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TECHNICAL MANUSCRIPT 315

**ELIMINATION OF
PLEUROPNEUMONIA-LIKE ORGANISMS
FROM TISSUE CULTURE**

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SEPTEMBER 1966

**DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland**

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ELIMINATION OF PLEUROPNEUMONIA-LIKE
ORGANISMS FROM TISSUE CULTURE

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ELIMINATION OF PLEUROPNEUMONIA-LIKE ORGANISMS FROM TISSUE CULTURE

ABSTRACT

Tissue cultures heavily contaminated with PPLO were treated with kanamycin (1 mg/ml of tissue culture medium for 48 hours), kanamycin in combination with heat (41 C for 18 hours) or novobiocin (50 mg/ml of tissue culture medium for 7 days) in combination with heat. Only treatment with novobiocin and heat was successful in ridding the cultures of the PPLO contamination.

Pleuropneumonia-like organisms (PPLO) are the crux of tissue culture. Many reports in the literature document 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 our tissue cells by a combination of treatment with novobiocin¹ and heat.²

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 of 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 Chnock, Hayflick, and Barile³ modified as follows: Bacto yeast extract was used in place of an extract of baker's yeast, and dextrose (10 g/liter) was added. Inoculated plates were incubated at 37 C in an atmosphere of 95% N₂ and 5% CO₂ for 14 days. A known culture of PPLO was inoculated each time as a positive control.

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 2 or 3 serial transfers of the cells PPLO could again be detected by culture. Only treatment 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* was unable to eliminate PPLO from the contaminated cells using novobiocin and kanamycin but no heat.

* Richard C. Carter, personal communication.

TABLE 1. TREATMENTS ATTEMPTED TO ELIMINATE PFLO FROM MONOLAYER TISSUE CULTURES^{a/}

Treatments	Antibiotic		Heat 41 C Exposure Time
	Level	Exposure Time	
1. Kanamycin ^{b/}	1 mg/ml	48 hr	-
2. Heat	-	-	18 hr
3. Kanamycin plus heat	1 mg/ml	48 hr	18 hr
4. Heat plus kanamycin	1 mg/ml	48 hr	18 hr
5. Novobiocin ^{c/} plus heat	50 µg/ml	7 days	18 hr

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 2. PFLO ISOLATIONS FROM CONTAMINATED CULTURES FOLLOWING ANTIBIOTIC OR HEAT TREATMENT

Treatment	Days after Treatment		
	14	28	240
Kanamycin (1 mg/ml) ^{a/}	Pos		
Heat (41 C for 18 hr)	Pos		
Kanamycin followed by heat	Neg	Pos	
Heat followed by kanamycin	Neg	Pos	
Novobiocin (50 µg/ml) ^{b/} followed by heat	Neg	Neg	Neg
Control	Pos	Pos	Pos

a. Kantrex[®], Bristol Laboratories, Syracuse, N. Y.

b. Cathomycin[®], Merck, Sharp and Dohme, West Point, Pa.

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,^{2,4} 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 because (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 now 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 its 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.

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