ULTRASTRUCTURAL AND CYTOCHEMICAL CHANGES IN TISSUE CULTURE INFECTED WITH TYPE 5 ADENOVIRUS

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M. E Aspiz, K. A. Troshin, and S.P. Ushakova

Cytology Laboratory (Chief, Professor I. A. Alov), Institute of Human Morphology of the Academy of Medical Sciences of USSR and Virology Laboratory (Chief, Dr. V. I. Marchenko), Moscow Regional Clinical Research Institute, Moscow
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Several workers [1, 3, 6, 7] have attempted earlier to relate the dynamics of ultrastructural cell changes to cytochemical changes observed under the effect of viruses. Because in most cases viruses do not have their own enzymes it was of interest to follow the changes in the activity of enzymes of tissue culture cells in the process of cell-virus interrelationship.

In this work we have studied changes in the activities of succinate dehydrogenase, DPN- and TPN-diaphorases, and acid and alkaline phosphotases, as well as some aspects of DNA and glycogen metabolism of tissue culture infected with adenovirus.

METHODS

Studies were made on monolayer tissue cultures of continuously maintained line HEp-2. Medium 199 with 10% bovine serum was used for culturing the cells. For cytochemical studies tissue cultures were grown on strips of cover slips in test tubes and these were infected on the third day with adenovirus type 5, (Rowe strain) in a dose of 10^{-1}. Prior to infection of the cultures the culture medium was removed and substituted with a medium containing horse serum instead of bovine serum. Infected cultures were examined after 5, 10, 24, 48 and 72 h following infection. Activities of succinate dehydrogenase and DPN- and TPN-diaphorases were determined according to Pearse, (1962) acid and alkaline phosphotases according to Gomori, DNA according to Feulgen, glycogen according to Shabadash, sulfhydryl groups according to Yakovlev and Nistratova, and total protein by means of bromphenol blue.

For electron microscopic studies the cells were grown on pads of Povitskaya. The cells were removed from glass by means of trypsin and were twice washed in saline. Fixation was done according to Caulfield [2], the material was then dehydrated in alcohol and embedded in metacrylate. Sections were made on ultramicrotome LKB and were studied by means of electron microscope JEM-5.

RESULTS

As revealed by electron microscopy during interphase non-infected cells of the HEp-2 line were usually rounded or slightly elongate in shape, with a relatively large nucleus. The nuclei contained evenly distributed fine reticular and granular material and usually one or two nucleoli of considerably greater electron density. In the cytoplasm there was a large number of somewhat elongate or oval-shaped mitochondria. The internal and external mitochondrial membranes and the membrane structure of cristae were clearly visible. The endoplasmic reticulum was seen less clearly, but in some areas of the cytoplasm it was possible to see the canals and vacuoles of the endoplasmic reticulum with ribonucleoprotein granules. A large number of microvilli were present on the cells.

Following the introduction of the virus the volume of the nucleus relative to the cytoplasm increased. The nuclear material became distributed irregularly and it was often possible to see electron-dense formations irregularly distributed throughout the entire nucleus. In some cells the nuclear membrane became swollen and the perinuclear space became widened. The nucleoli were practically unchanged.

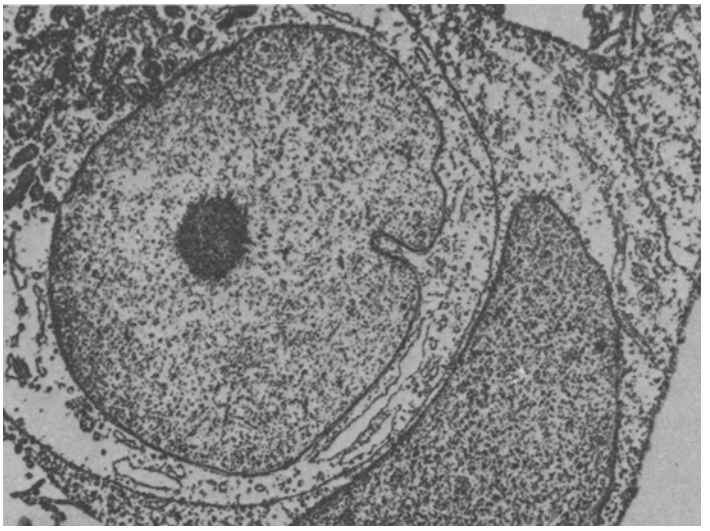

Fig. 1. Cytophagy in tissue culture HEp-2 infected with adenovirus. The nucleus of the cell in the process of engulfing a neighboring cell assumes a characteristic sickle shape. The mitochondria are greately damaged, some of them resembling vacuoles. Magn. 7000 ×.

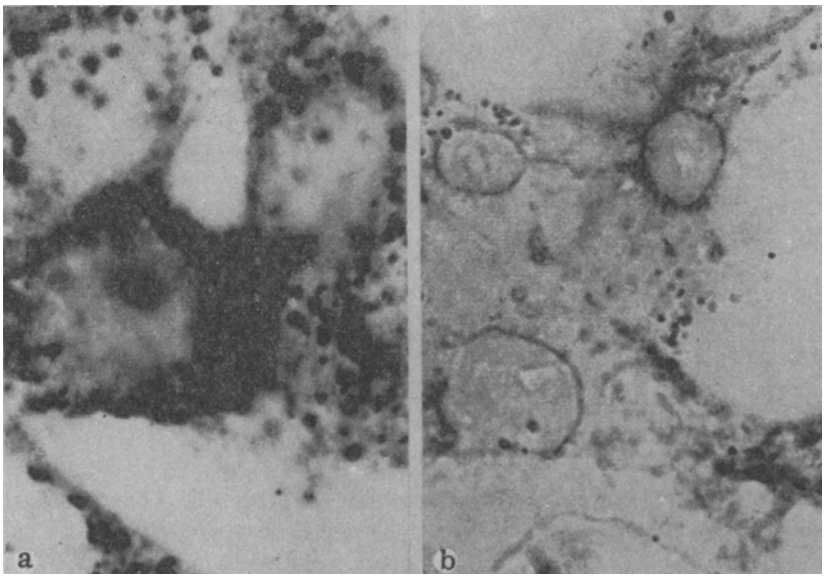

Fig. 2. Succinate dehydrogenase activity in tissue culture HEp-2). a) Noninfected culture; b) 72 h after infection with adenovirus (considerable fall in succinate dehydrogenase activity). Magn. 1080 ×.

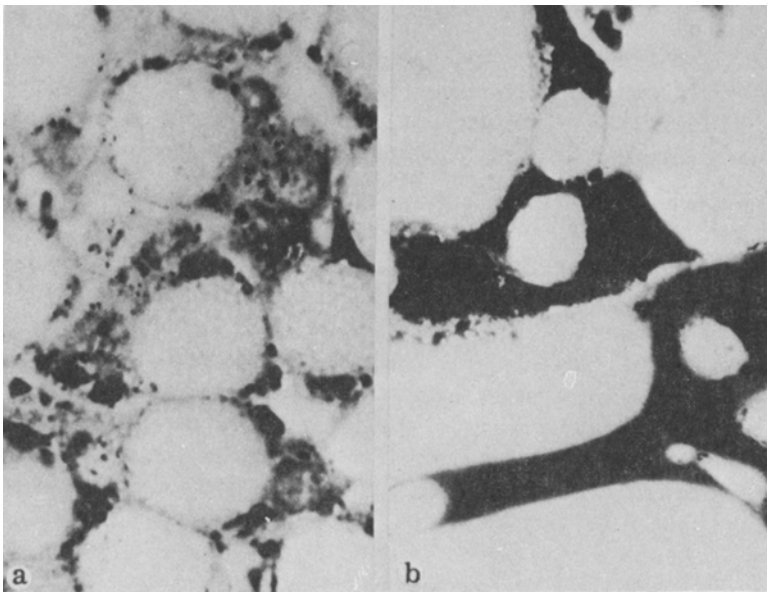

Fig. 3. Glycogen deposition in tissue culture HEp-2. a) Noninfected culture; b) 72 h after infection with adenovirus. Lyoglycogen globules occupy almost the entire cytoplasm. Magn. 1080 ×.

Significant changes occurred also in the cytoplasm. They consisted of a swelling and progressive deterioration of the cytoplasmic matrix. In many mitochondria the cristae were destroyed and the mitochondria themselves resembled vacuoles. Most significant changes were observed in the perinuclear zone. Cytophagy, i.e., one cell being engulfed by another, described in adenovirus infections [4], was observed only very rarely. When this occurred the nucleus of the engulfing cell usually became sickle-shaped and surrounded that of the engulfed cell (Fig. 1).

The results of a cytochemical study of DNA according to Feulgen have shown that, after 24 h, in the nuclei of some cells there appeared small to medium sized Feulgen-positive inclusions. At later stages the inclusions occupied a considerable portion of the nucleus. At the periphery of the nucleus there often appeared vacuoles which pushed the inclusions towards the center of the nucleus. These inclusions were apparently complex in structure: they gave strong reactions not only for DNA but also for total protein and for sulfhydryl groups.

Cytochemical studies on the activity of succinate hydrogenase and DPN- and TPN-diaphorases 5 and 10 h after infection have shown a certain increase in the activity of DPN- and TPN-diaphorases while that of succinate dehydrogenase was not significantly altered. The activity of succinate dehydrogenase became considerably lowered 24 h after infection; in the control and infected cells the activity of diaphorases at these periods of time remained practically at the same level. The activity of succinate dehydrogenase and of DPN- and TPN-diaphorases decreased considerably on the 3rd-4th day. In many cells this was accompanied by a change in the nature of formasan precipitate; frequently relatively larger and more coarse granules were encountered (Fig. 2).

In infected tissue cultures there was no apparent change in the activity of alkaline and acid phosphotases. Only on the third day the activity of these enzymes, especially that of acid phosphotase, became lowered. A change in the nature of deposition of glycogen was noted in infected cultures. There appeared large accumulations of amorphous lyoglycogen, which occupied a considerable part of the cytoplasm, and which appeared to diffuse through it (Fig. 3). The results of these observations have shown that enzymes taking a part in oxidative processes (dehydrases and diaphorases) and those which catalyze the hydrolytic disassociation of phosphorethers, are resistant in different degrees to the deleterious effect of the virus.

The later lowering of activity of DPN- and TPN-diaphorases is apparently related to their morphological localization. It is known that succinate dehydrogenase is concentrated exclusively in the mitochondria, highly labile structures, in which considerable changes could be observed as early as 24 h after infection. DPN- and TPN-diaphorases, in addition to mitochondria, are localized also in the membranes of the endoplasmic reticulum [8] and according to some authors also in the membranes of the Golgi apparatus [5]. The results of our observations have shown that the vacuolar system of the cell is more resistant to adenovirus infection than are mitochondria. The endoplasmic reticulum was destroyed only at later stages of infection. Possibly these properties of the cell components are responsible for the greater stability of diaphorases in adenovirus infection.

It was considered of interest to compare the changes in the enzyme systems of infected cells to the rate of viral reproduction. The results of titration of the virus have shown that the titer rose considerably on the third day following infection, in spite of the fact that at this time the ultrastructure of the cells was seriously damaged, and there was a considerable decrease in the activity of succinate dehydrogenase and DPN- and TPN-diaphorases, and some decrease in the activity of phosphotases. Apparently these enzymes, unlike, for example, viral polymerases, do not determine the process of viral reproduction. The above data raise a question relative to the problem of cell-virus relationships. If viral synthesis occurs during a sharp fall in the activity of oxidative enzymes of the cell, then what mechanisms contribute to the energy of viral reproduction? Possibly at early stages of viral infection certain energy resources become accumulated in the cell to compensate for the above mentioned activation of diaphorases, and these energy resources may be responsible for viral multiplication to later stages of development of the cytopathic effect.

SUMMARY

Cytochemical and electron microscopic methods were used to study changes in the HEp-2 tissue culture infected with type 5 adenovirus. The infected tissue culture was noted to have a considerable decrease in succinate dehydrogenase activity in 24, 48 and 72 h. The activity of DPN- and TPN-diaphorases somewhat increased 10 h after infection and sharply decreased only on the 3rd day of investigation. The activity of acid and alkaline phosphatases did not change substantially within the first 48 h of infection and somewhat declined in 72 h. Non-simultaneous decrease in the activity of succinate dehydrogenase and DPN- and TPN-diaphorases was connected with the morphological characteristics of the localization. Since the virus titer considerably increased on the 8th day of investigation it is supposed that these enzymes are not decisive for virus reproduction. The infected tissue culture was noted to have a change in glycogen deposition manifested by lyoglycogen. Feulgen-positive inclusions, constantly encountered in infected tissue culture have a complex structure: they give an intensive reaction not only to DNA but also to total protein and sulfhydryl groups.

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