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Further Observations on the Relationships between Gram-Negative Rods and Staphylococci Grown in the Presence of Penicillin

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SUMMARY: It has not been found possible to repeat the work of Briggs *et al.* (1957) in which Gram-negative rods were isolated from cultures of staphylococci treated with penicillin, and staphylococci were subsequently recovered from cultures of these rods when the latter were grown in the absence of penicillin. No flaw in technique has been revealed which would enable the earlier results to be readily explained in terms of contamination.

In a series of experiments designed to compare the effects of penicillin, cephalosporin C and bacitracin on *Staphylococcus aureus* (NCTC 6571) growing in aerated broth, Crawford & Abraham (1957) observed that Gram-negative rods frequently appeared and multiplied after most of the staphylococci had undergone lysis. When this phenomenon was first encountered we suspected that the Gram-negative organism was a contaminant which had entered an environment favourable for its selection. But when the phenomenon subsequently recurred we recalled that several groups of workers had previously reported that staphylococci underwent a transformation to Gram-negative rods on 'training' to resist high concentrations of penicillin, and that this transformation was reversible (Klimek, Cavallito & Bailey, 1948; Gale & Rodwell, 1948; McVeigh & Hobdy, 1952). We therefore decided that the phenomenon deserved further study under conditions more stringent than those employed by any of the groups which had described it previously. Single cocci were isolated from a culture of the Oxford staphylococcus. Gram-negative rods were isolated from a culture, derived from one of these single-coccus isolates, which had undergone partial lysis in the presence of penicillin; 12 separate rods were thus isolated. After serial subculture in the absence of penicillin 10 of these isolates gave rise to staphylococci of the parental phage type (Briggs, Crawford, Abraham & Gladstone, 1957). The strict precautions taken to exclude contaminants and the controls designed to show that the precautions were adequate were described in detail in that paper.

We concluded from these results that a transformation of the staphylococcus to a rod was responsible for the phenomenon. The evidence for this conclusion, in particular the repeated recovery of staphylococci from cultures of the Gram-negative organisms under conditions in which the staphylococci had no striking selective advantage, seemed at the time to be highly convincing.

During the summer of 1957 re-wiring of the department necessitated the

suspension of work for three months and the cultures available were freeze-dried. Professor S. Elek and Dr G. R. F. Hilson then informed us that they had obtained Gram-negative rods under conditions similar to those that we had used, but were unable to demonstrate a reversion of any strains of our rods to staphylococci. In an attempt to provide them with a freshly isolated strain of our Gram-negative rod we repeated our original experiments in November 1957 under conditions which were apparently identical with those used earlier. On this occasion, however, in repeated experiments we obtained no Gram-negative rods from the Oxford staphylococcus, nor from 14 other coagulase-positive strains of *Staphylococcus aureus*. Several strains of *S. aureus* were also grown on gradient plates containing 0.1 unit penicillin/ml. (Szybalski, 1953) and colonies which formed near the inhibition zone were examined and subcultured. Although many of these colonies contained a large proportion of Gram-negative cocci, and the Gram-positive cocci present were frequently enlarged and distorted, no rods were observed.

Attempts were then made, by the methods used earlier, to recover staphylococci from the cultures of Gram-negative rods which had been stored in the freeze-dried state. These attempts also were unsuccessful. On several occasions small numbers of Gram-positive cocci were observed in cultures of the rod which had been grown in the absence of penicillin in Mitchell flasks (Mitchell, 1949), but these cocci could not be isolated.

We have been unable to uncover any flaw in technique which would allow us to account for our earlier results in terms of adventitious contaminants. Paine & Daniel (1959) suggest that the isolation of single rods was not in fact achieved and that staphylococci were carried over (presumably on ten successive occasions) into the cultures of rod-shaped organism from which they were subsequently recovered. One of us (S.B.), who personally carried out micro-manipulations for the isolation of single rods, considers that this hypothesis is untenable.

Nevertheless, the present failure by Hilson & Elek (1959), by Paine & Daniel and by ourselves to repeat completely the experiments of Briggs *et al.* (1957) must cast doubt on the authenticity of the transformation which these experiments appeared to demonstrate. Since the phenomenon has now been observed by four groups of workers it may well be re-encountered. In that event we hope that our own experience will be helpful in further attempts to establish its true explanation.

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