Characterization of the epidermolytic toxins of *Staphylococcus aureus*

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Discovery of Epidermolytic Toxin

My interest in the role of staphylococcal toxins in blistering diseases of the skin arose directly from Dr Lyell's investigation of the Glasgow outbreak of staphylococcal impetigo and the scalded skin syndrome (SSSS) in 1968–9 (Lyell, Dick & Alexander, 1969). The absence of polymorphonuclear infiltration and of stainable organisms in the lesion suggested that the extensive splitting of the epidermis might be due to a diffusible product of the staphylococcus rather than local proliferation of the organism. Our initial study confirmed the low incidence of a positive egg yolk reaction among phage group II staphylococci which correlated with the high incidence of a bacteriocin active against species of *Corynebacterium*. It also revealed the interesting observation that approximately 50 per cent of isolates from extensive lesions produced δ-toxin in excess of α-toxin, whereas those from localized lesions produced α-toxin in excess of δ-toxin. Otherwise no unusual features were noted in the extracellular products of the isolates.

In 1970 Dr Marian Melish and Dr Lowell Glasgow, then working in Rochester, New York, published their seminal paper on the pathogenesis of SSSS (Melish & Glasgow, 1970). During a 16-month period from August 1968 to December 1969 they investigated 17 patients aged 5 days–5 years who presented with a range of symptoms including, extensive epidermolysis, localized bullous lesions or erythema without epidermolysis (the so-called scarlatiniform rash). Phage group II staphylococci isolated from all 17 cases, when injected subcutaneously or intraperitoneally into newborn mice at sublethal doses, uniformly produced a reaction strikingly similar to SSSS in young children. By 12–16 h after injection the skin became loosened when gently stroked (a positive

Nikolsky sign). Within a few hours bullae were evident and the skin began to peel, revealing a moist bright red glistening surface underneath. Fluid aspirated from the lesions was consistently sterile and on histological examination an intra-epidermal cleavage plane was seen at the level of the stratum granulosum.

Melish & Glasgow correctly recognized the significance of their observations in relation to the pathogenesis of SSSS and postulated '. . . a soluble product induces the dermatologic manifestations of this disease'. Confirmation of this hypothesis by isolation of a soluble extracellular toxic factor now known as epidermolytic toxin (exfoliative toxin (ET)) that caused intra-epidermal cleavage was soon reported by several workers (Melish, Glasgow & Turner, 1970; Kapral & Miller, 1971; Arbuthnott *et al.*, 1971). These early observations triggered an explosion of interest and within the next few years a large number of publications covering many aspects of ET appeared in the bacteriological, dermatological and paediatric literature and it was some time before a coherent picture emerged. The literature has been reviewed comprehensively (Elias, Fritsch & Epstein, 1977; Rogolsky, 1979) and the purpose of this contribution is to summarize briefly the characteristics of ET.

Production and Purification of ET

Factors affecting the production of epidermolytic toxin in culture are still poorly understood. Melish, Glasgow & Turner (1972) reported that several media failed to support toxin production *in vitro* and the method selected by this group was to culture strains in dialysis sacs, containing tissue culture medium, implanted in the peritoneal cavities of rats. Most other groups used *in vitro* methods, employing complex media in semi-solid agar or liquid shake cultures in the presence of CO_2 at a concentration of 10–20 per cent (v/v) in the gaseous atmosphere. However by making use of a yeast diffusate/casamino acids medium described for α-toxin production (Bernheimer & Schwartz, 1963), Arbuthnott, Billcliffe & Thompson, (1974) were able to achieve yields of 200–5000 units per ml of ET in 48 h shake cultures in the absence of added CO_2. Recently the consistency and yield of toxin production in this medium has been improved by buffering the medium to an initial pH of 7.4 with 0.07 M sodium phosphate.

Between 1973 and 1976, five groups published findings of purification studies (Kondo, Sakurai & Sarai, 1973; Arbuthnott *et al.*, 1974; Johnson, Metzger & Spero, 1975; Dimond & Wueper, 1976; Wiley, Glasgow &

Rogolsky, 1976). Molecular weight estimates ranged from 24 000–32 000 and there were conflicting opinions about heat sensitivity, metal ion dependence and the existence of multiple forms detected by electrophoresis and isoelectric focusing. Some of these discrepancies were resolved when it was shown that at least two serotypes of ET exist that differ in heat stability (Kondo, Sakurai & Sarai, 1974). More recently these serotypes, which have been designated as TA and DI, ExA and ExB and serotype 1 and 2 toxins by different authors, have been analysed in detail (Kondo, Sakurai & Sarai, 1976; Johnson *et al.*, 1979; Bailey, de Azavedo & Arbuthnott, 1980). Bailey *et al.* assessed the amino acid analysis data obtained in different laboratories; they concluded that the degree of similarity was such as to suggest that the three research groups had isolated the same two serotypes of ET and advocated the adoption of a common terminology. Following the nomenclature used for staphylococcal enterotoxins the two serotypes would be designated ETA and ETB and this is the notation that will be used in this paper. The main properties of ETA and ETB are summarized in table I.

TABLE I

The properties of staphylococcal epidermolytic toxins *

Property	ETA	ETB
Molecular weight	30 000	29 500
Isoelectric point	7.0	6.9
Heat sensitivity	Heat stable	Heat labile
N-terminal amino acid	Glutamic	Lysine
C-terminal amino acid	Lysine	Lysine
No. of tryptic peptides†	33	27
Genetic control	Chromosomal	Plasmid

* The physico-chemical presented here is take from Bailey *et al.*, 1980.
† Peptide mapping has revealed only 4 tryptic peptides are common to ETA and ETB.

Recently the amino acid sequence of the first 25 residues of each toxin has been determined (Johnson *et al.*, 1979) Although the residues showed no substantial length of identical sequence, there was evidence of a possible homologous relationship between ETA and ETB. However Bailey *et al.*, (1980) concluded from peptide mapping of tryptic digests of the two

serotypes that the amino acid sequences must be different. Clearly further amino acid sequence data are required to define the degree of relatedness with confidence.

Incidence of ET Production by *S. aureus*

Although the association of phage group II staphylococci with SSSS is well established and was first shown by Parker *et al.* (1955), epidermolytic strains belonging to phage groups other than group II have been described by several authors.

TABLE II

Summary of the incidence of different serotypes of epidermolytic toxin (ET) produced by strains isolated from impetigo and the scalded skin syndrome in Japan*

	No. of strains (per cent) with a particular ET serotype	
Toxin serotype†	Phage group II strains	Non-phage group II strains
ETA	9 (37.3)	0 (0)
ETB	0 (0)	15 (78.9)
ETA + ETB	14 (58.3)	2 (10.5)
No ET detected	1 (4.2)	2 (10.5)
Total no. of strains	24	19

* From Kondo *et al.* (1975).

† Toxin serotype was determined by screening strains on nutrient agar containing specific antiserum.

There have been relatively few studies of the incidence of ET production by clinical isolates. Kapral (1974) detected ET production in culture filtrates of 40 per cent of 200 randomly selected phage group II strains; also 19 non-group II isolates in a survey of 1000 randomly selected strains produced the toxin. Japanese workers have described a relatively high incidence of non-group II strains associated with SSSS and found that most non-group II isolates produced only ETB, whereas group II isolates produced either ETA or a mixture of ETA and ETB (table II).

In a survey of our collection of 116 strains isolated in the United Kingdom and Ireland over a number of years (de Azavedo & Arbuthnott,

1981) we found a different pattern of results (table III). Production of ETB alone was found in 15.5 per cent of the 84 phage group II isolates; the majority of strains produced either ETA alone or a mixture of ETA and ETB. The incidence of ET production by non-group II isolates from blistering conditions of the skin was low in our study but it is interesting to note that most of the toxinogenic isolates produced only ETA. As yet the reasons for the appearance of different ET phenotypes in different geographical locations is unknown.

TABLE III

Summary of the incidence of different serotypes of epidermolytic toxin (ET) produced by strains isolated from blistering conditions of the skin in United Kingdom and Ireland *

	No. of strains (per cent) with a particular ET serotype	
Toxin serotype†	Phage group II strains	Non-phage group II strains
ETA	31 (36.9)	6 (18.7)
ETB	13 (15.5)	1 (3.1)
ETA + B	28 (33.3)	3 (9.4)
No ET detected	12 (14.3)	22 (68.8)
Total no. of strains	84	32

* de Azavedo and Arbuthnott, 1981.

† Toxin serotype was determined by testing concentrates of culture filtrates against specific antiserum. Concentrates were also tested by bioassay in newborn mice. Non-toxin producing strains were checked by injecting live organisms subcutaneously in newborn mice.

Genetic Regulation of ET

Almost all the existing information on the genetic regulation of ET production stems from the work of Rogolsky and his co-workers. The capacity to produce ET was either completely or partly lost when strains were grown at high temperature in the presence of ethidium bromide. This suggested that a plasmid might be implicated in the expression of the toxin (Rogolsky *et al.*, 1974;); the possibility of ET being under the control of a lysogenic phage was excluded on several grounds. Many epidermolytic strains also

produced a bacteriocin and in some cases loss of ET coincided with loss of bacteriocin production suggesting that these two determinants might be linked on the same plasmid (Warren *et al.*, 1974). Moreover, a plasmid of '56 S' in the covalenty closed circular form identified by sucrose density gradient centrifugation correlated with the ET and bacteriocin markers (Warren *et al.*, 1975).

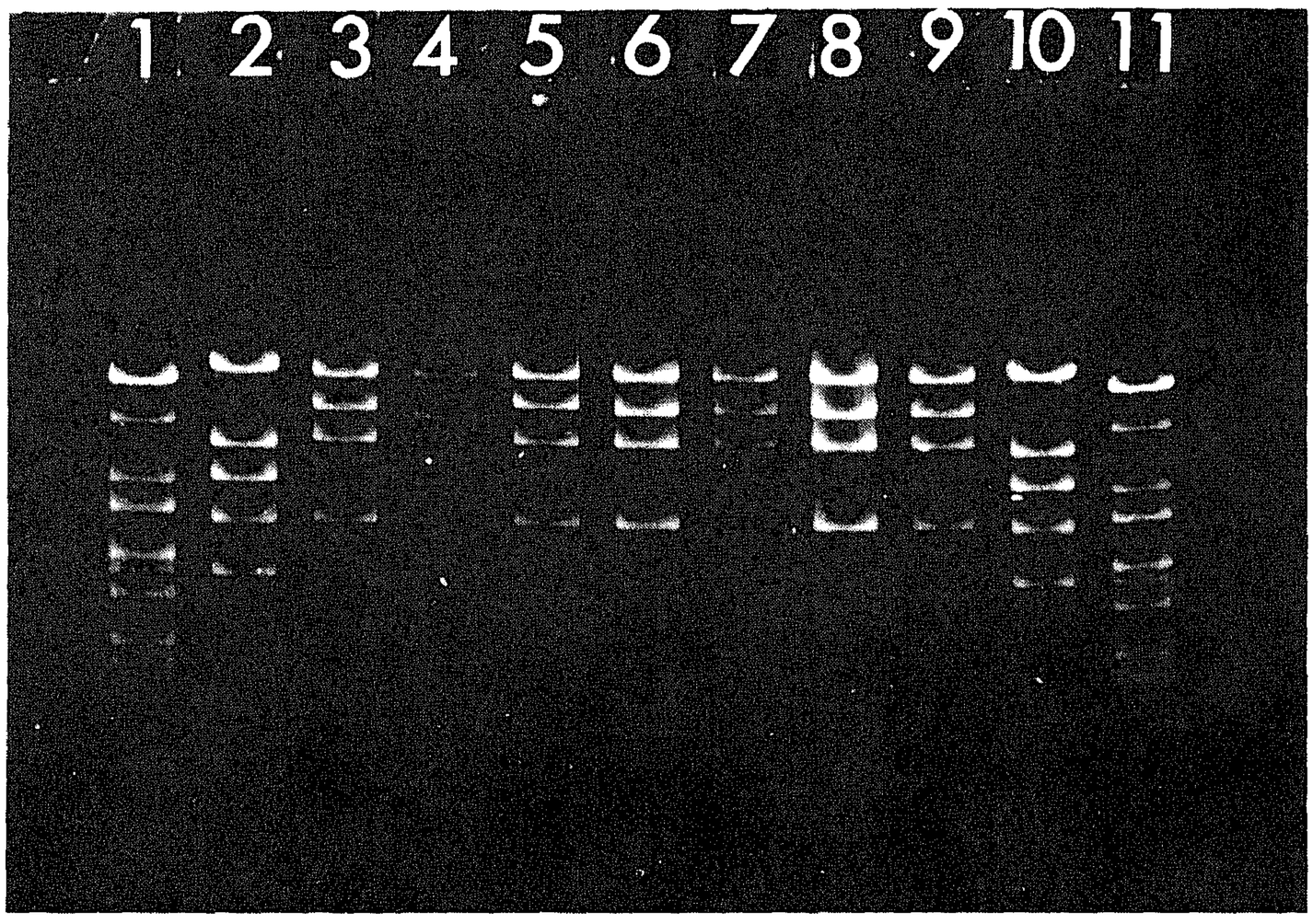

Fig. 1 Fragments produced by treatment with the restriction enzyme EcoRI of several 45 kilobase plasmids isolated from epidermolytic strains of differing pheno-type. Tracks 1 and 11 together with 2 and 10 show molecular weight standards produced by cleaving λ phage DNA with EcoRI and BamHI (tracks 1 and 11) and with EcoRI alone (tracks 2 and 10). The plasmids tested were isolated from strains with the following phenotypes: ETB+ Bac+ Cad^r (tracks 4, 5 and 8); ETA+ ETB+ Bac+ Cad^r (tracks 6 and 7); ETB+ Bac+ (track 3); ETA+ Bac+ (track 9). Identical patterns of fragments can be seen in tracks 3, 6, 7, 8 and 9. There is a difference in the position of 1 band in tracks 4 and 5 and it is interesting to note that the greatest difference is in track 4 which shows the plasmid from a phage group I strain.

In some toxin producing strains however the capacity to form ET could not be completely eliminated by curing which suggested that in some strains

both plasmid and chromosomal determinants might be involved in ET synthesis (Rogolsky, Wiley & Glasgow, 1976). Further evidence indicated that the chromosomally specified ET was antigenically distinct from that which was plasmid determined (Wiley & Rogolsky, 1977).

We have made use of highly specific antisera to ETA and ETB and the gel electrophoresis screening technique for plasmid DNA to investigate further the genetic basis of ET production (O'Reilly, Dougan, Foster & Arbuthnott, 1981). The plasmid content of a number of strains representing different toxin phenotypes and phage groups was determined. This allowed tentative assignment of phenotypic markers to particular plasmids and plasmid DNA molecules were compared by restriction enzyme analysis. These experiments revealed that ETB and/or bacteriocin production were linked to a *ca* 42 kilobase plasmid; loss of this plasmid on curing was associated with loss of ETB and/or bacteriocin. By contrast none of 12 ETA producers could be cured of toxin production and ETA is presumably chromosomally determined. It is interesting to note that some ETA producing strains also contain a 42 kilobase plasmid associated with bacteriocin production and that restriction enzyme analysis revealed similarities among several 42 kilobase plasmids isolated from strains of different phenotypes (Fig 1). Thus although there is now strong circumstantial evidence that ETB is a plasmid determined character, definitive evidence on the genetic regulation of ETB requires plasmid transfer experiments.

Mechanism of Action of ET

Perhaps the most challenging problem of all remains the mode of action of ET. The toxin exhibits a marked species specificity and of the many animals tested only humans, mice, monkeys and golden hamsters were found to be sensitive (Elias, Fritsch & Mittermayer, 1976; Fritsch, Kaaserer & Elias, 1979) and most experimental work has been done using the mouse. Initially it was thought that only newborn mice were susceptible to ET. However epidermal splitting can be demonstrated in the skin of adult mice, though with difficulty, and there is no doubt that glabrous areas of adult skin are sensitive as is the skin of adult hairless mice (Kapral & Miller, 1972; Arbuthnott *et al.*, 1973).

The ultrastructural changes in the epidermis that follow administration of ET have been well described (see review by Rogolsky, 1979). The first event involves the formation of fluid-filled gaps between cells in the stratum granulosum along a horizontal cleavage plane with the disappearance of the small vesicles normally present in the intercellular space. Subsequent

splitting of the desmosomes leads to the formation of extensive clefts in the epidermis. Despite the fact that the nature of the molecular mechanism that disrupts the normal forces of adhesion between keratinocytes in the epidermis is as yet unknown, some interesting negative findings have been reported. The toxin does not cause cytolysis of cells in the region of the epidermal split and it does not remove the glycocalyx from these cells (Ellias *et al.*, 1975). There is as yet no convincing evidence for the existence of a specific receptor for the toxin in mouse skin (Baker *et al.*, 1978) and no enzymic activity has been implicated. Several laboratories are currently involved in studies of this difficult problem and Kondo (1980) has recently described the partial characterization of an inhibitor of ET isolated by extraction of mouse skin with SDS.

Conclusion

The last decade has seen the description and characterization of a new staphylococcal toxin that is responsible for the histological changes seen in SSSS. Understandably this has created much interest in the field of staphylococcal research as only one other toxin, enterotoxin, has been shown to play a predominant role in causing the symptoms of staphylococcal disease. Progress in the characterization of ET has been rapid and the next few years should see further advances in our knowledge, especially in the genetic regulation of ET production and the mechanism of action at the molecular level. However the many other staphylococcal toxins should not be dismissed as unimportant and it is to be hoped that there will be continued attempts to evaluate the role of these factors in staphylococcal pathogenesis.

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