines of amnion and HeLa cells during cultivation for 7 days.

The object of the present investigation was to study the methods of multiplication of 2 transplantable (normal and malignant) lines of human cells—amnion and HeLa cells—and to determine the quantitative relationships between these methods at different periods of cultivation.

EXPERIMENTAL METHOD

A suspension of cells of the 2 lines mentioned above, taken from the bottom of the flask with a 0.2% trypsin solution of a 0.02% versene solution, was placed in Carrell's flasks in an initial concentration of 100,000 cells/ml. The cells were grown in synthetic medium No. 199 with the addition of 10% calf serum, and they settled on to mica plates or glass cover slips previously placed in the flasks, on which they multiplied in addition to growing at the bottom of the flask. Subcultures were made invariably on the 7th day. The nutrient medium was not changed before subculture. The mica plates or cover slips were fixed at certain times daily for 15 days in Bouin's fluid, stained with Meyer's hemotoxylin and eosin, and then mounted in Canada balsam. Some mica plates

TABLE 1. Coefficients of Mitosis, Amitosis, and Gemmation at Various Periods of Cultivation

Line of transplantable cells	Time from beginning of cultivation (in days)	Coefficient of method of cell division			
		mitotic	amitotic	gemmation	total
Amnion	2	29	16	5	50
"	4	30	23	0	62
"	5	17	34	16	67
"	7	4	21	30	55
HeLa	2	23	26	8	57
"	4	64	29	0	93
"	5	43	43	12	98
"	7	0	25	57	82

TABLE 2. Number of Binuclear and Multinuclear Cells Undergoing Amitotic Division on 5th and 7th Days of Cultivation

Line of transplantable cells	Time after beginning of cultivation (in days)	Cells with amitotic constriction of nucleus	Multinuclear cells	Binuclear cells	Total
		number of cells per 1000			
HeLa	5	4	9	30	43
"	7	21	0	4	25
Amnion	5	4	3	27	34
"	7	11	1	9	21

were fixed by Carnoy's or Shabadash's method and stained by Feulgen's method or for glycogen.

Besides the mitotic coefficient, the amitotic coefficient and the coefficient of gemmation were determined. The amitotic coefficient was deduced from the number of nuclei in a state of amitotic constriction, the number of binuclear cells with their surfaces in contact, the number of multinuclear cells, and the number of cells which had divided but were still connected by a thin nuclear bridge, per 1000 cells. The coefficient of gemmation is given by the number of budding nuclei per 1000 cells, and it was calculated separately for single and multiple gemmation in mononuclear and multinuclear cells. For each calculation, 4000 cells were counted and the mean value of 4 counts was taken.

EXPERIMENTAL RESULTS

As the data given in Fig. 1 and Table 1 show, the intensity of multiplication of the amnion cells was lower than that of the HeLa cells, although the character of the curves showed common features for all 3 coefficients.

From the 2nd day until the end of the 4th day an increase in mitotic activity was observed, steeper in the case of HeLa. The mitotic coefficient reached its maximal value on the 4th day. Then, starting on the 4th day, a decrease took place in the number of mitoses, falling to zero on the 7th day, or to 4 in the amnion cultures.

The curve of mitotic activity in the HeLa cultures obtained in this series of experiments corresponded in its essential details with the results described by S. Ya. Zalkind and L. G. Stepanova. The slight differences in the value of the mitotic coefficient could be attributed to a difference in the medium and to the fact that these authors seeded fewer cells in their experiments (lactalbumin hydrolyzate, 50,000 cells/ml).

The amitotic coefficient reached its peak on the 5th day and fell on the 7th day. The coefficient of gemmation was very low on the 2nd day, fell to zero on the 4th day, and then rose rapidly until the 7th day. It follows from these findings that after the 4th and 5th day a fall took place in the mitotic coefficient, which was accompanied by increases in the amitotic coefficient and the coefficient of gemmation. From the 5th until the 7th day the number of mitoses and amitoses fell sharply, while the curve of cell multiplication by gemmation rose equally sharply.

It must be noted that with the dynamics of multiplication of the HeLa cells as described above, on the 5th day a state occurred in which the number of mitoses was equal to the number of amitoses. The 2 processes of cell multiplication took place at the same intensity, but, as we shall see later, this state of equilibrium was temporary and unstable. Furthermore, these 2 methods of division were joined by a 3rd, that of gemmation. This corresponds to the polymorphism of the nuclei of this particular culture (Fig. 2). Cells are shown in Fig. 3 which have divided by amitosis, but which are still joined together by a thin nuclear bridge.

Counts on the 5th and 7th days showed a decrease in the number of binuclear and multinuclear cells and an increase in the number of nuclei with an amitotic constriction (Table 2). In our opinion, this process was associated with division of binuclear and multinuclear cells, continuing at these periods, before the formation of mononuclear cells from them; meanwhile, mitosis took place by constriction of the nuclei. The fact was noted that the nuclei of the cells dividing by amitosis were much larger than the nuclei characteristically present at these periods of cultivation in places where mitoses were predominant.

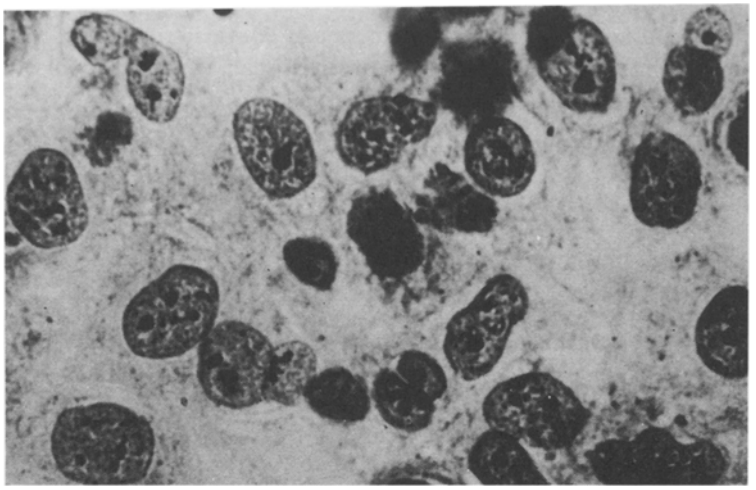

Fig. 2. Cells of an HeLa culture on the 7th day of cultivation; amitoses and budding of nuclei. Photomicrograph. Stained with hematoxylin-eosin. Objective 90, ocular 7 ×.

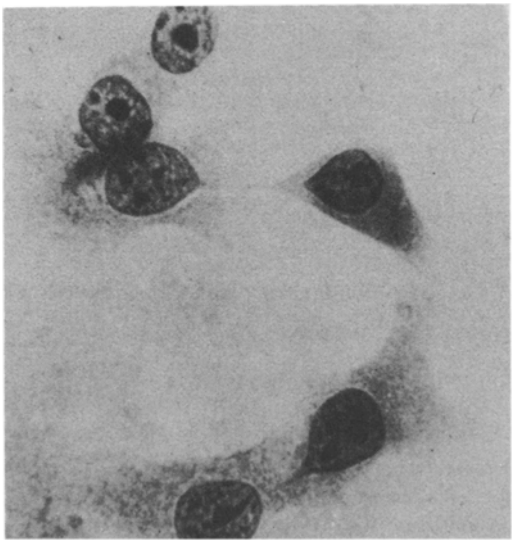

Fig. 3. Cells of an HeLa culture on the 5th day of cultivation. Dividing cells are still joined by a nuclear bridge. Photomicrograph. Stained with hematoxylin-eosin. Objective 90 ×, ocular 7 ×.

What explanation of the relationships observed between the various methods of multiplication of the cells can be given?

In our experimental conditions renewal of the cells in the cultures took place in an isolated environment in which, as a result of the growth and development of the cells, metabolic products accumulated, the concentration of the nutrient substances varied, and the pH of the medium moved consistently towards the acid side. For these reasons the conditions for growth and development of the cells were constantly changing in the course of the 7 day period of cultivation.

The conditions necessary for the appearance and continuation of mitosis are evidently not identical with the conditions favoring the development of amitosis and gemmation. The work of S. Ya. Zalkind and L. G. Stepanova has shown that the cell density and the mitotic coefficient are correlated, although their maxima are separated by 24 h. If it is remembered that the cell density observed in the experiments of these authors was maximal on the 4th day, and that the maximum of mitotic activity took place on the 3rd day, then the difference may naturally be ascribed to the fact that, in the case of the HeLa cultures on the 4th day, to the number of cells dividing by mitosis must be added the considerable number dividing by amitosis, which was not taken into account in the investigation cited.

No methods of cell multiplication were found to be peculiar to the HeLa culture and absent from the amnion cells. However, the number of irregular, multicentric mitoses with the chromosomes left in the stages of metaphase and with an atypical arrangement of the daughter chromosomes in ana- and telophases was greater in the amnion cultures than in the HeLa cultures.

Hence, the quantitative relationships between mitosis, amitosis, and gemmation have been studied during a 7 day period of cultivation. For the particular cell lines and the conditions chosen, differences inherent in their nature, and, also, an important measure of general agreement were observed in the dynamics of these indices.

SUMMARY

A study was made of modes of division of human cancer cells—HeLa strain—and of human amnion cells with the use of monolayer cultures in synthetic medium "199" with added calf serum. The mitotic, amitotic and nuclear gemmation coefficients were determined during the 7 days of cultivation.

A prevalence of mitotic division over amitosis and nuclear gemmation was noted during the first days of cultivation of amniotic cells, and on the 4th day cultivation of HeLa cells. On the 5th day, amitosis and nuclear gemmation were the prevailing forms of multiplication in the 2 cell strains.

The mitotic coefficient of the HeLa cells decreased by the 7th day; amitosis, as well as solitary and multiple budding of the nucleus proved to be the prevailing form in the 2 strains of cells.

Reduction of mitotic coefficient of the amniotic cells during the same period of cultivation was not so marked, but the prevalence of amitosis and nuclear budding was largely of the same character as in the HeLa cell cultures.

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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.
