Morphological Changes in a Susceptible Strain of *Streptococcus pyogenes* Treated with Streptocin A

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The production of bacteriocins is a property common to various strains of most bacterial species (Reeves, 1965). Bacteriocin-like inhibition associated with group A streptococcal cultures has been described (Kuttner, 1966; Overturf & Mortimer, 1970) and recently our laboratory has reported the isolation of a group A streptococcal bacteriocin, streptocin A, from cultures of *Streptococcus pyogenes* strain FF22 (Tagg, Read & McGiven, 1971). This bacteriocin was shown to be active against a wide range of different Gram-positive organisms but had no detectable action against Gram-negative species. Certain strains of *Staphylococcus aureus* have also been shown to produce bacteriocins (Dajani & Wannamaker, 1969; Gagliano & Hinsdill, 1970) with a range of activity and physical properties similar to those of streptocin A. The present report gives an account of investigations of the morphological changes produced in a susceptible strain of *S. pyogenes* following treatment with streptocin A.

The procedures adopted for the purification of streptocin A have been described in detail elsewhere (Tagg, Read & McGiven, 1973). One ml of a preparation of streptocin A (titre 8) was mixed with 1 ml (containing 1×10^9 colony forming units) of a logarithmic phase Todd Hewitt broth culture of strain FF38, washed twice in phosphate-buffered saline (pH 6.5). Streptocin A was shown to have a bactericidal effect on the susceptible streptococcus. Viability tests on samples taken at various times from mixtures of the streptocin A preparation (titre 8) and washed strain FF38 showed that there was an exponential decline in viability from 2×10^6 colony forming units/ml to no detectable viable organisms after 4 h at 37 °C. Control preparations of strain FF38 in phosphate-buffered saline (pH 6.5) showed no appreciable decrease in viability during a 4 h test period at 37 °C. Comparison of the change in extinction values at 600 nm of the test and control suspensions indicated no differences over 6 h and, hence, that the bacteriocin-treated organisms had not undergone any lysis.

Morphological changes produced in susceptible organisms were studied by electron microscopy of unfixed whole mounts, prepared by critical point drying using a previously described procedure (Cox, Pihl, Read & Nairn, 1972). Ultrathin sections were also prepared, essentially according to the method of Luft (1966), with fixation in 0.15 % (w/v) ruthenium red and 0.1 M-cacodylate buffer (pH 7.6) containing 3 % (w/v) glutaraldehyde, followed by post-fixation in 1 % (w/v) osmium tetroxide. Cultures of the sensitive streptococcus (strain FF38) were examined at time zero, 30 and 120 min after mixing with streptocin A.

Normal untreated organisms had the appearance shown in Fig. 1(a). The individual cocci, arranged in chains, each had a roughly circular outline and the cell contents appeared homogeneous in whole-mount preparations.

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Fig. 1. (a) Whole mount preparation of untreated *Streptococcus pyogenes* strain FF38 arranged in a chain and with a homogeneous appearance. (b) Streptococci after 30 min incubation with streptocin A; there is condensation of nuclear material surrounded by a halo. (c) After 180 min incubation with streptocin A the changes described in (b) are more marked. (d) Ultrathin section of bacteria treated as in (c). The changes in the nuclear material are similar to those seen in the whole mount preparation. Bar markers represent 0.5 μ m.

Within 30 min of contact with streptocin A, visible changes had developed within the central portions of some of the cocci (Fig. 1*b*), apparently consisting of a condensation of the nuclear material with an accompanying increase in the perinuclear space. After 180 min nearly all appeared to be affected (Fig. 1*c*). Ultrathin sections of such organisms (Fig. 1*d*) showed changes consistent with those observed in whole mount preparations. There was no apparent change in the morphological appearance of the walls.

The structural changes produced by streptocin A in a susceptible group A streptococcus seem similar to the changes observed following treatment of a susceptible streptococcus with a staphylococcal bacteriocin (Clawson & Dajani, 1970). There have been only a few other reports of ultrastructural changes in bacteria treated with bacteriocins. The observed changes have been produced by colicins (Beppu & Arima, 1971), pyocins (Ohnishi, Takade & Takeya, 1971) and also boticins (Ellison, Mattern & Daniel, 1971) and are not unlike those described in the present study. Recently it has been shown that streptocin A is also toxic for mammalian heart cells, possibly due to a sharing of specific receptors between sensitive micro-organisms and the heart cell membrane (Tagg, 1972). Our present studies are being directed towards an examination of the ultrastructural changes in heart muscle cells following treatment with purified streptocin A. Such investigations may provide evidence clarifying the possible role of streptocin A in the pathogenesis of rheumatic carditis (Tagg & McGiven, 1972).

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