The Cytopathic Effect of Viruses in Cell Culture
A Staging Method

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The present paper describes a modified method for cell culture staining with hemalum-eosin. Cell culture staining permits a cytological examination of bouth normal cells and of cells showing a marked cytopathic effect (CPE) caused by different viruses.

For a sharp contrastive staining of cell parts and a clear demonstration of viral CPEs such preparations have proven to be the most suitable demonstration teaching tool in medical course virology classes.

Key words: cytopathic effect, virus, staining technique

INTRODUCTION

Virological routine, i. e. isolation of viruses in cell culture, examines infected cells under a light a microscope. Despite the preparations involing nascent state, they allow making distinctions among different types of cytopathic effect (CPE) of respective virus groups. Nevertheless, a more accurate cytological study of the cell itself in a culture, and especialy of the CPE developed, requires staining. The staining enables observations of the cell membrane, cell nucleus and virus inclusive bodies in the cytoplasm or nucleus of the infected cells. Although there are different cell staining techniques, virological practice has proved that hemalum-eosin staining is one of the most suitable. In the literature several staining methods are described, such as Mayer's acid hemalum nucleus staining method, Weigeter's iron hematoxyllin nucleus staining method and hemalum-eosin staining method (2). For the cell culture staining we have used a modified staining method that combines the methods mentioned. This combined method has been used in virological laboratory for a number of years.

MATERIALS AND METHODS

In the study we used a continuous culture of green monkey kidney (GMK) cells. Cell cultures were grown in Breed-Demeter bottles. The culture medium was changed in five-day intervals. The culture was trypsinized in two-week intervals. The cells grew in Eagle's minimum essential medium (MEM) in Hank's salt solution, supplemented with 10%-fetal calf serum, 0.001%-arginine and antibiotica. The same culture medium, with 2.5%-fetal calf serum, served as a maintenance medium. To maintain GMK growth in tubes, we used the lactalbumin medium in Hank's solution with adding of 2.5% fetal calf serum, antibiotica and 8%-solution of $NaHCO_3$ for pH adjustment (1). Cell culture was trypsinized by 5 mL of a 0.25%-trypsin-versen solution. The bottles were closed rotating it half a circle round its longer axis to establish a contact between trypsin-versen and the cell layer. Trypsin-versen, which also served to wash the cells, was decanted after 2 mins. A very thin layer of trypsin remained in bottles to cover the cells. The cells were put in incubation at 37°C until they were noticed to begin to flake at rims. A small quantity of the culture medium was poured into the bottles and peeled cells were resuspended. These cell were then put into the prepared culture medium. Each tube received 1 mL of an inoculum which was a mixture of culture medium and cells. Cell incubation in a thermostat at 37°C proceeded for 3 to 4 days. When the cells achieved a good and uniform growth in a homogenous layer, the liquid medium was changed. Before inoculating culture medium with the cells, we fitted a sterile piece of covering slide.

Virus strains. Viruses used were isolated and identified in Virology Division, Croatian National Institute of Public Health.

DNA viruses:

1. Vaccinia virus, strain No. 3774/72 isolated from a crust.
2. Herpes simplex virus type 1, strain No. 1474/65 which was isolated from a vesicular smear.

RNA viruses:

1. Mumps virus, strain No. 933/78 isolated from a throat smear.
2. Coxsackie viruses, group A, type 16, strain No. 13359/77 which was isolated from a stool.

The viruses were propagated in cell culture to obtain the viral stocks that were kept at -20°C until being used. After the cells grew in the layer, the maintenance medium replaced the culture growth medium. Each tube was inoculated by 0.1 mL of the viral suspension. The cells were stained by a modified staining method described below.

The staining method.

The cells growing on slides were fixed by 96%-ethyl alcohol for 10 min and rinsed with absolute ethyl alcohol, separated by a 96%-ethyl alcohol, and once again with 70%-ethyl alcohol, and at last three times with water. Hemalum staining of the cells lasted 2 to 4 min; the cells were rinsed with water until the colour was lost. Staining with 0.1%-eosin followed for 3 mins. The cells were rinsed with distilled water, with 70%-ethyl alcohol, and with 96%-ethyl alcohol and with absolute alcohol two times. The slides were put in xylol and kept there for at least one hour. They were put on microscopic slides using Canada balsam as a mounting solution.

RESULTS

As CPE was observed, the slides were taken out and stained. The cytoplasm was rose and cell nucleus bluish-pink (Fig. 1). The staining procedure also made the nucleous and cytoplasm membrane visible.

Vaccinia virus. CPE occurred 24 hr after inoculation of the virus. Initially, small foci of hyperplastic cells arose in the layer. Then the cells rounded, very soon baloon-shaped cells were observed. Soon after pycnosis set in, and the following day the cells began to fall off the glass (Fig. 2).

Herpes simplex virus. The CPE was observed 48 hr after inoculating the virus into the cell culture. It appeared as individual foci in the layer. The cells rounded, enlarged and became brighter; they kept together in clusters for several days. Subsequently, they began to separate and to drop off the glass (Fig. 3).

Mumps virus. No CPE developed until the third day after inoculation. While cells in the layer started producing large round syncytial multinucleated cells, they also evolved a fair quantity of individual, widely dispersed, pycnotic dark cells. As CPE developed very rapidly, the cells fell off the glass after only two days (Fig. 4).

Coxsackie A virus. The CPE developed 48 hr after inoculation. Though changes usually occurred on layer rims, they were also occasionally seen in the cell layer. The cells appeared as small foci consisting of round pycnotic cells. As cell nucleus diminished, cytoplasm became more dense. With cells becoming smaller, holes emerged in the layer (Fig. 5).

DISCUSSION AND CONCLUSION

Cells can be examined in culture using different staining methods (2). Our modified hemalum-eosin cell staining method has proven optimal for our needs over many years. It allows the CPE of different viruses full expression. There are some CPEs that it shows especially well. For example, it shows the herpes virus as resembling a pink-blue grape which consists of round cells. As for parymyxovirus, its CPE takes the form of a syncytium with a multitude of peripherally located blue nuclei in a common pink cell cytoplasm. As, owing to differential staining, such preparations clearly differentiate between cytoplasm, nucleus, nucleolus and cytoplasmic membrane, they make noticing of the differences in the CPE of different viruses easy. They are thus best used as demonstration preparations during virology classes for medical profile students.

REFERENCES

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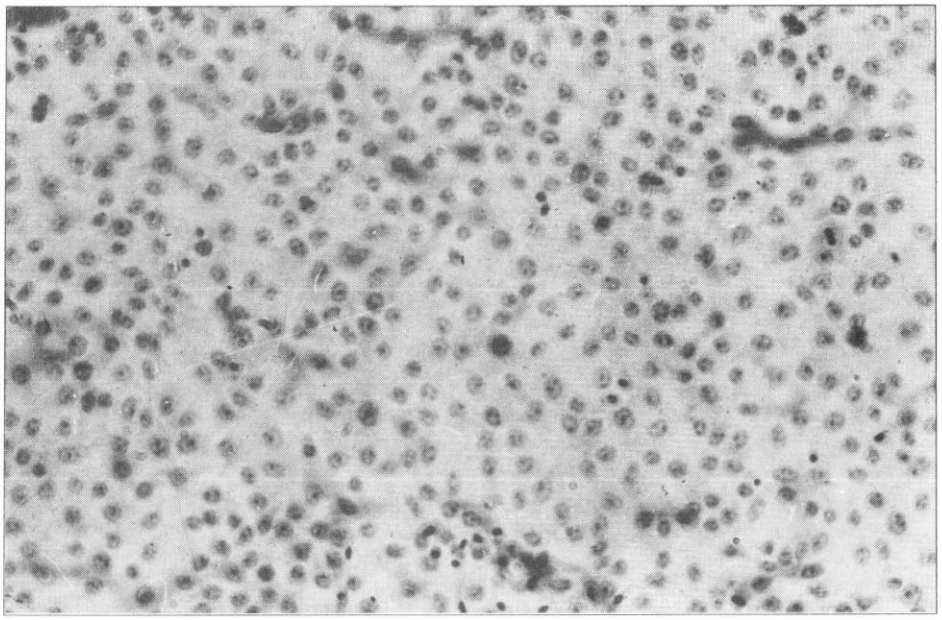


FIGURE 1.
Uninfected GMK cell culture
SLIKA 1.
Neinficirana stanična kultura GMK stanica (stanice bubrega zelenog majmuna)

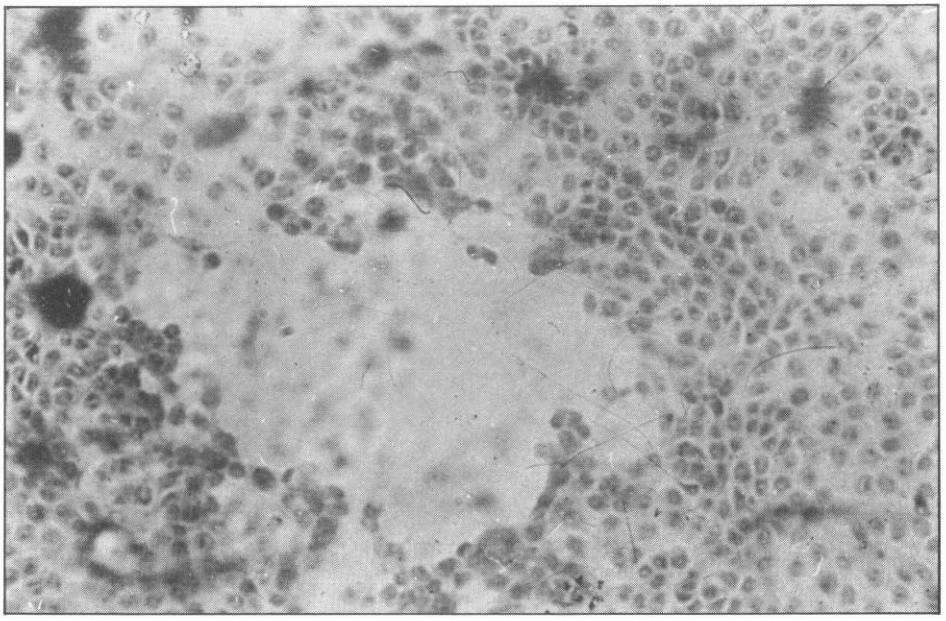


FIGURE 2.
CPE caused by vaccinia virus (GMK cells)
SLIKA 2.
CPE uzrokovan virusom vaccinia (GMK stanice)

FIGURE 3.
CPE caused by herpes simplex virus (GMK cells)
SLIKA 3.
CPE uzrokovan virusom herpes simplex (GMK stanice)

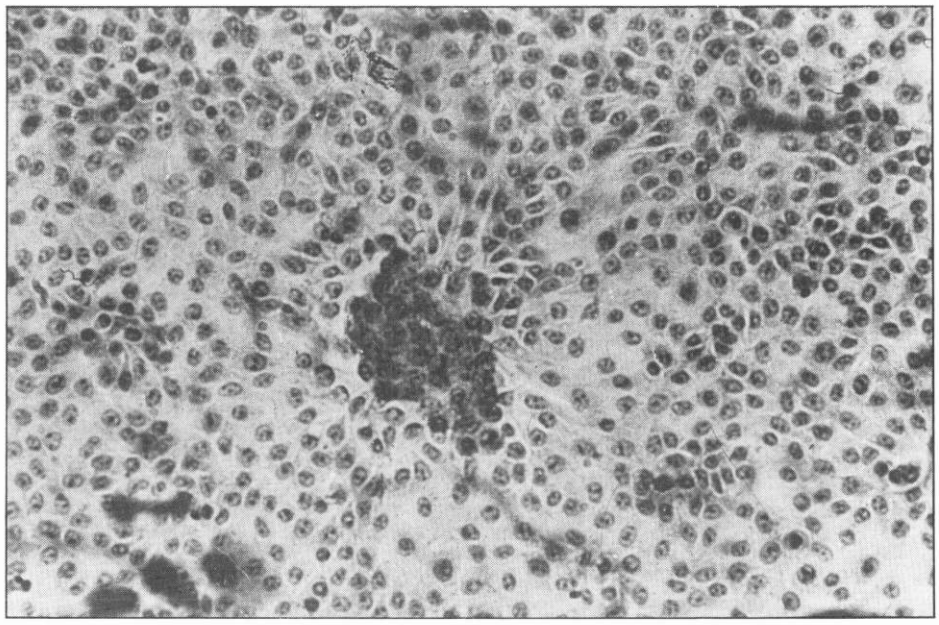
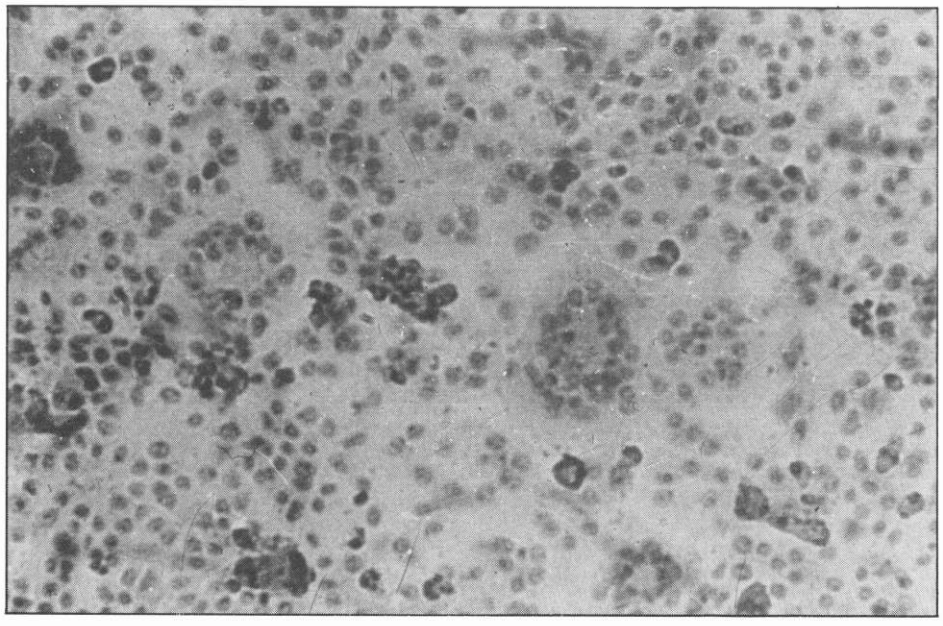


FIGURE 4.
CPE caused by mumps virus (GMK cells)
SLIKA 4.
CPE uzrokovan virusom mumpsa (GMK stanice)



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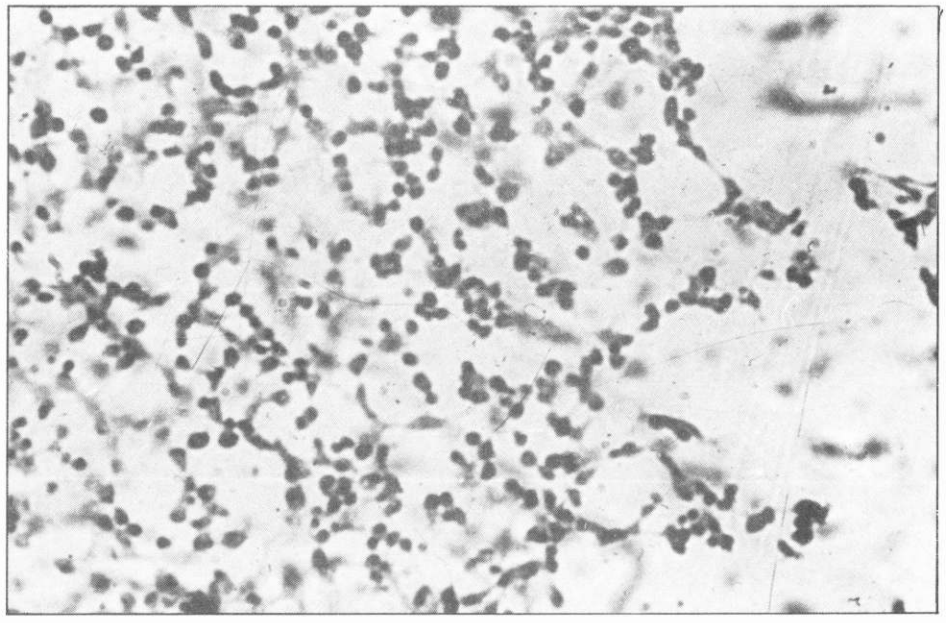

FIGURE 5.
CPE caused by enteroviruses (GMK cells).
SLIKA 5.
CPE uzrokovan enterovirusom (GMK stanice)

Sažetak

CITOPATSKI EFEKT VIRUSA U STANIČNOJ KULTURI

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U ovom radu prikazana je modificirana metoda bojanja stanica u kulturi hemalum-eozinom. Bojanje stanica kultura omogućuje citološki pregled zdravih stanica i onih s izraženim citopatskim efektom (CPE) raznih virusa. Zbog izrazito kontrastnog bojanja dijelova stanica i zornog prikaza CPE virusa, ovako pripremljeni preparati pokazali su se najpogodniji kao nastavni, demonstracijski materijal na kolegijima iz virologije, na studijima zdravstvenih profila.

Ključne riječi: citopatski efekt, virus, tehnika bojanja

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