AUTORADIOGRAPHIC STUDY OF THE EFFECT OF NEWCASTLE DISEASE VIRUS ON THE MITOTIC CYCLE OF CELLS

P. V. Glan

UDC 616.988.73-092.9-07:616-018.15-076.5

A primary culture of chick fibroblasts was infected with Newcastle disease virus. Starting from 7-8 h after infection, passage of the cells through the G_1 period and their transition into the S period was delayed. Entry of the cells into the S period ceased completely 20-24 h after infection. Passage through the S period and entry of the cells into the G_2 period was not significantly changed during the 20-24 h after infection of the culture.

* * *

Viruses usually inhibit cell division, but sometimes produce little change in mitotic activity and in certain cases they actually stimulate cell multiplication [2, 9]. Few investigations have yet been made of the effect of virus infection on the mitotic cycle, and these have dealt with the oncogenic viruses [4, 7].

The object of this investigation was to study changes in the mitotic cycle of a primary culture of chick fibroblasts after infection with Newcastle disease virus (NDV).

The method of labeled mitoses [10] could not be used in this case because the mitotic activity of chick fibroblasts does not exceed 6-8%, and 6-8 h after infection with virus it falls to 1%. We therefore attempted to study changes in the mitotic cycle indirectly, by using, first, the dynamics of changes in the index of labeled cells and second, changes in the mean intensity of label by counting silver granules above the nucleus.

EXPERIMENTAL METHOD

A primary culture of chick fibroblasts was infected with NDV (Kuz'minki strain) in a dose of 40 PFU per cell. An autoradiographic method with H^3-thymidine (0.5 μCI/ml) was used in the investigation. Two variants of the experiments were carried out: with continuous incubation of cells in medium containing H^3-thymidine, and with periodic addition of the cells to medium containing the isotope for short periods (20 min). The material was fixed in a 3:1 mixture of alcohol and acetic acid, after which the preparations were coated with type R liquid photographic emulsion and exposed in darkness for 1-2 days, and then developed and stained with hematoxylin. The index of labeled cells (in promille) was determined in the finished preparations. In addition, silver grains above the nucleus were counted and the mean intensity of label counted.

EXPERIMENTAL RESULTS

Changes in the index of labeled cells and in the mean number of granules above the nucleus in the infected culture were studied at various times after infection.

Dynamics of Changes in Number of Labeled Cells during Continuous Incubation with H^3-Thymidine. Virus was adsorbed on the cells for 1 h at 37°, after which the cells were placed for 24 h in medium containing H^3-thymidine, to compare the dynamics of accumulation of labeled cells in the experimental and control series (Fig. 1). The control curve shows that the number of labeled cells rose continuously for 24 h after addition of H^3-thymidine to reach 65% after which it remained constant because the reserve of cells capable of synthesizing DNA was exhausted. The proliferative pool of the primary culture of chick fibroblasts is thus 65%. The initial part of the experimental curve (0-6 h after addition of H^3-thymidine) coincided with the part of the control curve: probably during this period (7 h after infection) the virus had not yet produced appreciable changes in the speed with which the cells passed from the presynthetic G_1 period

Laboratory of Cytopathology, Moscow Research Institute of Virus Preparations (Presented by Active Member of the Academy of Medical Sciences of the USSR A. P. Avtsyn). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 66, No. 8, pp. 105-108, August, 1968. Original article submitted August 10, 1967.

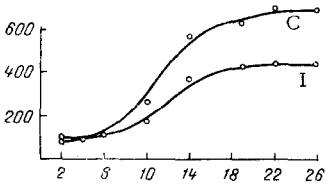

Fig. 1

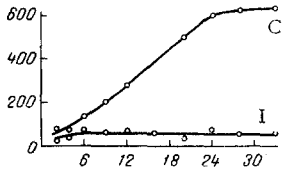

Fig. 2

Fig. 1. Changes in index of labeled cells during continuous incubation in medium with H^3-thymidine (1-24 h after infection). Abscissa, time after addition of H^3-thymidine (in hours); ordinate, index of labeled cells (in %); C, control culture; I, infected culture.

Fig. 2. Changes in index of labeled cells during continuous incubation in medium with H^3-thymidine (24-58 h after infection). Legend as in Fig. 1.

TABLE 1. Effect of Infection on Rate of Entry of Cells into S Period

	Index of labeled cells (in %) during continuous incubation in medium with H^3-thymidine		Rate of entry of cells into S period (in % per hour)
	13 h after infection	17 h after infection	
Control	25.9	56.0	4.1
Experiment	17.3	38.0	2.8

TABLE 2. Mean Number of Grains Above Nucleus in Cultures during Short Incubation in Medium with H^3-Thymidine

Time after infection (in hours)	Mean number of silver grains above nucleus	
	control culture	infected culture
8	40	39
12	40	31
16	38	40
20	40	40
24	40	39

into the S period of synthesis. From 8 h after the time of infection (7 h after addition of H^3-thymidine) the experimental curve began to lag behind the control, and by 24 h after infection the number of labeled cells did not exceed 40%. This was evidently because of delay in passage of the cells through the presynthetic period, because during the 18-20 h (duration of the G_1 period, according to our data) after initial addition of the isotope, accumulation of labeled cells took place mainly on account of transition of cells from the G_1 period. If the rate of entry of the cells into the S phase is expressed in percentages per hour [5], it is clear that this rate was much lower in the experimental series (Table 1).

In another experiment cells were infected with virus just as in the first case, but H^3-thymidine was added to the growth medium for 36 h starting 24 h after infection (Fig. 2). The curve reflecting accumulation of labeled cells in the control culture appeared just like the control curve in Fig. 1. The experimental curve was parallel to the abscissa: entry of the cells into the S period evidently ceased completely 24 h after infection.

Dynamics of Changes in Number of Labeled Cells during Successive Short Periods of Incubation of Cells with H^3-Thymidine. Every 4 h for 48 h after infection the cells of the control and infected cultures were placed for 20 min in medium containing labeled thymidine, after which they were immediately fixed. The number of cells in the S period in the control was approximately constant, while in the experiment it decreased starting from 7 h after infection (Fig. 3). We consider that these results confirm the view that cells are retarded as they pass through the G_1 period, and in addition they provide evidence against a block during transition of the cells from the S into the G_2 period: if such a block were present the number of labeled cells in the experiment would be greater than in the control, or if the cells were retarded during the transition from G_1 into S, the number of labeled cells would be more or less constant. In addition, after counting the grains of silver in this particular experiment we found no changes in the mean intensity of label until 20-24 h after infection by comparison with the control (Table 2). This also indicates that the rate of passage of the cells through the synthetic period remained unchanged [1].

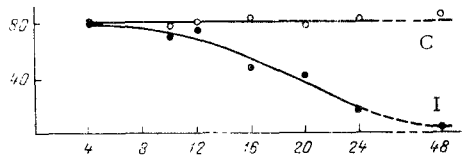

Fig. 3. Changes in index of labeled cells during successive short incubations in medium with H^3-thymidine (1-48 h after infection). Abscissa, time after infection. Remainder of legend as in Fig. 1.

Hence, 7 h after infection of the culture of chick fibroblasts with NDV transition of the cells from the presynthetic into the synthetic period is delayed, and it ceases altogether 20-24 h after infection. The rate of passage of the cells through the period of DNA synthesis is evidently not significantly altered (at least for 15-20 h after infection). Our preliminary results show that under the influence of virus infection the cells are delayed also in the postsynthetic period. However, this requires a closer study. We know that NDV inhibits the synthesis of cell protein [3, 8]. Delay in passage of the cells through the G_1 period under the influence of infection revealed by the present experiments is evidently associated with disturbance of the synthesis of these cells proteins, taking place mainly during the pre- and postsynthetic periods.

LITERATURE CITED

1. L. V. Erofeeva and Yu. M. Vasil'ev, Tsitologiya, No. 4, 549 (1966).
2. S.Ya. Zalkind, Arkh. Anat., Gistol. Embriol., No. 4, 95 (1964).
3. M. K. Kukhanova and N. V. Kaverin, Dokl. Akad. Nauk SSSR, 164, No. 6, 1417 (1965).
4. G. F. Makarova, Tsitologiya, No. 5, 577 (1967).
5. V. V. Terskikh, Dokl. Akad. Nauk SSSR, 163, No. 1, 217 (1965).
6. R. G. Tsanev and G. G. Markov, Biochemistry of Cell Division [in Russian], Moscow (1964), p. 56.
7. C. Basilico and G. Marin, Virology, 28, 429 (1966).
8. D. P. Bolognesi and D. E. Wilson, J. Bact., 91, 1896 (1966).
9. K. O. Habermehl and W. Diefenthal, Zbl. Bakt., I Abt. Orig., 199, 273 (1966).
10. H. Quastler and F. G. Sherman, Exp. Cell Res., 17, 420 (1959).