

## Antibiotic Effect of Tylosin on a Mycoplasma Contaminant in A Tissue Culture Leukemia Cell Line.\* (30950)

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In view of the frequent contamination of tissue cultures with organisms of the pleuropneumonia group (PPLO), we are reporting the effectiveness of tylosin tartrate in eliminating such a contaminant from our culture.

During the attempt to plate in agar one of our lines of murine leukemia cells(1), we detected a filterable microorganism which may be related to *Mycoplasma granularum*.†

Several chemotherapeutic and antibiotic agents, namely tetracyclin, kanamycin, erythromycin, chloromycetin, polymixin, stovarsol and iodine had been previously tested with uniformly negative results. Although the cultures frequently seemed "cured" when examined immediately after treatment, PPLO reappeared in the subsequent passages.

*Materials and methods.* Purina tylan soluble (tylosin tartrate), a product of Eli Lilly and Co., is distributed by Ralston Purina Co. Prior to the use of tylan in tissue cultures, it was tested with bacteriologic media for activity against *M. pulmonis* of murine infectious catarrh and *M. granularum*, recovered from tissue cultures. These two species are readily cultivable in 20% horse serum-nutrient bouillon and 48 hours-old cultures were employed. Transfers (.05 ml) were made to serum-bouillon and serum-agar containing respectively 100, 50 and 25  $\mu\text{g/ml}$  of tylan. The two higher concentrations completely suppressed growth of both mycoplasmas in both media, as did also the lowest one with *M. pulmonis*. Growth of *M. granularum* was not completely inhibited, however, in the presence of 25  $\mu\text{g/ml}$  and a few atypical colonies appeared on serum-agar. A concentration of 50  $\mu\text{g/ml}$  was used, therefore, as a trial

amount for tissue culture decontamination. The tylan solution containing 500 mg/100 ml distilled  $\text{H}_2\text{O}$  was filtered before use.

The contaminated cell culture had been maintained for 64 successive passages in Basal Medium Eagle with Earle's solution plus 10% Agamma calf serum, supplied by Grand Island Laboratories.

Each plate containing 10 ml of medium and  $10^6$  cells received 0.1 ml of tylan solution giving a final concentration of 50  $\mu\text{g}$  of tylan per ml of culture medium. This concentration was non-toxic for the cells.

*Results and discussion.* Since a single exposure to tylan (50  $\mu\text{g/ml}$ ) was insufficient to rid the cultures of PPLO, the cells were incubated in the presence of tylan for 2 consecutive passages, each one lasting for 4 days. For the 3rd passage, they were transferred to the same culture medium without tylan and kept untreated for 3 further passages. An untreated cell line was maintained the same way for control.

After the 3rd passage without treatment, the culture was checked for the presence of PPLO using special PPLO plates supplied by Hyland Laboratories. The tylan treated cell line was negative for PPLO, while the control line remained positive.

After being maintained without treatment for 4 additional passages, a second PPLO test was done. In contrast to the untreated cell line used for control, the cultures that had been treated with tylan remained negative.

In view of these findings, the recent paper of Newnham and Chu(2) is of particular interest. They reported the effect of 19 compounds against a variety of avian and mammalian strains of mycoplasma in *broth cultures*. Although we tested a number of these compounds, they proved to be ineffective in ridding our cell line of the PPLO contaminant. Tylosin, which at relatively low concentrations of 0.5 to 2  $\mu\text{g/ml}$  had the highest inhibiting action in Newnham and Chu's

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hands, was active against our tissue culture contaminant, but only when used at considerably higher concentration (50  $\mu\text{g}/\text{ml}$ ).

It would appear that the concentration of each compound reported to inhibit PPLO strains grown in *broth media* may not be the effective dose against these organisms growing in the *tissue culture* environment, where they are perhaps, protected by their close association with the cells. The advantage of tylan is that it can be used at the high concentration necessary to inhibit PPLO in tissue culture, yet it is non-toxic to the cells.

*Summary.* A tissue culture line of murine virus-induced leukemia cells was contaminated by a strain of mycoplasma (PPLO)

which may be or is related to *M. granularum*. This organism was resistant to treatment with tetracyclin, kanamycin, erythromycin, chloromycetin, polymixin, stovarsol and iodine, but sensitive to Tylosin. Two consecutive treatments with Tylosin (Purina tylan) at a concentration of 50  $\mu\text{g}/\text{ml}$  were non-toxic to the cells and were effective in ridding the cultures of PPLO.

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1. Friend, C., Patuleia, M. C., de Harven, E., J. Nat. Cancer Inst., 1966, in press.

2. Newnham, A. G., Chu, H. P., J. Hyg. Camb., 1965, v63, 1.

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### Dissociation of Adult Mouse Liver by Sodium Tetraphenylboron, A Potassium Complexing Agent.\* (30951)

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The dispersion of mammalian tissues into a suspension of single cells has usually been effected by the use of enzymes or of the chelating agent, ethylenediamine tetraacetic acid (EDTA). These agents are in general unsatisfactory, since many tissues are refractory to them and even in the case of those which are dissociated, the cells which are liberated are morphologically damaged.

We report here the dissociation of adult mouse liver *in vitro* into a suspension of single cells by sodium tetraphenylboron (TPB). TPB has also been found to dissociate adult mouse brain. Unlike the action of EDTA or trypsin, the cells released retain the gross morphology of the various cell types *in situ*.

It has generally been accepted that cells in tissues are aggregated by some mechanism in which a divalent cation, usually considered to be  $\text{Ca}^{2+}$ , 'bridges' the negatively charged surfaces of the adjacent cells. TPB is an agent which specifically complexes  $\text{K}^+$  (1)

and does not complex the divalent cations. The dissociation of tissue by a potassium complexing agent suggests that  $\text{K}^+$  is the major cation promoting cell-cell adherence in liver. A coordination mechanism for cellular aggregation is presented and extended to a consideration of the problems of orderly cell movements and of contact inhibition.

*Materials and methods.* Male C3H mice between 2 and 4 months of age were used. The mice were killed by breaking the neck. The livers were removed and placed in a solution containing .05 M sucrose, 0.14 M of NaCl, and .005 M sodium phosphate buffer, adjusted to pH 7.8. The agents to be tested were added to this solution and when necessary the pH re-adjusted with NaOH.

Tetraphenylboron ( $\text{Na}^+$  salt) is obtained from K and K Laboratories, Inc. It is kept frozen as a 1% stock in water and diluted into the dissociation solution just before use. The "sucrose-salt" solution contained .05 M sucrose, .14 M NaCl and .002 M  $\text{Na}_2\text{HPO}_4$ . The pH was adjusted by addition of  $\text{NaHCO}_3$  as indicated.

*Experimental. Dissociation at 4°C.* The

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