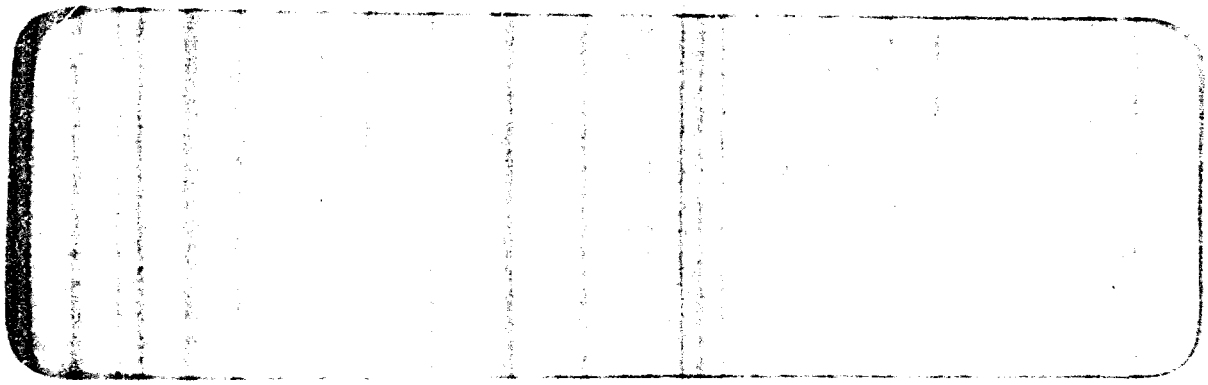

APPLIED MICROBIOLOGY, Jan., 1967, p. 209-210
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Vol. 15, No. 1
Printed in U.S.A.

Elimination of Pleuropneumonia-like Organisms from Tissue Culture

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Pleuropneumonia-like organisms (PPLO) are the crabgrass of tissue culture. Many reports exist in the literature of successful attempts to eliminate these organisms from tissue cultures, but usually such reports were stimulated by the failure of some previously described method to be successful in the hands of the investigator.

Although our cultures exhibited no overt manifestations of contamination, upon culture for PPLO we found that they were heavily contaminated with PPLO. This report describes the successful elimination of PPLO from tissue cells by a combination of treatment with novobiocin (P. Balduzzi and R. Charbonneau, Experientia 20:651, 1964) and heat (L. Hayflick, Nature 185:783, 1960).

TABLE 1. *Treatments attempted to eliminate PPLO from monolayer tissue cultures*[a]

Treatment	Antibiotic		Exposure (hr) to heat (41 C)
	Level	Exposure time	
Kanamycin[b]	1 mg/ml	48 hr	—
Heat	—	—	18
Kanamycin plus heat	1 mg/ml	48 hr	18
Heat plus kanamycin	1 mg/ml	48 hr	18
Novobiocin[c] plus heat	50 μg/ml	7 days	18

[a] Volume of growth medium (medium 199 supplemented with 10% calf serum) was 50 ml for all treatments.
[b] Kantrex, Bristol Laboratories, Syracuse, N.Y.
[c] Cathomycin, Merck, Sharp and Dohme, West Point, Pa.

Table 1 describes the treatments to which monolayer cultures of L (Earle) cells, grown in 32-oz prescription bottles, were subjected in attempts to eliminate the PPLO. After the treatments, the cultures were fed fresh growth medium (medium 199 plus 10% calf serum) and were grown free from antibiotics. Cultures were tested for PPLO about 14 days after treatment, and at periodic intervals thereafter.

PPLO were detected by growth on the agar medium described by R. Chanock, L. Hayflick, and M. Barile (Proc. Natl. Acad. Sci. 48:41, 1962) modified as follows: yeast extract (Difco) was used in place of an extract of baker's yeast, and dextrose (10 g per liter) was added. Inoculated plates were incubated at 37 C in an atmosphere of 95% N_2 and 5% CO_2 for 14 days. A known culture of PPLO was inoculated each time as a positive control.

TABLE 2. *Pleuropneumonia-like organism isolations from contaminated cultures after antibiotic or heat treatment*

Treatment	Days after treatment		
	14	28	240
Kanamycin (1 mg/ml)[a]	Positive		
Heat (41 C for 18 hr)	Positive		
Kanamycin followed by heat	Negative	Positive	
Heat followed by kanamycin	Negative	Positive	
Novobiocin[b] (50 μg/ml) followed by heat	Negative	Negative	Negative
Control	Positive	Positive	Positive

[a] Kantrex, Bristol Laboratories, Syracuse, N.Y.
[b] Cathomycin, Merck, Sharp and Dohme, West Point, Pa.

The results of these experiments are shown in Table 2. Neither kanamycin nor heat treatment alone was capable of ridding the cultures of PPLO. PPLO could not be isolated from cultures treated with a combination of kanamycin and heat 14 days after this treatment. However, after two or three serial transfers of the cells, PPLO could again be detected by culture. Only treat-

ment with novobiocin and heat was effective in ridding the cultures of PPLO. These cultures are still negative for PPLO 8 months after treatment. Another investigator (Carter, *personal communication*) was unable to eliminate PPLO from the contaminated cells with novobiocin and kanamycin but no heat.

That PPLO are difficult to eliminate from tissue culture is obvious from the number of methods described in the literature for their elimination. It appears that not all strains of PPLO are susceptible to the same treatments. The failure to eliminate PPLO from our cultures by either kanamycin or heat alone, techniques successful in the hands of others (J. Foch and C. Hacker, Exptl. Cell Res. 21:242, 1960; L. Hayflick, Nature 185:783, 1960), supports this view. The reappearance of PPLO in the cultures treated with both kanamycin and heat suggests that these organisms in their life cycle might be protected in some way from this adverse environment, and might remain dormant for a period.

It is unlikely that the reappearance of PPLO in these cultures represents reinfection, as (i) the treated cultures were handled only after swabbing the working area with hypochlorite solution and prior to handling untreated cultures, and (ii) PPLO failed to appear in the cultures treated with novobiocin and heat. If PPLO were to appear 8 months after treatment in the cultures treated with novobiocin and heat, they would probably have arisen from an exogenous source.

Treatment of our cultures with novobiocin and heat suffers from being a "shotgun" treatment. However, our primary concern was to eliminate PPLO from our cultures, and this we were successful in doing by this technique.

Although selection cannot be ruled out, the growth characteristics and morphology of the surviving cells, and their ability to produce virus, did not appear to be altered by the treatment.