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Characterization of Staphylococcal Exfoliative Toxin

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Coagulase positive staphylococci of phage group II have been associated with a spectrum of dermatologic changes ranging from bullous impetigo, localized at the infection site, to generalized skin Involvement with or without bulla formation and exfoliation. The primary staphylococcal infection associated with generalized exfoliation may be a site distant from the skin and its clinical manifestation termed the staphylococcal scalded skin syndrome (14,15). This syndrome is characterized by exfoliation of the superficial layer of the epidermis within the granular cell layer (11).

A generalized dermatitis Occurring in foals, infants and children, with lesions which Strikingly resemble those produced by scalding, has been associated with these organisms. This exfoliative dermatitis has been known as

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Ritter's Disease when occurs in the new born infant and as Toxic Epidermal Necrolysis (Lyell's disease) in older Individuals (Fig.1) (12,13). Although the association of staphylococci with these diseases had been noted, only recently did Melish and Glasgow (16) report the development of an experimental animal model and clearly establish an etiologic relationship. Subsequently, Arbuthnott et al (1) and Kapral and Miller (6) reported that culture filtrate of staphylococci belonging to the phage group II could cause a wide-spread exfoliation of the epidermis when injected subcutaneously or intraperitoneally to neonatal mice within 5 days of age. On the other hand, Melish and Glasgow(16) proposed that the generalized exfoliation in Ritter's disease and Bullous impetigo were different clinical manifestation resulting from the same etiology. More recently, Kapral and Miller (6) demonstrated an extracellular product of Staphylococcus aureus responsible for the generalized exfoliation in neonatal mice and named it exfoliative toxin (ET). Arbuthnott et al (1) also reported that epidermal necrolysis could result from the action of a diffusible toxic product of phage group II staphylococci

In this communication an attempt was made to review the research work on staphylococcal exfoliative toxin, development of an experimental model for detecting the exfoliative toxin production, purification and chemical characterization of exfoliative toxin and finally, the nature of the genetic Determinant controlling exfoliative toxin production in Staphylococcus aureus.

Assay of exfoliative toxin activity

An experimental mouse model for the detection of exfol-

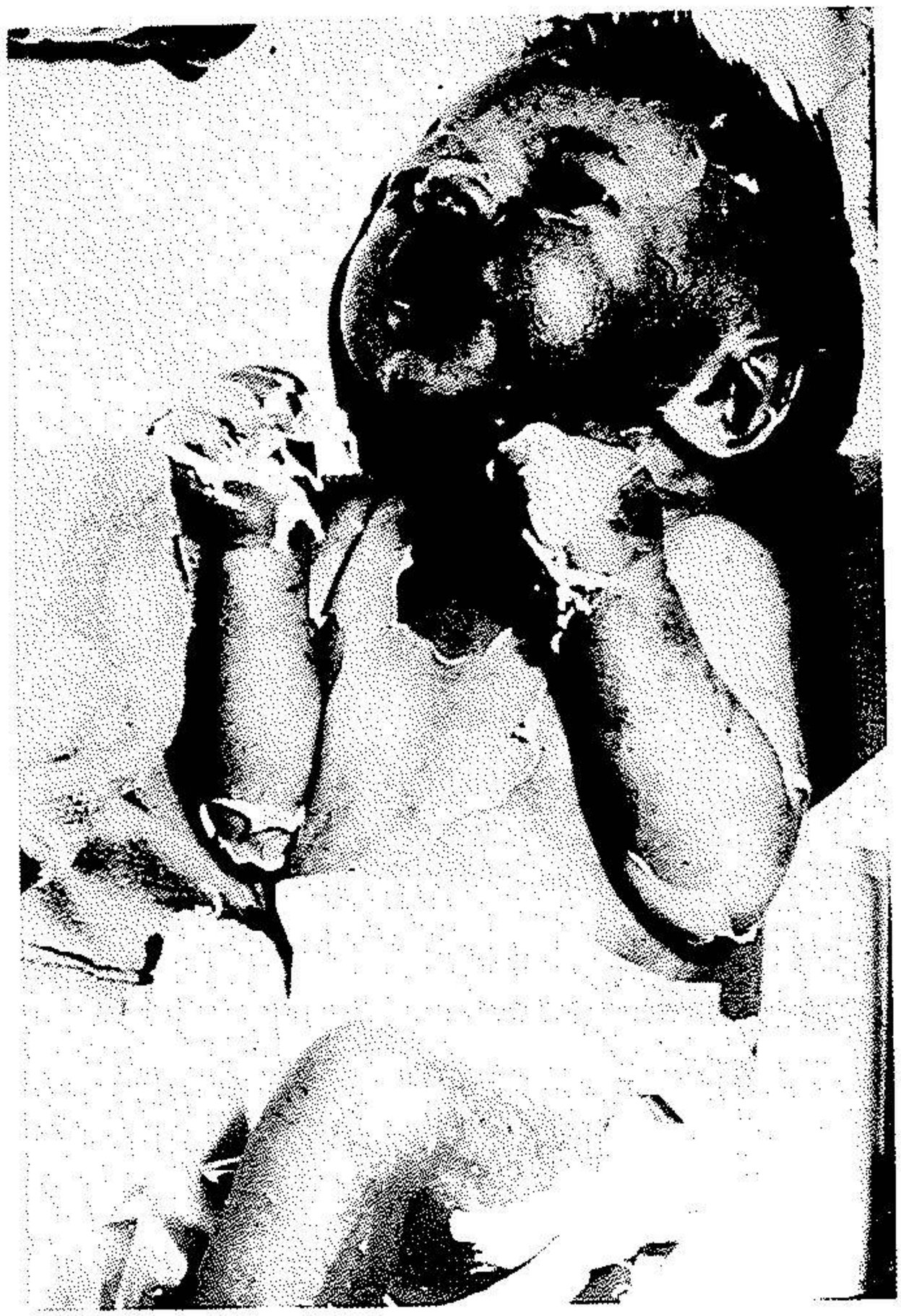

Fig.1. Exfoliative toxin of Staphylococcus aureus produces exfoliation in new born infant

iative toxin is developed by Melish and Glasgow (14,15). Exfoliative toxin (ET) producing staphylococcal strains as opposed to ET negative strains produce epidermal exfoliation within 18 hours subcutaneous or intraperitoneal inoculation into new born mice (Fig. 2). In order to carry out experiments for the detection of exfoliative toxin production, eighteen -day pregnant mice are housed in individual cages. After delivery, the new born mice are selected at random and redistributed to the lactating females. Each new randomized litter consisted of 10 to 12 mice and constitute a single experimental group. The response of new born mice to injection of staphylococci is dose related. For each strain a characteristic dose of staphylococci is lethal within 10 hours of injection. This lethal dose range from 10^7 organisms to as high as 10^{10}. The animals die without any visible reaction at the site of injection or in the skin. A 10 fold dilution of the lethal dose of staphylococci produces a reaction of severe scalded skin syndrome in mice one to five days old. This dose is referred to as the effective dose. A further 10 fold dilution " subeffective doses" produces no gross evidence of disease in the mice. After 16 to 18 hours of the injection the skin appears intact, but light stoking reveals exfoliation of the skin. When staphylococci are inoculated by the intraperitoneal route, a typical exfoliative reaction is seen in animals receiving phage group II staphylococci. The effective intraperitoneal dose is 10 times higher than the subcutaneous dose.

The author has presented a modified assay system(7) for detection of exfoliative toxin in toxin negative

staphylococcal stains as determined by standard method of Melish and Glasgow (14). In this modified method, the extracellular Supernatant material from naturally Occurring ET negative strains is concentrated 20 fold and inoculated into new born mice, exfoliative toxin activity could be detected. The modified assay system is more sensitive to detect ET activity in staphylococcal strains which do not produce great enough exfoliative toxin to be detected by the standard method of Melish and Glasgow.

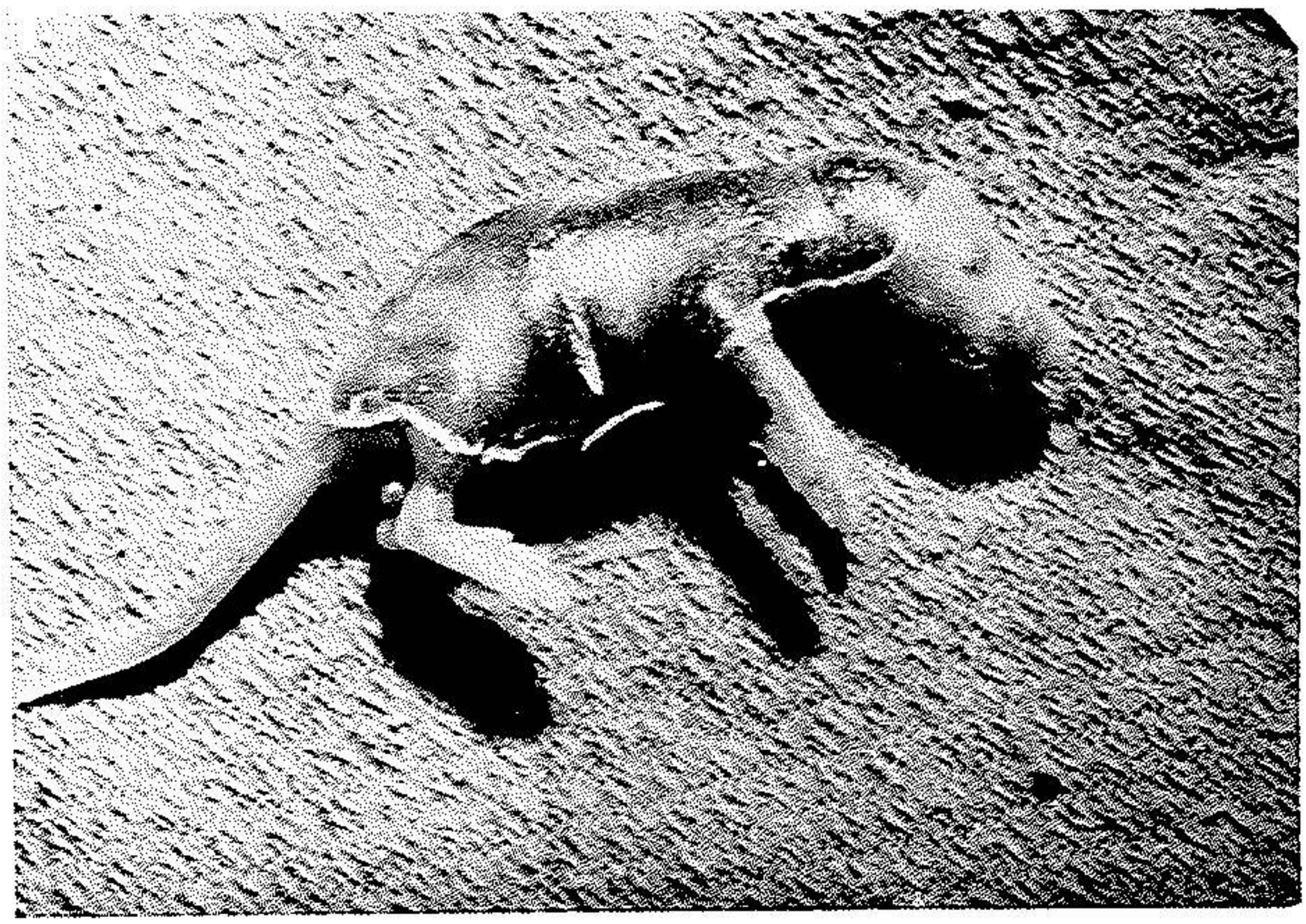

Fig.2. Response of newborn mice to exfoliative toxin of Staphylococcus aureus after subcutaneous injection.

Preparation and purification of Exfoliative Toxin

Different methods for the production and purification of exfoliative toxin have been reported, ranging from production in dialysis bags implanted in the Pretoneum of the rabbit (17) to in vitro production in shaking flasks (9). The author used the following method for production and purification of exfoliative toxin (8). Staphylococcal strains are grown in 250 ml flasks containing 100 ml of heart infusion broth (Difco). The flasks are incubated with shaking at 37 C. on a new Brunswick model G 76 gyrotory water bath shaker covered by a plexiglass hood that is flashed with 100 % CO^2 twice daily. After 72 h. incubation,the cells are separated by centrifugation at 10,000 X g for 30 min. and discarded. Ammonium sulfate is added to a liter of cell-free supernatant to a final concentration of 80% and ET is purified from the resulting crude material by the electrofocussing procedures described by Melish et al (17) (Fig.3.).Electrofocused ET give a single band when electrophoresed on polyacrilamide gel indicating that it is free of other contaminating staphylococcal extracelluar products (Fig.4). Purified preparation of ET is shown to be free of alpha toxin activity by their inability to lyse rabbit erythrocytes.

Another successful method of production and purification of exfoliative toxin has been reported by Johnson et al (5). The exfoliative toxin is produced under Controlled conditions of fermentation. A 2 Gram portion of the crude lyophilized toxin is dissolved in 200 ml of 0.01 M phosphate buffer, pH 6.0. A column (2.5 by 40 cm) is filled with carboxymethyl cellulose and the toxin is run

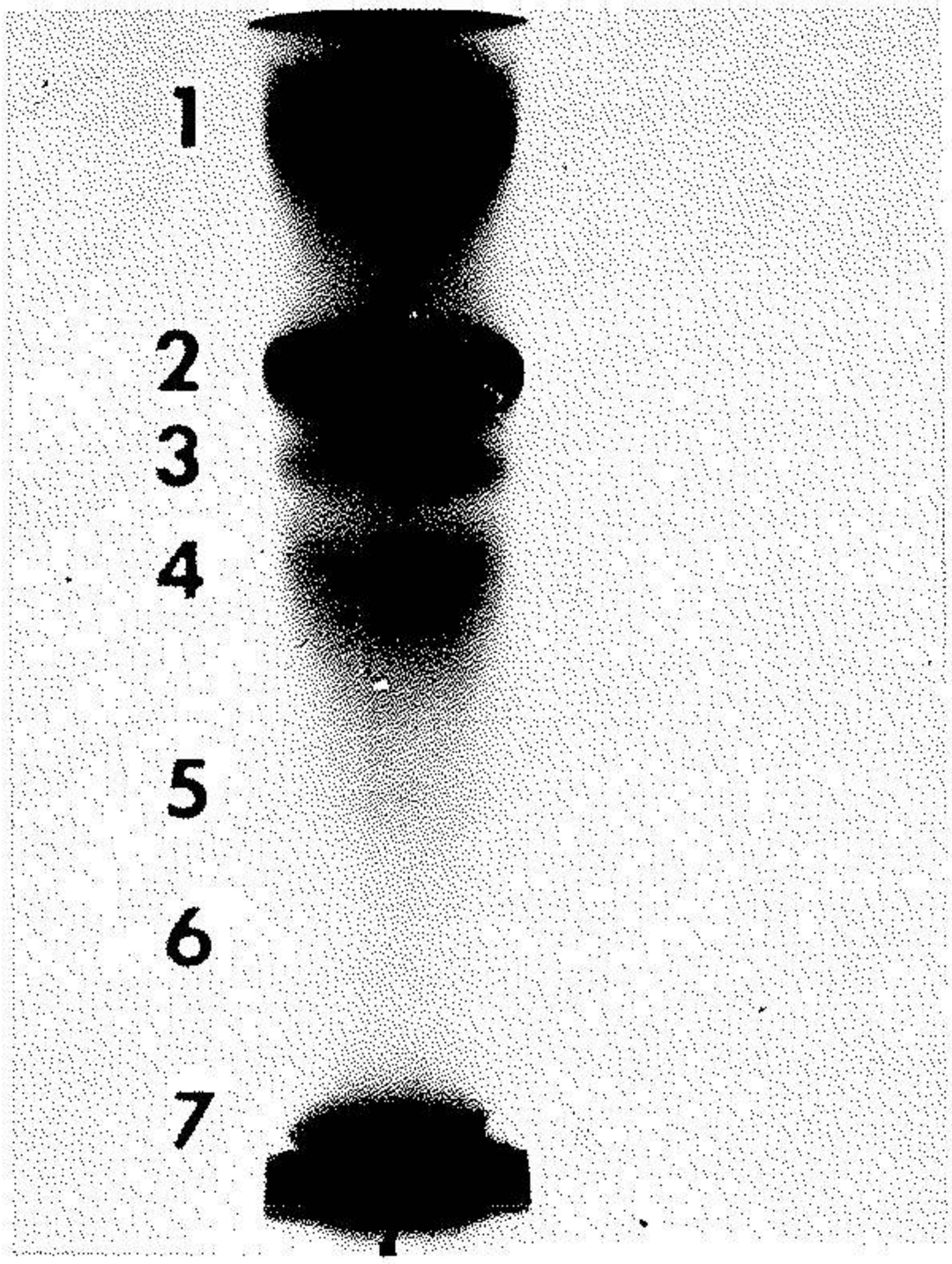

Fig. 3. Different band produced by electophoresis of crude exfoliative toxin on polyacrylamide gel. The second band was determined to be the staphylococcal exfoliative toxin.

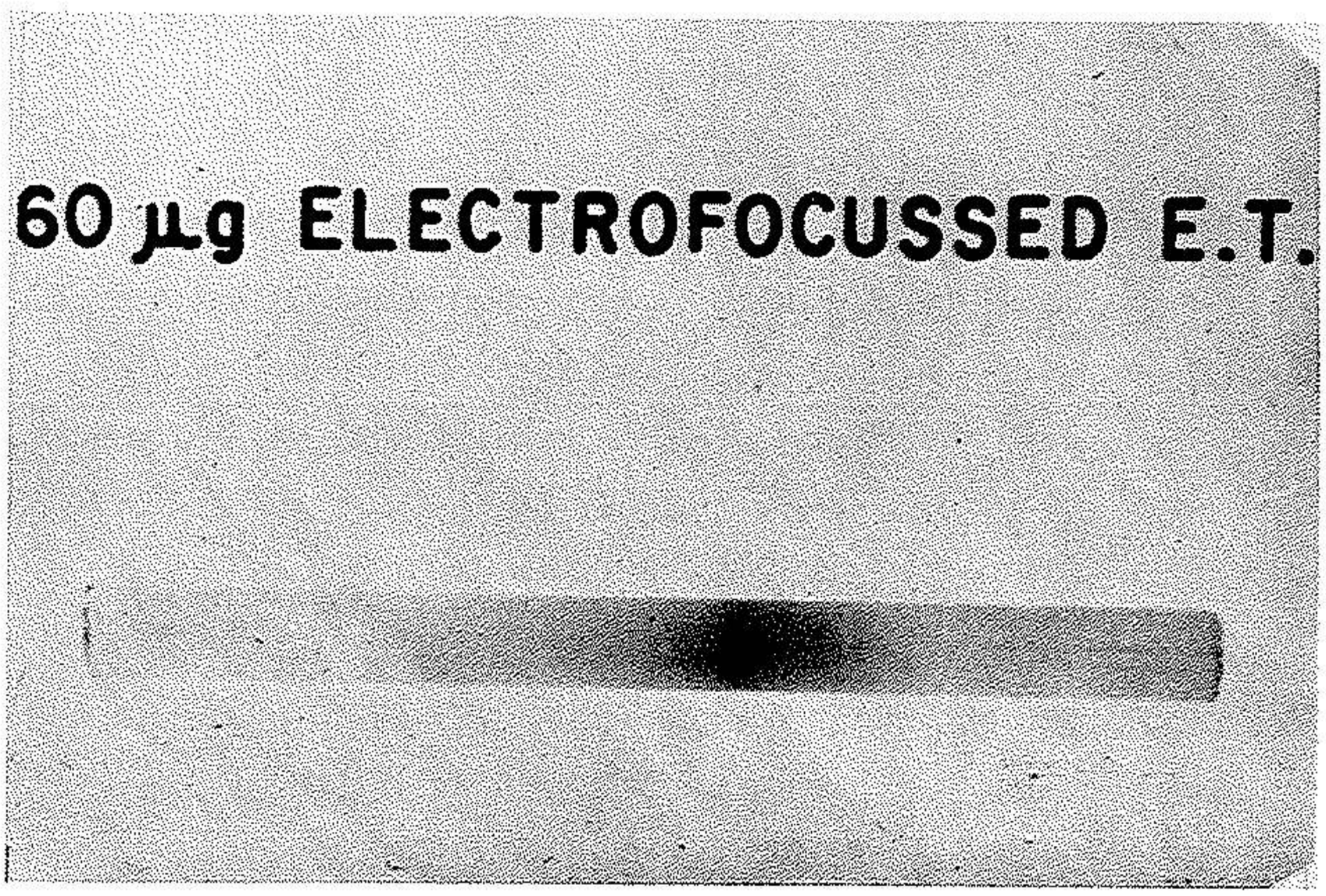

Fig. 4. Electrofocussed staphylococcal exfoliative toxin gave a single band on polyacrylamide gel, indicating that the ET preparation was purified and free from other staphylococcal extracellular products.

slowly onto the column. The toxin is eluted from the column with 0.05 M sodium phosphate, pH 6.8, at a flow rate of 1 ml/min. Fractions of 10 ml each are collected. The peak eluted from the Column contained alpha-hemolysin activity as well as exfoliative activity. A column(1.5 by 30 cm) is filled with hydroxyapatite and the peak fractions from carboxymethyl cellulose column are run slowly onto the column. The column is washed with 0.03 M phosphate buffer pH 5.7 until 2 column volumes had passed through. Two distinct peaks are eluted from the column. The first peak contains the exfoliative toxin and is completely free from alpha hemolysin. The second, smaller peak Contains The alpha hemolysin and is completely free from any exfoliative toxin .

Earlier reports of purification methods have relied on either Disc electrophoresis (10), starch block electrophoresis (16), or isoelectric focuscing (10,16)as a final necessary step in separating the exfoliative toxin from contaminating amount of alpha-hemolysin. These procedures, by nature are restrictive in the sample size that can be applied. The purification method described by Johnson et al (5) is superior and is virtually unlimited in the quantities of exfoliative toxin that can be isolated.

Physicochemical properties of exfoliative toxin

The extracellular staphylococcal product responsible for epidermal exfoliation has been isolated and purified and termed exfoliative toxin (5,9,10). The purified exfoliative toxin Shows only a single band (Figure 4) in sodium dodecyl sulfate gels (5,10). From migration of exfoliative toxin on polyacrylamide gels, the molecular

weight of the toxin has been determined to be 26000 by using standard marker. The kjeldahl nitrogen content of exfoliative toxins is 16 % and it has a maximum absorption of 278 nm (10). The chemical nature of purified staphylococcal ET has been reported to be a simple protein (10,17).

Johnson et al (5) demonstrated that exfoliative toxin is a single protein composed of 17 amino acids and is responsible for exfoliation of new born mice. These results Support the findings of other investigators in this field (9,17). The results of Johnson et al (5)from gas chromatographic analysis indicate the absence of any monosaccharides or disaccharides. These findings are in opposition to the data reported by Rogolsky et al(21) which stated that the exfoliative toxin is a glycoprotein composed of 9 per cent carbohydrate. In addition,Johnson et al (5) reported that the exfoliative toxin, like alpha and beta hemolysin has no cysteine. All of the staphylococcal enterotoxins have a half-cysteine in their molecular structure. No lipid has been detected in the exfoliative toxin preparation (21).

In Ouchterlony double diffusion (20) the purified exfoliative toxin shows a single line of percipitate with its specific Antiserum, The Antiserum prepared to the exfoliative toxin does not neutralize the effects of reference alpha-hemolysin. Commercially available antiserum to the alpha-hemolysin does not neutralize the activity of exfoliative toxin, neither does it form a precipitin line with the toxin in Ouchterlony diffusion.

Genetic determinant controlling ET production

Important source of Variation in Staphylococcus

aureus strains is the presence from time to time, or extrachromosal genetic elements, or plasmids. These elements are now known to consist of double-stranded DNA that replicate independently of bacterial chromosome (2,3). All staphylococcal cells probably carry one or more of these plasmids at any instant in time (22).

The establishment of viral influence on the production of bacterial toxins raises the possibility that staphylococcal exfoliative toxin production might be phage mediated. This possibility is unlikely in view of the inability to demonstrate any association between specific phages of ET positive strains and ET production (24). However, important criteria have proven that ET production is plasmid mediated. The identification of a plasmid controlling ET production include a high temperature spontaneous rate of loss of a genetic marker, the effect of specific chemical agents such as ethidium bromide or sodium dodecyl sulfate on rate of exfoliative toxin loss and plasmid elimination (23).

Rogolsky et al (23) demonstrated that exfoliative toxin production is not phage regulated and ET synthesis is controlled by a plasmid-linked gene.Most staphylococcal strains carry one or more Plasmids. These plasmids usually harbor one or more genetic determinants tor resistance to inorganic ions or to antibiotics (18).For instance, staphylococcal penicillinase plasmids can carry in addition to the genes that regulate penicillinase biosynthesis, genes for resistance to erythromycin, cadmium ions, mercury ions, arsenate ions and arsenite ions (18,19). Another type of staphylococcal plasmids are irreversibly lost from cells by various physical and chemical agents and also by spontaneous mutation. When

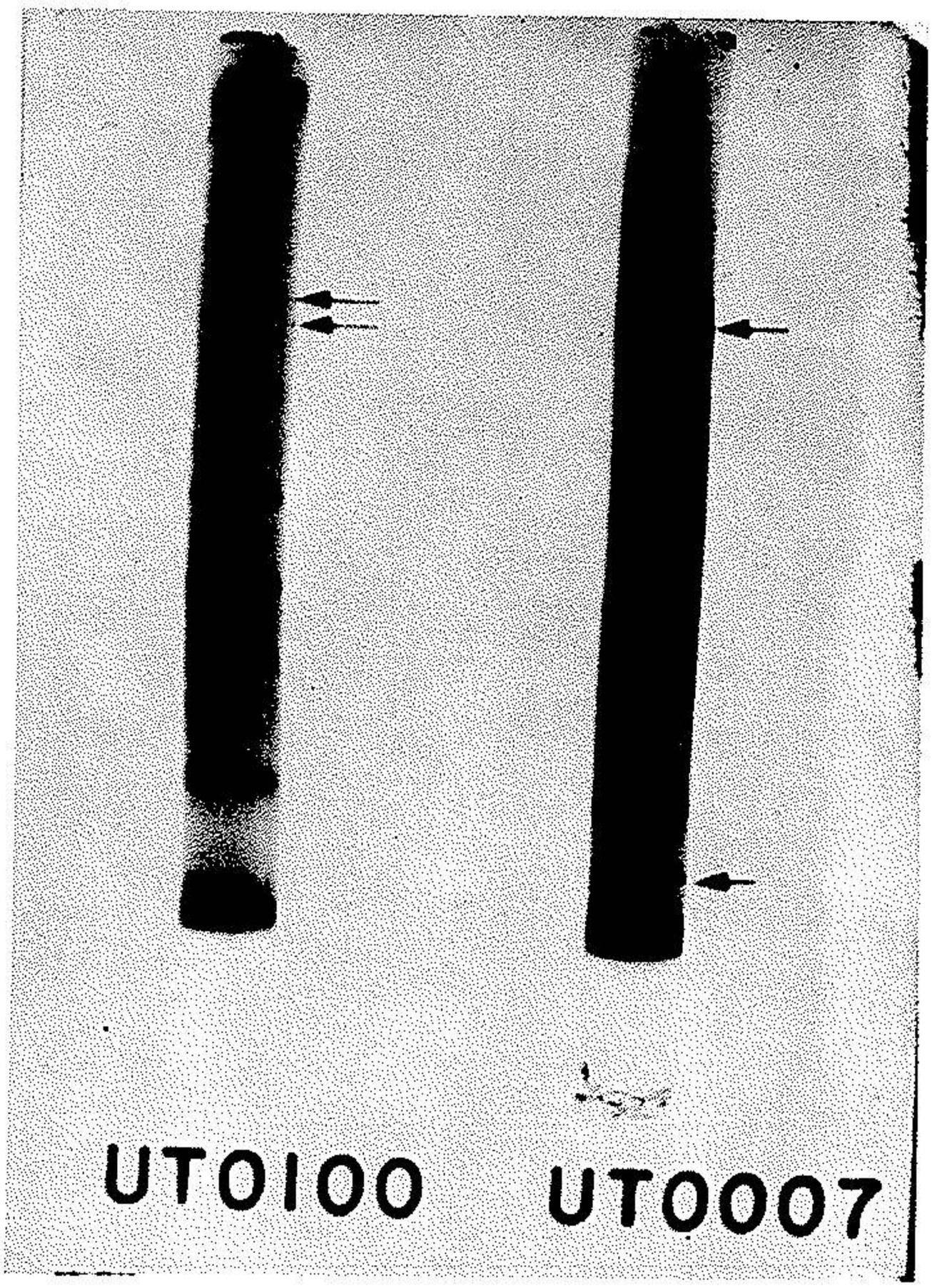

Fig.5. Polyacrylamide disc electrophoresis of the 20 fold concentrated culture supernatant fluids isolated from toxin^{+} strain UT0007 and its toxin^{-} plasmid cured derivative UT0100. Black arrows indicate those bands with exfoliative toxin activity.

a specific plasmid is lost from a cell, all markers borne on this plasmid are concomitantly eliminated and, when a plasmid is trasduced into a recipient, all markers on the plasmid are usually jointly transferred. Further studies by Keyhani et al (8) demonstrate that exfoliative toxin negative staphylococcal strains which lake any plasmid DNA, produce small amount of exfoliative toxin (Figure 5) (7,8). The production of exfoliative toxin in staphylococcal strains which lack plasmid is regulated by chromosomal loci. Therefore, it can be stated that exfoliative toxin production is largely controlled by the plasmid gene,but production of small amount of ET is controlled by chromosomal gene.

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