SHORT COMMUNICATIONS

The Removal of Mu Particles from *Paramecium aurelia* (Stock 540) by Penicillin

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A number of endosymbionts, such as kappa, mu and lambda, exist in the cytoplasm of the protozoon *Paramecium aurelia* (Sonneborn, 1959; Preer, 1967; Beale, Jurand & Preer, 1969; Jurand & Selman, 1969). The mu particles, responsible for the phenomenon of mate-killing (Beale, 1957), have been studied in relative depth, and considerable information on their structure and maintenance in the cytoplasm of the paramecia has been published (Gibson & Beale, 1962; Jurand & Selman, 1969).

A major problem concerns the ancestry of mu. Most evidence points to mu being procaryotic endosymbionts as their morphology resembles that of such procaryotes as rickettsiae (Jurand & Selman, 1969; Franklin, 1971), and their composition includes proteins, carbohydrates, nucleic acids and lipids, as well as having diamino-pimelic acid in their cell walls (Stevenson, 1965, 1969). Furthermore, recent work has established that mu can survive in an axenic medium (Gibson, Chance & Williams, 1971).

Some research has also been directed at the effect these particles have on their host cells. Comparative studies on paramecia with and without mu are therefore required, and methods to produce mu-free paramecia have been developed. Unfortunately most of these methods involve treatment, such as growth at elevated temperatures, which also affects the paramecia (Sonneborn, 1959; Franklin, 1971), and it would therefore be advantageous if methods known to affect only mu could be used. Penicillin affects the synthesis of bacterial cell walls (Park, 1966), and it is generally accepted that it does not affect eukaryotic cells. The effect of penicillin on the maintenance of mu in paramecia was therefore investigated, with the hope that it might lead to their loss and so provide a further method of producing mu-free paramecia and also give further evidence as to the bacterial nature of mu.

METHODS

Stock 540 *Paramecium aurelia* were used, kindly supplied by Dr I. Gibson, School of Biological Sciences, University of East Anglia, Norwich. The cells were grown at 27 °C in bacterized medium consisting of grass infusion broth inoculated 24 h before use with *Aerobacter aerogenes* (Sonneborn, 1950). For treatment, the organisms were pelleted by centrifugation in an M.S.E. Oil Testing centrifuge, and washed three times with sterile Dryl's solution (Dryl, 1959). Individual paramecia were then placed in a known quantity of Dryl's solution in a depression slide, and penicillin (Benzyl-penicillin, B.P. Glaxo), dissolved in Dryl's solution, was added to produce a concentration of 1000 units/ml.

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Fig. 1. Photomicrographs of mu particles in the cytoplasm of paramecium stained with lacto-orcein, after fixation with osmium tetroxide vapour. Phase-contrast microscopy. Bar represents 1 μ m. (a) Rod-shaped particles in untreated cell; (b) spherical particles in the cytoplasm of paramecium which had been treated with 1000 units/ml penicillin for 48 h.

The fission rate of the paramecia was calculated from the number produced from one cell in 24 h. After treatment for 48 h, the paramecia were stained with lacto-orcein (Beale & Jurand, 1966) which normally shows mu as purple rod-shaped organisms.

RESULTS

The experiments were repeated three times. The mu particles suffered no visible damage during the initial 24 h of treatment but after 48 h some (25, 36 and 29 % respectively) of the paramecia contained no rod-shaped mu. The host paramecia appeared quite normal but contained a number of small, purple-stained, spheres, interpreted as mu protoplasts (Fig. 1 *a, b*). These were lost from the cytoplasm of the host paramecia within three fissions, apparently due to a combination of their destruction and dilution as the host cells continued to divide.

Some of the treated paramecia did not become mu-free. Detailed examination of these failed to show any organisms containing both rod-shaped and spherical mu particles.

The paramecia suffered no visible damage, as determined by phase-contrast microscopy, during the treatment. They swam and divided at the same rate as untreated paramecia. Those organisms that became mu-free were collected and grown in bacterized medium at 27 °C for 18 months. Regular checks on these cultures showed that none of the organisms contained mu.

DISCUSSION

This treatment provides a quick method of producing mu-free paramecia which is both easy to administer and apparently harmless to the host cell; the loss of mu is irreversible.

The change in morphology of the mu indicates that the mu cell wall is either destroyed, or prevented from forming, during the division cycle of the mu. The resulting naked proto-plasts would probably have lost the ability to divide. They were apparently being destroyed within the host cell, possibly due to the alien environment of the host cytoplasm. Thus the mu cell wall appears essential for survival of the mu in the cytoplasm of the paramecia. The fact that no host cell contained both normal and spherical mu suggests that those cells that retained mu did not allow the penicillin to enter their cytoplasm in sufficient concentration to affect the mu.

These results endorse the work of Stevenson (1965) who showed that the chemical make-up of the mu cell wall was similar to that of certain bacteria. It is perhaps interesting to note that kappa, a related endosymbiont found in other strains of *Paramecium aurelia*, are unaffected by penicillin (Williamson, Jacobson & Stock, 1952).

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