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Preliminary Report

Effects of Cytoplasmic DNA Synthesis upon Nuclear DNA Synthesis in Poxvirus-infected Cells

Several studies (Kato *et al.*, 1960a, 1960b; Magee *et al.*, 1960; Cairns 1960) have been reported independently on cytoplasmic DNA synthesis in poxvirus infection using autoradiographic techniques with H^3-thymidine. Kato *et al.* (1960a, 1960b, 1961, 1962) have emphasized that the site of incorporation of H^3-thymidine in the cytoplasm of cells infected with poxvirus corresponds exclusively to the "B" type inclusions which are common to all kinds of poxvirus infection. Furthermore, they conclude that 1) host nuclear DNA is not transferred to cytoplasmic DNA, 2) the host nucleus is not the site of viral DNA synthesis, 3) cytoplasmic DNA synthesis occurs independently of host nuclear DNA synthesis, 4) host nuclear DNA synthesis is suppressed after virus infection. This paper deals with further investigations on the last point.

Quantitative studies were carried out by silver grain counts on autoradiographs. Cowpox virus (LB red strain) was used. Cowpox virus was chosen for the study since; 1) On multiplication it shows distinct two types of inclusion formation which gives the basic model of the development of poxvirus inclusions (Kato *et al.*, 1959a, 1959b). 2) Cowpox virus, as a rule, has little ability to form giant cells, whose formation complicates determination of the exact number of inclusion bearing cells and studies on the nucleo-cytoplasmic relationship at the single cell level. 3) Although vaccinia virus is the most common poxvirus used in laboratory study, the definition and origin of most vaccinia viruses are still obscure, as Downie (1959) pointed out. He mentioned that "While certain strains are alleged to have been derived from smallpox, it seems to the writer that the properties and the characters of most strains now in use suggest that they have been derived from cowpox virus, *i.e.*, the virus responsible for the pox disease of cows." Thus vaccinia virus does not denote a definite virus. We think we should not use undefined term "vaccinia virus" especially in morphological and biochemical study.

A low inoculum of virus (10^4 - 10^5 PFU per ml) was put into a Leighton tube containing about 3×10^5 cells (the FL strain of human amnion cells). Twenty four hours after infection, there were every stage of inclusion bearing cells and about 40 per cent of the cells had no inclusions. At this stage, the cells were exposed to 2 ml of a medium containing H^3-thymidine (5μc/ml, specific activity 4.4c/mM) for 2 hours. After fixation with Carnoy's fluid, the dipping method for autoradiography was applied using Kodak liquid emulsion NTB2. The preparations were treated with 2 per cent perchloric acid for 40 min at 4°C to remove acid soluble

fraction before autoradiography was carried out. After exposure to the emulsion for a week, the samples were developed, fixed by routine autoradiographic procedures and stained with Giemsa solution. As reported previously, all "B" type inclusions were labelled. Some of cells show nuclear incorporation of H^3-thymidine. The morphological sequence of development of inclusions in poxvirus has been well established (Kato *et al.*, 1959a, 1959b, 1962). According to the size of the inclusions, the sequence of the development of inclusions were divided into 4 stages as shown in Fig. 1. To study the relation between the development of

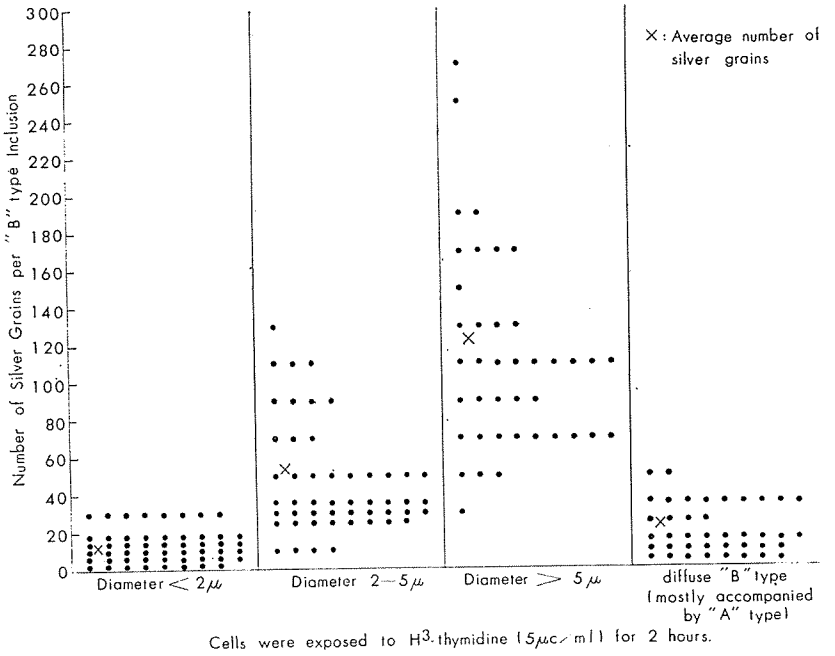

Fig. 1. Relation between Development of Inclusions and Their Ability to Synthesize DNA

inclusions and their ability to synthesize DNA, the silver grains appearing on a single "B" type inclusion were counted in more than 40 cells at each stage. As shown in Fig. 1, incorporation of the H^3-thymidine occurred at all 4 stages. The most intensive incorporation, however, was found in well developed "B" type inclusions with diameters of more than 5 μ. The degree of incorporation decreases at the final stage of development of diffuse "B" type inclusions which is usually accompanied by developed "A" type inclusions. It is interesting that the number of the silver grains at the first stage of development of small "B" type inclusion had a very limited distribution. The distribution of the number of the silver grains became wider as the inclusions developed. These facts seem to show that the ability to synthesize cytoplasmic DNA initially is the same regardless to

the condition of the host cell. However, the degree of synthesis of further DNA probably varies with the metabolic condition of the host cell.

The effects of cytoplasmic virus DNA synthesis upon nuclear DNA synthesis were investigated. The percentage of cells with labelled nuclei in noninclusion bearing and inclusion bearing cells was compared in a single preparation and control cells in a tube without virus. As shown in Fig. 2, most of the inclusion

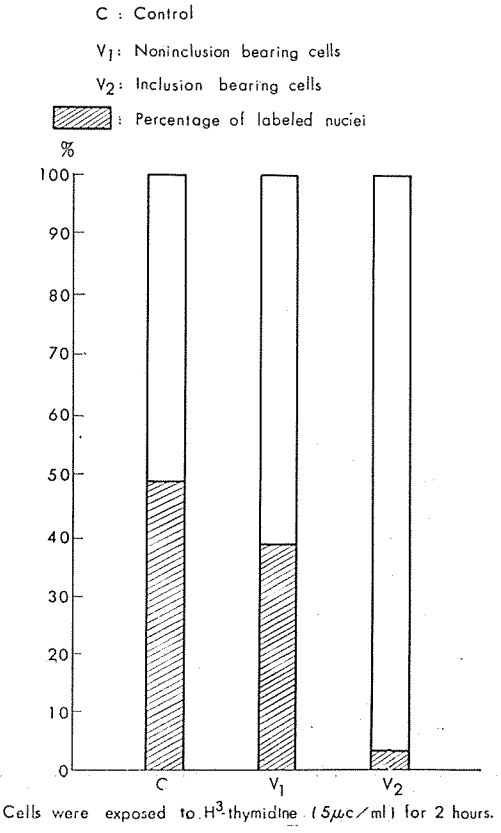

Fig. 2. The Effects of Cytoplasmic Virus DNA Synthesis upon Nuclear DNA Synthesis (I)

bearing cells did not show any nuclear DNA synthesis, while there was hardly any significant difference between control cells and noninclusion bearing cells. The latter must have included many infected cells which had not yet produced "B" type inclusions. A comparison was also made of the number of silver grains in labelled nuclei of noninclusion bearing and inclusion bearing cells. As shown in Fig. 3, there is a definite difference in the degree of the incorporation in the two. Thus the nuclei of the inclusion bearing cells not only showed a low percentage of labelling, but also a very low degree of the incorporation. The experiment reveals that

immediately cytoplasmic DNA synthesis begins, nuclear DNA synthesis is suppressed, and cytoplasmic DNA never comes from nucleus. Comparing of the Fig. 1 with Fig. 3, the velocity of the incorporation of H^3-thymidine into nucleus of noninclusion bearing cell per two hours seems to be nearly identical with the one into cytoplasm of the cells having "B" type inclusion with a diameter of more than 5 μ. The result suggests that the efficiency of cytoplasmic DNA synthesis of poxvirus is as high as that of nuclear DNA synthesis of noninclusion bearing cell.

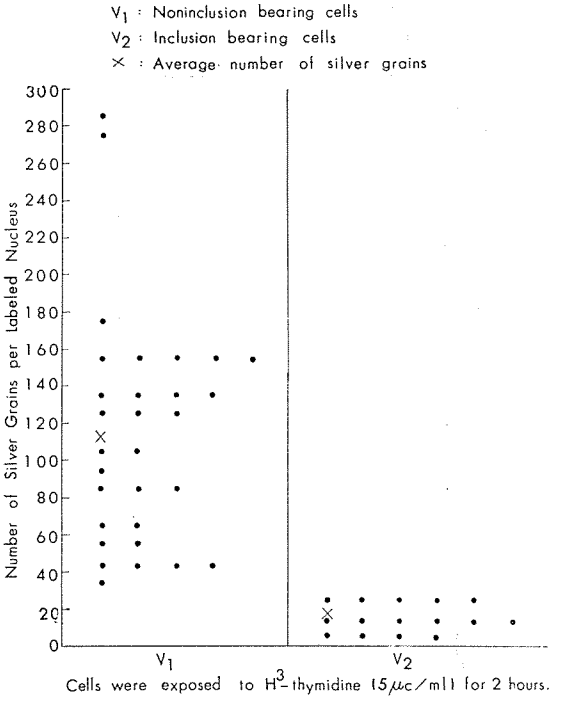

Fig. 3. The Effects of Cytoplasmic Virus DNA Synthesis upon Nuclear DNA Synthesis (2)

Summary: Silver grain counts were made on the "B" type inclusion bodies of cowpox virus. All "B" type inclusions, no matter how small, were labelled after exposure to H^3-thymidine. The most intensive DNA synthesis occurred in "B" type inclusions with a diameter of more than 5 μ. However the degree of cytoplasmic DNA synthesis decreased in diffuse "B" type inclusions which are mostly accompanied by "A" type inclusions. Immediately cytoplasmic DNA synthesis begins, nuclear DNA synthesis is suppressed.

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