Thymidine Kinase Activity in Human Amnion Cell Cultures Infected with Shope Fibroma Virus*

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One of the ‘early’ effects of cellular infection by DNA-containing viruses is an increase of various enzymic activities concerned in DNA synthesis, which in most instances seems dependent upon the synthesis of virus-coded enzymes (Keir, 1968).

As far as poxviruses are concerned, relevant information relates almost exclusively to the vaccinia subgroup and very little is known about other poxviruses (Joklik, 1966).

The Shope fibroma virus is an oncogenic pox virus of the myxoma subgroup (Joklik, 1966) and has only recently been subjected to systematic study in tissue cultures. It has been shown to replicate in primary cell cultures of human amnion (Hodes et al. 1966; La Placa et al. 1967; Hodes & Chang, 1968) where it causes a peculiar cytopathic effect characterized by the appearance of pock-like lesions (La Placa et al. 1967). The time-course of nucleic acid synthesis (Ewton & Hodes, 1967) and the induction of DNA nucleotidyltransferase (Chang & Hodes, 1967) in cells infected with Shope fibroma virus have also been reported. A study of thymidine kinase activity in human amnion cell cultures infected with Shope fibroma virus is reported in this paper.

Human amnion cell cultures in tubes and Kolle's bottles were prepared as previously described (La Placa, 1966). The cultures were infected with a dose of Shope fibroma virus, BOERLAGE strain, calculated so as to cause the formation of virus inclusions only in 1% to 1.5% of the cells by 48 hr after infection. The relatively small virus inoculum was chosen with the aim of obtaining information not only on the possible increase of thymidine kinase activity in cells infected with Shope fibroma virus but also on the time-course of thymidine kinase activity in relation to the spread of the infection among the cellular population.

The virus inoculum was in a volume of 2 ml. for the human amnion cell cultures in bottles and of 0.2 ml. for cultures in tubes. After adsorption for 2 hr at 36° the inoculum was removed and the cultures re-fed with maintenance medium (Eagle's basal medium without serum) and incubated at 36°.

At the times indicated in Fig. 1, two infected and two uninfected control culture bottles were processed for thymidine kinase assay and permanent preparations stained with haematoxylin and eosin were made from two infected cell culture tubes (Enders & Peebles, 1954) for counting virus inclusions/1000 cells.

Two bottle-cultures were infected with vaccinia virus (strain derived from the vaccine produced by the Istituto Sieroterapico Toscano, Siena, Italy) at a multiplicity of infection of 0.01 TCD 50/cell and harvested 24 hr after infection to provide positive control samples for use in each thymidine kinase assay. Values of thymidine kinase activity ranging from 525 to 547 μmoles of thymidine phosphorylated/200 μg. of proteins/15 min. were observed in cell cultures infected with vaccinia virus.

Thymidine kinase assay was performed according to McAuslan (1963). The cells

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were harvested and washed in 0.05 M-tris+HCl buffer, pH 7.8, containing 0.002 M 2-mercaptoethanol and resuspended in 1 ml. of the same buffer. They were then disrupted by five consecutive cycles of freezing and thawing and the resulting suspension was centrifuged at 100,000 g for 30 min. The sediment was discarded and the protein content of the supernatant fluid was determined by the method of Gornall, Bardawill & David (1949). A sample containing 200 μg. of protein was used for the enzyme assay, details of which are given in the legend to Fig. 1. After 15 min. at 37°C the reaction was stopped by immersing the tubes in a boiling-water bath for 3 min. The samples were then centrifuged, and 25 μl. portions of the supernatant were applied to DEAE-cellulose paper discs (Whatman DE-81), which were washed as described by Bresnick & Karjala (1964). The discs were placed in counting vials containing 10 ml. of scintillation fluid [0.5% 2,5-diphenyloxazole and 0.01% 1,4-bis-2-(5-phenyloxazolyl)-benzene in toluene] and counted in a Nuclear Chicago Mark I spectrometer.

![Graph](image-url)
with a counting efficiency of about 80%. Values of samples boiled immediately after the addition of thymidine (zero time) were subtracted.

The data pertaining to thymidine kinase activity in cell cultures uninfected and infected with Shope fibroma virus are shown in Fig. 1 together with the number of virus inclusions/1000 cells and the number of new inclusions at each time tested.

The small virus inoculum used in our experiments enabled us to follow the time-course of thymidine kinase activity in the infected cell cultures, up to 10 days after infection in the presence of only slight destructive cytopathic changes. Indeed, destructive cytopathic changes in human amnion cell cultures infected with Shope fibroma virus were delayed and progressed slowly in comparison with the cell cultures infected with vaccinia virus where, at the time of harvesting (24 hr after infection), the cytopathic changes involved more than 75% of the cell population.

The shape of the curve of thymidine kinase activity, although somewhat influenced by the time intervals selected, clearly demonstrates that, in cells infected with Shope fibroma virus, the enzyme reached levels significantly higher than those observed in uninfected controls. Moreover, it also indicates (1) that the rise in thymidine kinase activity was not maintained but was followed by a decrease (switch off) of the enzyme activity, and (2) that the peaks of thymidine kinase activity were coincidental with the peaks of new inclusions observed at each time. During the entire observation period, three peaks of thymidine kinase activity were observed.

From these data it seems that the replication cycle of the BOERLAGE strain of Shope fibroma virus in human amnion cell cultures is rather prolonged when compared with what is known about the replication cycle of the vaccinia subgroup of poxviruses (Joklik, 1966). A longer replication cycle of Shope fibroma virus in comparison with vaccinia virus has been observed also by Chang & Hodes (1967).

However, the differences observed in our experiments between the absolute values of thymidine kinase levels obtained in cells infected with vaccinia virus and Shope fibroma virus respectively correlate well with the different rate observed in the progression of the respective virus-specific cytopathic changes.

In conclusion, in cells infected with Shope fibroma virus there is an increase in thymidine kinase activity coincidental with the highest points of new inclusion production, followed by a decrease of enzymic activity toward normal levels.

The coincidence between the fluctuations of enzymic activity and those of the number of new virus inclusions seems to suggest the dependence of the higher thymidine kinase activity of the cells infected with Shope fibroma virus upon the synthesis of a virus-induced enzyme.

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REFERENCES


Short communications


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