CYTOLOGICAL CHANGES IN CELLS OF LINE RES* CAUSED BY THE VIRUS OF TICK-BORNE ENCEPHALITIS

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The literature on the question of the cytopathogenic action of the virus of tick-borne encephalitis on cell cultures contains much that is contradictory. According to some reports [17-19, 21] the virus of tick-borne encephalitis causes destruction of HeLa cells. Other workers [2, 8, 10, 14, 15] were unable to confirm this statement. The virus of tick-borne encephalitis causes a cytopathic effect in only a few cell cultures. The most conclusive results in this respect have been obtained in primarily trypsinized cultures and in transplantable cell lines obtained from the kidneys of pig embryos [1, 11, 16]. Because of the difficulty in demonstrating the cytopathic effect, it is necessary to make a detailed study of cell cultures infected with the virus of tick-borne encephalitis to assist the development of methods of early indication and diagnosis. The object of the present investigation was to study the changes in the fine structure of cultures of line RES, sensitive to the virus of tick-borne encephalitis, in the early periods of infection.

EXPERIMENTAL

The object used for infection with the virus of tick-borne encephalitis was monolayer cultures of the transplantable cell line RES obtained from the kidneys of pig embryos [5]. This cell line is sensitive to the viruses of the herpetic group, viruses of Coxsackie group B, and also the virus of tick-borne encephalitis [9]. Meanwhile, line RES is insensitive to poliomyelitis virus of all three types.

The cultures were infected with tick-borne encephalitis virus of strain Ix-10, isolated by A. K. Shubladze in 1948 from the tick Ixodes persulcatus. The virus was adapted to a culture of the embryonic kidneys of a sheep and used in the investigation after 6-8 passages.

The RES cells were grown on cover slips placed in penicillin flasks containing 2 ml of medium No. 199 with 10% normal ox serum. The seeding concentration was 200,000 cells/ml. A continuous cell layer was usually formed by the end of the second day. The 48-h cultures were inoculated with 0.2 ml of virus-containing liquid to each flask. A dose of virus equal to 0.2 ml of undiluted virus-containing fluid contained $10^{5.6}$ LD_{50}. The following dilutions of virus were used: 1:1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-5}. The duration of incubation of the inoculated RES cultures was 20-48 h. The infected cultures grown on the cover slips were then fixed by A. L. Shabadash's method [12] for 2-24 h and stained by various histological methods (hematoxylin and eosin, iron hematoxylin, methyl green), with pyronine by Brachet's method to detect RNA, and by the Feulgen method for DNA. A parallel series of control experiments was performed. A mixture of various dilutions of virus with specific rabbit serum against the virus of tick-borne encephalitis (neutralization index 10,000) was added to the flasks with the RES cultures. Preliminary contact between the virus and immune serum (in an incubator at 37°C) lasted for 45 min. The second control consisted of uninfected RES cultures.

*RES, an abbreviation for Ren Embryonis Suis, the kidney of the pig embryo.

RESULTS

The RES line differs from most cell strains used in virological practice by its marked monomorphism. RES cultures consist only of cells of epithelioid character, regular and polygonal in shape, with well defined borders, and containing one round or oval nucleus (Fig. 1). In contrast to the heteroploid cell lines (SOTs, HeLa, A-1, KEM-1, and so on), multinuclear cells are hardly ever seen in RES cultures, and even binuclear cells are rare. The mitotic activity of RES cultures is much lower than the activity of most heteroploid cell lines, and does not exceed 20-25 mitoses per 1,000 nondividing cells. In addition, in cultures of the RES line atypical division figures are practically never seen (multipolar mitoses, mitoses with chromosomal bridges, etc.), so characteristic of aneuploid cell strains [4, 6]. Karyological investigations of the RES line undertaken after various numbers of passages [3] have shown that from 70 to 90% of RES cells contain 38 chromosomes (Fig. 2), i.e., they correspond to the diploid karyotype characteristic of the somatic cells of the pig [13, 20, 22]. Besides diploid cells, the populations of line RES contain hypodiploid cells with 37, 36, 35, or 34 chromosomes. The proportion of hyperploid cells is very small—not more than 5% of the total number of cells. Cells of RES cultures are further characterized by their cytochemical stability, regular distribution of glycogen and RNA in their cytoplasm and an abundance of lipids.

In the first experiments the cultures infected with virus were incubated for 42-48 h. At the end of this period most of the cell layer had undergone total destruction. The cells fell from the cover slip, and in the areas remaining intact a picture of intensive degeneration of the cytoplasm and nuclei of the great majority of cells was seen—karyorrhexis, karyopycnosis, plasmarrhexis, and complete cytolysis (Fig. 3). These phenomena took place not only after infection with virus in high concentrations, but also after infection in dilutions of 10^{-2} and 10^{-3}. Under the influence of dilutions of 10^{-4} and 10^{-5} the changes which developed were less severe, but considerable areas of the RES cultures consisted of cells with pycnotic nuclei and cytoplasm in the form of large eosinophilic droplets (pyroninophilia of part of the droplets was demonstrated by Brachet's method). In circumscribed areas cells with well marked signs of karyorrhexis were found.

By means of Feulgen's method it was possible to detect the initial stages of the changes in the nuclei of the RES cells infected with virus of tick-borne encephalitis—regrouping of the chromatin granules, the formation of large aggregates of Feulgen-positive material, and a marginal arrangement of the chromatin. As a result of the concentration of the DNA-containing material in the central parts of the nuclei, in some cases irregularly shaped conglomerates were observed, somewhat resembling in their appearance the inclusions in adenovirus infections.

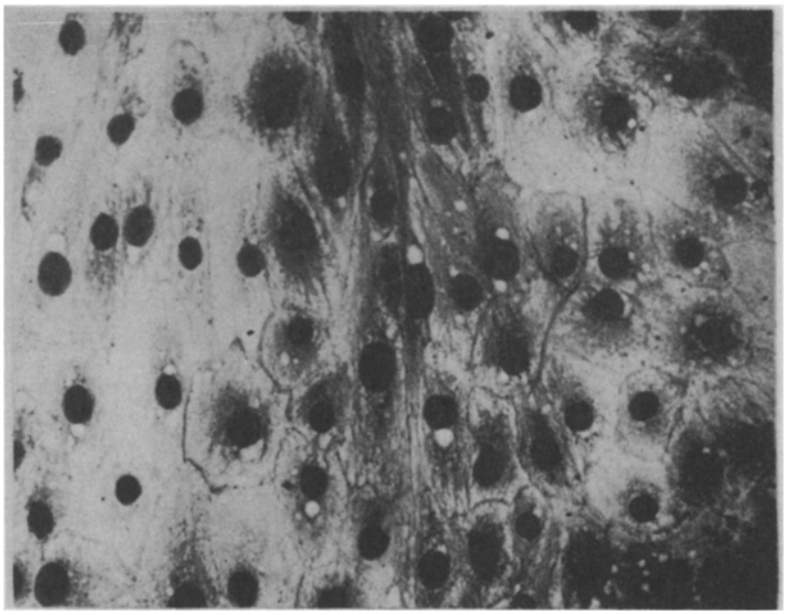

Fig. 1. 48-hour culture of line RES. Normal cells of polygonal shape with clear cell borders. The spherical vacuoles correspond to the localization of lipid droplets characteristic of normal RES cells. Staining with hematoxylin and eosin. Objective 25 ×, ocular 10 ×.

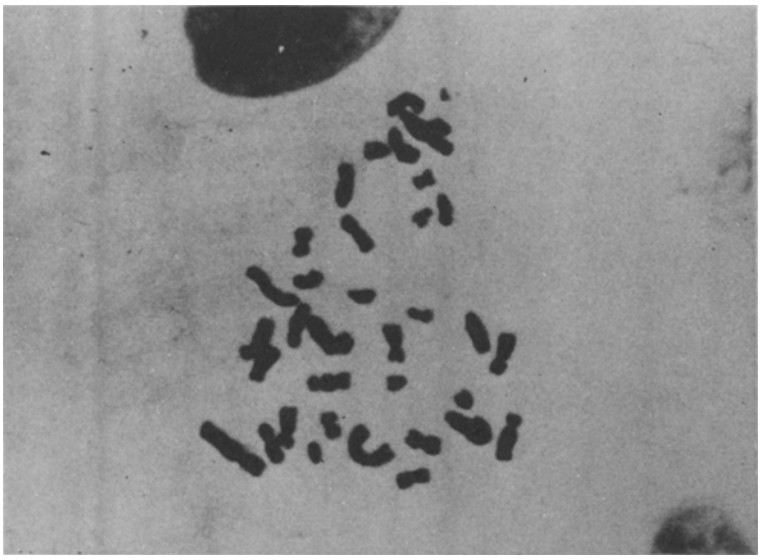

Fig. 2. Set of chromosomes from a cell of an RES culture (38 chromosomes). The cell culture was previously treated with colchicine. Feulgen's method. Objective 90 ×, ocular 10 ×.

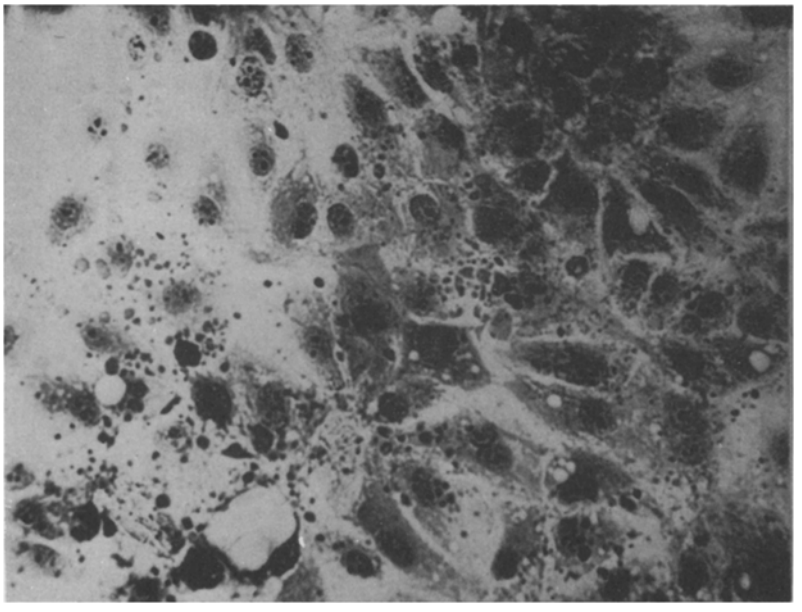

Fig. 3. RES culture infected with virus of tick-borne encephalitis, strain Ix-10 (dilution 10^{-2}). Period of incubation 42 h. Massive cell destruction. Stained with iron hematoxylin and eosin. Objective 25 ×, ocular 10 ×.

Following the introduction of a mixture of virus with immune serum into the RES cultures, no cytopathic changes developed, and cytologically they were indistinguishable from the cultures of the second control group (not treated with virus or with a mixture of virus with serum). Having demonstrated conclusively that 42-48 h after infection with virus of tick-borne encephalitis the cytopathic changes in the cultures of line RES were very clearly defined, the next step was to carry out experiments with shorter incubation periods.

Changes in the structure of the RES cells were observed 20-26 h after infection with virus-containing fluid, either whole or in a dilution of 10^{-1} or 10^{-2}. The processes of destruction affected single cells or small groups of cells. In the affected cells mixing of large and small eosinophilic droplets was observed in the cytoplasm, together

with regrouping of chromatin and pycnosis of the nuclei. The cytoplasm of individual cells showed total destruction, leading to the appearance of bare, deformed nuclei. In the control preparations of groups 1 and 2 no destructive changes were found. In dilutions of 10^{-3}-10^{-5}, the virus had no clearly defined cytopathic action on the RES cells.

The changes in the RES cultures incubated with Ix-10 virus (dilutions 1:1–10^{-4}) for 30 h were more obvious than in the cultures incubated with virus for 20-26 h, and rather less obvious than after incubation for 42-48 h. The cultures incubated for 30 h were characterized by the presence of numerous small foci of cell destruction in the RES cultures, scattered throughout the cell layer. In some cases these foci merged to form larger destructive foci. In some places the necrotic cells were detached from the surface of the glass. The changes in the structure of the nuclei and cytoplasm of the RES cells corresponded to those described above (karyopycnosis, regrouping of chromatin, mixing of eosinophilic droplets in the cytoplasm, and so on). After incubation of the RES cultures with a mixture of various dilutions of virus and immune rabbit serum, no cytopathic changes were found.

Some investigations [1, 11] have yielded results showing the cytopathogenic action of the virus of tick-borne encephalitis on preliminarily trypsinized cell cultures obtained form the tissues of the pig embryo, and also on a transplantable cell line obtained from the kidney of the pig embryo. However, there are few reports in the literature of the fine cytological changes arising in the cell structures under the influence of the virus of tick-borne encephalitis. The number of investigations in which the cytopathogenic action of the virus has been studied on fixed and stained preparations of cell cultures is particularly small.

We studied the cytopathogenic action of the virus of tick-borne encephalitis (strain Ix-10) on a highly sensitive tissue system, cultures of line RES obtained from the kidney of the pig embryo. As stated earlier, this transplantable cell line is characterized by high cytological and karyological stability. In its karyological characteristics the RES line resembles cell strains of hypodiploid character (obtained from the kidneys of the pig embryo [2], but it differs from the latter in its higher percentage of cells possessing a diploid set of chromosomes. The karyological characteristics (closeness to the diploid level) distinguish the RES line sharply among the heteroploid cell strains used in virology. The properties of the RES line such as the epithelioid character of the cultures, the clarity of the cell borders, and the marked monomorphism enable even very slight changes developing in the cytoplasm and nuclei of the cells to be detected early.

Infection of the RES cultures by different concentrations of virus of tick-borne encephalitis led after 30-38 h to the development of typical cytopathogenic phenomena in many cells, and sometimes in most. Under these circumstances the structures of the cytoplasm and of the nucleus were affected approximately to the same degree and almost at the same time. The ordinary method of staining with hemotoxylin and eosin is perfectly adequate for diagnosis of the cytopathic action of the virus of tick-born encephalitis. In some cases the use of the Feulgen method may speed up the detection of the cell damage caused by the virus, but the use of this method is not obligatory. Of course the specificity of the cytopathogenic action of the virus could not be determined without a suitable control (the action of a mixture of virus and immune serum on the culture). The cytopathic changes which we observed in the RES cells were essentially indistinguishable from those described earlier [7], but they were more massive and they affected a higher percentage of cells than in primary cultures of pig's kidneys.

The fact that the tissue system used in these experiments is a transplantable cell line, together with the cytological and karyological stability of the RES cultures and their high sensitivity to the virus of tick-borne encephalitis, should make possible the wide use of the RES line in diagnostic work.

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