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ADAPTATION OF HORSE SICKNESS VIRUS TO TISSUE CULTURE

by

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In a previous paper it was briefly stated that the horse sickness virus (HSV) may be adapted to the hamster kidney cells (1). The purpose of this communication is to describe the interaction between virus and cells and the development of infectivity during a single growth-cycle of the virus.

Cells were obtained from minced fresh hamster kidneys by a modification of Youngner's method (2), and trypsinized overnight at 4° C. The cells were suspended in the growth medium in a concentration of 300,000 cells per ml. The cell suspension was seeded in 1-ml. amount into screw capped 16×125 mm. 'Pyrex' tubes or in 2-ml. amount into 20-ml. rectangular serum bottles. The growth media was *CSV* 6 (ref. 3) containing 20 per cent calf serum. For the maintenance of infected cells, the same medium with 10 per cent horse serum free from horse sickness antibodies was used. The cells usually formed complete monolayers after incubation at 37° C for 5-6 days. All media had a final *p*H of 6.9-7.0. 100 units penicillin, 100 gamma g streptomycin, and 100 units mycostatin per ml. were added to all media.

Strain No. 28 of virus isolated from the blood of a horse during the recent outbreak of the disease in Iran (4) was passed four times intracerebrally in mice before being transferred to hamster kidney cells. Prior to inoculation of culture the medium was removed. The infecting virus was then added as 0.1 ml. of the 1/20 suspension of infected mouse brain in saline containing 3×10^{5} *LD 50* of virus.

After an adsorption period of 45 min at 37°C nutrient medium was

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added, in 0.9-and 1.9-ml. amounts respectively, to the tube and bottle cultures. For serial passage of virus 0.1 ml. of undiluted fluid collected from the former passage was used. All these fluids were stored at—40° C.

Table 1. Serial Transfers of HSV in Hamster Kidney Cells (HKC)

HKC passage	Titre of collected fluid at 96h log ID_{50} /ml.
I	4.2
II	5.0
III	6.2
IV	6.14
V	5.08
VI	6.22
VII	4.62
VIII	5.18
IX	4.07
X	5.25

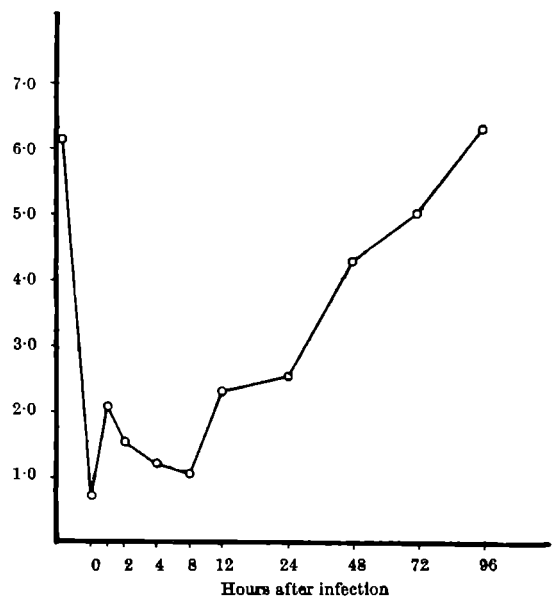

Fig. 1

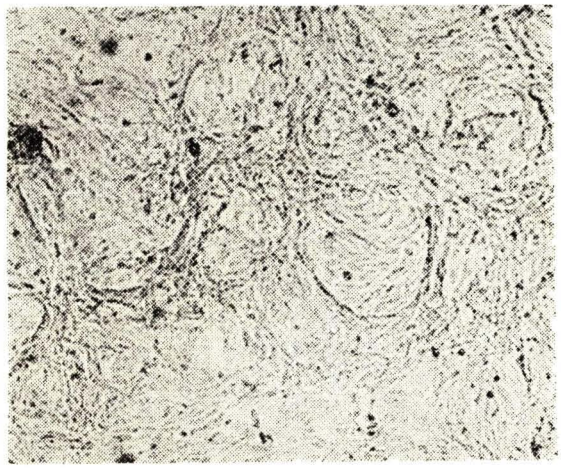

Fig. 2. Hamster kidney cells culture at 8 days. (× 125)

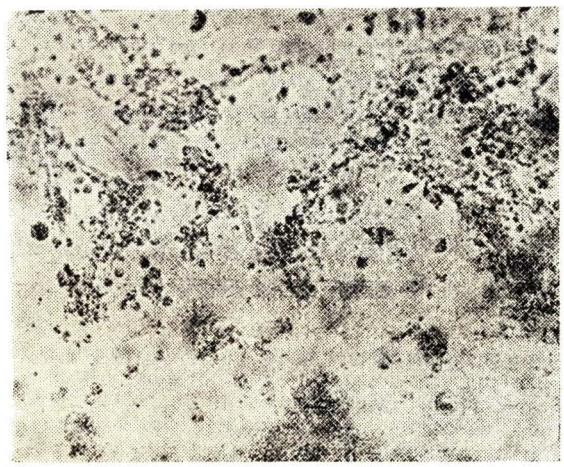

Fig. 3. Hameser kidney cells infected with HSV, fourth passage, third day after infection. (× 125)

During the course of these investigations 10 serial transfers of HSV in hamster kidney cells culture were carried out and the infectivity of all the cultures was measured. The first cellular changes were noticed in the second transfer, 36 h after infection. Infected cells lost their characteristic shape and became rounded and distorted. This change increased on the third day when

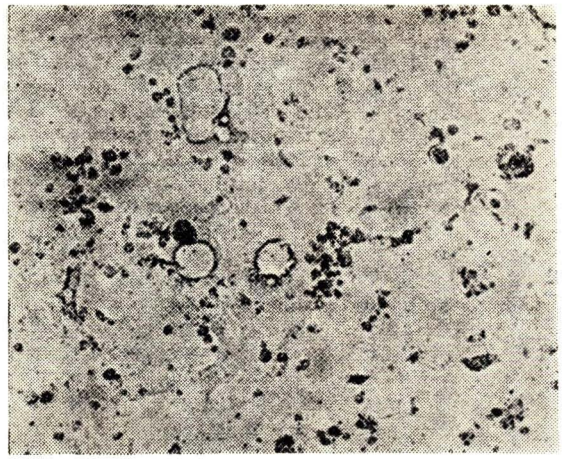

Fig. 4. Same as Fig. 3, fourth day after infection. (× 125)

in all parts of the infected sheet a marked cytopathological effect was noticed. At this stage the cytoplasm was granular and contracted. The nucleus was pycnotic and disintegrated. This progressive degeneration was complete on the fourth day when the infected sheet was practically entirely detached from the glass.

During the 10 serial transfers in hamster kidney cells the original mouse brain was diluted beyond the extinction point of the original infectivity (dilution factor 10^{-15}). The *LD 50* of ten passages in hamster kidney cells are given in Table 1.

In two different experiments 60 tubes of hamster kidney cells were infected. After an adsorption period of 45 min at 37° C, with 0.1 ml. of undiluted culture of the fourth passage, the culture was washed with two changes of phosphate-buffered saline, 1 ml. of fresh medium was then added and incubation at 37° C was continued.

Immediately after the second washing and at fixed intervals thereafter, four tubes were removed from the incubator and the pooled medium inoculated in mice (Fig. 1).

It can be seen that in the first hour of incubation there is an increase in titre and from then a decrease up to 8 h. A rapid increase in titre then occurs between 8 and 12 h. These data suggest the occurrence of an eclipse period with HSV. This eclipse phase is followed by the release of a large amount of a new virus, the maximum yield being obtained at the fourth day.

The morphological changes in infected cell sheet advance gradually until the complete degeneration of the cells, which occurs on the fourth day.

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References

1 Mirchamsy, H, and Taslimi, H., *C.R. Acad. Sci., Paris,* 255, 424 (1962).
2 Younger, J. S., *Proc. Soc. Exp. Biol., N.Y.,* 85, 202 (1954).
3 Cooper, P. D., Wilson, J. N., and Burt, A.M., *J. Gen. Microbiol.,* 21, 702 (1959).
4 Rafyi, A., *Bull. Off. Int. Epiz.,* 29, R.N., 587 (1961).