*Short Communication*

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# *De novo* Synthesis of Streptolysin S (SLS)

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Streptolysin S (SLS) is the non-antigenic, oxygen-insensitive cytolysin which is produced by Group A streptococci. It is largely responsible for a zone of beta-haemolysis surrounding colonies on blood agar media [1]. For the production of SLS, it always requires the addition of a carrier to the culture or resting cell suspension [2]. However, Loridan and Alouf [2] reported for the first time the purification of RNA-core-induced SLS and isolation and haemolytic characteristics of the carrier-free toxin.

The present study was undertaken to investigate whether SLS released by successive inductions with carriers could originate from either (a) presynthesized SLS associated with cell surface layers or in the cytoplasm or (b) *de novo* synthesized SLS during the period of each induction.

In order to test which of these mechanisms was operating the following method [2] was adopted. The culture medium Brain Heart Infusion Broth supplemented with 2% sodium bicarbonate and 1% maltose (BHIB-BM) used for toxin production was that of Loridan and Alouf [2]. *Streptococcus pyogenes* group A Strain C203S (because it produces a high level of SLS, [2]) obtained from Professor Joseph E. Alouf, Institut Pasteur, Paris, was grown in BHIB-BM and after 6h growth, the culture was divided into two aliquotes of 250 ml each (namely 'cells A' and 'cells B') and centrifuged and washed. After each being suspended in 8 ml induction buffer (IB, 100mM-$KH_2PO_4$, 2mM-$MgSO_4$; adjusted to pH 7.0 with NaOH supplemented with 30 mM-maltose), both were induced with RNA-core for SLS release. The presence of SLS was determined by haemolytic assay using trypan blue and cholesterol as controls [2]. The 'cells A' were suspended in induction buffer containing 200 µg chloramphenicol/ml and 'cells B' were induced with induction buffer alone. After induction and centrifugation [2], supernates were collected and analysed for haemolytic activity.

Weak haemolysis was observed at 1/10 dilution of material obtained from 'cells A' but at no higher dilutions, whereas a titre of 1000 HU/ml was found in material obtained from 'cells B' which was suspended in induction buffer without added chloramphenicol (Table 1). On resuspension and induction for a second time, no haemolysis was observed in material obtained from 'cells A' whereas 'cells B' gave a titre of 1000 HU/ml. Induction 3 gave the similar results.

In order to study the reversibility of SLS synthesis, 'Cells A' were suspended and induced in induction buffer alone on the 4th induction cycle, following 3 cycles of induction in the presence of chloramphenicol. On removal of chloramphenicol, the titre of induction cycle 4 was 100 HU/ml. This was also the case after a further induction cycle in the absence of chloramphenicol (in induction 5). Upon induction in the presence of an increased concentration of chloramphenicol (400 µg/ml, in induction 6), no haemolytic material was released thereafter. Removal of chloramphenicol from the

TABLE 1. EFFECT OF CHLORAMPHENICOL ON SLS PRODUCTION.

| Induction no: | Cells A* (250 ml) | HU/ml | Cells B* (250 ml) | HU/ml |
|---|---|---|---|---|
| | IB + 200 µg/ml Cm | | Only IB | |
| 1 | | 10 | | 1000 |
| 2 | | - | | 1000 |
| 3 | | - | | 1000 |
| | Only IB | | | |
| 4 | | 100 | | 1000 |
| 5 | | 100 | | 1000 |
| | IB+400 µg/ml Cm | | | |
| 6 | | - | | 1000 |
| | Only IB | | | |
| 7 | | - | | 1000 |
| 8 | | - | | 1000 |
| 9 | | - | | 1000 |
| 10 | | - | | 1000 |
| 11 | | - | | 1000 |
| 12 | | - | | 1000 |
| 13 | | - | | 1000 |
| 14 | | - | | 1000 |
| 15 | | - | | 100 |
| 16 | | - | | 100 |
| 17 | | - | | 100 |

C2035 was grown in 500 ml BHI-BM . * Culture was divided into two samples of 250 ml named A and B. IB = Induction Buffer. Cm= Chloramphenicol.
The table shown is a mean of three observations and is representative of five separate experiments.

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subsequent induction cycles (induction 7 and 8) yielded no further haemolytic material. The loss of reversibility in the effects of chloramphenicol after induction 6 in 'cells A' indicated an irreversible loss of protein synthetic ability (possibly cell death).

Afterr 8 cycles of induction in control ('cells B'), the titre was 1000 HU/ml. These findings are in keeping with those of Bernheimer [3], who demonstrated by using variety of enzymes poisons that SLS is formed only when the cocci are actively metabolizing and that SLS was not released from a preformed pool.

As it is evident from Table1 that SLS is synthesised *de novo* during each induction cycle and that inhibitors of protein synthesis such as chloramphenicol block its formation. An alternative, but more complex interpretation might involve synthesis of a protein (possibly catalytic) which promotes release of presynthesised SLS. The two possible interpretations were not differentiated by this experimental approach. According to Akao *et al.* [4] the production of SLS by streptococci was inhibited by treatment with the protease inhibitor, tosylphenylalanine chloromethyl ketone (TPCK), even in the presence of the inducer oligonucleotides. Trypsin reversed the effect of TPCK or TLCK. The reversal was dependent upon the amount of added trypsin and the incubation time at 37° suggesting that a protease activity was involved in haemolysin formation. The effect of trypsin was not observed if chloramphenicol was also added, suggesting that a precursor of SLS was processed as it was synthesized and released into medium as the active haemolysin, by the concerted action of a protease and inducer oligonucleotides.

Chloramphenicol blocked protein synthesis of 'cells A' while cells B' (used as control) not exposed to antibiotic were not affected and yielded the same haemolytic titre after each induction (1000 HU/ml). As the activity of such haemolytic material was inhibited by trypan blue but not by cholesterol, it was considered to be due to SLS.

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***Key words:*** Streptolysin S, chloramphenicol, Induction, Haemolysis and Protein.

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