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CYTOCHEMICAL STUDIES WITH AFRICAN HORSE SICKNESS VIRUS BY FLUORESCENT MICROSCOPY (*)

(Brief Report)

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With 5 Figures

(Color plate)

In a previous report (1) using fluorescent antibody technique, it became evident that replication of African Horse Sickness virus (AHS virus) occurs in the nucleus. In a recent report (2) it was found that actinomycin D inhibited the yield of this virus and it was suggested that AHS virus is either a DNA containing virus or an RNA virus whose replication is DNA dependent. Results of studies with Feulgen reaction (3) provided additional information on the presence of a DNA moiety in this virus. The experiment reported here represents an attempt to present the cytochemical study on developing AHS virus in tissue culture.

Line MS of monkey kidney were grown on cover slips (22×9 mm.) in screw-cap culture tubes. Growth medium and maintenance medium were the same as previously used for fluorescent antibody technique. 5×10^4 cells were seeded in each tube. After 3 days of incubation at 36° C the growth meduim was discarded and 0.2 ml. of virus dilution containing 1000 plaque forming units (PFU) were placed on each cover slip culture. After 90 minutes adsorption at 36° C the virus fluid was removed and replaced by 1 ml. of maintenance medium. Non-infected MS cell cultures, treated in the same manner, served as controls. The virus used was OD strain of AHS virus type 2. The 10th passage in MS cell cultures was lyophilized, stored at —40° C and used for this experiment. The titer of this virus was 2×10^7 ml. PFU.

For acridine orange (AO) staining cover slip preparations of infected cells were fixed in Carnoy's fluid for 5 minutes. They were then transferred directly to

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absolute alcohol, hydrated in ethyl alcohol series and after 5 minutes in McIlvaine's citric acid-sodium phosphate buffer at pH 4.0 stained for 8 minutes in 0.01% AO solution in McIlvaine's buffer. After rinsing 4 times McIlvaine's buffer, the cover slips were mounted in the same buffer on standard microscope slides.

For digestion tests Carnoy-fixed coverslips were incubated for 30 minutes at 36° C either in 0.05% 5× crystallized RNase (Pentex Inc.) at pH 4.0 in McIlvaine's buffer or in 0.01% 1× crystallized DNase (NBC) in 0.025 M veronal buffer containing 0.003 M MgSO4 at pH 7.5. Control coverslips were incubated in the corresponding vehicle alone. All coverslips were brought to pH 4.0 in McIlvaine's buffer before AO staining.

A Zeiss fluorescent microscope with an Osram HBO 200 W, BG 12 exciter and UG 5 (41/50) barrier filter was used. The film used was Kodak high speed ektachrome. Exposure time ranged from 3 to 6 minutes, depending upon the amount of fluorescence. All photographs were taken with oil immersion objective (Planapo 100/1.3) and 10× oculars.

In coverslips stained with AO the first change was observed, 12 to 16 hours after infection, in the nucleus. The nucleoli were enlarged, with a much intensified red fluorescence indicating a possible rôle of nucleoli RNA in the early stage of viral synthesis. Small yellow-green bodies were noticed around the nucleoli (Fig. 2). 24 hours after infection more yellow green stained bodies were present in the nucleus and occasionally in the cytoplasm. At this time cytoplasm stained in brillant red showed several vacuolations (Fig. 3). In preparations stained with AO, 48 hours after infection clusters of yellow-green stained particles were dispersed in the cytoplasm. Most cells were full of these particles (Figs. 4, 5) which were released from the exhausted cells. The cytoplasm at this stage was unable to take the RNA color.

The distribution and development of yellow-green particles seems to parallel the accumulation of viral antigen as observed by immunofluorescence. Pretreatment of the infected cells with RNase or DNase did not prevent the intranuclear particles to acquire the yellow-green color when subsequently stained with AO although fluorescence of normal nuclear DNA was prevented by DNase treatment. However, when specimens were first incubated for 90 minutes in 0.02% pepsin at pH. 2.0 at 36° C resistance to DNase was abolished. The change of resistance to susceptibility to DNase may be the result of digestion of the protein coat of virus inside the nucleus and consequently liberation of nucleic acid which is stained in yellow-green.

These observations furnish additional evidence that DNA is likely the predominant nucleic acid in African Horse Sickness viruses and that the virus develops within the nucleus.

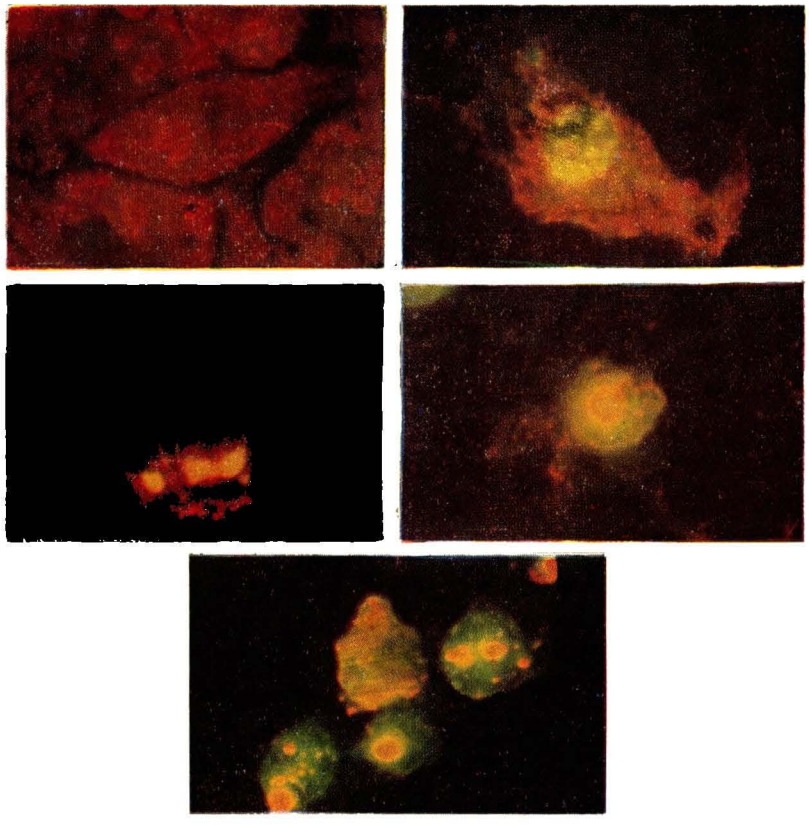

Fig. 1. Normal MS cells. Note Yellow-green color of nuclear DNA, flame red cytoplasmic RNA and reddish tinge in the nucleoli indicating presence of RNA.

Fig. 2. Single MS cell 16 hours after inoculation with AHS virus type 2 (O.D) strain. Appearance of DNA staining particles in the nucleus.

Fig. 3. 24 hours after infection: DNA staining bodies and vacuolation are observed in cytoplasm.

Fig. 4. and 5. 48 hours after infection: Cell rupture and release of DNA staining particles with total disappearance of the RNA staining properties of the cytoplasm.

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