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DECONTAMINATION OF BACTERIAL INFECTION OF MONOLAYER CULTURES WITH A SPECIFIC BACTERIOPHAGE

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SUMMARY

A few cell lines and primary monolayer cultures were accidentally infected by bacteria. These cultures were successfully decontaminated by means of the specific bacteriophage virus after quick identification of the responsible bacteria. This method presents a practical interest for preservation of valuable cultures.

Key words: bacteria; contamination; decontamination; bacteriophage.

INTRODUCTION

Cell growth media may sometimes become contaminated by bacteria in spite of all precautions taken against such infections, including the introduction of antibiotics into the media. When this happens, the cultures are lost regardless of prolonged treatment with antibiotics. In order to decontaminate valuable cultures thus infected, we employ the phenomenon of bacteriophage as described by d'Herelle (1).

MATERIALS AND METHODS

Cells. Primary cultures used were: TC 176, human astrocytoma (frontal tumor) in culture for 3 months; TC 177, human multiform glioblastoma (frontal tumor) in culture for 2½ months; and TC 162S12, human glioblastoma grafted into the brains of mice (2). Cell lines used were: MCF7 (Michigan Cancer Foundation), human breast tumor cell lines from pleural effusions (3); RBA, rat mammary adenocarcinoma cell line derived from DMBA (induced tumor) (4); and NMU, rat mammary tumor induced by nitroso-methylurea. (These three strains were loaned to us by the Biochemistry Laboratory of the INSERM U 148, Pr. Rochefort.)

Media. The cultures were grown in Puck's N-16 medium modified (GIBCO) or Eagle's medium (Hanks' and Earle's salts), supplemented with glutamine, fetal bovine serum (10%) and antibiotics (penicillin-streptomycin or gentamicin).

Bacteriophage stock. Different strains of bacteriophage in suspension in peptoned medium (Laboratoire du Bactériophage, Paris) were used: *Bacté-Staphy-Phage*—5.10^9 bacteriophages per ampul (5-ml) selected and adapted for lysis of diverse substrains of staphylococcus (aureus, citrine, albicans). *Bacté-Pyo-Phage*—5.10^9 bacteriophages per ampul (5-ml) selected for lysis of staphylococcus, streptococcus, pyocyaneus, proteus, pseudomonas, coli, paracoli and enterococcus.

Electron-microscope examination of the bacteriophage viruses. This examination was used only to verify the quality of the bacteriophage stock and the integrity of the viruses. A drop of bacteriophage stock suspension was placed on Formvar carbon-coated grids glow-discharged according to Dubochet et al. (5). Then the grids were stained in 2% uranyl acetate, rinsed quickly in water, and shadowed with chromium. Electron-microscope examination followed. Electron micrographs were taken at high magnification with 80 kV on a Philips EM 200. The exact magnification was determined by means of a Polaron grating replica.

Method of decontamination. Examination of a drop of accidentally infected culture (turbid medium) through Gram reaction was sufficient for rough identification of the contaminant bacteria and subsequent determination of the bacteriophage stock to be used. The pH of the medium also was measured. The exact quantity of glucose present (less than 1 g per l) was provided by the laboratory that prepared the medium. The infected flask was rinsed with the usual medium

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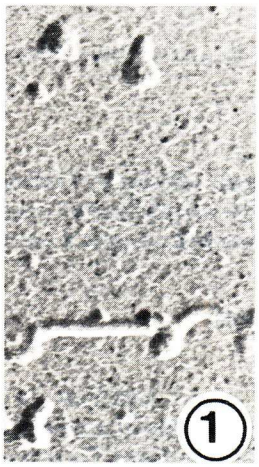
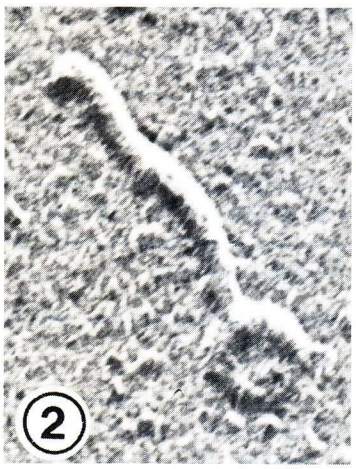
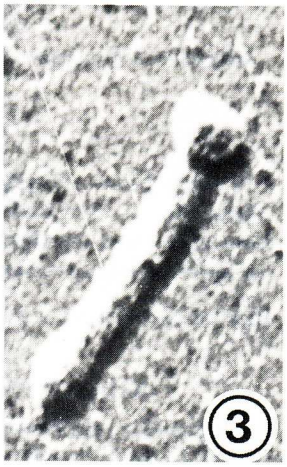
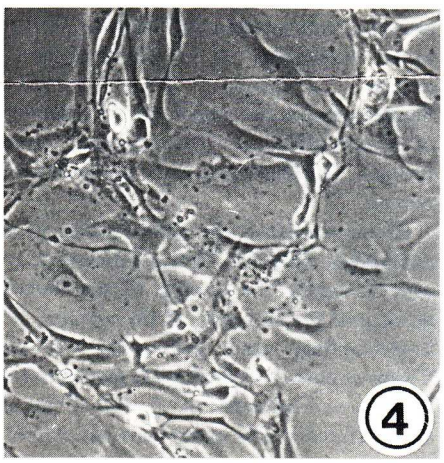
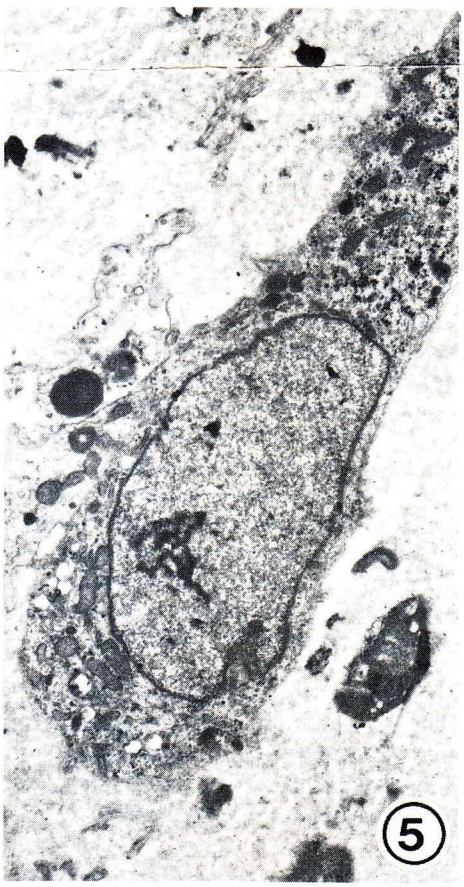
solution for the culture, with the addition of a bacteriophage stock but without antibiotics. This medium solution was left in contact with the cells of the cultures for 5 min and then discarded. The same process was then repeated, this time replacing the culture's usual medium (5-cc) to which was added glutamin, fetal bovine serum and the same strains of bacteriophages (2-cc) as used in the first rinse. The treated cultures were placed in an incubator at 37° C and observed from time to time as normal. When the decontamination was completed (between 6 and 12 hr), the cultures











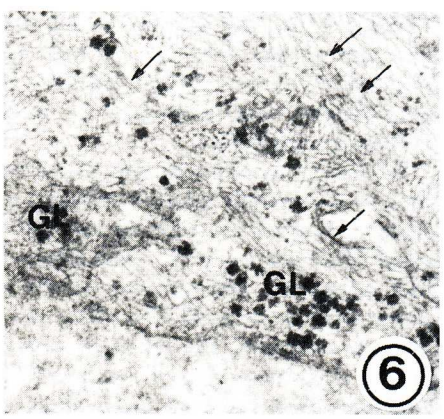


TABLE 1

DELAY OF CONTAMINATION

Cell Strain and Medium	Contamination		Decontamination	
	Date	Bacteria identified	Bacteriophage	Delay of decontamination[a]
TC 176 Puck's N 16 6 flasks (25-ml)	10/12/77	Staphylococcus (Cocci Gram +)	Bacté-staphy-phage	6 hr
TC 177 Eagle 6 flasks (25-ml)	10/24/77	Pseudomonas (Bacillus Gram-)	Bacté-pyo-phage	6 hr
162 S 12 Puck's N 16 4 flasks (25-ml)	10/28/77	Streptococcus (Cocci Gram +)	Bacté-pyo-phage	6 hr
MCF 7 Eagle 20 flasks (50-ml)	11/12/77	Staphylococcus	Bacté-staphy-phage	Decontaminated the following day
RBA Eagle 20 flasks (50-ml)	11/17/77	Streptococcus	Bacté-pyo-phage	Decontaminated the following day
NMU Eagle 10 flasks (50-ml)	11/12/77	Streptococcus	Bacté-pyo-phage	Decontaminated the following day

[a] Delay of decontamination was variable, but apparently was not related to the nature of the culture medium or to a particular type of bacteria. At any rate, it is always very quick (from 6 to 24 hr).

were placed in a normal medium with fetal bovine serum as usual.

Method of electron-microscope examination of the cells after decontamination. After treatment with EDTA, the pellets of each strain of cells in culture were fixed with 2% glutaraldehyde and then with 1% OsO_4 in phosphate buffer at pH 7.2. After araldite embedding, thin sections were stained with uranyl acetate and lead citrate (6) before electron-microscopy studies. Morphological studies were supported with daily observation of the cultures through a Leitz phase-contrast microscope.

RESULTS

Table 1 lists the cell strains and media used, the bacteria identified, the bacteriophage used, and the delay in decontamination observed.

Integrity of bacteriophage. The bacteriophage stock was examined through the electron microscope. With the glow-discharge apparatus, the bacteriophages were more numerous on the grids. The characteristics of each strain of bacteriophage were recognized. The micrographs show the different types of bacteriophages used with their classic morphological features (7). In Fig. 1, in the same drop of "Bacté-pyo-phage" three different strains of bacteriophages (staphylococcus phage, streptococcus phage, coli phage) can be seen. In Figs. 2 and 3, at high magnification, a good conservation of the shape of the phages is shown.

Morphology. Examined under a light microscope, the cells exhibited the same morphological features after decontamination as before the infection. For example, in TC 176 (Fig. 4), the general

FIG. 1. In the same bacteriophage stock: a coliphage, a staphylococcus phage, and a streptococcus phage. ×70,000.

FIGS. 2, 3. Integral phages seen at high magnification. ×200,000.

FIG. 4. Astrocytoma cells (TC 176) 8 days after decontamination. ×30.

FIG. 5. Astrocytic cells (TC 176) without cytological alteration. ×7,000.

FIG. 6. The same field as in Fig. 5 at high magnification exhibiting characteristic features of an astrocyte: scattered microfilaments (*arrows*) and glycogen (GL). ×34,000.

shape of the cells was conserved, their cytoplasm exhibited no granulation, and the nucleus of each cell remained unaltered.

Electron-microscope examination of the cells after decontamination of the cultures with this method verified the absence of morphological alteration and the absence of viruses. The cells observed (TC 176) present typical organelles such as, for example, the microfilaments and the glycogen granulations of the astrocytoma cells (Figs. 5, 6). Moreover no chromosome damage can be found through classical karyological studies.

Discussion

The phenomenon of bacteriophagia is a biological event that occurs when the concentration of glucose is less than 1 g per l and only in alkaline media (8, 9). The bacteria are subsequently destroyed by bacteriophage viruses which attack them and reproduce within them. The bacteriophages are in turn destroyed by the acidification of the media which results from the normal cell metabolism, thus rendering the culture free from bacterial and viral infections (8–10).

Obviously, it is essential to begin this decontamination as soon as possible after the discovery of the infection so that the bacteria will not have been present long enough in the culture medium to injure the cells. Although Gram reaction is not sufficiently precise to identify a specific bacterium, it is, however, an adequate indication for our method because the bacteriophage stock used is a mixture of different strains.

As seen above, this method does not endanger the cells of the cultures. Even if an integration of the nucleic acid of the bacteriophage virus in the genome of the eucaryotic cells in culture was possible, the gene expression of the bacteriophage is hardly probable. As far as we know, no such case has ever been reported. This method has practical value for saving infected cultures because of its rapidity, efficacy and reliability without any apparent cellular damage.

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