A COMPARATIVE CYTOLOGICAL ANALYSIS OF THE TISSUE CULTURE CELLS UNDER NORMAL CONDITIONS AND ACTED UPON BY THE POLIOMYELITIS VIRUS

REPORT II. CYTOLOGICAL CHANGES IN THE GROWING CELLS ACTED UPON BY THE POLIOMYELITIS VIRUS

S. Ya. Zalkind and L. G. Stepanova

Moscow Scientific Research Institute for Viral Preparations, Ministry of Health Preservation of the USSR
(Presented by Active Member AMN SSSR, G. V. Vygodchikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 12, pp. 76-80, December, 1960
Original article submitted January 22, 1960

In our first report [3] we presented data on the dynamics of normal growth changes for 4 cell lines cultured outside the organism, kidney cells from Macacus rhesus (2nd generation), a strain of heart cells from Macacus cynomolgous, and strains of the malignant cells HeLa and HEp-2. In this report we present the results of studying externally cultivated cells acted upon by the poliomyelitis virus.

A considerable amount of work was devoted to studying the morphological changes caused in the tissue culture cells by the different viruses. A survey of the existing data may be found, in particular, in the abstract by Syverton [9]. However, these investigations were not carried out on a comparative basis, and the authors were much more interested in the phenomenology of the cytopathogenic effect than in its dependency on the properties and the state of the effected cells. This situation stimulated us to investigate the problem from its comparative-cytological aspect and to study the cultures at different stages of their life cycle.

METHOD

The cells were cultured on layers of mica (moscovite) in a fluid nutrient medium. The cultivation conditions and the histological technique employed have been described in our previous report [3]. The cultures, containing 50,000 cells per ml, were inoculated with the poliomyelitis virus (type I, Brunden strain), 100-50 TsPD. The virus was added to the suspension of cells prior to the beginning of cultivation, and also to the growing cultures at various stages of cultivation. Material for the morphological investigation was taken at 1, 4, 8, 12, and 24 hr after inoculation with the virus and then daily until the end of the cultivation. Fixation was carried out in Bouin's solution, and hematoxylin and eosin were used for the staining.

RESULTS

I. Addition of Virus to the Cell Suspension Prior to the Beginning of the Cultivation

Despite the action of the virus, the cells attached themselves to the mica and formed a significant layer on its surface. The changes observed in their morphology have been considered for each culture individually.

1. The Culture of Renal Cells of Macacus rhesus. One day after inoculation of part of the cells we observed significant changes, predominantly concerned with the structure of the nucleus: its membrane lost turgor, and in it there appeared compact accumulations of chromatin, lying principally under the membrane. The shape of the cells also changed: they became rounded or acquired branch-like formations, which gave them a certain similarity to neurons. The protoplasm was often eosinophilic, and inclusions could be seen in it, distributed either compactly or diffusely (Fig. 1a). With a greater degree of damage we encountered abnormal accumulations of chromatin in the cytoplasm, sometimes simulating the picture seen with mitosis. In the later stages, after 2 days, only groups of single, markedly altered, rounded cells, their "shadows," remained in the layer (Fig. 1b).

2. The Culture of a strain of Cells from the Heart of Macacus cynomolgous (SOTs). The effect of the virus appeared differently from that seen in the culture of kidney cells. After cultivation with the virus for a day changes were manifested mainly in the peculiar round form of the cell clumps, appearing like "beads" (Fig. 2a). At the same time, the first signs of specific degeneration, described above, appeared in the nuclei of several cells. On the second day the number of cells altered in this way had increased, and by the 3rd and 4th days we observed only single, markedly altered cells in the layer.

3. HeLa Strain of Cells. In the course of the first days of viral action the typical changes described above were not present; all that was noted was the appearance of a small number of compact and rounded cells, isolated from the layer (Fig. 2b). On the 3rd day of cultivation there appeared a significant number of pathologically altered cells. The normal cells remained only in the form of small islets.

4. HEp-2 Strain of Cells. A similar pattern of changes took place in the cells of the culture HEp-2 as well. A small number of typically altered cells appeared only on the 2nd day. On the third day approximately 50% of the cells were pathologically altered. By the 4th day only a small number of markedly changed cells were left in the layer.

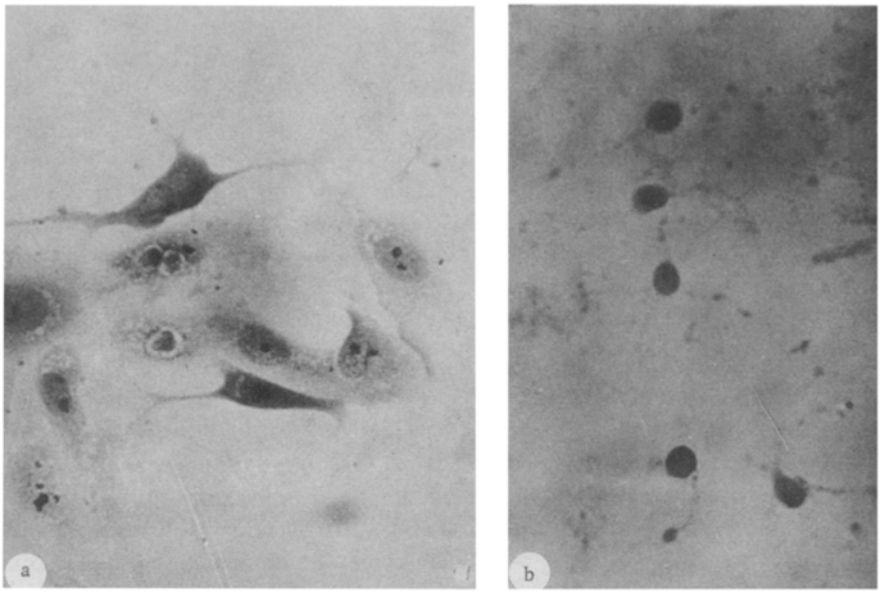


Fig. 1. The effect of Poliomyelitis virus (Type I, Brunden strain) on the cells of a subculture from the kidney. a) After a 24 hr exposure to its action; b) after a 96 hr exposure. Fixation was carried out in Bouin's solution; staining was performed with hematoxylin and eosin. Photomicrograph. Magnification 350×.

II. The Effect of the Virus on Growing Cells

In this series of experiments we studied the effect of the virus on cells that had been growing for varying lengths of time (1–8 days) in the cultures. We noted significant morphological changes in the cells, qualitatively

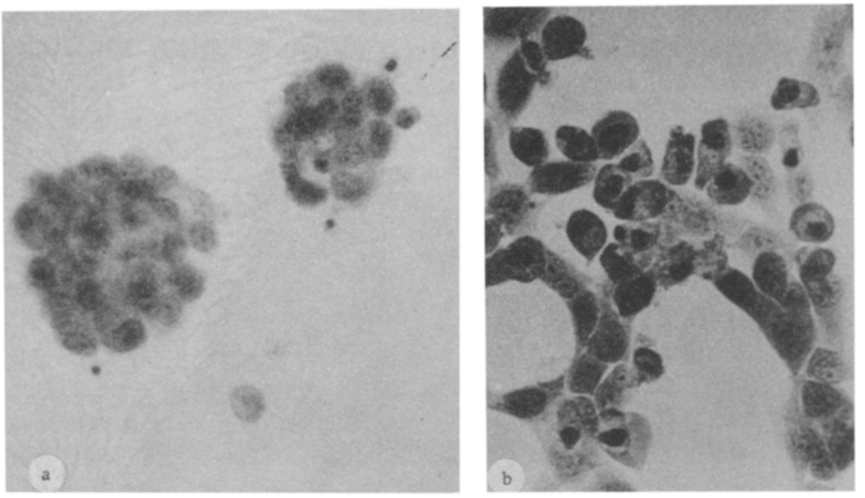


Fig. 2. The effect of Poliomyelitis virus (Type I, Brunden strain). a) On cells of the SOTs culture after a 24 hr exposure. Fixation was carried out in Bouin's solution; staining was performed with hematoxylin and eosin. Photomicrograph. Magnification 350 ×.

TABLE 1

The Dynamics of Changes in the Number of Monkey Renal Cells Damaged by the Poliomyelitis Virus as Related to the Duration of Exposure and the Age of the Culture (per 1,000 Uninvolved Cells)

Age of the culture (in days)	Duration of exposure to the virus (in days)			
	1	2	3	4
1	244	207	Almost all involved	Almost all involved
2	232	210	444	342
3	165	273	743	Almost all involved
4	125	123	124	151
5	183	—	200	—

TABLE 2

The Dynamics of Changes in the Number of SOTs Cells Damaged by the Poliomyelitis Virus as Related to the Duration of Exposure and the Age of the Culture

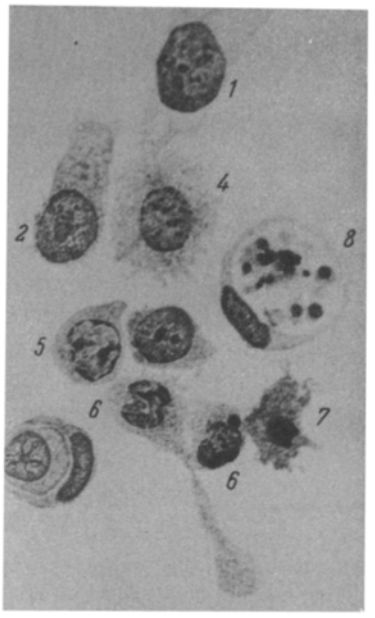
Age of the culture (in days)	Duration of exposure to the virus (in days)		
	1	2	3
1	206	Almost all cells involved	No. cells left on the layer
2	64	The same	The same
3	56	175	—

similar to those described above but differing from them, somewhat, principally in their dynamics.

1. The Culture of Renal Cells of Macacus rhesus. The first statistically reliable changes due to the virus were observed only after 12 hr following the beginning of the exposure. However, isolated typical cell changes were encountered in the culture after exposure to the virus for 8 hr. One day after the initiation of the viral action we noted a considerable number of typically changed cells. The number of these cells gradually increased over the next few days of the exposure. By the 5th day we found only a small number of cell "shadows." Beginning with the 6th day there was complete absence of the culture treated with the virus from the layer.

2. The Heart Strain of Cells (SOTs). As in the exposure of the cells in suspension, the changes were reflected primarily by alterations in the form of the cell agglomerates. Changes in the morphology of the separate cells could be noted only on the 2nd day. They decreased in size, became rounded, their cytoplasm became markedly vacuolated, the nuclei, more compact and occasionally containing compact accumulations of chromatin, and, in several instances, clumps of chromatin lay directly in the cytoplasm.

3. The HeLa Strain of Cells. Beginning with the 2nd day of exposure changes were noted in the character of cell distribution: there were no heavy layers, the cells were compact, and by the 2nd day the signs of typical degeneration appeared in isolated cells (Fig. 3). The number of altered cells gradually increased. Nevertheless, a small number of cells were observed on the layers even at the end of the experiment (7 days).



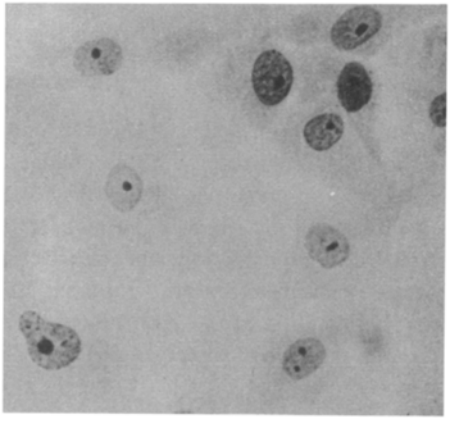


Fig. 3. Successive stages (1–8) in the development of degenerative changes of the nucleus and cytoplasm in HeLa cells (above). Drawing apparatus. Magnification 315×.

TABLE 3

Stimulation of Mitotic Activity in Cells Cultivated Outside the Organism Under the Influence of the Poliomyelitis Virus (Type I, Brunden strain, 100-50 TsPD). Number of Mitoses per 1000 Culture Cells

Age of the culture (in days)	SOTs	HeLa	HEp-2
1	27	41	22
2	85	78	87
3	42	31	21

4. The HEp-2 Strain of Cells. As in the case of the HeLa cells, compact rounded cell accumulations already appeared by the 1st day. However, they did not show the typical changes in the nucleus or protoplasm. On the 2nd day the typical signs of degeneration appeared in isolated cells.

In all the cultures investigated the damaged cells arranged themselves in compact groups between the morphologically normal cells.

So as to elucidate the dependency of the morphologically changed cells, exposed to the virus, on the age of the culture, we added the virus to the culture after varying durations of growth and fixed material each day, beginning with the 1st day of exposure and continuing until the occurrence of senile degeneration in the control cultures. Hoping to study the relationship of the involved cells to the age of the culture objectively, we counted the number of damaged cells in the preparation per 1,000 uninvolved cells. The data obtained for the cells from the renal cultures is presented in Table 1.

We observed similar dynamics for the changes in the culture of SOTs exposed to the virus (Table 2).

Analysis of the material in Tables 1 and 2 shows that sensitivity to the action of the virus, manifested by the morphological changes, was highest in the comparatively young cultures, under 3 days old. High sensitivity was indicated by two signs: 1) the number of damaged cells in the young cultures, after any given time interval, was higher than in the older cultures; 2) in the young cultures the effect of the involvement developed more rapidly and by the 3rd day had already led to massive degeneration of the cells. In contrast, cell damage in the 5-day old cultures was manifested in a comparatively small number of cells (12–18%) during the first few days (see Fig. 3), and that picture lasted, without particular changes, up to 4 days of cultivation with the virus. The reason for the decreased vulnerability of the cells in the several-day-old cultures is still not clear, although it may be postulated that the intense metabolic processes peculiar to cells in the first stages of cultivation potentiates the elevation in their sensitivity to the viral action.

III. Changes in the Mitotic Behavior of the Cultures Subsequent to the Action of the Poliomyelitis Virus

The action of the virus on a suspension of the cells being cultivated (especially the cells for the cultures of SOTs, HeLa, and HEp-2, distinguished by high numbers of mitoses) led, by the 2nd day, to a marked elevation in mitotic activity (Table 3). It may be postulated that this increase in mitotic activity is a reaction connected with

either a protective stimulation of the cells' metabolism when they are exposed to the viral action, or with the direct injurious action of the latter, which leads to local necrotic processes, which, in turn, may also serve as sources of stimulation for the mitotic activity [10]. It should be pointed out that, according to certain data, when cultures of monkey kidney cells are acted upon by the poliomyelitis virus, it results in a temporary increase in glycolysis [8], and an elevation in the acid phosphatase activity [7]. There are indications in the literature that the action of pathogenic and reactive factors causes a temporary stimulation of mitotic activity. Thus, it has been shown that injecting mice with tetanus toxin causes an elevation of the mitotic activity in the corneal epithelium after 36 hr [4]. The injection of anti-plague vaccine leads to an increase in the mitotic activity of the cells in the spleen [2]. The stimulation of mitotic activity in the culture cells was of a short-term nature; even by the 3rd day we noted its intensity subsiding, caused, apparently, by the fact that at that time the growth of the culture almost completely ceases.

An elevation in the mitotic activity by the second day of exposure to the virus was also noted in the cultures to which the virus was added when they had already begun to grow.

The activity of the poliomyelitis virus on a suspension of the cultured cells did not impede their attaching onto the layer or their first stages of growth, but, after several days, led to their death. Morphologically the action of the virus manifested itself by typical, successively developing, changes in the nucleus and cytoplasm, described in a number of works [6], particularly in the recent investigation by A. I. Drobyshevskaya and V. P. Mikhailov [1]. On the basis of morphological data, the authors came to a conclusion regarding the resultant changes in the virus-infiltrated cells which coincides with our hypothesis, the latter based on our observing the stimulation of mitotic activity.

Besides noting changes in the individual cells of the transplanted strains SOTs, HeLa, and HEp-2, we also observed changes in the character of their distribution. In place of uniform layers there appeared separate groups, containing several "tens" of cells. This, obviously, was a result of weakening the intercellular bonds. The observed disturbances, occurring as a result of the poliomyelitis virus, were morphologically different in the cells of the different cultures. In the cells from the culture of monkey kidney the changes had already begun within the first days of exposure, immediately effecting a considerable portion of the cells and rapidly increasing. By the third day there were almost no cells left on the layer. In the SOTs culture the changes did not appear in a significant number of cells until the second day of exposure, but the rate of subsequent development of the cell involvement was about the same for the two cultures. Cells from the strains that originated from malignant tumors reacted to the action of the poliomyelitis virus in a morphologically similar manner, albeit somewhat slower and less pronounced than the specimens from the suspension of normal cells. A sufficient number of typically altered cells appeared in these cultures only by the third day; complete cessation of growth in these cultures occurred approximately a day later than in the kidney and SOTs cultures.

It must be noted that the capacity of cultures to manifest morphological impairments under the influence of a virus does not always directly correspond to their sensitivity to the action of the virus as determined by virological methods. There are indications in the literature [5] that there is no coordination between disturbances in the structure of HeLa cells acted upon by the poliomyelitis virus and the accumulation of this virus in the culture. Derivatives of fluorine, for example, impede the multiplication of the virus, yet do not inhibit the appearance of the cytopathogenic effect. The authors came to the conclusion that the processes leading to the elevation of the viral concentration in the culture and the processes leading to the pathological changes in the cells were, to a significant degree, independent of one another.

The sensitivity of the growing cells to the virus was lower than that of the cells in the suspension. This was demonstrated by the preservation of a certain number of undamaged cells within the layer, even after extended intervals of cultivation. The high sensitivity to the viral action shown by the cells in the early stages of development, as compared with those already growing, is apparently related to the more intense metabolism which is characteristic for cells in their first stages of growth outside the organism.

SUMMARY

The authors studied the effect of polio virus upon the morphology of the cells obtained from subculture of the renal tissue (Macacus rhesus), heart (Macacus cynomolgus) and HeLa and HEp-2 malignant cells. The changes become noticeable in individual cells after an 8–12-hour action and are marked in 24 hr. These changes are

manifested in the chromatin redistribution, the loss of nuclear membrane turgor, the change of the cellular shape and cytoplasmic eosinophilia. The most sensitive to the action of the virus are the cultures during the initial stages of growth, and the youngest and the most active cells in a given culture. The following changes occur in the cultures of transplantable strains acted upon by the virus: at first decomplexation of the stratum is noted with the formation of roundish cellular accumulations, and only later the changes in the individual cells are seen. Temporary stimulation of mitotic activity is noted in the cells of the transplantable strains on the second day of the viral action

LITERATURE CITED

1. A. I. Drobyshevskaya and V. P. Mikhailov, in: Annual of the Institute of Experimental Medicine of the AMN SSSR [in Russian] (Vilnyus, 1957) p. 438.
2. S. Ya. Zalkind, Doklady Akad. Nauk SSSR 119, 2, 365 (1958).*
3. S. Ya. Zalkind and L. G. Stepanova, Byull. Éksp. Biol. Med., 6, 110 (1959).*
4. I. A. Utkin, Works of the Fifth All-Soviet Congress of Anatomists, Histologists and Embryologists [in Russian] (Leningrad, 1951) p. 436.
5. W. W. Ackermann, A. Rabson, and H. Karz, J. Éxper. Med. 100, 437 (1954).
6. T. W. Dunnebacke, Virology 2, 811 (1956).
7. E. Kovacs, J. Éxper. Med. 104, 589 (1956).
8. H. Levy and S. Baron, Nature 178, 1230 (1956).
9. J. T. Syverton, J. Nat. Cancer Inst. 19, 687 (1957).
10. H. Teir, Acta Pathol. et Microbiol. Scand. 30, 158 (1952).

*Original Russian pagination. See C. B. translation.