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Changes in Cellular Nucleic Acids during Infection with Poliomyelitis Virus as studied by Fluorescence Microscopy

Remarkable similarities have been found between the biological properties of animal viruses containing ribonucleic acid and those of viruses containing deoxyribonucleic acid (such as bacteriophages). The question has thus been raised whether the mechanism of duplication of the two types of virus may involve common steps[1]. One possibility is that viruses containing ribonucleic acid have an intracellular deoxyribonucleic acid phase. With this point in mind, extensive cytochemical investigations of the changes occurring in the nucleic acids of cells infected by poliomyelitis virus have been carried out.

Monolayer cultures of monkey kidney cells grown on coverslips were infected with type 1 Mahoney virus in a concentration adequate to infect almost all the cells in half an hour. The virus release from these cells is known to begin between five and six hours after infection[2]. At various times after infection, specimens were collected and examined, either living or after methyl alcohol or Carnoy's fixation, and some also after freeze-drying. In all cases the samples were stained for 3 min. with diluted acriflavin (10^{-4} or 10^{-5} in weight in phosphate buffered saline, *p*H 7·4). After thorough washing and subsequent differentiation in 0·5 *M* calcium chloride, they were observed under the fluorescent microscope (cf. ref. 3).

The following observations were made. Normal uninfected cultures, or infected cultures up to 3 hr. after infection, show only a very faint fluorescence, dull green in the nucleus, reddish yellow in the nucleolus, faint red ochre in the cytoplasm. Three hours after infection a hazy greenish fluorescence appears in the nucleus. Four hours after infection the nucleus shows a bright green fluorescence, while the cytoplasm takes on a brighter red colour. The red fluorescent material shows a marked tendency to lump, and is discarded from the cells into the medium at this and subsequent hours as coarse granules and bigger clumps. Five hours after infection the bright green fluorescence of the nucleus disappears in more than 50 per cent of the cells; the nuclei appear empty except for the clearly visible nucleolus. The cytoplasm is mostly depleted of the red fluorescent material, and instead a bright green fluorescence is seen, at first as a ring around the nucleus and later spreading over the central part of the cell. Often a bright green, spherical body can be seen lying para-nuclearly, corresponding to the well-known acidophilic inclusion, described by different authors, in fixed and stained preparations.

The use of enzymes shows that the red fluorescence disappears after treatment with ribonuclease, and that the green fluorescence disappears or is greatly attenuated by the action of deoxyribonuclease; controls treated with the buffer solutions in which the enzymes were dissolved showed no changes.

The observations made on fixed and stained preparations confirm the finding of Reissig *et al.*[4], that the disappearance of the chromatin network in the nucleus is one of the earliest visible changes observed in cells infected with poliomyelitis virus.

The behaviour of fluorescence and the effect of specific enzymes suggest that a modification of the nuclear deoxyribonucleic acid is an early consequence of infection. The changes seen a little later in the cytoplasmic red fluorescent material would suggest changes in the cytoplasmic ribonucleic acid. The appearance of a green fluorescence sensitive to deoxyribonuclease in the cytoplasm suggests a marked alteration in the permeability of the nuclear membrane occurring 3–4 hr. after infection. This is supported by the finding that while the nuclear membrane is of normal appearance in living and frozen dried infected cells, it appears greatly distorted and wrinkled when fixed in methyl alcohol or Carnoy's fluids. It is likely that the wrinkling is produced by a quick loss of nuclear materials during fixation, secondary to the permeability changes.

The significance of the observed changes in deoxyribonucleic acid cannot be assessed at present. It is suggestive that the increased fluorescence of the nuclear deoxyribonucleic acid reveals an essential step in virus reproduction, since it is one of the first visible signs of infection. The subsequent migration of deoxyribonucleic acid to the cytoplasm may either indicate the migration of virus precursors in the same direction or may be a consequence of the alteration of the nuclear membrane and not primarily connected with the reproduction of the virus. A better understanding of the observed phenomena must await further work. Details of this investigation, which was supported by a grant from the National Foundation for Infantile Paralysis, will be published elsewhere.

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