VIROLOGICAL STUDY OF EMBRYONIC CELLS OF GUINEA PIGS AND GOLDEN HAMSTERS INFECTED WITH ROUS VIRUS (CARR-ZIL'BER AND SCHMIDT-RUPIN STRAINS)

D. M. Martirosyan and V. Ya. Shevlyagin

UDC 576.858.63

After introduction of Rous fowl sarcoma virus into a culture of embryonic tissue of hamsters and guinea pigs, only the initial stage of virus synthesis (the provirus stage) is observed. The virus is not synthesized in its infective form and can be isolated from the medium and cells only during the first two days after infection.

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After the discovery of the pathogenicity and oncogenicity of Rous virus for rats and rabbits [1,3] the relationship between this virus and the tissues of various mammals was studied by many investigators [5-8]. However, even now the possibilities and essential conditions for synthesis of mature virus in mammalian tissues, and also the forms in which virus is present in them, are not absolutely clear.

It has been reported that complete synthesis of virus takes place in mouse embryonic tissue infected with Rous virus in vitro, and that small numbers of virus particles are excreted into the surrounding medium [3].

In the present investigation the fate of Rous virus was studied in embryonic tissue of hamsters and guinea pigs in vitro.

EXPERIMENTAL METHOD

Experiments in vitro were performed on 30-day guinea pig embryos and 2-week golden hamster embryos. In parallel series, two strains of Rous virus were used: Carr-Zil'ber and Schmidt-Rupin strains. Cell-free extracts from these tumors were used as virus material for infecting the cells. The cells were infected in suspension for 16-18 h at 5-10° on a magnetic mixer (10-15 million cells were infected with 15 ml tumor extract) and in a monolayer at 37° for 1 h.

Infective virus was determined in the cells and medium by titration in chicks aged 2-3 days. Virus antigen in the cells was determined by the direct Coons' method using chick antisera, labeled with fluorescein isothiocyanate, against Rous virus. The γ-globulin fraction of the sera, obtained by electrophoresis in agar, was exhausted with powdered rabbit's liver and also with normal fibroblasts of the corresponding animal.

A more complete description of the experimental method will be found in our previous paper [2].

EXPERIMENTAL RESULTS

Embryonic Tissue of Guinea Pigs. Bright, diffuse fluorescence of the cytoplasm of most cells was found in the suspensions of cultures infected with Carr-Zil'ber and Schmidt-Rupin strains in the first 2-3 days after infection (Fig. 1, I). The fluorescence in the cells was much weaker by the 5th-6th days (II). By the 7th day, fluorescent cells with dark nuclei could be seen only occasionally. By the 14th-20th day, very

Department of Virology and Immunology of Tumors, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR L. A. Zil'ber). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 65, No. 4, pp. 73-76, April, 1968. Original article submitted October 10, 1966.

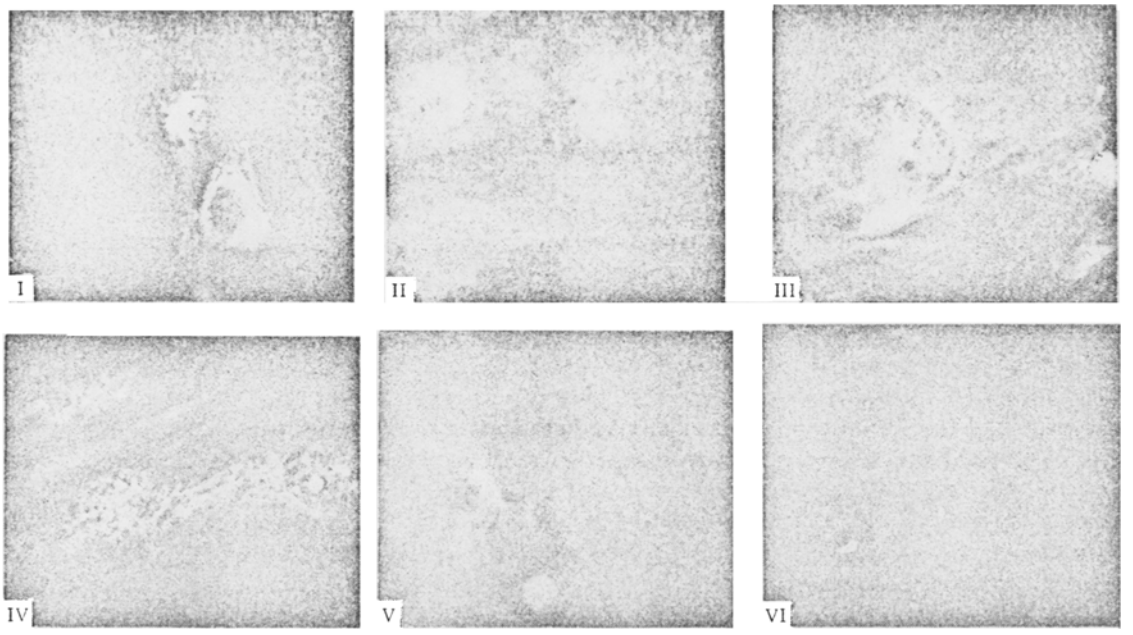

Fig. 1. Guinea pig embryonic tissue. I) In first two days after infection (bright fluorescence of cytoplasm); II) on 6th-10th day after infection (weak fluorescence of single cells); III) on 14th-20th day after infection (considerable enlargement of nuclei); IV) on 14th-20th day after infection (fluorescence in nuclei and nucleoli of single cells); V) guinea pig embryonic tissue treated with extract of methylcholanthrene tumor on 14th-20th day of growth (no fluorescence; control); VI) in first days after infection (blocking of fluorescence with unconjugated antiserum; control). 720×

few cells with fluorescent cytoplasm were present, but cells with greatly enlarged nuclei had appeared (III). A fluorescent chromatin network could be seen in the nuclei of solitary cells (IV). In uninfected tissue, and also in tissue treated with extract of a methylcholanthrene fowl tumor, no such changes were observed (V).

Preliminary treatment of the infected tissue with unconjugated antiserum (the reaction of blocking specific fluorescence) completely abolished the fluorescence (Fig. 1, VI).

The same stages of development (fluorescence of the cytoplasm during the first 2-3 days, appearance of enlarged nuclei in the cells, weakening of fluorescence) was observed in the cultures infected on a monolayer as in those infected in suspension, but many fewer fluorescent cells were found in the first 2-3 days, and enlargement of the nuclei was observed in the cultures only toward the 20th day.

Mature infective virus was found in the culture fluid and cells in both series of experiments only during the first two days after infection, and on subsequent days infective virus was never isolated.

The morphological changes observed in guinea pig cultures infected by both strains consisted essentially of the appearance of foci of multilayered growth in them on the 14th-16th day after infection. However, these cells were evidently not very viable and were readily detached from the glass, disappearing during subculture.

In cultures infected on a monolayer, no foci of multilayered growth were present, although solitary round granular cells also appeared.

Embryonic Tissue of Golden Hamsters. When embryonic hamster cells were infected in suspension with Schmidt-Rupin and Carr-Zil'ber strains, virus antigen was also found in the first 2-3 days after infection in the form of diffuse, bright fluorescence of the cytoplasm of most cells. On the following days (4th, 7th, and 10th) the number of fluorescent cells diminished considerably.

By the 14th-16th day, greatly enlarged nuclei appeared in the cells; in contrast to preceding and succeeding days, when the nuclei were completely dark and resting, at these times granules could be seen

in them. A delicate fluorescent network could be seen in the nuclei of occasional cells, but such cells were very few. Cells with a fluorescent nuclear membrane and a very few with fluorescent cytoplasm were also observed. In the control cultures no fluorescence could be seen in the cells. The nuclei were dark and were not enlarged.

On the subsequent days (25th-30th) cells with enlarged nuclei had almost disappeared; the cells of preparations at this period resembled the control cultures.

After infection on a monolayer the dynamics was the same, but far fewer fluorescent cells were present in the first days. Infective virus was isolated from the cells and culture fluid in both series of experiments only in the first two days after infection. No morphological changes were observed in infected cultures of hamster tissue.

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