DUAL VIRUS INFECTION OF SINGLE CELLS *

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The hypothesis that activity of one infectious agent within a host may modify the course of disease instituted by another agent in the same host has long been proffered. Such an idea was hypothecated by Edward Jenner [1] after he had observed that the clinical course of vaccinal infection may vary with concurrent herpes simplex. The obligately intracellular parasitic nature of viruses makes the demonstration of multiple viral infections in single cells of some theoretical interest.

The following experiments establish the possibility of dual infection of single cells with different combinations of several viruses. Infections with the viruses of fowlpox, laryngotracheitis of fowls, vaccinia, herpes simplex and rabies are associated with the presence of specific intracellular inclusion-bodies.[2,3] Dual infection of single cells with certain combinations of these viruses have been determined by microscopical recognition of such specific inclusions. This concrete evidence of the activity of two viruses within one cell as presented by the occurrence of two different and characteristic kinds of inclusions in that cell establishes cytological evidence that single cells can become infected with more than one virus. These observations concur with those of Syverton and Berry [4,5] in which they describe coexistent infections of individual cells. These investigators induced specific inclusions of two viruses (herpes simplex and vaccinia) in single cells of rabbit's cornea.[6,7]

EXPERIMENTAL PROCEDURES

Technic

Chick embryos, 11 and 12 days old, served as experimental hosts. With the exception of one experiment in which nervous tissue was required, the chorioallantois was the organ in which dual viral infections were established. The source of each inoculum except that of rabies was a chorioallantoic membrane previously infected with a single virus. A suspension of embryonic chick brain infected with rabies virus † was used to establish rabic infections.[8]

Development of membranal lesions following inoculations with two viruses was watched through a coverglass over an opening in the egg

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shell.[9] At varying intervals infected tissues were fixed in Zenker's fluid (5 per cent acetic acid). Histological sections were stained routinely with hematoxylin and eosin.

Fowlpox-Herpes Simplex Virus Infections

Chorioallantoic membranes of 11-day embryos were inoculated with fowlpox virus and incubated at 37° C. for 48 hours. The grossly evident fowlpox lesions were then inoculated with herpes simplex virus and were allowed to develop during 72 additional hours. During the last 3 days of incubation characteristics of fowlpox infection progressed and dominated the gross appearance of the lesions.

Microscopical examinations of sections presenting 5-day fowlpox lesions and concurrent 3-day herpetic infection showed intranuclear inclusions of herpes simplex and intracytoplasmic inclusions of fowlpox within single cells (Figs. 1 and 2). As is characteristic of infection with fowlpox virus, the ectodermal epithelium was markedly hyperplastic and hypertrophic. Almost all of the cells contained typical fowlpox inclusions. In some areas the intranuclear inclusions of herpes simplex occurred in the superficial layers of hyperplastic fowlpox-infected epithelium. At other foci the herpetic infection had spread through the whole epithelial layer of cells and had invaded the chorioallantoic mesoderm. It was determined that dual infection of single epithelial cells had occurred abundantly throughout the lesion.

It was more difficult to judge how the activity of one of these viruses in a cell had modified the influence of the other virus on the same cell. The appearance of the fowlpox inclusions and the effect of that virus on the cells appeared to be entirely typical of pure fowlpox infection. This is reasonable since the fowlpox infection, which develops more slowly and with less destruction, was given a 48-hour advantage over the herpetic infection. The intranuclear herpetic inclusions appeared larger and more basophilic than usual. The herpetic infection as a whole was less extensive and decidedly less destructive to cells than commonly. Fowlpox virus usually does not invade mesodermal cells, and herpetic infection of mesoderm apparently had not facilitated its invasion by fowlpox virus. In one case metastatic foci of herpetic infection were observed in the embryonic heart, as is characteristic of herpetic infection in chick embryos, without evidence of an accompanying metastasis of fowlpox.

Fowlpox-Laryngotracheitis Virus Infections

Chorioallantoic membranes were inoculated with fowlpox virus 24 hours before laryngotracheitis virus of fowls was superimposed, and lesions were fixed 72 hours later.

Microscopically the fowlpox infection appeared to have developed normally. The laryngotracheitis infection appeared well developed but was obviously imposed upon an abnormally hyperplastic epithelium. Intranuclear inclusions of laryngotracheitis and intracytoplasmic inclusions of fowlpox were readily determined to be present in single cells (Fig. 3). Laryngotracheitis virus normally stimulates the development of large multinucleated cells, each nucleus of which contains an inclusion. The characteristic feature of the combined infections was the occurrence of large syncytia whose nuclei contained typical laryngotracheitis inclusions and whose cytoplasm was filled with large masses of Bollinger bodies (Fig. 4).

If fowlpox and laryngotracheitis viruses are inoculated simultaneously, each virus invades epithelium and produces its characteristic inclusion. Dual infection of single cells is evident but the fowlpox lesion which normally develops more slowly is dominated by the more rapidly progressive lesion of laryngotracheitis.

Fowlpox-Vaccinia Virus Infections

Seventy-two-hour fowlpox lesions were inoculated with vaccinia virus and incubated for 48 and 72 hours longer. The epithelium was hyperplastic and practically every cell contained a fowlpox inclusion. Very few Guarnieri bodies were seen and it could not be determined that the cells were doubly infected. On the other hand, 24-hour fowlpox lesions inoculated with vaccinia virus and allowed to develop 48 hours longer showed exceptionally extensive vaccinal lesions with very few fowlpox inclusions. In this case, also, dual infection of single cells could not be determined with certainty. It appears that in the first series of experiments the vaccinia virus found an unfavorable environment for growth within cells well infected with fowlpox virus. In the second series one may reasonably speculate that fowlpox virus had parasitized a large number of epithelial cells and had stimulated a hyperplastic reaction; that vaccinia virus, gaining entrance to these hyperactive cells before the slowly forming fowlpox inclusions were developed, found a favorable medium for growth and that the rapid development of Guarnieri bodies prevented almost entirely the appearance of fowlpox inclusions. Since inclusions of both these viruses are cytoplasmic, a dual infection of single cells is more difficult to recognize.

Herpes Simplex-Vaccinia Virus Infections

Membranes inoculated with mixed suspensions of herpes simplex virus and vaccinia virus were fixed after 48 hours of incubation. There was considerable necrosis and ulceration of epithelium. Microscopically, infection with each virus was evidenced by the occurrence of

characteristic inclusions. Areas in the epithelium where the infection appeared to be only vaccinal merged into other foci which were purely herpetic. It was at these margins where dual infection of single cells could be most clearly demonstrated (Fig. 5). Such doubly infected cells occurred but rarely. Although the development of an herpetic and a vaccinal inclusion within one cell did occur, it was an unusual rather than a characteristic feature of these lesions. Each virus metastasized from these complicated membranal lesions to the liver of the embryos. Vaccinal foci and herpetic foci in the liver seemed to occur independently of each other.

Rabies-Herpes Simplex Virus Infections

Twelve-day-old embryos were inoculated intracerebrally with 0.03 cc. of 1:20 suspension of chick embryo brain infected with rabies virus. After 72 hours of incubation the same embryos were given a similar inoculation of chorioallantoic tissue infected with herpes simplex virus. After another 72 hours of incubation the embryos were sacrificed for histological study. Grossly the embryos showed a marked hydrocephalus, which Dawson [10] has reported as being characteristic of rabic infection in chick embryos.

Microscopically there was extensive destruction of brain substance with somewhat less hemorrhage than is found in herpetic encephalitis. Sections stained with eosin, fuchsin and methylene blue showed intranuclear herpetic inclusions in a great many cells that also contained typical Negri bodies in their cytoplasm (Figs. 6 and 7).

Other Attempted Dual Infections

On the assumptions that the growth of a virus within a cell depends upon the furnishing by that cell of a favorable nutritional and physiological environment for the activity of the virus, and that the growth of a virus in a cell alters the normal physiological activity of the host cell, the possibility that an already parasitized cell might be susceptible to infection by a virus to which a normal cell is resistant was considered. In these experiments, fowlpox virus was used as the primary infectious agent because it is slow to cause necrosis of individual cells.

The viruses of varicella, herpes zoster, of abortion of mares and the agent of an inclusion pneumonia in human beings have not as yet been shown to be cultivable on embryonic chick tissues. Material that might have contained one of each of these viruses was inoculated onto chorioallantoic membranes infected with fowlpox virus with the idea that a fowlpox-infected cell might offer an altered and favorable environment for the growth of one of these other viruses.

Microscopical sections showed no evidence of the growth of any of these agents except fowlpox virus itself.

Discussion

By using viruses that form recognizable intracellular inclusions, we have been able to demonstrate cytological evidence that individual cells may be invaded by, and become hosts to, two different viruses. These observations have been concerned chiefly with the parasitized cells themselves and not with the influence that one infection may have on another and concurrent infectious process in the same host. The combinations of viruses; *viz.*, fowlpox with herpes simplex, with laryngotracheitis and with vaccinia, and herpes simplex with vaccinia and with rabies, were chosen so that the occurrence of intranuclear and intracytoplasmic inclusions in one cell could be assuredly interpreted as representing dual infection of that cell.

The viruses of herpes simplex and laryngotracheitis appear to grow well within fowlpox-infected cells and these secondarily imposed infections are progressive. The same is true of herpetic infection in rabies-infected cells. Fowlpox and rabies viruses are characterized by their slow destruction of cells. On the other hand, two viruses like herpes simplex and vaccinia that very rapidly change the physiological activity of cells are by the method used only rarely found to infect one and the same cell. In no instance has it appeared that the activity of one virus within a cell rendered it more readily susceptible to invasion by another virus.

Findlay and MacCallum [11] presented as "a possible explanation of the interference shown by the neurotropic strain of yellow fever virus with the pathogenic action of the pantropic strains of yellow fever and Rift Valley fever" the idea that "when certain cells are already occupied by actively multiplying virus particles they cannot be invaded by certain other virus particles." The same interference phenomenon has been observed in experiments with certain related plant viruses.[11] Such an interference phenomenon is but rarely encountered, and investigators agree that only strains of the same virus or closely related viruses are likely to exhibit it. The mechanism of the interference is obscure.

The viruses used in this study were not related, nor does infection with any one of them protect a natural or experimental host against invasion by any other of them. These experiments show that certain different viruses may invade and multiply within single cells. It is only to this extent that the present discussion has any bearing on an interpretation of the interference phenomenon.

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DESCRIPTION OF PLATE

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FIG. 1. An ectodermal epithelial cell of chick chorioallantois showing an intranuclear inclusion of herpes simplex and a cytoplasmic inclusion of fowlpox. Five-day fowlpox lesion with concurrent 3-day herpes simplex. × 3000.

FIG. 2. A single epithelial cell showing a diffuse, granular, herpetic inclusion in the nucleus and a characteristically lobulated fowlpox inclusion in the cytoplasm at the left of the nucleus. Five-day fowlpox lesion with concurrent 3-day herpes simplex. × 3000.

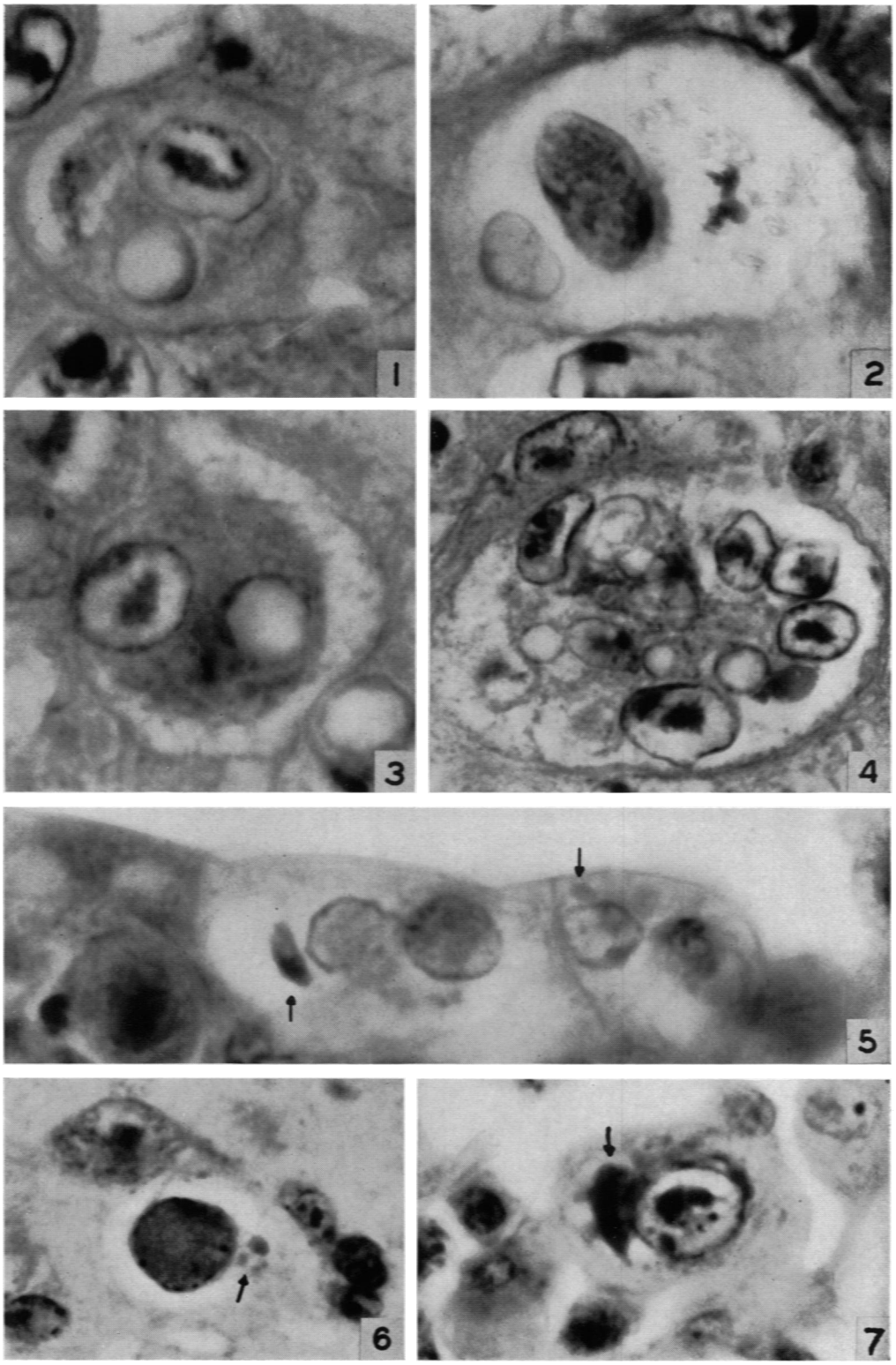
FIG. 3. A single epithelial cell showing intranuclear inclusion of laryngotracheitis and cytoplasmic inclusion of fowlpox. Four-day fowlpox lesion with concurrent 3-day laryngotracheitis. × 3000.

FIG. 4. A multinucleated cell showing five nuclei containing inclusions of laryngotracheitis, with numerous fowlpox inclusions in its cytoplasm. Four-day fowlpox lesion with concurrent 3-day laryngotracheitis. × 2200.

FIG. 5. Epithelial cells showing two intranuclear herpetic inclusions of the diffuse type and two Guarnieri bodies of vaccinia. One cell infected with herpetic virus has in its cytoplasm a large vaccinal inclusion within a vacuole. Forty-eight-hour infection. × 3000.

FIG. 6. A single neuron in the brain of an embryonic chick showing a diffuse, intranuclear, herpetic inclusion and a small Negri body in its cytoplasm. Six-day rabic infection with concurrent 3-day herpetic encephalitis. × 2200.

FIG. 7. A single ganglionic nerve cell showing an intranuclear herpetic inclusion and a large Negri body in its cytoplasm. Six-day rabic infection with a concurrent 3-day herpetic encephalitis. × 2200.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