Characterization of the Abortive Infection of Chick Embryo Cells by Herpesvirus Type 1

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The failure of type 1 herpesvirus to form plaques in chick embryo cells distinguishes the type 1 virus from the type 2 virus. Herpesvirus type 2 forms plaques in these cells at titres equal to those obtained in rabbit kidney cells, while most type 1 strains fail to form plaques in chick embryo cells (Figueroa & Rawls, 1969). However, most type 1 virus isolates contain a small proportion of virus which does form plaques in chick embryo cells, and this proportion increases with passage of the isolates in tissue culture. The type 1 variants capable of forming plaques in chick embryo cells retain the biological and antigenic characteristics of the parental type 1 virus, but acquire a relative resistance to the DNA inhibitor ara-A (Lowry, Melnick & Rawls, 1970). The present study was undertaken to characterize the type 1 abortive infection in chick embryo cells.

The failure of herpesvirus type 1 to replicate in chick embryo cells was not due to lack of adsorption of the virus to the cell. The adsorption of a plaque-purified strain of the type 1 virus incapable of replicating in chick embryo cells (ch- virus) and a plaque-purified strain of the type 1 virus which replicated well in these cells (ch+ virus) was examined. Chick embryo monolayers were exposed to approximately 100 p.f.u. of ch- or ch+ virus. At various intervals, the inoculum was removed from three cultures and the unadsorbed virus was assayed in rabbit kidney cells. After removal of the inoculum, the chick embryo monolayers exposed to ch+ virus were overlayed, and the appearance of ch+ plaques was compared with the disappearance of inoculum virus. After 15 min. of exposure, 36% of the ch+ virus and 50% of the ch- virus had adsorbed. By the end of 2 hr, 67% of the ch+ and 90% of the ch- virus had adsorbed. There was good correlation between the disappearance of ch+ virus from the inoculum and the appearance of ch+ plaques.

The capability of ch- viruses to penetrate and be uncoated was implied by the production of virus antigens in cells abortively infected. Cells were infected with ch+ or ch- virus at equal multiplicities, and the cultures were tested for complement fixing (CF) antigens at 0, 3, 6, 12, 18, 24 and 48 hr after infection (Table I). The production of virus antigens was similar for both viruses in rabbit kidney cells. When the production of antigens was followed in chick embryo cells, the kinetics of antigen formation was the same for the ch+ and ch- viruses, but the ch+ virus produced eight times more antigen by 24 hr after infection. The production of virus antigen detected by the CF test appeared not to be dependent upon virus DNA synthesis. Using 20 μg./ml. of ara-C, approximately equal titres of CF antigens were produced in the presence and absence of ara-C. This dose of inhibitor was found to inhibit infectious virus production by more than 99%, and the synthesis of DNA was inhibited by 83%, as measured by the incorporation of tritiated thymidine into DNA.

Immunofluorescence tests using coverslip cultures of chick embryo cells fixed for 10 min. in acetone also showed the presence of cytoplasmic and nuclear antigens when the cells were infected with ch+ or ch- viruses. No antigen was detected until 6 hr after infection, and by 16 hr after infection strong fluorescence of these antigens could readily be detected. No fluorescence was detected in controls using serum lacking antivirus antibody or uninfected cells. No difference could be detected between ch+ and ch- strains in the localization of the antigens or the number of cells showing fluorescence.
Virus antigens associated with the surface of infected cells were examined by the indirect immunofluorescence test (Tevethia, Couvillon & Rapp, 1968). Infected cells were incubated for 24 hr, monodispersed, reacted with human serum containing antibody to herpesvirus type I and then reacted with fluorescein-labelled goat anti-human immunoglobulin. Cells showing a bright fluorescent ring around the surface of the cell were considered to be positive. In rabbit kidney cells 24 hr after infection, both ch+ and ch- viruses produced virus-specific antigen on the surface of infected cells. Surface antigens were detected in approximately 75% of chick embryo cells infected with ch+ virus (Fig. 1a), but only 5% or fewer of the ch- infected cells had detectable surface fluorescence (Fig. 1b). The addition of 20 μg./ml. of ara-C to the medium of cultures of chick embryo cells infected with ch+ virus prevented the appearance of surface antigens.

The synthesis of virus DNA was next examined. Cell monolayers containing approximately 10⁶ cells/ml were infected with a multiplicity of 0.3 to 1 p.f.u./cell, and the cells were incubated for 48 hr at 37°. The cells were then scraped into the culture fluid and sedimented by centrifugation at 1000 rev./min. for 10 min., washed twice with TNE buffer (10⁻² M-tris chloride pH 7.4, 0.1 M-NaCl and 1 mM-EDTA) and resuspended in 10 ml. buffer to which sodium N-lauroyl sarcosinate was added to a final concentration of 1%. After the suspension was gently shaken at room temperature for 15 min., the preparation was treated for 30 min. at 37° with 50 μg./ml. of pronase. Buoyant densities of the DNA in the lysed cell preparation were determined by centrifugation in CsCl (refractive index = 1.400) in a Spinco Model E analytical ultracentrifuge equipped with a monochromator and photelectric scanner. Centrifugation was continued for 20 to 24 hr at 25° at 44,000 rev./min. with the four-cell ANF rotor. Cells were scanned at a wavelength of 265.4 nm. The densities of the virus and cellular DNA were determined from the scanner tracings, and were calculated against an internal standard of Escherichia coli DNA with an assumed density of 1.71 g./cm.³ (Schildkraut, Marmur & Doty, 1962). DNA with a density of that of herpesvirus was readily detected in the ch- and ch+ infected rabbit kidney cells (Fig. 2). In chick embryo cells, virus DNA was detected in ch+ infected cells, but none was detected in cultures infected with ch- virus (Fig. 3). Incorporation of [3H]thymidine into DNA with a buoyant density of that of herpesvirus was observed in chick embryo cells infected with ch+ virus but was not observed in these cells infected with ch- virus.

Evidence for the production of defective virus particles was investigated by electron microscopic studies. Culture fluids from chick embryo cells infected with either ch- or ch+ virus were examined for virus particles by a previously described method (McCombs, 1968).
Benyesh-Melnick & Brunschwig, 1966). When the cells were infected with ch+ virus, \(1.5 \times 10^9\) particles/ml were found in the extracellular fluid; however, no particles could be detected in the extracellular fluids of cultures infected with ch- virus. Thin sections of chick embryo cells infected with ch+ and ch- virus were made and examined by electron microscopy.

Fig. 1. Immunofluorescent photomicrograph of unfixed chick embryo cells infected with herpesvirus type 1 strains. (a) ch+ infected cells with surface fluorescence. (b) ch- infected cells with no detectable surface fluorescence.

Fig. 2. Equilibrium sedimentation patterns of DNA extracted from herpesvirus type 1 ch+ and ch- infected rabbit kidney cells.

Fig. 3. Equilibrium sedimentation patterns of DNA extracted from herpesvirus type 1 ch+ and ch- infected chick embryo cells.
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(McCombs et al. 1966). Replicate cultures were examined simultaneously for virus antigen by the immunofluorescence test. Cultures infected with ch- synthesized virus antigen in 50% or more of the cells. In 200 cells examined by electron microscopy, a few particles could be detected in only two cells. None of the virus particles present in these two cells had dense cores. In contrast, virus particles were readily detected in the nuclei of cells infected with ch+ virus.

Other studies have demonstrated the existence of another type of conditional lethal mutant in herpesvirus strains. Aurelian & Roizman (1964) described virus strains (dk-) that replicated well in HEp-2 cells, but underwent an abortive infection in dog kidney cells. Mutants of these virus strains which could replicate in dog kidney cells were obtained by continued propagation of the infected dog kidney cells (Roizman & Aurelian, 1965). The possible identity of the dk- and ch- strains was excluded by growth studies which indicated that the dk- virus replicated well in chick embryo cells (unpublished observation). Aurelian & Roizman (1964) demonstrated that the dk- virus was able to penetrate dog kidney cells and to produce virus antigens and virus DNA; but the capsid proteins and some non-structural virus proteins malfunctioned in dog kidney cells and the virus products needed for virus envelopment were not made (Roizman & Aurelian, 1965; Sydiskis & Roizman, 1967). Our investigation of the products of the ch- abortive infection and the ch+ productive infection in chick embryo cells has provided evidence that the failure of the ch- virus to replicate in chick embryo cells is related to defects in DNA synthesis. The ch- virus adsorbed to and penetrated the chick embryo cells and some virus antigens were made. Eight times less antigen was produced in chick embryo cells infected by ch- virus than in cells infected by ch+ virus, as detected by the complement fixation test. In addition, virus antigens detectable on the surface of ch+ virus-infected chick embryo cells by the indirect immunofluorescence test were not produced in cells infected by ch- virus. Inhibition of DNA synthesis by ara-C does not inhibit the production of all virus antigens (Ben-Porat, Shimono & Kaplan, 1969; Géder et al. 1966); however, the inhibitor prevented the synthesis of the virus antigens associated with the cell surface. Furthermore, chick embryo cells infected with ch- virus failed to synthesize detectable virus DNA, although herpesvirus DNA was readily isolated from chick cells productively infected with ch+ virus. Roizman, Aurelian & Roane (1963) found that protein synthesis must precede virus DNA synthesis in herpesvirus replication in HEp-2 cells, and that the replication of virus DNA begins when one or more specific proteins needed for DNA synthesis reach a critical level. The lack of production of an early protein by ch- virus in chick embryo cells could account for its defective DNA production and subsequent abortive infection. Electron microscopic studies of the abortively infected cells also indicate that the defect in virus production occurs before virus assembly.

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