A Method for Direct Demonstration of Pleuropneumonia-Like Organisms in Cultured Cells.* (29731)

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There has been an urgent need for a rapid and simple method of demonstrating pleuropneumonia-like organisms (PPLO) in infected cell cultures. It has become increasingly evident that interpretation of research in which cultured cells are employed may be strongly affected by unknown PPLO contamination of the cells(1,2,3,4). The routine examination of cell strains and lines for presence of PPLO has been found difficult and time consuming by many investigators with the previously available methods, and has therefore often been ignored.

We wish to present the following new method by which the organisms are demonstrated directly in infected cultures. The original observations which led to the development of this method were made of cell cultures prepared for chromosome analysis by the method which our laboratory uses for continuous amnion cell lines(8). The technique includes hypotonic treatment of the cells(9) and is a modification of 2 other chromosomal preparation methods(10,11). A characteristic deposit of darkly stained, granular material was observed in cultures later found to be PPLO-contaminated; this material was absent from PPLO-free cultures.

Materials and methods. Reagents. Sodium citrate solution 0.6%. Use 6.84 g sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$,F.W.294.12). Add distilled water to 1000 ml.

Carnoy's fixative. 1 part glacial acetic acid, 3 parts absolute ethyl alcohol.

Orcein stain. A 2% solution of natural orcein (Gurr's) in 60% glacial acetic acid is prepared by dissolving the orcein in boiling glacial acetic acid with stirring. Cool to 55°C. Add distilled water to make the acetic acid concentration 60%. Cool to room temperature. Filter 2 times through Whatman filter paper No. 1.

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Procedures. Cells are cultured on a cover slip in a suitable container. [We have found the "Demuth cup" (Demuth Glass Works, Division, Brockway Glass Co.) with inserted round cover slip convenient for this procedure.] When a light cell sheet has developed, the medium is poured off and is replaced by the 0.6% sodium citrate solution. Three ml of this solution is proper for the Demuth cup. Subsequently, 1 ml of distilled water is added, drop-wise, making the final concentration of sodium citrate 0.45%. The slide is left in this solution for 10 minutes, then an equal amount of Carnoy's fixative is added slowly. The fluid in the cup is poured off and is replaced with 2 ml of Carnoy's fixative. Ten minutes is allowed for fixation. The slide is taken out of the container and left in the air for 5 minutes or until absolutely dry. The cells are stained for 5 minutes with the orcein stain and then washed 3 times in absolute alcohol. The slide is mounted in Euparal (Flatters & Garnett Ltd., England). All the procedures are carried out at room temperature.

Results and discussion. By microscopic examination using phase optics, the PPLO infected cultures clearly show an abundance of the individual mycoplasma organisms located primarily at the cell borders, and apparently in part, attached to the peripheral parts of the cytoplasm, and in the intercellular spaces (Fig. 1). Their location agrees with previously published micrographs of thin sections (1). Uninfected cultures do not show similar particles or granules (Fig. 2). We recommend the use of cultures of FL human amnion cells (12) for the best demonstration of PPLO with the technique described, since various cell types have shown some variation in response to the hypotonic treatment. A certain amount of cytoplasm must be maintained for the most remarkable demonstration of the pleuropneumonia-like organisms. For this

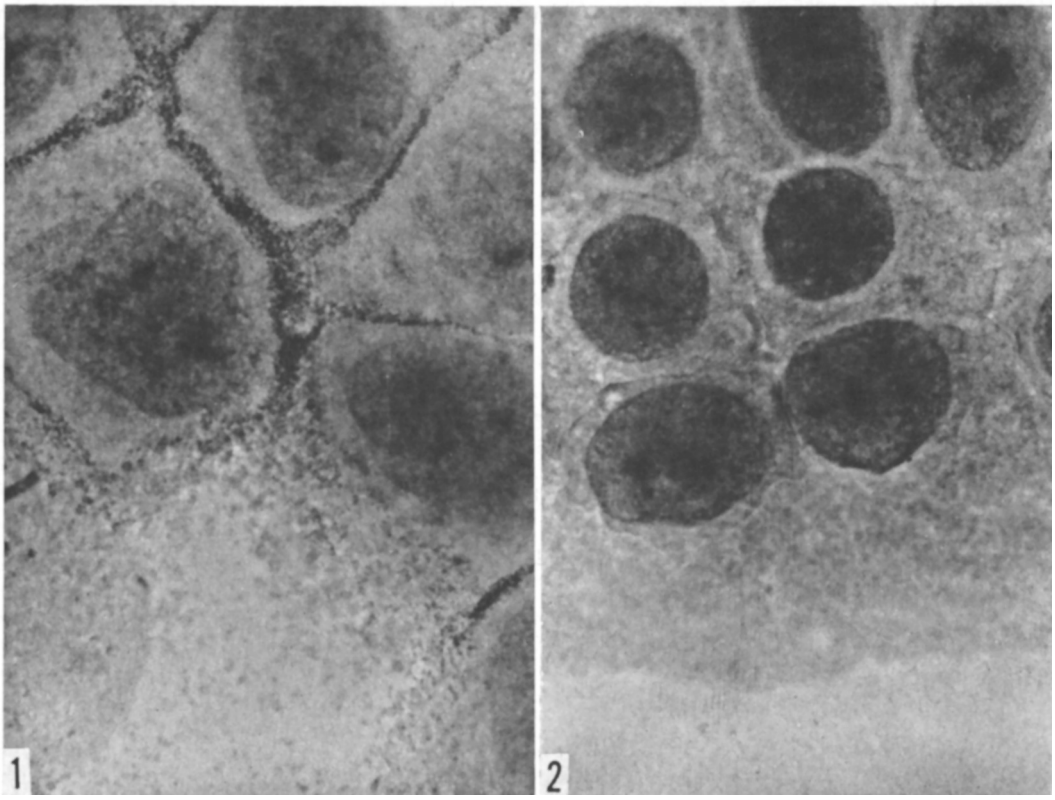

FIG. 1. Typical appearance of cultured cells of FL line of human amnion infected with pleuropneumonia-like organisms, after preparation and staining according to the technique described here. Magnification: 1224 ×.

FIG. 2. Uninfected FL cells prepared with the same technique as the culture shown in Fig. 1. Magnification: 1224 ×.

reason, the use of FL cells as "indicator cells" is easier and gives the most reliable results. Our experience has shown that the LY tissue culture medium(12) containing 20% human serum supports the growth of various strains of PPLO's to an unusually high titer. A proper method is to inoculate young cultures of FL cells propagated in LY medium with 20% human serum with a 0.2 ml amount of the supernatant of cell cultures to be tested for the presence of PPLO. With this technique we have found in all contaminated cell lines high concentrations of the organisms. Preparation and staining of the FL cell cultures as early as 24 hours after inoculation with PPLO containing supernatants, have demonstrated remarkably well the organisms at the cell borders.

A suitable magnification is obtained with a 40× phase objective for demonstration of the typical phenomenon in the PPLO infected cultures; with 100× oil immersion phase objective, the organisms may be observed individually, appearing as round bodies of sharp contrast. The data obtained in our laboratory with this method agree fully with the results of the best available PPLO-agar culture methods. The demonstration can be accomplished in approximately 30 minutes. The method has demonstrated PPLO contamination of 30 different cell lines of animal or human origin obtained from several laboratories; the isolates include PPLO strains with different characteristics. We have also applied this method for PPLO observation in experimental work and have found advantages, especially in time-saving, over previously described methods, based on cultivation(5,6) or chemical reaction(7).

The different size, and in many cases shape

and location of other bacterial cell culture contaminants which may be detected by this technique, and their capacity to grow in simple bacteriological media, distinguished them easily from mycoplasma.

Summary. Direct microscopic observation of pleuropneumonia-like organisms (PPLO) in cell cultures is easily accomplished following hypotonic treatment, air-drying and staining with orcein. A rapid technique using FL human amnion cells, inoculated with supernatant from suspected cultures, is described. The demonstration of PPLO contamination of 30 cell lines by this rapid method was in complete agreement with results of PPLO-agar techniques.

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Subclasses of Human IgG Molecules Differing in Heterologous Skin Sensitizing Properties. (29732)

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Subclasses of normal IgG (γ_2-immunoglobulin) molecules have recently been detected (1-6). These subclasses are based on differences in antigenic determinants, and G-myeloma proteins related to each subclass have been identified.

IgG molecules are composed of 2 different types of polypeptide chains, the heavy (γ) and light (κ or λ) chains. The distinctive characteristics of molecules in the IgG subclasses designated γ_{2a}, γ_{2b} and γ_{2c}-globulins* are due to antigenic differences among the heavy chains(3,6). These differences have been further localized to the Fc (fast migrating) fragment, obtained after papain digestion(3,6).

Studies in other species have shown that the Fc fragment of IgG molecules is responsible for several biologic properties, including their ability to sensitize skin of heterologous species for the passive cutaneous anaphylaxis (PCA) reaction(8-10). Since the γ_{2a}, γ_{2b} and γ_{2c} subclasses of IgG differ in Fc fragment antigenic characteristics, possible differences in the skin-sensitizing properties of these molecules were investigated. The γ_{2a}, γ_{2b} and γ_{2c}-globulins have not yet been individually purified from normal serum. However, G-myeloma proteins representing each of the subclasses were available. The ability of these myeloma proteins to sensitize heterologous (guinea pig) skin was investigated with the technic of reverse passive cutaneous anaphylaxis (RPCA).

Materials and methods. Normal human

* This terminology is consistent with the earliest designations of IgG subclasses(1,3). When information is available concerning the interrelationships of these subclasses, more appropriate designations, conforming to the recommendations of the Committee on Human Immunoglobulin Nomenclature(7) can be made.