ACTA MEDICA IRANICA
Vol. XIX. 1976, P. 47-52

The Staphy lococcal Exfoliative Toxin Development of a Modified Assay System

MASSOUD KEYHANI, D.V.M., M.S.

INTRODUCTION

Certain strains of **Staphylococcus aureus** produce a toxin which has been associated with specterum of clinical disease entities termed the staphylococcal scalded skin syndrome (SSS). Scalding of skin is characterized by exfoliation of the superficial layer of the epidermis within the granular cell layer (6,7). This exfoliative dermatitis has been known as Ritter's disease when it occurs in newborn infant and as toxic epidermal necrolysis (Lyell's disease) in older individuals (4,5,9). The toxin responsible for exfoliation of the skin in the scalded skin syndrome has been purified and termed exfoliative toxin (ET) (1,8). ET activity is stable upon storage at 4 C. for five months and is heat stable to 59 C. for 30 min (8). The exfoliative toxin has been found to be antigenic, acid labile and have a molecular weight of approximately 24000 (2,3). An experimental mouse model for the detection of ET was developed by Melish and Glasgow (6). Exfoliative toxin producing **Staphylococcus aureus** or unconcentrated material from these bacterial cells cause epidermal exfoliation after being injected either subcutaneously or intraperitoneally into newborn mice. **Staphylococcus aureus** strains which are ET negative do not cause exfoliation under these conditions.

Associate professor, Department of Microbiology and Infections Diseases of Animals, Faculty of Veterinary Medicine, University of Tehran.

In this communication attempts were made to develop a more sensitive assay system for detection of ET in strains that activity could not be detected by the standard assay procedure of Melish and Glasgow (6).

Materials and Methods

Bacterial Strains

Staphylococcus aureus strains of phage group, I, II and III were used in these studies. These strains are listed in Table 1. The staphylococcal strains came from the culture collection of B. Wiley, University of Utah. Exfoliative toxin positive strains are classified by their ability to produce epidermal exfoliation in newborn mice after being tested by the assay procedure described by Melish and Gasgow (6), whereas exfoliative toxin negative strains produce no exfoliation in newborn mice if assayed by this procedures.

Culture medium

The medium used for growing cultures and toxin production was heart infusion broth (Difco). Microorganisms were cultured in this medium with stirring at 37 C. under an atmosphere of 10 % CO2.

Trypticase soy agar medium was used as the solid medium for maintaining the cultures.

Mice

Eighteen-day pregnant mice were housed in individual cages with food and water. After delivery, the newborn mice were selected random and redistributed to the lactating females. Newborn mice one to five days old were exclusively used in this experiment.

Assay of exfoliative toxin

Staphylococcal strains were inoculated in heart infusion broth and incubated for 72 hours in a CO2 atmosphere at 37 C. on a new brunswick model G76 Gyrotary water bath shaker. Exfoliative toxin from positive

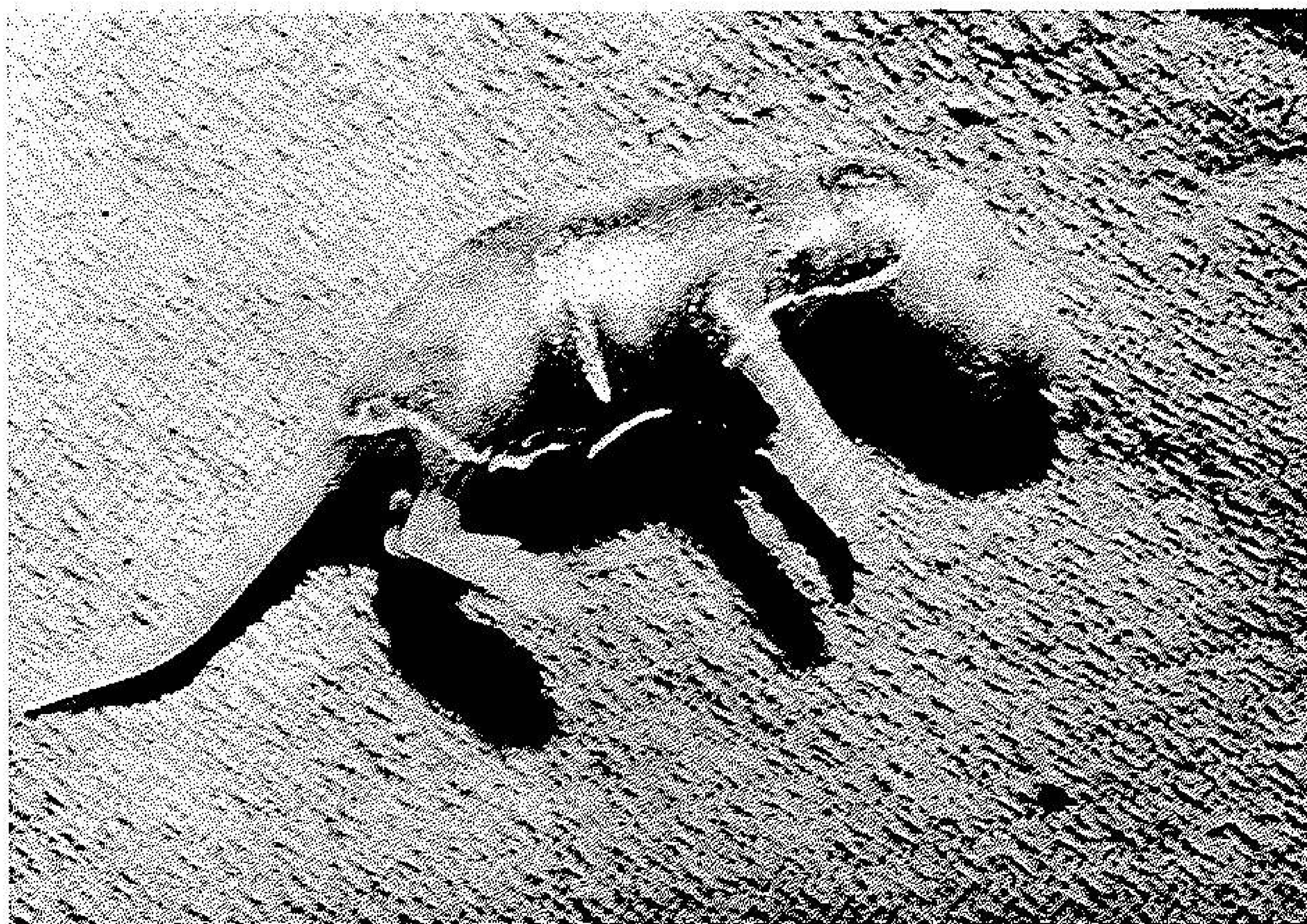
strains was then injected into the scapular area of a mouse under 5 days age. Eighthee n to 24 hours after injection of ET producing staphylococci, the epidermal area at the site of injection remained wrinkled after gentle stroking, producing evidence of Nikolsky sign which is characteristic of SSS. The affected epidermal area could then be easily rubbed loose to reveal underlying erythematous moist, glistening tissue.

RESULTS

Phage group I staphylococcal strains 1001 and 1002, phage group II strains 0013, 0014, 0015, 0018 and 04081 and group III 0639 and 0645 are all exfoliative toxin negative if assayed for ET activity by the standard method of Melish and Glasgow (6). However, if these strains were assayed by the modified assay system presented in this paper all produced epidermal exfoliation within 18 hours after injection into newborn mice (Table I).

In the modified assay system for detecting exfoliative toxin production, staphylococcal strains to be tested were grown in 250 ml flasks containing 100 ml of heart infusion broth (Difco). The flasks were incubated with skaking at 37 C. on a new Brunswick model G 76 Gyrotory water bath shaker covered by a plexiglass hood that was flushed with 100 percent Co2 twice daily. After 72 hours of incubation, the cells were separated by centrifugation at 10,000 X g for 30 minutes and discarded.

Ammonium sulfate was added to 500 ml of cell free supernatant to a final concentration of 80 percent. Crude extracellular material from exfoliative toxin negative strains were concentrated 20 fold by disolving the precipitated material from 500 ml culture into 25 ml of phosphate buffered saline prior to subcutaneous injection into newborn mice. Twenty fold concentrated extracellular material from all of these ET negative strains produced exfoliation when tested by the modified assay system (Fig. 1).



DISCUSSI5N

Exfoliative toxin producing staphylococcal strains as opposed to ET negative strains produce epidermal exfoliation within 18 hours subcutaneous or intraperitoneal inoculation into newborn mice. However if extracellular supernatant material from the naturally occuring ET negative strains was concentrated 20 fold and inoculated into newborn mice, ET activity could be detected (Table I). From these observation it can be concluded that the modified assay system presented in this paper is more sensitive to detect ET activity in staphylococcal strains. The results of these studies shows that the naturally occuring ET negative strains do not produce great enough exfoliative toxin to be detected by the standard method of Melish and Glasgow (').

Table 1

Assay of exfoliative toxin activity in strains of staphylococci by the standard and modified assay system

Strains	Phage group	ET activity as measured by Standard method A	ET acivity as measured by modified assay system B
1001	I		+
1002	I	-	+
0013	II	-	+
0014	II	-	+
0015	II	-	+
04081	II	-	+
0639	III	-	+
0645	III	-	+
0007	II	+	+
0001	III	+	+

A. The standard assay procedure invoves direct inoculation of staphylococci into newborn mice as described by Melish and glasgow (6).

B. Tne modified assay system involve the inoculation of 20 fold concentrated extracellular supernatant material into newborn mice as described in the results.

SUMMARY

Certain strains of **Staphylococcus aureus** produce a toxin which cause epidermal exfoliation within 18 hours after direct subcutaneous or intraperitoneal injection into newborn mice. The extracellular product responsible for exfoliation is termed exfoliative toxin (ET). In the present studies a modified assay system has been developed for detection of exfoliative activity in toxin negative strains as determined by standard method of Melish and Glasgow. If extracellular supernatant material from naturally occuring ET negative strains was concentrated 20 fold and inoculated into newborn mice, exfoliative toxin activity could be detected.

RÉSUMÉ

Certaine souches de **Staphylococcus aureus** produit un toxin qui provoque épidermale exfoliation pendant 18 heures aprés l'injection sous-cutanée ou intraperitoneale directe chez la souris nouveau-né.

Le produit extracellulair responsable de cette exfoliation est nomé toxine exfoliative (TE). Dans cette étude un système modifié d'essai a été développé pour la detection d'exfoliative dans les souches negative-toxin qui étaient negatives par la méthode standarde de Melish et Glasgow. Si la substance supernatante extracellulaire des souches naturellement TE negatives est concentrée 20 fois et inoculée chez la souris nouveau-né l'activité de toxin exfoliative peut-être detectée.

This work was carried out by the author who was the recipient of a Senior Fullbright Fellowship 73-1î5-A at the Univeraity of Utah, U.S.A.

REFERENCES

1. Kondo, I., S. Sakurai and Y. Sarai, 1973. Purification of exfoliation produced by **Staphylococcus aureus** of bacteriophage group 2 and its physiochemical properties. Infect. and Immu. 8: 156-164.

2. Kapral, F. A. and M.M. Miller, 1971, Product of **Staphylococcus aureus** resposible for the scalded skin syndrome. Infect. and Immun. 4: 541-545.

3. Lyelle, A. 1967. A review of toxic epidermal necrolysis in Britain. Brit. J. Derm. 79: 662-671.

4. Loweny, ED., JV. Baublis and GM. Kreye, 1967. The scalded skin syndrome in small children. Arch. Derm. (Chicago) 95: 359-369.

5. Lyell, A., HM. Dick and JO. D. Alexander 1969. Outbreak of toxic epidermal necrolysis associated with staphylocci. Lancet 1: 787-790.

6. Melish, M. E., and L.A. Glasgow, 1970. The staphylococcal scalded skin syndrome. Development of an experimental model. New Eng. J. Med. 282: 1114-1119.

7. Melish, M. E., L. A. Glasgow, and M. D. Turner. 1972. The staphylococcal scalded skin syndrome: Isolation and partial characterization of the exfoliative toxin. J. Infect. Dis. 125: 129-140.

8. Melish, M. E., and L. A. Glasgow, 1971. Staphylococcal scalded skin syndrome: The expanded clinical syndrome: J. Pediat. 78: 958-967.

9. Tyson, RC., SC. Ushinski and R. Kisilevsky, 1966. Toxic epidermal necrolysis (The scalded skin syndrome): Its association in two cases with pathogenic staphylococci and its similarity in infancy to Ritter's disease. American J. Dis. Child. 111: 386-392.