A COMPARATIVE CYTOLOGICAL ANALYSIS OF TISSUE CULTURE CELLS UNDER NORMAL CONDITIONS AND EXPOSED TO THE ACTION OF POLIOMYELITIS VIRUS

COMMUNICATION I. THE TREND OF THE CYTOLOGICAL CHANGES IN 4 VARITIES OF CELLS CULTIVATED IN NORMAL CONDITIONS

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Since the publication of work which showed that the method of tissue culture could be used for virological purposes [3, 9-12], an extensive literature has accumulated concerning the various aspects of this subject. The cultivation of a number of normal and tumor tissues in liquid nutrient media has, in particular, fully justified itself. Under these circumstances the cells are arranged in the form of a continuous layer on the walls of the flask and they are usually studied unstained, through the glass, under the low power of the microscope. The action of the virus is judged in these conditions by its cytopathogenic effect, i.e., by the injury and death of the affected cells [6, 7].

This method of study gives only the most general picture of the changes in the culture and sheds no light on the details of the processes taking place.

It is desirable to make a detailed study, by means of cytological and cytochemical methods, of the changes taking place in the culture from the moment of transfer to fresh nutrient medijm to its natural degeneration (in the absence of subculture) both in normal conditions and under the influence of the virus.

We considered it necessary to conduct this investigation from the comparative cytological point of view, by studying the changes in two varities of normal tissues (kidney cells of Macacus rhesus and heart cells of Macacus cinomolgus) and two varieties of malignant tumor cells (HeLa cells and Hep-2 cells).

The practical importance of our investigation was associated with the fact that all the varieties studied are used in the cultivation of viruses.

EXPERIMENTAL METHOD

A suspension of trypsinized cells of the varieties mentioned above was placed (in an initial concentration of 50,000 units/ml) in Carrel or Povitskaya flasks containing nutrient medium.

We tried to create so far as possible identical conditions for the cells, in the sense of the composition of the nutrient media. The cultures which we used were divided into two groups in accordance with the composition of the nutrient media: kidney cells and HeLa cells. The latter were grown on a medium consisting of a 0.5% solution of lactalbumin hydrolyzate in Hanks's solution with 2-5% of ox serum; the heart and Hep-2 cells were cultivate in medium No. 199 with 10% calf serum.

At the same time mica disks were placed in the vessels, on which cells settled from the suspension, remaining here until degeneration, when they came off the mica and fell into the liquid medium. In our

experiment, which lasted not more than 7-8 days, the cells remained on the mica until the end. The nutrient medium was not changed throughout the period of investigation. The mica disks were fixed every day for 15 minutes in Bouin's fluid, and then stained with hematoxylin-eosin and mounted in Canada balsam.

In addition to the morphological analysis, we studied the mitotic activity in the cultures. This was expressed by the number of mitoses per 1000 dividing cells in the different areas of the preparation.

EXPERIMENTAL RESULTS

We shall mainly give a description of the stages of development of all the varieties together, pointing out the special features of each.

Before the beginning of cultivation the suspension consisted of round cells with round, light or darkly stained nuclei. Measurement with the ocular micrometer showed that the cells of the monkey's kidney were larger than those of the other three varieties. This suspension was nonhomogeneous. In the heart culture, for example, two types of cells were found: larger (cell diameter~14 μ, diameter of nucleus~9 μ) and smaller (cell diameter~8 μ, diameter of nucleus~5 μ). In some cells a picture of dying mitoses was observed, incapable of completion in the course of the preceding cultivation in the nutrient medium.

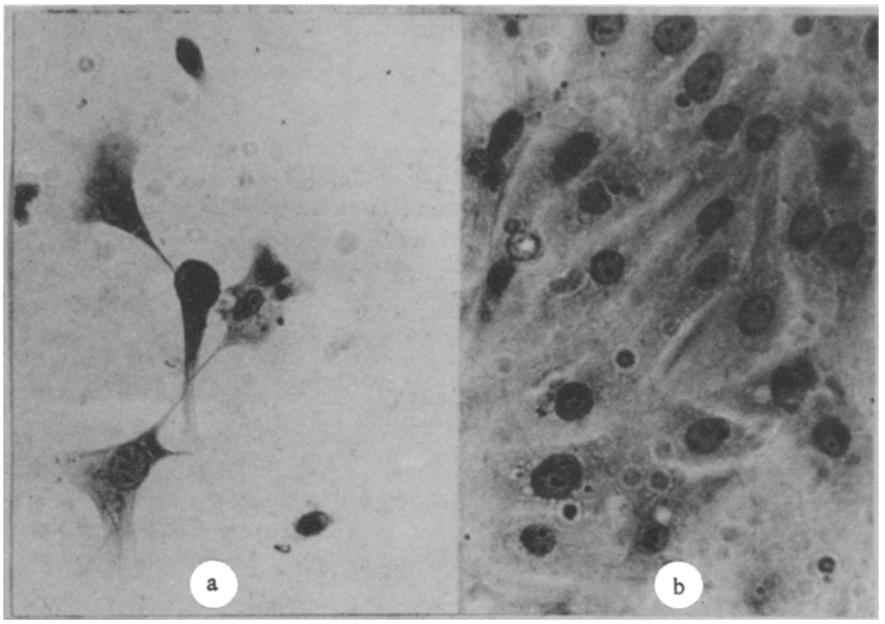

Fig. 1. Cells of a culture of the kidney of Macacus rhesus. Stained with hematoxylin-eosin. Microphotograph. Magnification: ocular 7 ×, objective 20 ×. a) 1st day of cultivation; conversion of the round cells of the suspension into elongated cells with processes is seen; b) 3rd day of cultivation.

Only 24 hours after the beginning of cultivation in fresh nutrient medium, considerable changes were observed in the shape of the cells (instead of round, they became elongated, and acquired processes), and proliferation of the cells led to an increase in their number. At first they formed small groups, and later, extensive films, covering the entire surface of the mica disk. At this stage it could be seen that the round cells of the suspension were converted immediately into elongated cells (Fig. 1, a).

This process of "regeneration" followed an almost identical course in all the cultures, although certain differences were present between the different varieties as revealed by a difference in their biological properties, in the intensity and means of their proliferation and so on.

Culture of kidney cells of Macacus rhesus. On the 2nd day of cultivation the total number of cells increased, and they became more varied: they developed a whole series of forms, reflecting a transition from the round cell to a large, flat cell with ill-defined edges. Full development of the culture took place on the 3rd-4th day. At this period the cells covered the mica disk with a continuous layer (see Fig. 1, b). Beginning with the 5th day the picture observed was one of breaking up of the films, appearance of large cells, differentiation and degeneration. These changes gradually increased in intensity, and on the 7th-8th day the cells began to come away from the mica disk. In the period of growth the following categories of cells could be found in the kidney culture: 1) cells of the suspension not yet undergoing development; 2) cells (usually with a single process) starting to undergo development; 3) elongated cells with two processes, with clearly defined edges; these forms evidently showed maximum activity and were most characteristic of the fully developed culture; 4) flat, large cells with pale, oval or round nuclei, with ill-defined edges; 5) multinuclear cells of ordinary dimensions; 6) giant cells, appearing in the middle and later periods of cultivation. In the cytoplasm of many cells, even at early stages of development, a large number of vacuoles was observed.

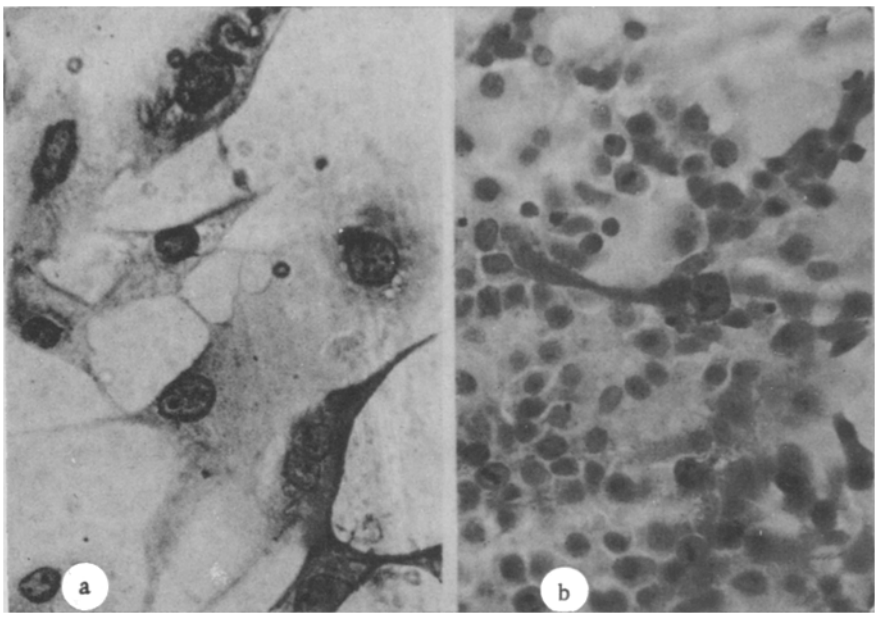

Fig. 2. Cells of a culture of the kidney of Macacus rhesus on the 6th day of cultivation (a) and of a culture of the heart of Macacus cinomolgus on the 4th day of cultivation (b); in the center an irregular tripolar mitosis is seen. Stained with hematoxylin-eosin. Microphotograph. Magnification: ocular 7×, objective 20×.

Degeneration of the culture of kidney cells was expressed primarily by the appearance large, flat cells, sometimes with several nuclei (Fig. 2, a).

Some of these were joined together to form structures enclosing empty spaces. A characteristic feature of the culture of kidney cells was the comparatively very small number of mitoses; the maximum mitotic activity on the 3rd-4th day did not exceed 30/1000. In this connection there arises the problem of the mode of proliferation of this culture.

Distinctive features of the culture of kidney cells depending on its origin. The question of the relationship between the morphology and the trend of the changes in the culture of kidney cells and its past history was one which merited special attention. We studied cultures of kidney cells directly (a few hours) after removal of the organ and subsequent treatment with a 0.25-0.3% solution of trypsin in order to separate the cells from each other, and also subcultures obtained from the cultures grown for 6-7 days, cells of which were detached by means of a 0.25% solution of trypsin or a 0.02% solution of versene.

The cells taken from the animal body were slower to undergo development, but preserved their viability for a far longer period. The full development of the culture taken from the body accordingly took place on the 4th-5th day after the beginning of cultivation. The duration of life of these cultures, without changing the nutrient medium, was also considerably longer than that of the cells of the subculture; even on the 13th day of cultivation a large number of relatively normal cells was observed. The mitotic activity was the same, irrespective of the past history of the culture. Morphologically, considerable differences between the cells were found, depending on their origin.

Culture of the heart cells of Macacus cinomolgus. Already on the first day of cultivation there appeared relatively large collections of up to 10 cells. In later stages of the investigation the number of cells increased sharply, and on the second day they covered the whole mica disk in a continuous film. These cells, too, could be subdivided into two groups during cultivation: small and lightly stained (cell diameter~31 μ, diameter of nucleus~26 μ) and large, lightly stained cells (cell diameter~52 μ, diameter of nucleus~35 μ). In all the cells large nuclei were seen, as a rule with several nucleoli surrounded by lightly stained margins. Full development of the culture took place on the 3rd-5th day of cultivation. Beginning on the 5th day, in addition to the films of flat cells, cells with processes were encountered, forming reticular structures or surrounding round, empty spaces. Beginning on the 4th day an ever-increasing number of small cells with oxyphilic, granular cytoplasm and a compact, small nucleus, were seen, and were in various stages of degeneration.

The mitotic activity was high even on the first day (49/1000), reaching still higher figures (70/1000) on the second day, and maintained at a high level until the end of cultivation (on the 7th day — 44/1000). Irregular tri- and quadripolar mitoses, arrest of a proportion of the chromosomes at the metaphase stage and an irregular mutual arrangement of the daughter chromosomes in the anaphase (see Fig. 2b) were found here in greater number than in the culture of kidney cells.

Culture of HeLa and Hep-2 cells. The cells of both cultures showed considerable morphological and functional resemblance. They proliferated intensively by mitosis and on only the 2nd-3rd day they covered the mica disk with a continuous film. The cells were polyhedral, often with irregular nuclei, forming processes, and with oxyphilic cytoplasm. The highest mitotic activity (up to 95/1000) was observed on the 3rd-4th day of cultivation. On the 6th-7th day it fell sharply, and in the HeLa culture it reached zero. At the same time morphological degeneration began, as shown by the appearance of reticular structures, pyknotic cells with oxyphilic cytoplasm and multinuclear giant cells. In both cultures many irregular mitoses were found.

The distinctive features of the cultures of the different varieties of cells were shown by a study of their mitotic activity. This mainly agreed with the intensity of the increase in the number of cells counted by means of the Goryaev counting chamber (Fig. 3). The differences which were found in the peak periods (of 24 hours) were evidently associated with the fact that in the second case an indication was given of the density of distribution of cells in the culture, i.e., the end result of previous proliferation.

As can be seen from Fig. 3, the culture of kidney cells was distinguished by its comparatively low mitotic activity. At the same time numerous patterns of nuclear structure were observed in this culture, which could be interpreted as different forms of amitotic division (typical amitosis, budding and fragmentation of the nucleus). The fact that this culture proliferated mainly by amitotic division was shown by the comparatively large number of amitoses present (60/1000), which remained constant throughout the whole period of cultivation.

We consider that these findings are of very great theoretical importance. It may now be regarded as established that amitoses are a physiological method of cell proliferation [1, 2]. So far as we know, however, there are no reports in the literature of the importance of amitotic methods of division in the proliferation of tissue culture cells.

The three remaining cultures investigated showed high mitotic activity, although there were some characteristic differences in its trend, as shown in Fig. 3.

Cytological study showed the special feature of the heart culture. The high intensity of proliferation of the cells of this culture must be associated with the possibility of its spontaneous malignant change, similar to that described for some normal tissue cultures [4, 5, 8].

On the other hand, the heart culture differed from cultures of malignant cells in several respects: the lower intensity and characteristic trend of its mitotic activity and the more regular, round shape of its nuclei.

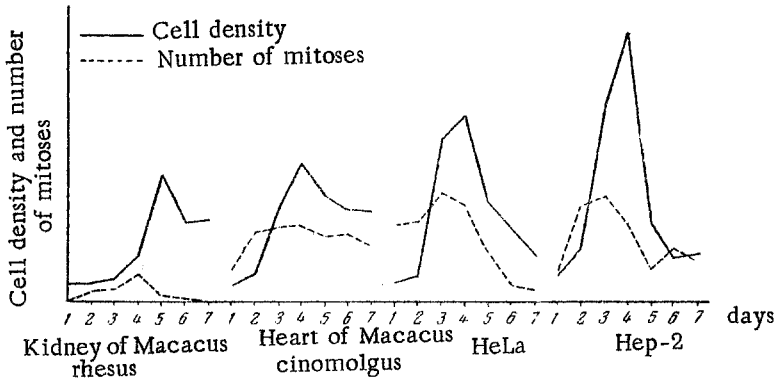

Fig. 3. Graph showing the trends of growth and mitotic activity of cells of four varieties of tissue culture during 7 days of cultivation.

The small number of irregular mitoses in this culture could not be regarded as evidence in support of its malignant change, since in the kidney culture also, which was known to be normal, a variety of irregular forms was found among a small number of mitoses. The solution of the important problem of the future prospects of using heart cultures for virological purposes must await special and comprehensive investigation.

SUMMARY

The authors studied the morphology of the following cells: renal cells of Macacus rhesus, cardiac cells of Macacus cinomolgus, and of different lines of malignant HeLa and Hep cells. All these cells were cultured on mica films in the liquid nutrient medium. Although the dynamics of the cytological changes of the cultures were similar, each one of them had certain growth peculiarities reflected in the curves of mitotic activity. In 122 experiments this activity in the renal cells was low. Evidently, the cellular reproduction here occurs by amitosis. Irregular mitoses are encountered in all four cultures as a manifestation of intensive cellular reproduction. The growth dynamics and morphology of the renal culture vary depending on the cells originating directly from the body or from a subculture. The culture of a cinomolgus heart differs by a number of signs (dynamics of mitotic activity, the shape of the nuclei) from the cultures of malignant cells, with which it has certain morphological similarities.

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