CHANGES IN ROUS VIRUS AND POLYOMA VIRUS DURING THEIR SYNTHESIS IN CELLS OF UNNATURAL HOSTS

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A line of human embryonic fibroblasts, transformed by Rous sarcoma virus (Schmidt–Ruppin strain) contained and produced Rous virus, as was shown by the complement fixation and immunofluorescent tests, by electron-microscopic investigation, and by the presence of an isotope peak in a sucrose gradient. By its biological properties the synthesized virus differed from the original Schmidt–Ruppin strain for, in particular, the range of cells sensitive to the virus and its protein membrane were changed. Similar data indicating a change in the biological properties of a virus produced in the tissue of an unnatural host also were obtained for polyoma virus, synthesized in human embryonic fibroblasts transformed by it.

KEY WORDS: human embryonic tissue; Rous fowl sarcoma virus; transformation; polyoma virus.

The problem of overcoming intraspecific virus specificity and the possibility of synthesizing oncogenic viruses of animals in human embryonic tissue are among the urgent problems in present-day virology. In this connection another interesting problem is that of variation of oncogenic viruses in cells of an unnatural host, man in particular, and the acquisition of pathogenicity for the new host by such viruses. Data in the literature on variation of oncogenic viruses synthesized in heterologous cells are contradictory [6, 7, 9]. In the writers' laboratory the possibility of transformation of human embryonic tissue, transformed by Rous fowl sarcoma virus (line 23) and polyoma virus (line P-2) were obtained [4].

The object of the present investigation was to study the morphological and karyologic characteristics of cells of lines 23 and P-2.

EXPERIMENTAL METHOD

Cell cultures were grown on Eagle's medium with 20% bovine serum in Carrell flasks and in Leighton tubes with coverslips.

The immunofluorescence investigation of cell line 23 was carried out by the direct Coons' method [8] using chicken hyperimmune serum against Rous virus. Cell line P-2 was investigated by the indirect method, using immune rabbit antipolyoma serum [11]. The complement fixation test was carried out by the method of Nartsissov et al. [2] with hamster serum from animals with tumors. The electron-microscopic investigation was carried out by A. F. Bykovskii by the method described previously [1].

EXPERIMENTSL RESULTS

Karyologic analysis of line 23 showed the presence of the human set of chromosomes. The mixed hemadsorption test confirmed that cells of line 23 are of the human type.

Cells of line 23 had all signs of transformed cells which had undergone malignant change: epithelioid shape of the cells, stratified, irregular growth, ability to grow in semiliquid nutrient agar, loss of contact inhibition, ability to induce tumors when injected into the brain and into the retrobuccal pouch of golden hamsters [4].

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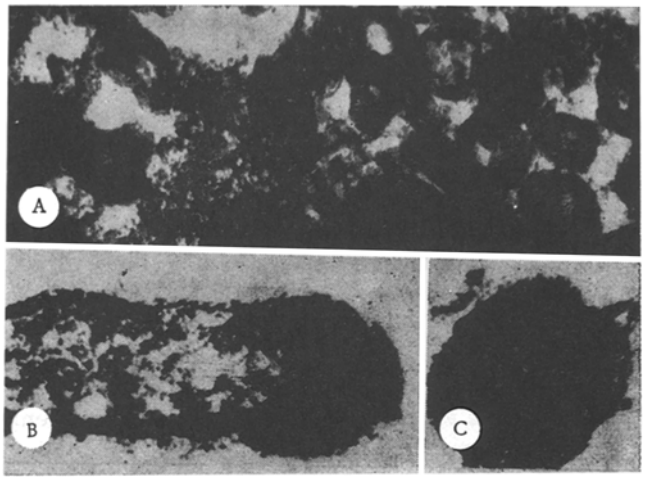

Fig. 1. C-particles of Rous virus in line 23 cells. A) Extracellular particles of C-type (120,000 ×); B) particles budding on cell surface; C) mature virus (380,000 ×).

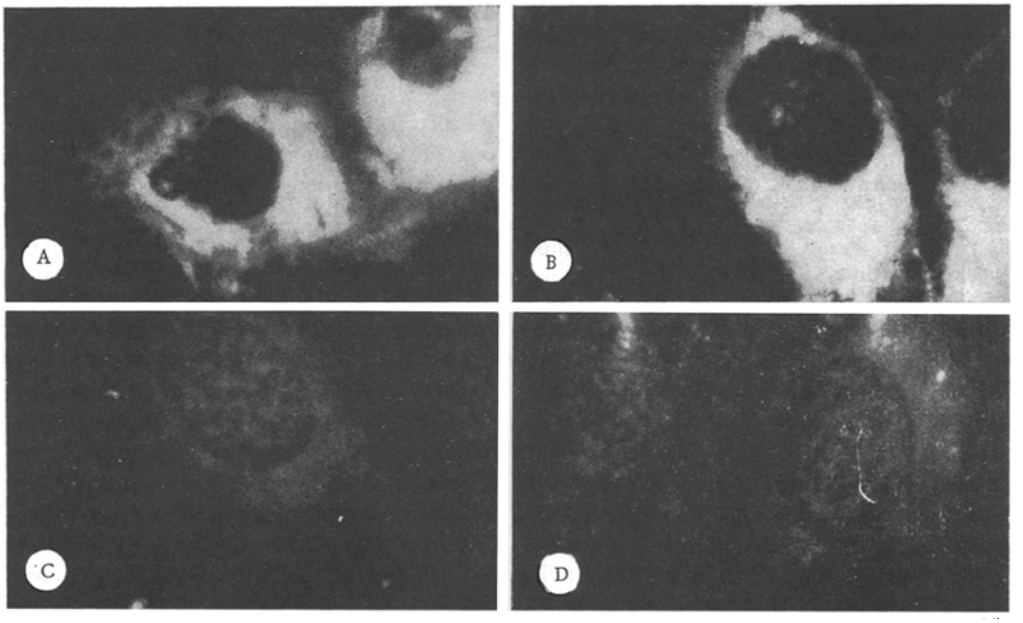

Fig. 2. Detection of antigens of Rous and polyoma viruses in line 23 and P-2 cells by immunofluorescence method (350 ×). A) Specific fluorescence of line 23 cells with immune serum against Rous virus; B) specific fluorescence of line P-2 cells with immune serum against polyoma virus; C) normal human fibroblasts after treatment with immune serum against Rous virus; D) normal human fibroblasts after treatment with immune serum against polyoma virus.

Electron-microscopic investigation of the line 23 cells showed C particles characteristic of Rous virus, located in the cytoplasm, budding on the cell membrane, and situated extracellularly (Fig. 1).

The immunologic study of the line 23 cells showed that antigens of Rous virus were detected in the complement fixation test with the sera of hamsters with tumors and also by the immunofluorescence test with hyperimmune antiserum against purified preparations of Rous virus in the cytoplasm of 96% of cells (Fig. 2A). In the control (human embryonic tissue and also cells of line P-2) fluorescence of not more than 2-3% of cells was obtained (Fig. 2C).

Cultures of line 23 cells produced virus which, labeled with [^{3}H]-uridine, was detected in a sucrose gradient within the zone 1.16-1.17 g/cm^3.

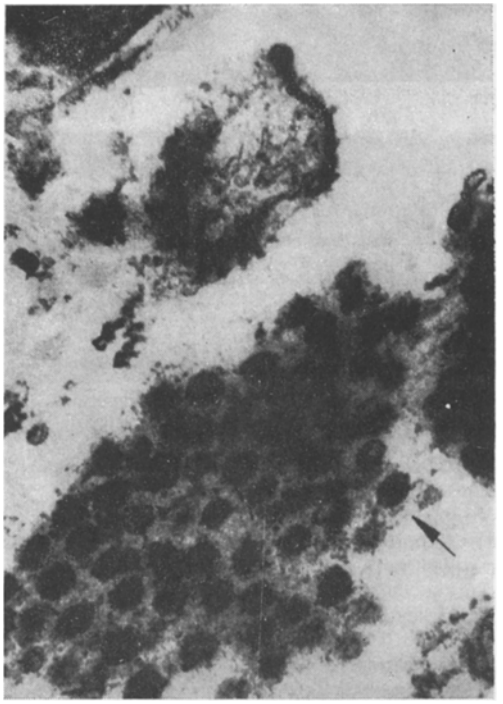

Fig. 3. Concentration of polyoma-like particles in cells of line P-2 (120,000 ×).

A study of the biological properties of Rous virus synthesized in line 23 cells showed that it differs from the original Schmidt–Ruppin strain. In particular, the range of cells susceptible to it was altered: When unconcentrated and filtered through a millipore filter (45 nm) the virus remained capable of transforming chick embryonic tissue in vitro and, in addition, it still induced epithelioid transformation without additional treatment with Sendai virus in human, mouse, rat, and hamster embryonic tissues. In the same tissues it formed foci of transformation beneath the agar. Chicken immune serum against the original Rous virus (Schmidt–Ruppin strain, neutralization index 10^6 when titrated in chicks) did not neutralize the virus synthesized in line 23 cells in experiments in vitro on human embryonic tissue.

It is interesting to note that the ability of the line 23 cells to induce tumors in chickens became increasingly repressed in the course of cultivation. This was shown by the following fact: In experiments carried out 10 months after line 23 cells were obtained, the minimal number of cells required to induce tumors in chickens was $2 \cdot 10^6$ per point of injection, and after 5 years the number was 100 times greater, namely $2 \cdot 10^8$ cells.

Cells of line P-2 (human embryonic fibroblasts, transformed by polyoma virus with the aid of Sendai virus) were also of the human type and had all the properties of transformed cells which had undergone malignant change [4].

Electron-microscopic investigation of the cells of line P-2 revealed concentrations of crystal-like structures in the cytoplasm, consisting of particles morphologically similar to particles of polyoma virus (Fig. 3).

In the immunofluorescence study antigens of polyoma virus were found in the cytoplasm of more than 98% of line P-2 cells (Fig. 2B). In the controls, consisting of human embryonic cells and line 23 cells, 1-2% of cells gave fluorescence (Fig. 2D). T antigen also was found in the line P-2 cells. To detect it by the immunofluorescence test, the sera of hamsters with tumors induced by polyoma virus, not containing virus-neutralizing antibodies, were used. T antigen was found in the cytoplasm of more than 90% of cells.

The concentrated liquid phase of the line P-2 cells caused agglutination of guinea pig erythrocytes in the cold to a dilution of 1 : 256. This reaction was abolished by antiserum against polyoma virus. Cells and the liquid phase of line P-2 cells gave a positive plaque assay test specific for polyoma virus. The liquid phase induced a powerful cytopathic effect on mouse embryonic tissue and also caused transformation of mouse and rat embryonic tissues. The ability to produce a cytopathic action and transformation of mammalian tissues persisted after filtration of the liquid phase of the P-2 cells through millipore filters with a pore size of 45 nm.

It thus follows from these experiments that mature infectious viruses are synthesized in cells of an unnatural host (man), transformed by heterologous viruses (lines 23 and P-2). However, compared with the original strains of Rous and polyoma virus, the properties of these viruses were changed: They showed increased affinity for mammalian tissues and changes in the protein membranes of the viruses. It is interesting to note that polyoma virus caused both productive (synthesis and liberation of virus particles) and integrative (conversion of cells from normal to malignant) forms of infection when acting on human embryonic cells.

In recent years several workers have shown that the infectivity of an oncovirus depends on the degree of homology of its genome with the nucleic acid of the host cell and also that genetic recombination of the oncovirus with the cell DNA is possible [10]. Accordingly it can be suggested that the genomes of Rous and polyoma viruses, as a result of prolonged replication in cells of the new host (man), have incorporated part of his genetic material, thus leading to their greater homology with the new host, as a result of which their affinity for human cells was increased and their protein membrane modified.

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ROLE OF THE LIVER IN DEVELOPMENT OF DYSHORMONAL DISEASES OF THE MAMMARY GLAND IN RATS

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Intermittent administration of CCl_4 combined with permanent illumination led to a decrease in the frequency of mastopathy and mammary gland tumors in sexually immature female rats and lengthened the period of their development. It is suggested that during regression of cirrhosis the liver may lose its ability to activate estrogens.

KEY WORDS: estrogens; cirrhosis of the liver; mastopathy.

According to data in the literature, prolonged and intermittent administration of CCl_4 to mice and rats causes cirrhosis of the liver, which is accompanied by hyperestrogenization of the animal, leading to the subsequent development of mastopathy and tumors of the mammary gland [1, 2, 4, 8]. It has also been shown that after exposure to CCl_4 ends, the structure and function of the damaged liver are restored [3, 7, 9].

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