The versatile staphylococcus

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Today we are celebrating the centennial of the discovery of the *Staphylococcus* in Aberdeen by Alexander Ogston (1880; 1881; 1882). This is a memorable occasion, and it is a special privilege to deliver the Marnoch Lecture honouring Alexander Ogston for his great discovery which has since become so significant in human life. It is noteworthy that this wily rugged micro-organism continues to occupy a position of pre-eminence as a cause of infection (Altemeier, 1971; 1972). Unlike the beta-hemolytic *Streptococcus* and the *Pneumococcus* that have remained highly sensitive to the action of the sulphonamides and penicillin, it has been able to acquire significant resistance to both of these agents, as well as to almost all other antibacterial agents developed since.

John Marnoch (Obituary notices, 1936) succeeded Alexander Ogston as Regius Professor of Surgery in 1909, a Chair which he held until 1932. Among his many interests was inflammation, its results, and its treatment. This included inflammation caused by bacteria. He always stressed fundamentals and facts, and we shall keep this in mind in presenting our data today.

After Pasteur's (1860) contribution of the germ concept of fermentation and disease and Lister's (1867) development of the antiseptic principle in surgical practice, it was logical for Ogston to become interested in the possibility of bacteria being the cause of wound infections. He reasoned that specific bacterial infection caused suppuration, and wrote, 'Often I meditated on the subject and became the more convinced that there was a single cause, and that this cause was some special germ' (1881; 1882).

It has been fascinating to review his clear reasoning and methodical investigations which included the microscopic identification of cocci in pus stained with aniline violet solution of first one and then over 100 abscesses in the early 1870s, inoculation of pus into mice with the production of

This paper includes the substance of the Marnoch lecture delivered by Professor Altemeier.

abscesses containing cocci, the demonstration that the injection of pus after treatment with heat or phenol did not cause abscesses in animals, the successful cultivation of the bacteria after inoculation into eggs, and the proof of the pathogenicity of pure cultures of such bacteria after animal inoculation with the production of abscesses.

These brilliant observations and his advanced and accurate interpretations were promptly accepted by Virchow, Mikulicz & Czerny, and his investigations were reaffirmed by Rosenberg. There was some confusion, however, as to whether Ogston's discovery was of the *Staphylococcus* or the *Streptococcus,* but in 1882 he differentiated the two types of cocci, one arranged in chains corresponding to that described by Billroth as the *Streptococcus,* and the other occurring in clusters or bunches as the *Staphylococcus,* a name which he used at the suggestion of W. D. Geddes, then Regius Professor of Greek at Aberdeen.

Thus Ogston made solid and lasting contributions to the world during the decade of 1876–86, a period often recognized as the 'Golden Age' of the science of pathogenic bacteria.

Apparently he was unaware of the versatility of the *Staphylococcus* and its ability to adapt successfully to circumstance and various forms of treatment directed against it with one possible exception. In his report (1881) upon 'Microorganisms in Surgical Diseases', which he presented to the Scientific Grants Committee of the British Medical Association, he reported that 'micrococci in (infected) wounds withstand most antiseptic applications'.

Since the introduction of the senior author (W. A. A.) to staphylococci as a medical student in 1930 and as a special student in bacteriology in the laboratory of William H. Wherry, Professor of Bacteriology at the University of Cincinnati, he has become more and more impressed not only with the pathogenicity of the staphylococci, but also with their remarkable versatility. In the period between 1930 and 1939, it was evident that this microorganism was the aetiologic agent of approximately 60–65 per cent of postoperative (in hospital) surgical infections. In addition, it had the ability to produce a large number and variety of other infections in patients, and these represented a major part of surgical practice (table I). All too frequently they were serious and were characterized by extended morbidity, high mortality, loss of function with disability, loss of limb, cosmetic disfigurement, frequent recurrences, and epidemic potential (Altemeier, 1945; Altemeier & Helmsworth, 1945; Altemeier & Wadsworth, 1948; Altemeier, 1946; Altemeier, 1949/50; Altemeier, 1944). The high mortality of staphylococcal septicaemia (90 per cent) and the gravity of the metabolic and pathophysiologic effects of acute established

TABLE I

Clinical infections caused by *S. aureus* indicative of its versatility

Skin and Appendages

1. Furuncles
2. Impetigo contagiosum
3. Paronychia
4. Pyoderma gangrenosum
5. Carbuncles
6. Conjunctivitis
7. Cellulitis
8. Wound abscess
9. Botryomycosis

Respiratory Tract

1. Tracheobronchitis
2. Laryngitis
3. Pneumonia
4. Pneumonitis
5. Empyema
6. Lung abscess

Circulatory System

1. Lymphangitis
2. Lymphadenitis
3. Thrombophlebitis
4. Bacteremia
5. Septicemia
6. Acute Vegetative endocarditis
7. Septic shock
8. Pylephlebitis

Genito-Urinary Tract

1. Cystitis
2. Pyelitis
3. Renal abscess
4. Endocervicitis
5. Salpingitis
6. Tuboovarian abscess
7. Parametritis

Oropharyngeal and Cervical Areas

1. Tonsillitis
2. Sinusitis
3. Retropharyngeal abscess
4. Cervical abscess
5. Otitis externa
6. Otitis media
7. Mastoiditis
8. Parotitis
9. Acute thyroiditis

Musculo-fasciąl-skeletal tissues

1. Osteomyelitis
   (a) Hematogenous
   (b) Post-trauma
2. Septic arthritis
3. Tenosynovitis
4. Necrotizing fascitis
5. Metastatic abscesses to muscle

Alimentary Tract

1. Acute gastroenteritis
2. Pseudomenbranous enterocolitis
3. Peritonitis
4. Intraabdominal abscesses
5. Retroperitoneal abscesses
6. Visceral abscesses
   (a) Pancreatic
   (b) Hepatic

Central Nervous System

1. Meningitis
2. Brain abscess

infections were particularly impressive (Altemeier & Helmsworth, 1945; Altemeier, 1967).

With the advent of modern antimicrobial therapy and the introduction of the sulphonamides, there was great anticipation that the pathogenic power of this formidable adversary had finally been conquered. This was abetted by the enthusiastic reports of Ravdin, Professor of Surgery at the University of Pennsylvania (Long & Ravdin, 1942a; Ravdin & Long, 1942c) and Long, Professor of Preventive Medicine at the Johns Hopkins Hospital (Long & Ravdin, 1942a; 1942b; Long, 1943), who visited Pearl Harbour immediately after the attack to observe firsthand the effectiveness of prophylactic sulphonamide therapy on casualties. Their reports to the Committee on Wounds and Burns at the National Research Council in Washington, D.C. in January of 1942, indicated that the answer to the prevention of surgical infection in war wounds and wounds of violence had been found. Ravdin reported the nearly complete absence of infection in wounds, and Long (1943) concluded that 'if the basic principles of surgical care are combined with the adequate and thoughtful utilization of sulphonamide compounds in the prophylaxis of wounds, there is good reason to believe that the incidence of wound infection will become practically zero'.

The capability of the hemolytic *S. aureus,* however, to resist the effect of sulphonamide was recognized early and with great disappointment (Lockwood & Lynch, 1940). On the other hand, its effectiveness in preventing and controlling infections by beta-hemolytic streptococci and pneumococci was heralded as a great advance in surgery (Meleney & Whipple, 1945; Zinninger & Altemeier, 1945).

When penicillin was introduced for clinical trials in late 1942 and early 1943, hope again ran high that an anti-staphylococcal agent had been found which would both control established infections and prevent infections in wounds caused by this organism (Meleney & Whipple, 1945; Zinninger & Altemeier, 1945).

Five observations made in our Surgical Research Bacteriology Laboratory refuted this concept and predicted that staphylococci again would have the capability of overcoming the effectiveness of the new antimicrobial agent, penicillin. These included the following:

1. The demonstration in the spring of 1943 that only 96 per cent of staphylococci passing through our laboratory were sensitive to two units or less of penicillin *in vitro.* The remaining 4 per cent were resistant.
2. The demonstration on blood agar plates containing troughs or

cylinders of penicillin solution that colonies of resistant *S. aureus* could develop within the zone of effective inhibition for sensitive staphylococci isolated from infected wounds. These colonies were 'rough' in appearance (Altemeier, 1945).

3. A progressive overall increase in the incidence of resistant staphylococcal isolates was observed in continuing studies of the *in vitro* sensitivity patterns of all isolates of the *S. aureus* in our laboratory between 1943 and 1950. By 1950 an average of 51 per cent of the strains had become penicillin-resistant (Altemeier, 1956–7).

4. The dynamics of infection in 1,683 burn wounds under penicillin treatment was studied between 19 January 1942 and 1 September 1960 at the Cincinnati General Hospital. This study indicated that the bacteria most important in producing serious wound infections after thermal injuries were *S. aureus, Pseudomonas aeruginosa,* and *Proteus*. It also revealed a disturbing development of penicillin-resistant isolates in these wounds (Altemeier & MacMillan, 1962). It was particularly noteworthy that beta-hemolytic Streptococci were not the cause of a single case of septicaemia since the introduction of the use of penicillin, although it was still present in burns in a definite number of cases. This confirmed the value of penicillin for the prevention of invasive hemolytic streptococcal infections in the treatment of the burned patient, but at the same time, emphasized again the ability of staphylococci to successfully overcome the effect of this antibiotic agent.

5. In a further and more detailed study of the incidence of staphylococcal resistance to penicillin over a five-year period between 1 July 1951, and 30 June 1956, the results were grouped in periods of three months rather than one year. A curious and unexpected cyclic variation with peaks and recessions of 19 to 81 per cent in penicillin-resistant patterns became apparent (fig. 1) (Altemeier, 1956–7), in the data of 1422 consecutive strains. This latter observation suggested that either there was some inconsistency of methods of testing for sensitivity or possibly that some significant cyclic change was occurring in the staphylococcal community at intervals of two, three, or more years for unknown reasons.

Reports of some of these studies were made in 1948 with predictions that within ten years there would be a hospital reservoir of penicillin-resistant staphylococci and, consequently, a greater problem in the management of staphylococcal infection in the United States. This came to pass, and in 1958, the first International Conference on Hospital-Acquired Disease was held at the Center for Disease Control in Atlanta (Altemeier, 1959).

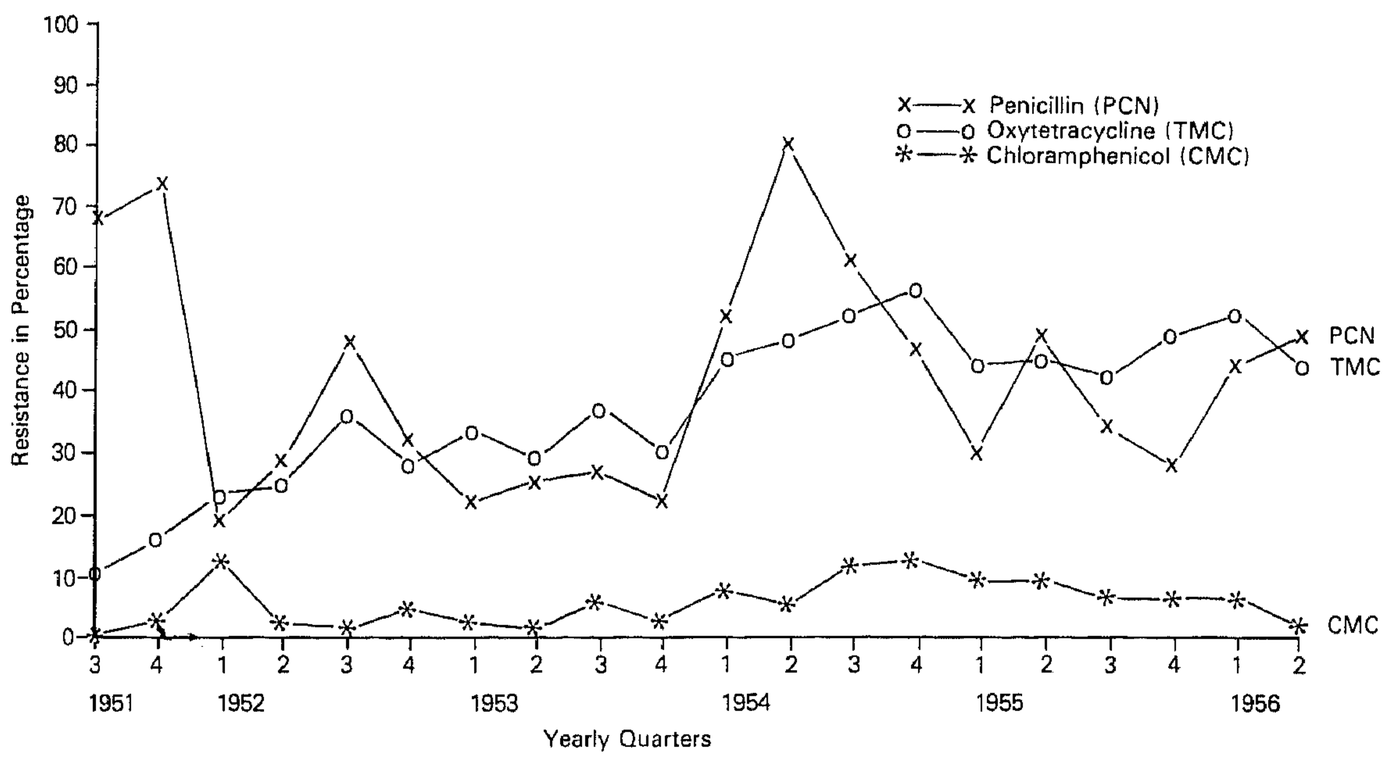

Fig. 1 The cyclic variation of isolates of *S. aureus* to penicillin between 1951 and 1956.

Similar studies were reported (Altemeier, 1963) and others were started in an attempt to find an explanation for the cyclic variations in penicillin resistance. The first was a study of the accuracy of the disc method of sensitivity testing by comparing the disc and tube dilution methods of testing (Hill *et al.*, 1958). A marked discrepancy in the antibiotic concentrations in the discs sold by three different manufacturers became apparent, and a 42.0 per cent error for measuring the resistance of staphylococci to penicillin was found. (Hill *et al.*, 1958). After appropriate changes in testing techniques, however, cyclic variations again became evident.

Emerging Bacteriophage Types

The second group of experiments included attempts to identify changes in specific bacteriophage types of staphylococcal isolates using the International Series of bacteriophages. With this new technique in the mid-1950s, variations in the percentage incidence of two phage types of staphylococcal isolates were identified at different periods of time. The first of these was type 53,77 which accounted for 38 per cent of the staphylococcal isolates in 1955–6, and the second was type 80, 81 which was

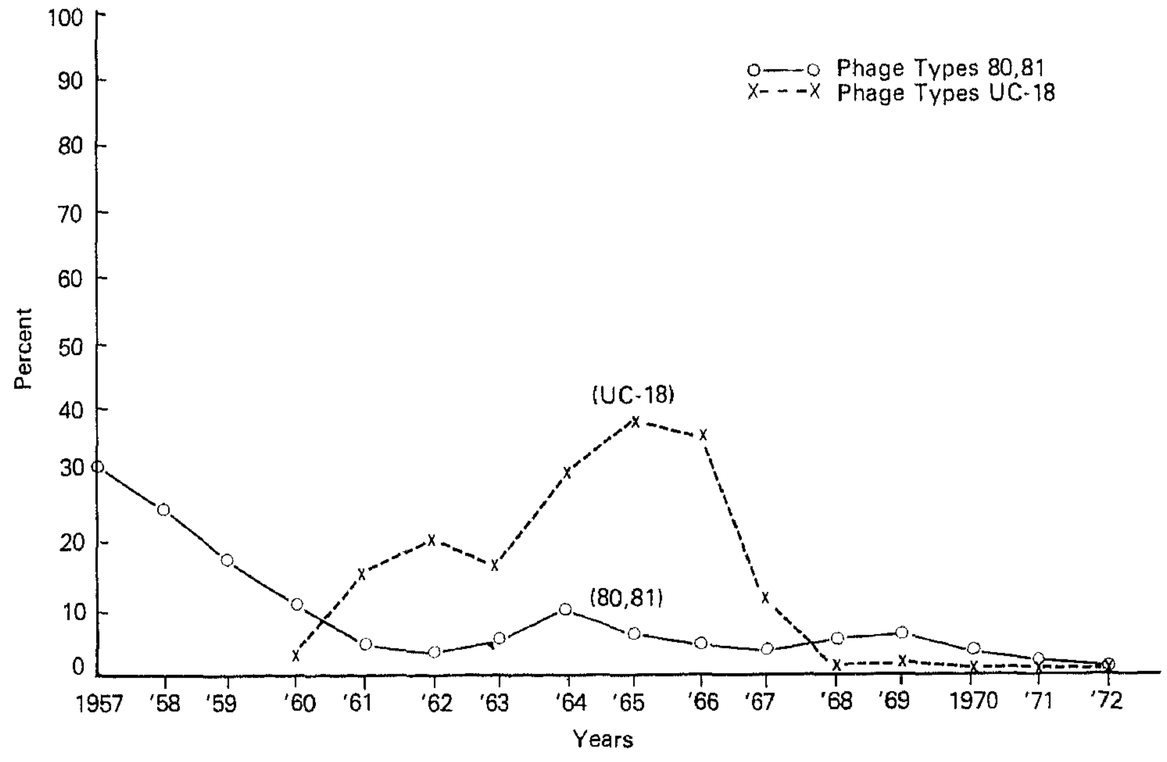

Fig. 2 The decline in incidence of 80, 81 phage type staphylococci between 1957–61, and the emergence and prevalence of UC-18 phage type between 1960–7.

TABLE II

Comparative incidence of *S. aureus* phage types UC-18 and 80, 81

Year	80, 81 (per cent)	UC-18 (per cent)
1957	29.8	—
1958	23.6	—
1959	18.3	—
1960	11.2	5.8
1961	5.2	15.0
1962	4.1	19.0
1963	5.4	16.0
1964	10.2	29.6
1965	6.7	38.0
1966	5.2	35.1
1967	4.2	12.0
1968	5.7	0.6
1969	6.1	0.8
1970	3.6	0
1971	2.1	0 to 1
1972	0.4	0 to 1

the most frequently isolated strain during 1957, 1958, 1959 and 1960. In 1957 the latter's incidence was 29.8 per cent and thereafter it declined to 23.6 per cent in 1958, 18.3 per cent in 1959, and 11.2 per cent in 1960 (Altemeier *et al.*, 1966; Hummel *et al.*, 1962). Subsequent to 1960, its incidence has remained at a low level, reaching 0.4 per cent in 1972 (Hummel *et al.*, 1962). (fig. 2 and table II).

Of considerable interest, also, was the observation that of a total of 1465 isolates of *S. aureus* tested in the Surgical Research Bacteriology Laboratory during the first nine months of 1958, 91 per cent were coagulase positive, but only 68 per cent of these were typable with the 32 bacteriophage strains available with 32 per cent being untypable. Of the untypable strains, 65 per cent were resistant to penicillin (Altemeier *et al.*, 1966; Hummel *et al.*, 1962; Thomas *et al.*, 1960). This suggested to the senior author (W. A. A.) the possibility that one or more new bacteriophage types of staphylococci were hidden in these high peaks, and a research grant proposal was submitted to the National Institute of Health. A visit was made to Dr Blair in New York City to explore possible methods of developing new identifying phages. Our grant proposal was turned down, and our investigations were discouraged as being highly improbable.

Nevertheless, the investigations were pursued to explore possible methods of staphylococcal phage conjugation for the purpose of developing new phages for typing otherwise untypable staphylococcal isolates. Within six months, 20 bacteriophages were developed in this way (Thomas *et al.*, 1960; Hill *et al.*, 1964). Six of these were found to be new, and two were highly significant as typing agents. These were named UC-18 and UC-13.

The new phage strains were reacted with four hundred isolates of *S. aureus* along with the phages of the International Series. Of this number, 223 or 56 per cent were untypable with the phages of the International Series. With the new phages, 38 per cent of the untypable strains became typable, the total number of all strains typed being 260 or 65 per cent. Thus, typability in this series was increased from 44 per cent with the International Series to 65 per cent with the International Series plus the two new bacteriophages (Hill *et al.*, 1964).

The new bacteriophages were then added to the basic set in the International Series, and all staphylococcal isolates between 1 January 1960, and 1 September 1979, have thus been typed. At the same time, their anti-microbial susceptibilities were determined using the improved disc method. Currently the phages in the basic set used in this study are 29, 52, 52A, 79, 80, 3A, 55, 71, 6, 42E, 47, 53, 54, 75, 77, 83A, 81, 84, 85, 94, 95, 96, UC-13, and UC-18. Pertinent to this study was the inclusion of phages 84 and 85 in 1968 and 94, 95, and 96 in 1977 (Altemeier & Lewis, 1978).

The data obtained from this investigation, which is still ongoing, have been most interesting. At the beginning, the 80–81 phage type was on the decline (fig. 2). As indicated earlier, its incidence in 1960 was 11.2 per cent as compared with 18.3 per cent in 1959, 23.6 per cent in 1958, and 29.8 per cent in 1957. Subsequent to 1960, the incidence progressively fell throughout the next ten years to reach a level of 0.4 per cent in 1972.

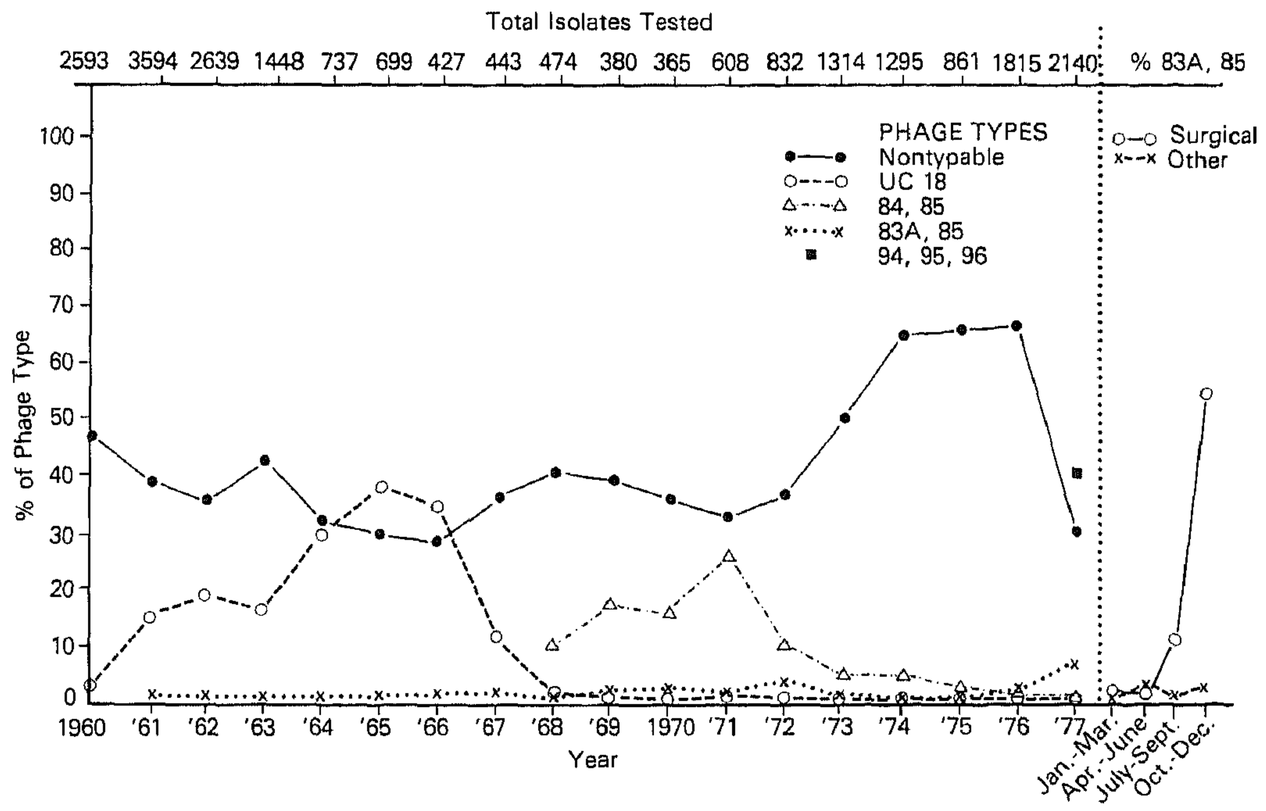

Fig. 3 Emergence and decline of new phage type 84, 85 staphylococci in 1968–73.

In the period between 1961 and 1967, the spontaneous emergence, increase, and decrease in incidence of the new phage type *S. aureus,* UC-18, were noted (table II and fig. 2.) The inclusion of this phage in the International Series increased the overall typability by 38 per cent in 1965. By 1968, however, the UC-18 type *S. aureus* had also rapidly and spontaneously disappeared. This strain was shown by us and others to be an important nosocomial agent with epidemic potential for producing infections in surgical patients (Hill *et al.,* 1964; Altemeier & Lewis, 1978). The percentage of resistance to penicillin shown by the UC-18 strain was 95.4 per cent, but it remained sensitive to the synthetic penicillins. It showed an increase in resistance to other antibiotic agents. From 1968 to 1972, another strain, phage type 84,85 emerged and predominated (fig. 3). By 1971, 27 per cent of all isolates tested were of this phage type (Altemeier & Lewis, 1978; Lewis & Altemeier, 1976).

In 1977, another phage type was noted to have emerged and to have assumed considerable importance. It was the complex of phages 94, 95, and 96, which were added to the group of phages used in our laboratory beginning 1 January 1977. A surprising total of 40 per cent of the staphylococcal isolates tested in 1977 were of this type (Altemeier & Lewis, 1978). Before the last three months of 1976, our laboratory had not seen a gentamicin-resistant isolate of *S. aureus*. During this period, however, 1.3

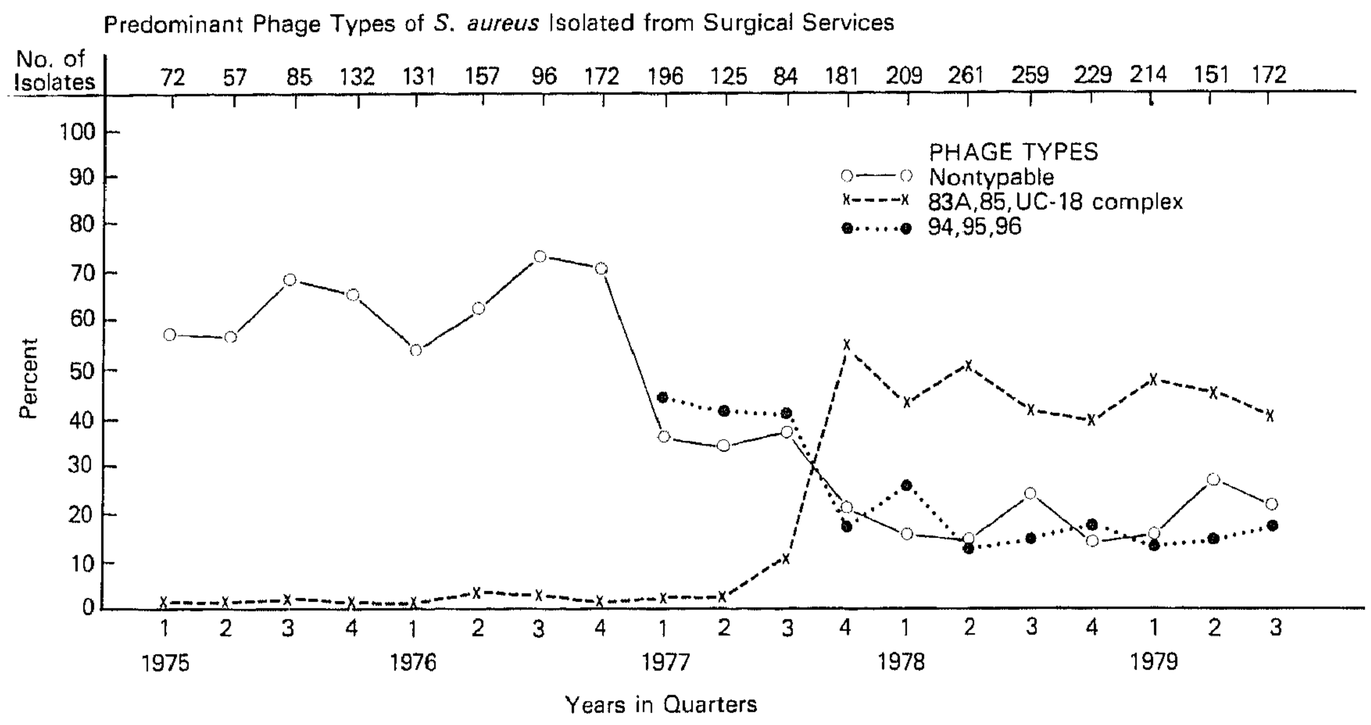

Fig. 4 Decline in incidence of phage types 94, 95, 96 *S. aureus* and emergence and maintenance of high level of phage types 83A, 85, UC-18 between Sept. 1977–9.

per cent of all isolates were found to be resistant to gentamicin, and this trend increased during 1977 when it was noted that the gentamicin-resistant strains correlated with phage types 94, 95, and/or 96. Phage type 95 predominated on the surgical service whereas phage type 94, 96 predominated on other services at the University of Cincinnati Medical Center.

A more dramatic emergence of another phage-type of staphylococcus 83A, 85 was monitored and noted to have suddenly occurred during the last three months of 1977 and has continued at a high level up to 30 September, 1979 (fig. 4 and 5). In addition to its rapid increase and a continued high level of incidence, this latest strain of *S. aureus* has also been shown to have acquired a marked increase in resistance to almost all available antibiotics, most notably to penicillin, the semisynthetic penicillins, and gentamicin. (table III) This has been a frightening development to thoughtful and concerned surgeons.

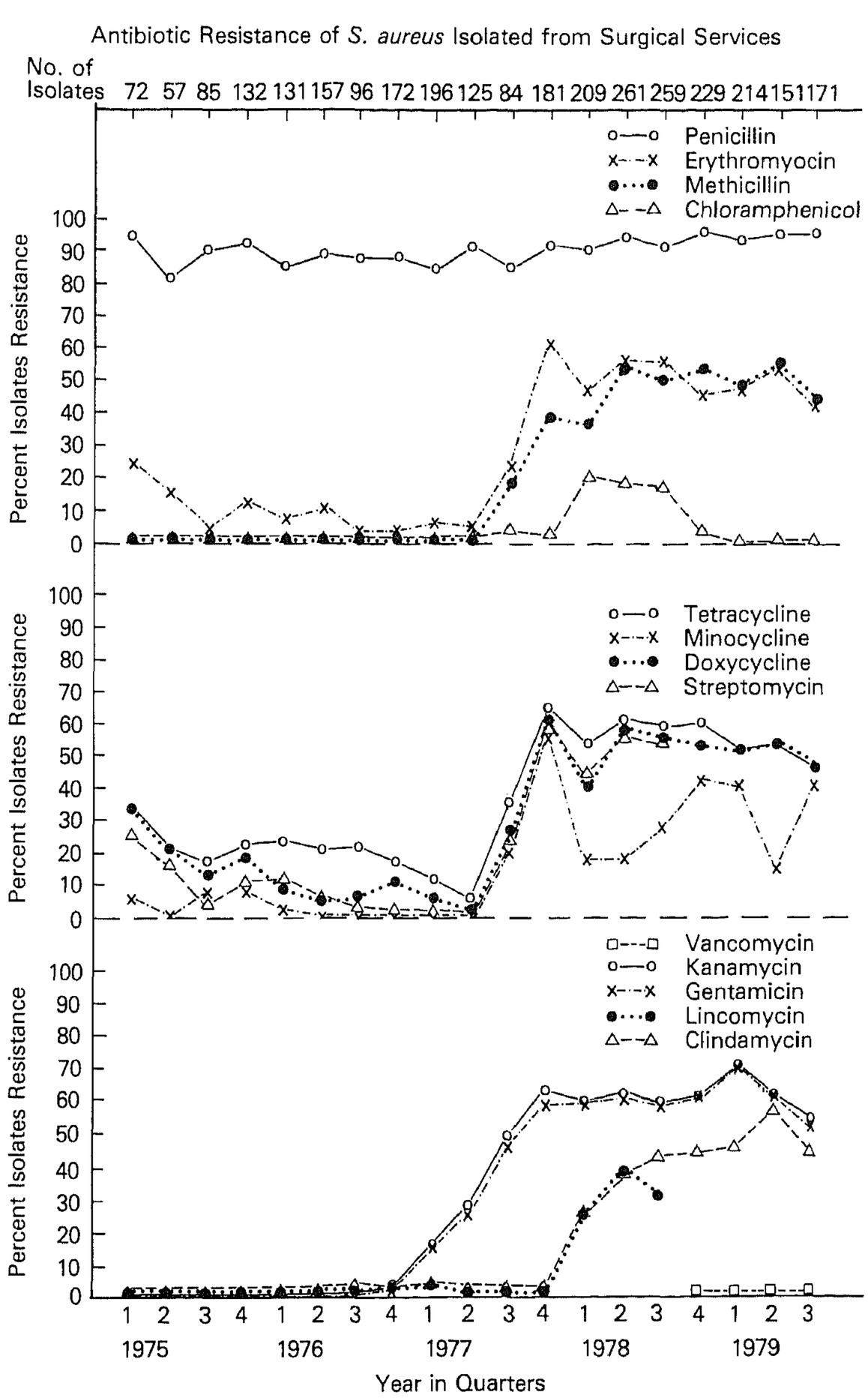

Fig. 5 a, b, c. Antibiotic resistance pattern of *S. aureus* which developed in 1977 with the emergence of phage types 83A, 85.

TABLE III
Staphylococcal Toxins

Exotoxins—types	*Action*
1. Alpha	Lethal Toxin, Dermonecrotic, Hemolytic, Leucolytic
2. Beta	Hemolytic
3. Gamma	Hemolytic, Dermonecrotic
4. Delta	Hemolytic
5. Enterotoxin	Gastrointestinal tract, Unknown cerebral centres
6. Leucocidin	Leucolytic
7. Exfoliative Toxin	Scalded Skin Syndrome
Extracellular enzymes	
1. Coagulase	Thrombokinase-like
2. Staphylokinase	Fibrinolytic
3. Hyaluronidase	Spreading Factor (?)
4. Phosphatase	— — —
5. Proteinase	— — —
6. Lipase	— — —
7. Gelatinase	— — —

Except for the peak incidences described above, the majority of the other types studied in our laboratory have been scattered and have not fallen into any specific pattern during the past 20 years.

These data have suggested to the senior author (W. A. A.) that there has existed in the hospital environment during the past 25 years an interesting and important phenomenon which plays an important role in the aetiology of surgical wound and other nosocomial infections. With the emergence of the different phage-type strains of *S. aureus,* the evidence also indicates that epidemics of infection by these microorganisms have occurred in hospitalized patients (Hummel *et al.,* 1962; Hill *et al.,* 1964; Altemeier & Lewis, 1978; Lewis & Altemeier, 1976; Rosendal *et al.,* 1976; Williams & Dean, 1974; Wallmark & Finland, 1961). The causes of these changes are still unknown, and this newly recognized phenomenon, another manifestation of the unusual versatility of *S. aureus,* has the following characteristics:

1. The spontaneous emergence of different phage types of staphylococci in the hospital environment with a rapid rise to peak incidence, its

maintenance at a high plateau level for two to five or six years, and its sudden and precipitous decrease and essential disappearance.

2. As one phage type disappears, however, a new phage type usually emerges and follows a similar pattern.

3. The antibiotic resistance pattern of each new emerging phage type changes significantly.

4. The causal relationship of this phenomenon is unknown. We have not been able to establish a clear relationship between the types of antibiotic therapy currently in use and the emergence and particularly with the spontaneous and precipitous disappearance of these phage types.

5. The epidemic potential of the different phage types can vary. Some have been particularly important in clinical surgical practice such as the 80, 81, UC-18, and 83A, 85. Some have had high antibiotic resistance patterns and considerable epidemic potential.

It will be interesting to follow this phenomenon further and to see if the present epidemic strain, 83A, 85 will also disappear only to be replaced by another.

The emergence and disappearance of a local epidemic caused by a specific phage type of staphylococci have been documented by others. Rosendal *et al.* (1976), noted a decrease in antibiotic resistance along with a decrease in epidemic strains. A similar observation was noted by Williams & Dean (1974). Between the years 1955 and 1960, Wallmark & Finland (1961), found a decrease in group III strains and an increase in 80, 81 and nontypable strains. Most of their nontypable strains were resistant to antibiotics. A study by Klastersky, Beumer & Daneau (1971) showed that heteroresistant strains of *S. aureus* were predominantly group III phage types and that these strains were isolated only from within the hospital environment.

In our studies of the yearly mortality rates for all types of septicaemia between 1955 and 1965, it was also noted that three separate peaks of 75 per cent, 100 per cent, and 97 per cent occurred in those caused by *S. aureus* in 1955–6, 1957–8, and 1961–3 (Altemeier *et al.*, 1966) (fig. 6). These peaks have suggested the possibility of some correlation with the emergence of the staphylococcal phage-types 53–77, 80–81, and UC-18 noted in our studies during those periods.

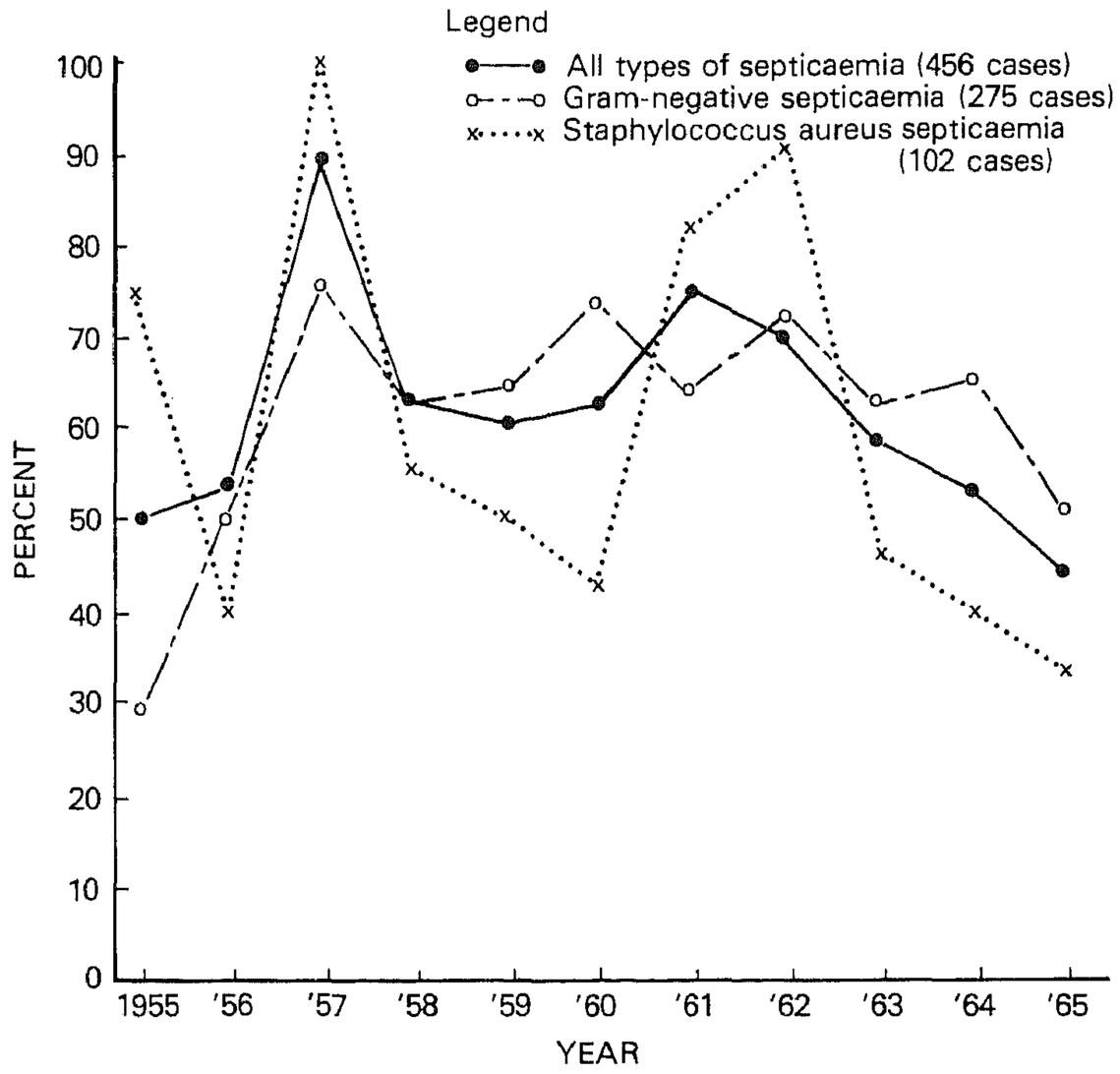

Fig. 6 Comparative curves of yearly mortality rate of all cases of septicaemia with *S. aureus* and gram-negative bacilli between 1955–65. Note peaks of incidence.

THE TOXIC SHOCK SYNDROME

The most recent indication of staphylococcal versatility in the production of disease has been the newly described 'Toxic Shock Syndrome' (Todd *et al.*, 1978). This new and intriguing clinical syndrome has been under study in the United States during the past two years. The Center for Disease Control in Atlanta, Georgia, has become greatly interested in this syndrome and has been actively collecting information on reported cases. As of 8 September 1980, approximately 300 cases have been reported since the first paper by Todd *et al.*, in 1978.

The characteristics of this syndrome are fascinating as indicated by the data gathered thus far (Center for Disease Control, May 1980–June 1980; Richmond, 1980).

1. It affects primarily previously healthy younger women who use tampons during their menstrual period. It has been reported only in the United States except for one and possibly ten cases in Canada.
2. The mean age of those contracting the disease has been 26 years, with 96 per cent having the onset during menses. Only a few children (non-menstruating females) and a few males apparently have developed the toxic shock syndrome. These last two groups have had focal infections.
3. It has a sudden onset with high fever, vomiting, diarrhoea, rapid dehydration, and hypotension and shock.
4. *S. aureus* has been found in the vaginal tract of women having this syndrome.
5. There is still uncertainty as to what bacteriophage class, or type the isolates represent, some being reported as phage group I and others as group III.
6. An erythematous macular rash has occurred and has been followed later by desquamation, particularly of the palms and soles.
7. Disorientation and combative attitude have been noted.
8. Blood cultures have been negative.
9. The case fatality ratio has been between 3 and 10 per cent.
10. Up to 42 per cent of women who have had toxic shock syndrome are at risk of developing recurrences.
11. Treatment recommended has included the immediate infusion of intravenous fluids, supportive therapy, and probably a beta-lactamase resistant antibiotic.

The case definition currently recognized by the Center for Disease Control now requires all five of the following criteria (Center for Disease Control, 1980; Richmond, 1980):

1. Fever to or greater than 102°F.
2. An erythematous macular rash followed by desquamation.
3. A fall in blood pressure to 90 mm Hg or less.
4. The involvement of at least four organ systems.
5. The absence of meningococcemia, bacteremia, or Rocky Mountain Spotted fever.

The symptoms of this syndrome and its association with the *S. aureus* suggests the etiologic agent to be a powerful and probably complex staphylococcal toxin (Schlievert *et al.*, 1979). The emergence and course of

this syndrome suggests the possibility of a newly emerging phage-type. Time and further study will be required for clarification of the nature of this disease and its relationship to the toxins of *S. aureus*.

Electron Microscopic Studies

Since antimicrobial therapy plays such an important role in staphylococcal infections, our interest has also turned to electron microscopy and scanning microscopy in an effort to obtain a better understanding of the versatility of the organism in acquiring resistance to penicillin and methicillin. Penicillin resistance is believed to have been primarily due to the production of a penicillinase which catalyses the hydrolysis of the beta-lactam ring of the penicillin molecule and in that way destroys its antimicrobial activity (Davis *et al.*, 1967; Borowski *et al.*, 1964; Rountree & Beard, 1968; Parker & Hewitt, 1970; Altemeier *et al.*, to be published; Avakyan *et al.*, 1972). Semisynthetic penicillins have been developed which resist the action of penicillinase, but unfortunately mechanisms other than penicillinase production can be acquired by mutation to resist the action of methicillin (Davis *et al.*, 1967). This capability is yet another example of the versatility of the *Staphylococcus*, illustrating the repeated experience that there has been no antimicrobial drug used so far to which it has not been able to develop resistance (Davis *et al.*, 1967).

It has been recognized that *S. aureus* has been most susceptible to the action of penicillin during active microbial growth and cell division. An explanation of this was sought through the use of electron microscopic and scanning microscopic techniques to study the antimicrobial effect of penicillin and methicillin on sensitive and resistant strains of the hemolytic *S. aureus*. Osmium-formaldehyde fixed cultures of a penicillin-sensitive strain (S-2928) after exposure to penicillin were examined in our laboratory using the Jelco electron microscope and scanning scope at a magnification of × 150 000. The following were our findings (Altemeier, Brackett & Lewis, to be published):

1. Evidence that the greatest penicillin-induced changes occurred in cell bodies of the *S. aureus* during the process of active division. Other cells not dividing showed little or no evidence of penicillin induced change (fig. 7).
2. The early penicillin-related findings included changes in the cell wall with reduction in the inner layer of the cell wall and the development of multiple distinct layers in the cell membrane. These findings were greatest in the vicinity of the area of cell division.

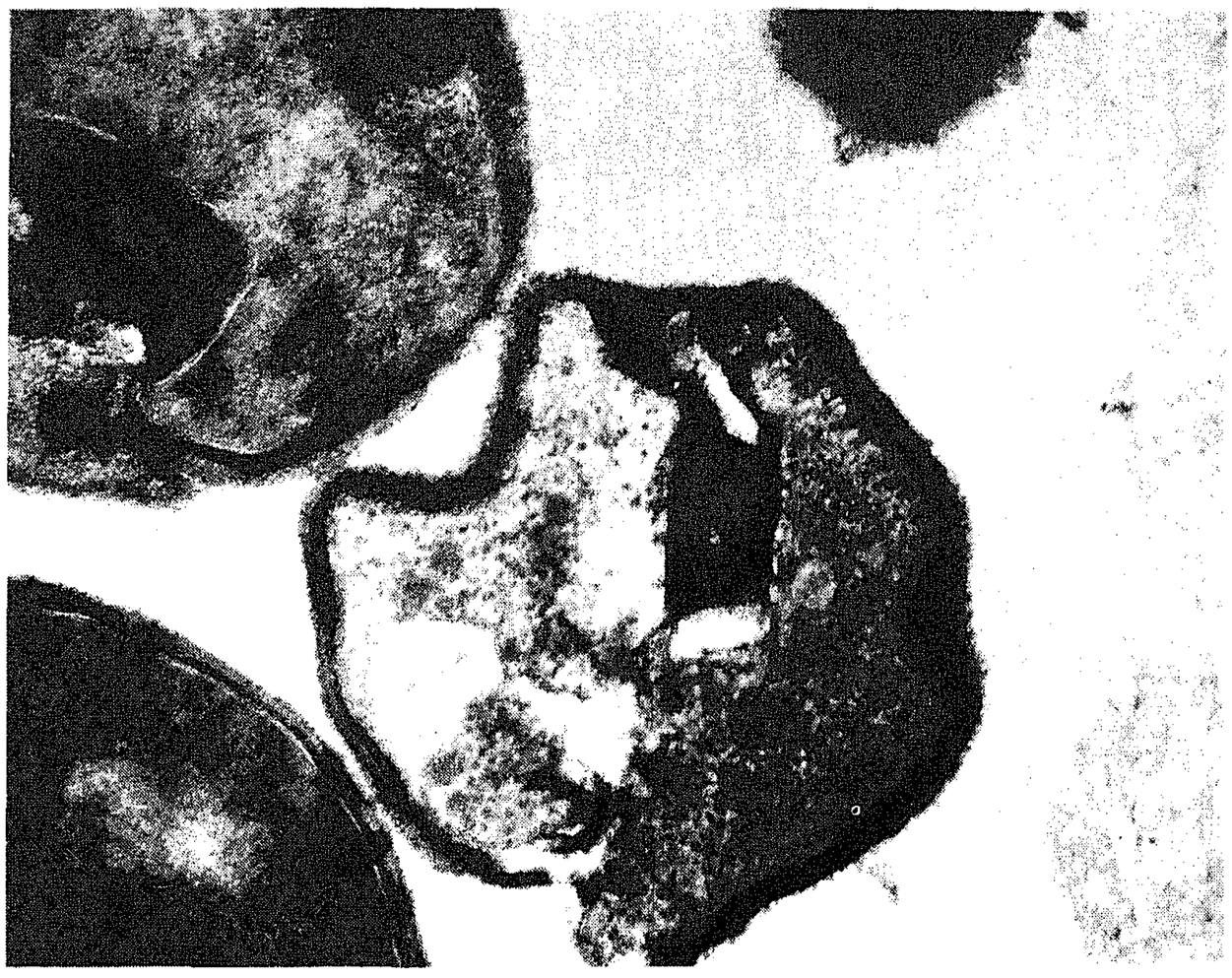

Fig. 7 Electron microscopic photograph (× 150 000) showing effect on penicillin-sensitive strain of *S. aureus* on cell wall at zone of division, and on cell membrane.

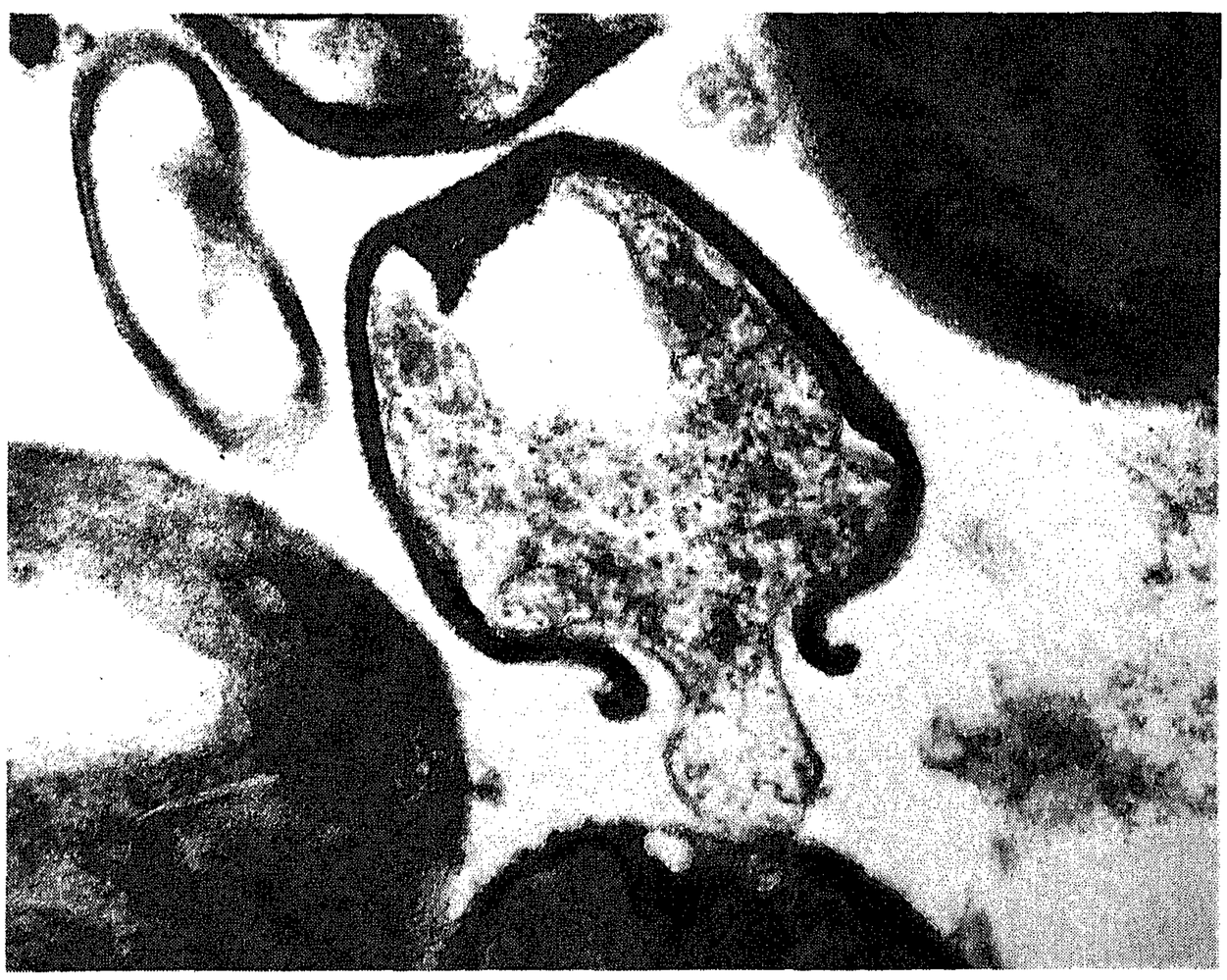

Fig. 8 Marked changes produced in *S. aureus* by penicillin with enlargement of size of body, increased thickness of cell wall and its inward extensions, herniation of cytoplasm, and disintegration of the cytoplasm.

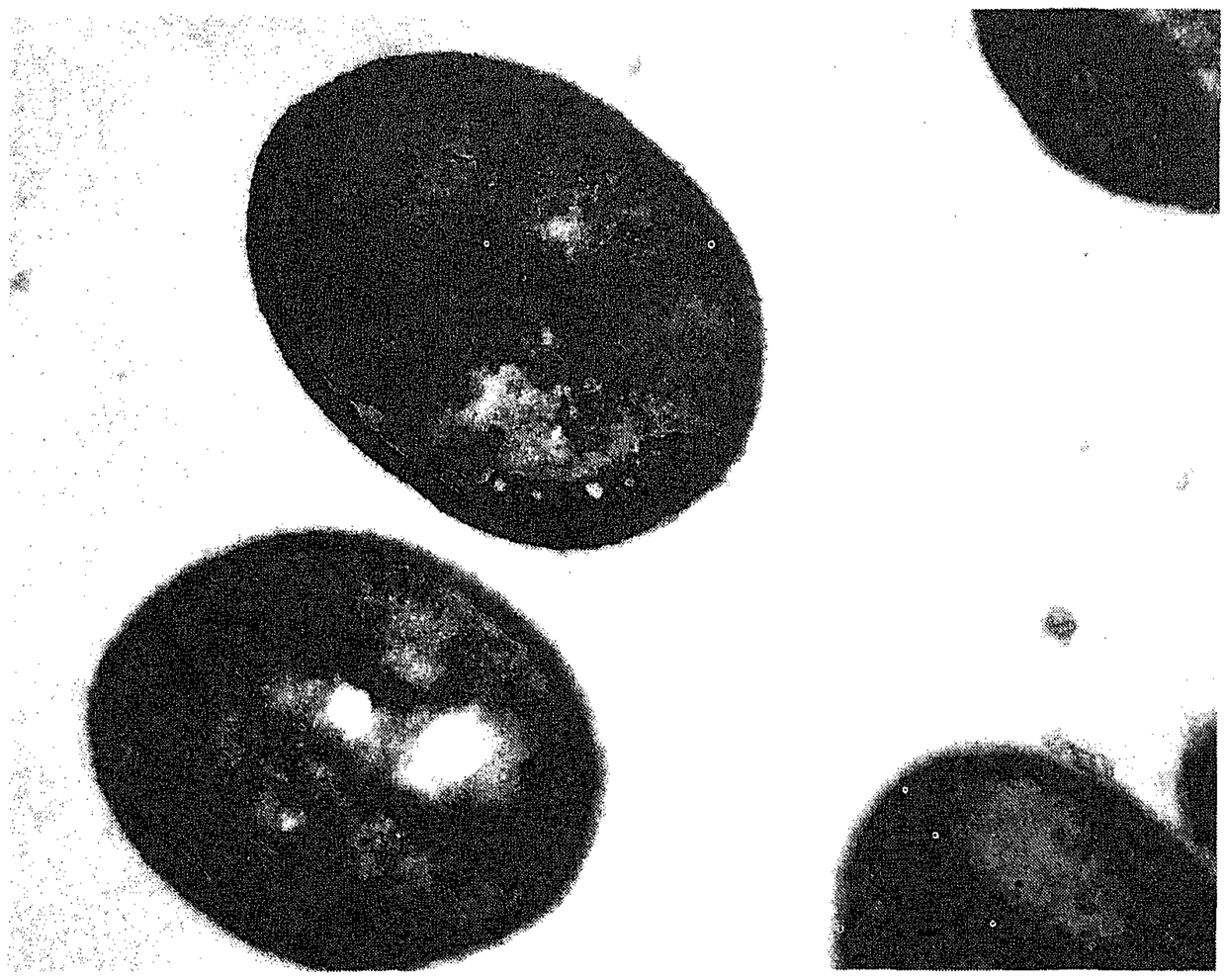

Fig. 9 Showing lack of changes in penicillin, methicillin, and gentamicin resistant *S. aureus* after exposure to methicillin.

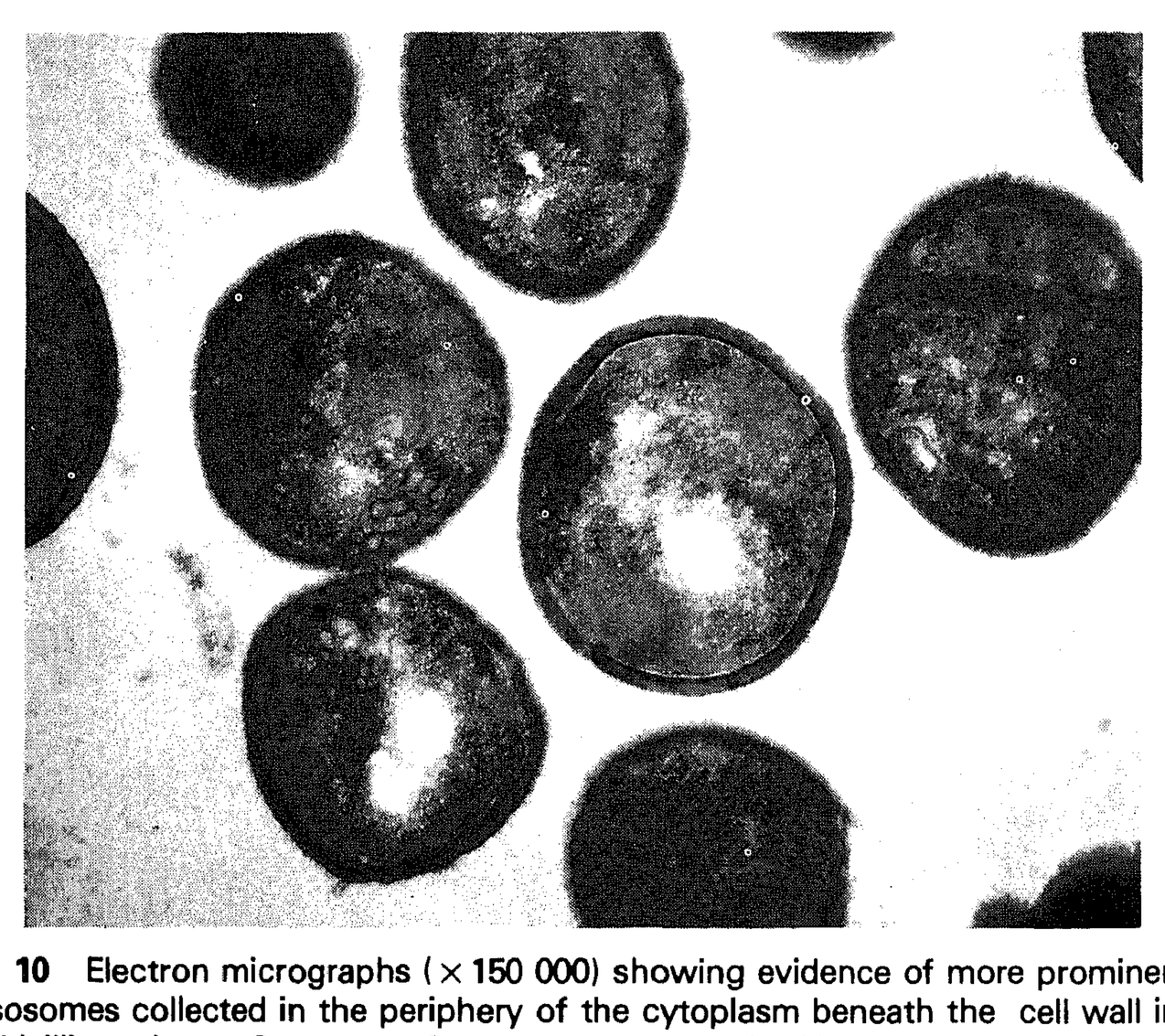

Fig. 10 Electron micrographs (× 150 000) showing evidence of more prominent mesosomes collected in the periphery of the cytoplasm beneath the cell wall in methicillin resistant *S. aureus* after exposure to methicillin.

3. The later changes (fig. 8) which occurred included:

(a) Breaks in the cell wall.
(b) Marked increase in size of the penicillin affected *Staphylococcus*.
(c) Increased thickness of the cell wall and its inward extensions at the line of division and with evidence of its destruction.
(d) Marked changes in the granular component, the ribosomes and the mesosomes which progressed to complete degeneration and necrosis of the cell. These findings suggested either the continuing intracellular action of penicillin or spontaneous autolysis of cytoplasm. The retraction of the disrupted and thickened cell wall often in a partially curled manner suggested the presence of some elasticity.
(e) Herniation and escape of cytoplasm through points of disruption in cell wall.
(f) Intact cell membrane frequently remaining for extended periods during the process of degeneration.
(g) Curling of fragments of cell wall of cell membrane which persisted after complete destruction of cytoplasm.

Similar findings to these have been described in the excellent studies of Avakyan, Kats & Pavlova (1972). After exposure of the penicillin, methicillin, and gentamicin-resistant *S. aureus* (Strain S-28914) to penicillin or methicillin, little or no changes in these bacteria were noted (fig. 9).

(a) Essentially no change in the size of the staphylococcal bodies.
(b) Little or no change in the thickness or density of the cell wall.
(c) No evidence of multiple layering of cell membrane.
(d) The granular component remained dense and evenly distributed.
(e) No evidence of destruction of cell bodies.

However, the mesosomes were noted to become more prominent and more easily visible with collections in the periphery of the cytoplasm beneath the cell wall. There also appeared to be a connection between the cell membrane and the mesosomes (fig. 10). The significance of this finding has not been understood.

Electron microscopic studies of the effects of exposure of beta-hemolytic *Streptococcus* and *Pneumococcus* to penicillin demonstrated findings similar to those noted with penicillin-sensitive strains of the *S. aureus*:

1. Greatest effect during cell division.
2. A thinner less densely staining cell wall.
3. Elongation of penicillin effected cells.
4. Supernumerary areas of cell wall disruption or beginning division.
5. Extensive destruction of the granular endocytoplasm (fig. 11).

It was noteworthy that no evidence of resistance of the hemolytic *Streptococcus* or *Pneumococcus* was seen, in contrast to that demonstrated by the *S. aureus*.

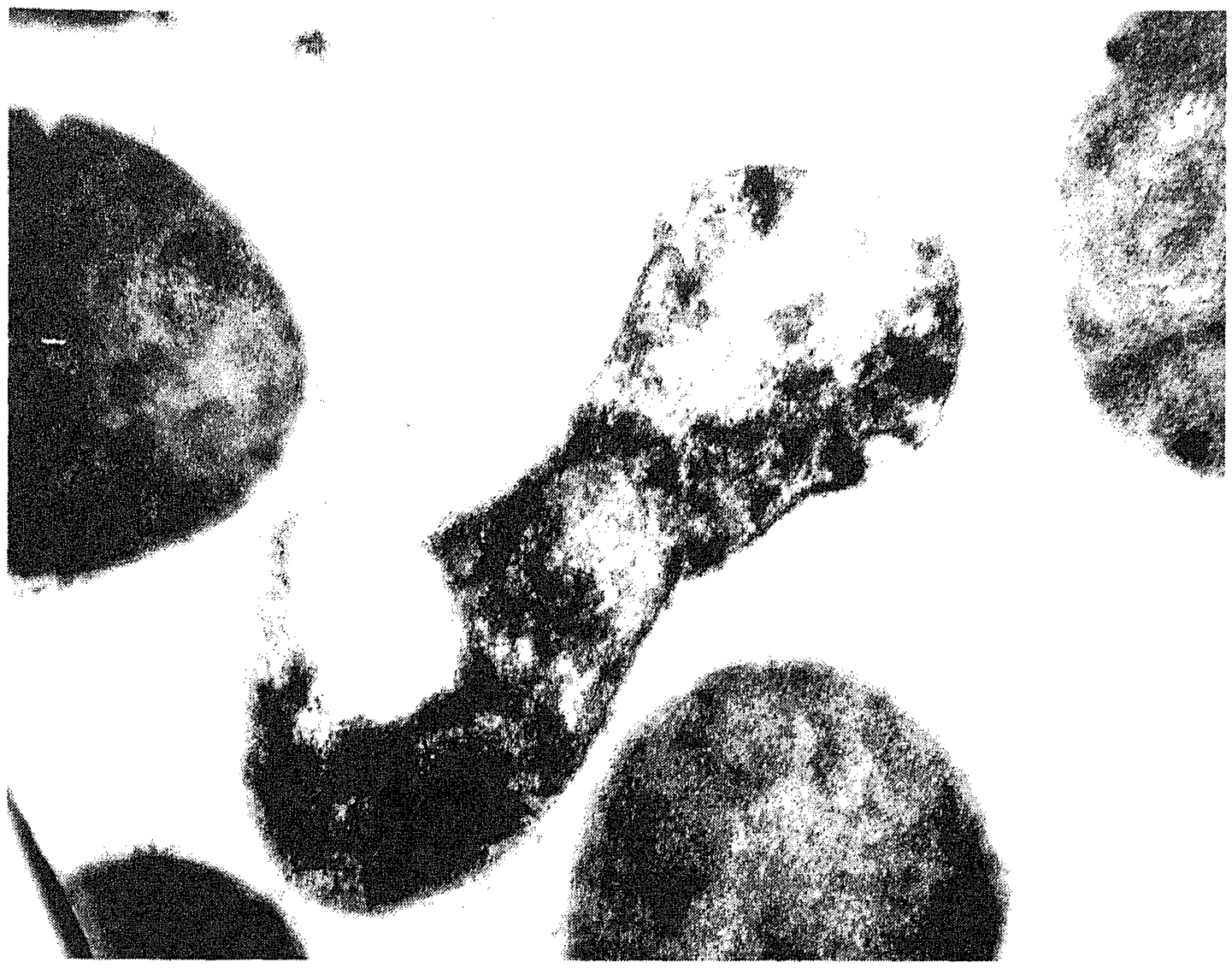

Fig. 11 Marked destructive changes produced by penicillin in beta-hemolytic streptococci shown by EM studies (× 150 000).

SUMMARY

The experience of the author and his associates during the past 50 years as well as that of other investigators has repeatedly demonstrated not only the multiple facets of the virulence and pathogenicity of the hemolytic *S. aureus*, but also its remarkable ability to develop or acquire effective methods of resisting and overcoming all antimicrobial agents used in its control.

As a pathogenic agent it has continued to produce a wide variety of diseases by means of complex mechanisms and by utilizing a number of exotoxins and extracellular enzymes.

Of special interest in understanding its unusual pathogenic activity has

been a new phenomenon which the authors have recognized and described. It is characterized by the sequential spontaneous emergence of new bacteriophage-types of staphylococci which reach high levels of incidence, cause local epidemics, persist for two to six years, and then recede rapidly or precipitously for reasons not clearly understood.

These different phage types have had different antibiotic-resistant patterns, and the two strains which have emerged most recently are demonstrating unusual resistance to almost all antistaphylococcal agents used in clinical practice today. The two types are now causing significant local epidemics.

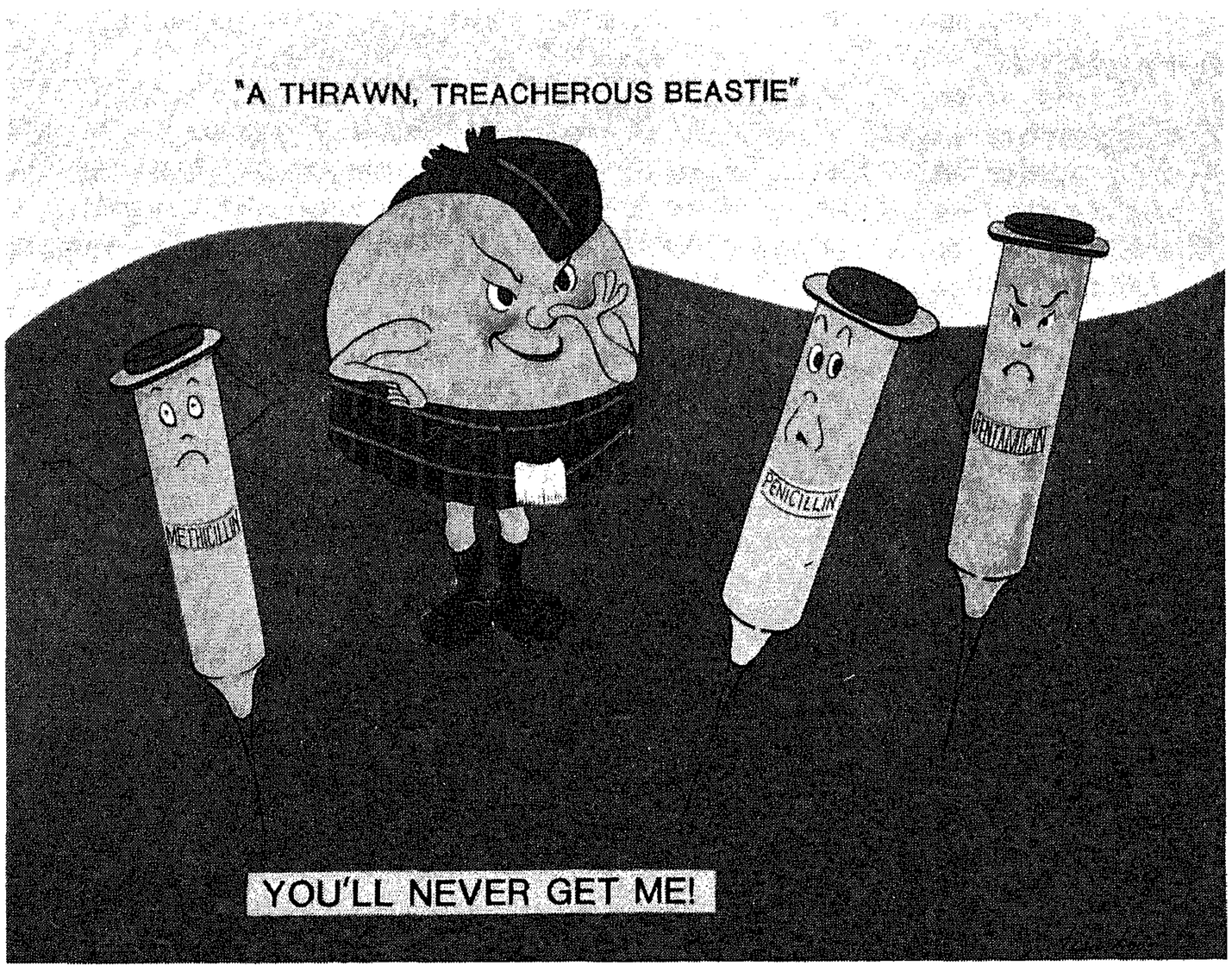

Fig. 12 *S. aureus*—a thrawn and treacherous beastie.

It has been believed by some that *S. aureus* has receded in incidence to such a degree that it no longer is an important cause of wound infections. While the gram-negative bacilli have assumed increasing importance in this regard, it has been our experience that the versatility of *S. aureus* has

permitted it not only to remain as a significant cause of wound infections and other staphylococcal diseases, but also to become the etiologic agent of some newly recognized infections such as toxic shock syndrome. In any event, the *Staphylococcus* has remained with us in many areas, and it can be expected to 'come back' in others.

All in all, I believe Professor Ogston and Professor Marnoch would both agree with me today that the *Staphylococcus* is a 'thrawn and treacherous beastie' (fig. 12), and that we can expect this formidable adversary to be around for many years to come—perhaps even for the next Centenary Conference.

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