A STRAIN OF HAMSTER EMBRYONIC FIBROBLASTS TRANSFORMED BY ROUS VIRUS IN VITRO

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The properties of Cricetulus migratorius, obtained by transformation of the fibroblasts by Rous virus, were investigated. The transformed cells, adapted for growth in tissue culture, were highly oncogenic for hamsters but not for fowls, because they did not contain infective virus.

The action of oncogenic viruses on primary trypsinized cultures of cells from the Armenian hamster (Cricetulus migratorius) has received little study. In particular, there is no information in the literature on interaction between Rous virus and cells of the Armenian hamster in vitro, on the possibility of their transformation by this virus, or on prolonged cultivation of the resulting cells.

The object of the present investigation was to study the properties of transplantable cell line KhÉT-ASa obtained by malignant transformation of normal hamster tissue by Rous virus (Carr–Zil'ber strain) in vitro.

EXPERIMENTAL METHOD

A culture of normal Armenian hamster fibroblasts obtained by trypsinization from the skin and muscle tissues of embryos was grown in Povitskaya flasks on medium No. 199 with 10% inactivated bovine serum until a well-developed monolayer was obtained. After the first passage, hamster fibroblasts were mixed with tumor cells obtained from a fowl sarcoma (Carr–Zil'ber strain) by trypsinization, in the ratio of 2:1 and cultivated on a growth medium consisting of medium No. 199 (2 parts), lactalbumin hydrolysate in Hanks's solution (1 part), and 20% inactivated bovine serum. Cells were removed from the glass mechanically without the use of versene at weekly intervals during the first month of growth, and every 4 days thereafter.

The transplantable line KhÉT-ASa was formed after the lag phase, which lasted about 2 months from the beginning of transformation (approximately from the 25th passage). After this time, subcultures were obtained by means of a suspension containing 150,000 cells/ml, whereas in the first passages the suspension contained 150,000 cells/ml.

The oncogenicity of the culture was tested by subcutaneous injection of the cells into 2-week-old and adult hamsters. Tumors induced by cells of line KhÉT-ASa in the hamsters, when cultivated in vitro, became the sources of new cell lines produced without a lag-phase and morphologically identical with the original culture.

Infective virus was detected by a biological test, by inoculation of the culture fluid and cells into fowls.

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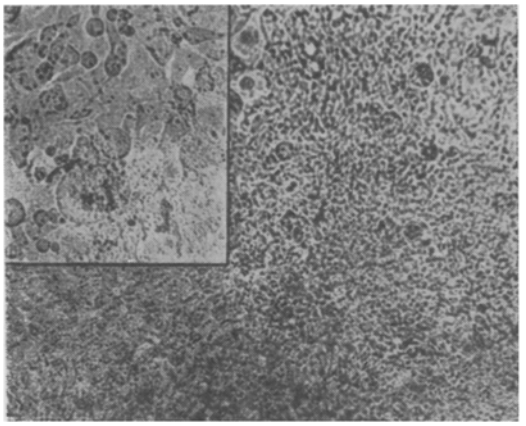

Fig. 1

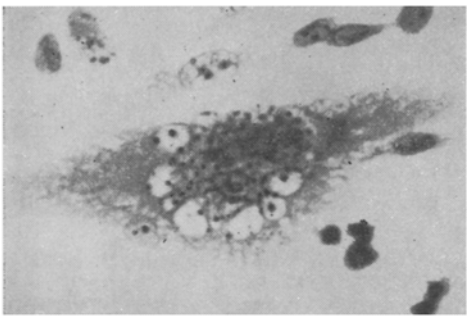

Fig. 2

Fig. 1. Focus of stratified growth of transformed cells of line KhÉT-ASa (112×): a) large granular cells (250×). Native unstained preparation.

Fig. 2. Giant multinuclear syncytial cell. Azure-eosin, 500×.

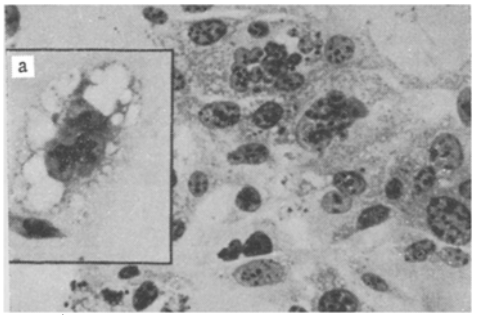

Fig. 3. Culture of KhÉT-ASa cells (30th passage): hyperplasia of nuclei (250×). a) Vacuolated multinuclear cell (500×). Azure-eosin.

For the cytologic study, 120,000 cells were grown in penicillin flasks on growth medium. The cover slips with growing cultures were fixed after 24 and 48 h in Shabadash's neutral fixing fluid or Nikiforov's mixture. The specimens were stained with azure-eosin (by the Romanowsky–Giemsa method) and with hematoxylin-eosin. The mitotic index was expressed in pro mille [3]. The generation time was determined by the method of Epifanova et al. [2]:

$$TM = (MI \times A)/MI_{cloch},$$

where TM is the duration of mitosis; MI the mitotic index (in $^0/_{00}$); MI_{colch} the mitotic index of the same culture after the addition of colchicine; A the duration of action of colchicine. The specimens were incubated with colchicine in a final concentration of 0.5 μg for 1.5 h. The generation time was calculated by the formula

$$T = TM/MI,$$

where T is the duration of the mitotic cycle, i.e., the generation time.

The dynamics of division of the KhÉT-ASa cells was determined by counting the number of living cells daily for 7 days in individual tubes which had been seeded simultaneously with 150,000 cells.

EXPERIMENTAL RESULTS

After about 25-30 days of growth foci of stratified growth morphologically similar with those in cultures of chick fibroblasts infected with Rous virus appeared in the mixed culture [6]. During combined cultivation of normal and tumor cells (the most effective method of infection [4, 5]) repeated infection of the normal cells with virus liberated during destruction of the tumor cells took place.

Three types of cells were found in the stained preparations: round basophilic cells growing in foci; diffusely growing fibroblast-like cells, and giant multinuclear cells. Strain KhÉT-ASa was distinguished by its marked polymorphism, and at different stages cells first of one type, then of another, were predominant (Fig. 1). At all periods of growth the culture was characterized by the presence of giant, vacuolated multinuclear (30 nuclei) cells with nuclei of different sizes, or of mononuclear cells with a large, lobular nucleus (Fig. 2). Most cells showed a marked degree of vacuolation and hyperplasia of the nucleoli (Fig. 3). With the appearance of foci of transformation the medium quickly began to turn acid, and the rate of growth of the cells was increased by 2-3 times over the control.

After passage of normal hamster fibroblasts for 1 month they began to degenerate. Despite repeated attempts, they could not be cultivated for a longer time, whereas the transformed culture has already gone through more than 150 passages (in about 2 years).

A feature distinguishing line KhÉT-ASa, like line CA-SV-40-63-1 described by Blyumkin et al. [1], is the rapid transition from vigorous growth to "old-age" destruction, on account of which frequent passages (at least once every 3-4 days) and daily replacement of the medium were necessary. The dynamics of propagation of these cultures showed a period of rapid growth from the 1st day after attachment of the cells to the glass, with an increase in their number by 4, 6, 8, or even 10 times during approximately the first 4-5 days of growth, followed by a period of degeneration and destruction of the cells. Line KhÉT-ASa possessed a high mitotic index (about 30 $^0/_{00}$), whereas the mitotic index of the control cultures averaged 8 $^0/_{00}$. The duration of mitosis in the transformed culture was 40 min, while the duration of its mitotic cycle (the generation time) was 27 ± 2 h.

Injection of cells of the mixed culture after the 5th-6th passage into homologous animals did not induce the development of tumors. Starting with the 12th passage, however, high oncogenic activity of the cells was observed and their injection in a dose of $2.5 \cdot 10^6$ into hamsters now induced tumors consisting morphologically of spindle-cell sarcomas after 6 days. Neither living nor destroyed KhÉT-ASa cells induced tumors in chickens. Although infective virus was present in the culture fluid during the first passages, starting with the 4th-5th passage it could no longer be detected. Line KhÉT-ASa, although it has still maintained the same intensity of growth, has now modified slightly its oncogenic properties. Tumors appearing in hamsters after injection of the same or even larger doses of cells ($10 \cdot 10^6$) are mainly absorbed. Because of this, it is interesting to note the appearance of plaques, which are areas of total lysis of the cells with clearly defined edges, varying in size, in the growing monolayer of KhÉT-ASa cells at about the same time (starting with the 64th passage). These plaques, which appeared and disappeared in successive passages, did not, however, lead to destruction of the cells outside the plaques, and they resembled those formed by infective viruses in sensitive tissues.

It can be postulated that during cultivation in vitro activation of a certain virus, possibly a "helper" virus, takes place, as a result of which lysis of only certain cells of the culture (possibly the giant cells mentioned above) occurs. The number of these cells as a proportion to the total number of cells seeded in that passage (only one-hundredth of all the cells taken from the flask were seeded) determines the irregularity of appearance of plaques from one passage to the next. It is intended to study the presence and formation of cells of this type in cultivation by a microfilming method. The cytologic properties of strain KhÉT-ASa described above – its cellular polymorphism, its tendency toward syncytium-formation, and the presence of giant multinuclear cells, as well as the oncogenicity of the cells for homologous animals – are the result of malignant transformation of normal fibroblasts in vitro under the influence of Rous virus and they are, in the main, analogous to those found during malignant transformation in vivo.

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