THE ISOLATION OF MICROORGANISMS (PPLO) OF PLEUROPNEUMONIA-LIKE ILLNESS FROM TISSUE CULTURES

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It has been established that several tissue cultures used in many laboratories are inected with PPLO organisms which cause pneumonia-like illness and can be found in 57 to 59% of all tissue cultures investigated of different origins [3, 6, 7]. It is interesting that the etiologic agent of primary atypical pneumonia in man which was first considered to be a virus belongs to this group of organisms [1].

PPLO is a peculiar organism which in many respects is similar to the L-forms of bacteria. Both of them require for their growth special media, have peculiar morphology, and are similar to the etiologic agent of pneumonia in cattle. They consist of large spherical forms with tiny granular formations, pass through bacterial filters, and are capable of intra-cellular existence. Their extreme resistance to penicillin is a characteristic peculiarity of PPLO and L-forms. According to the data of Hayflick [4], PPLO in tissue culture evokes no apparent cytopathogenic effect. The source of infection of tissue cultures by these micro-organisms is not known at this time.

The aim of the present report is the study of various long surviving tissue cultures and primary cultures, as well as attepmts to establish the possible source of infection of cultures by PPLO organisms.

EXPERIMENTAL METHOD

A continually growing HeLa cell culture, Hep-2 culture, Cave culture, Sots culture, and human amnion were used for these investigations and they were grown in media No. 199 with 10% human serum or bovine serum.

Primary cultures were prepared from embryonal tissue of mouse and chick embryos with 0.25% trypsin. The cells were suspended in medium No. 199 with 10% bovine serum.

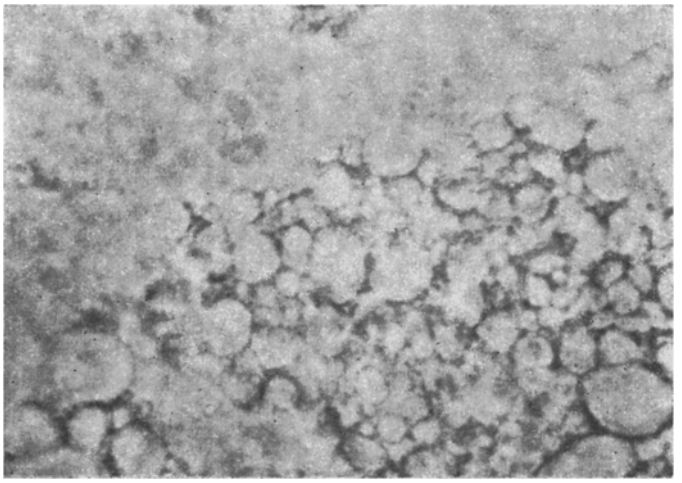
For the isolation of PPLO, the material under study was cultured on 0.3% semi-solid agar which was prepared on a tryptic digest of bovine heart, 10% sucrose, 0.1% magnesium sulfate, or on 0.3% agar without added sucrose and magnesium. In all cases, 10% normal horse serum was added, cultures were set up by culturing the horse serum, magnesium sucrose solutions, and other solutions used in the experiment. The cultures were grown aerobically at 37°.

EXPERIMENTAL RESULTS

The HeLa, Hep-2, Cave, Sots, and human amnion cultures from the 3rd to the 7th day were noted to contain tiny colonies with flat centers characteristic of L-forms and PPLO. They were seen to consist, on phase contrast microscopy, of very tiny granular formations of spherical and vacuolar form (see figure). Sometimes delicate small reticular formations were noted, the presence of which in the opinion of several investigators [2] is a characteristic sign of PPLO.

The results of our investigation confirm the fact that the presence of fibrillar structures in PPLO cultures should not be considered as a distinguishing feature between PPLO and L-forms, for in the majority of the PPLO forms the fibrillar structures were absent.

In order to identify completely the cultures and differentiate them from the cells of the culture, the PPLO was transferred on to 1.3% semi-solid agar containing sucrose, magnesium, and normal horse serum in the afore-mentioned amounts. The PPLO cultures grew out in 3-5 days in the form of very tiny, hardly visible, flat colonies which are characteristic of PPLO.



Colonies of PPLO organisms isolated in tissue cultures.

Morphologically, the colonies were characterized by the presence of tiny spherical and vacuolated forms; sometimes at the edge of the colonies, delicate, hardly noticeable reticular formations were noticed. The growth of the cultures did not depend on the presence of penicillin in the media: they grew well on media without penicillin. PPLO, isolated from HeLa, Sots, and Hep-2 cells were easily grown on subcultures; reversion to bacterial forms was not noticed. The characteristic peculiarity of most PPLO cultures was the extreme resistance to penicillin and sensitivity to several other antibodies such as streptomycin (Pulvertaft, 1953). As our investigators indicated, cultures of PPLO isolated from HeLa and Cave cells were not only resistant to penicillin, but also to streptomycin. They grew well on media containing 10,000 units of streptomycin/ml, apparently as a result of prolonged growth in the presence of this antibiotic in the tissue culture.

PPLO was not isolated from 10 cultures of chick embryonal tissue (each prepared from a single chick embryo).

In studying 12 primary cultures of mouse embryonal tissue from SZN strain, PPLO was observed in three instances. In two of them, colonies were formed consisting of spherical vacuolated forms as well as elongated reticular forms, and in some instances atypical forms appearing like flakes which consisted of tiny, motile, granular bodies.

In order to elucidate the means of entry of PPLO into primary cultures of mouse embryonal tissue, we undertook the following experiments: in separating the embryos and preparing the cellular suspension from them, the presence of PPLO was investigated in the tissue of pregnant mice and of the embryos yielding the cellular suspension as well as all the solutions and nutritive media used in the preparations. In the course of each experiment the following were seeded separately: heart blood, liver, placenta, amniotic fluid and embryonic pulp (after mincing the embryos), a suspension of the resulting cells in trypsin and in growth medium (medium No. 199 + 10% ox serum), and also Hanks's solution, a solution of trypsin and antibiotics, medium 199, and ox serum.

Cultures were grown at 37° for several days in cultures of material from mouse No. 32 of SZN strain and of mouse No. 52. Growth of PPLO was observed on the basis of the fact that there was no reversion in bacterial culture. In mouse No. 32, liver, blood, embryonal pulp grew out atypical colonies on penicillin-free media consisting of spherical forms and clearly outlined vacuoles and elongated reticular forms. In mouse No. 52, on media without penicillin containing sucrose and magnesium, cultures of the cell suspension and of the amniotic fluid yielded a superficial, mutating, diffusing growth which consisted of spherical forms and small granular bodies. The cultures obtained did not survive subcultures and did not revert to bacterial forms. PPLO isolated from mouse organs probably does not cause infection of the animal organs but can serve as a source of infection in primary cell cultures.

On the basis of the data obtained by us, it was concluded that one of the sources of infection of cell cultures can be PPLO which are found in animal organs from which tissue cultures are prepared.

SUMMARY

PPLO were isolated from the transplantable cellular strains HeLa, CaVe, Cois, Hep-2 and human amnion, as well as from the primary tissue of mouse embryo. Microorganisms isolated from HeLa and CaVe were highly resistant to penicillin and streptomycin. Not in a single case was it possible to isolate PPLO from the 10 cultures of chick embryos. One of the sources of primary culture contamination with PPLO are organs of animals from which tissue cultures are prepared.

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