

The Bacteriology of the Staphylococci

Staphylococcus aureus

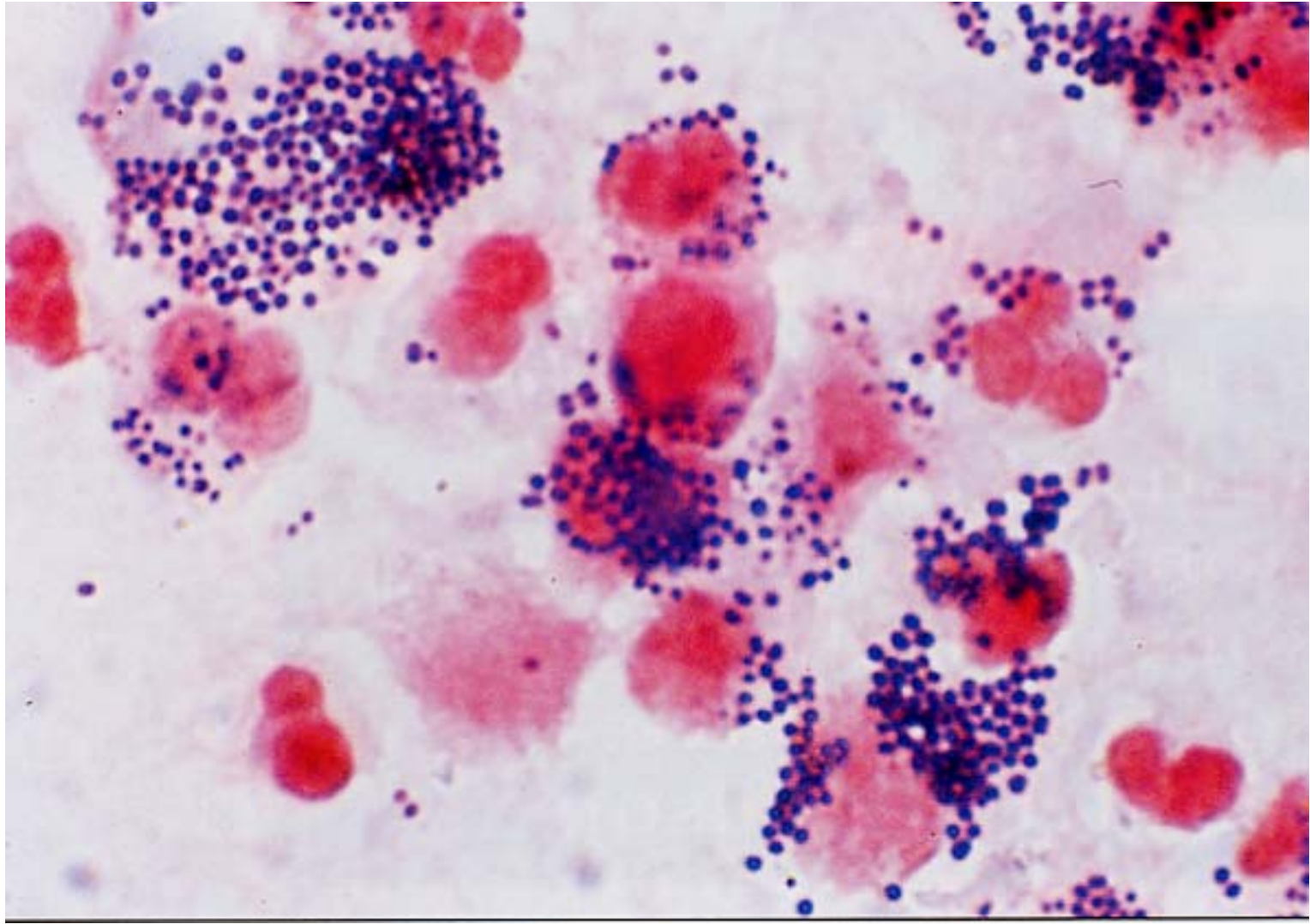
- Many neonates, most children and adults become transiently colonized by *S. aureus*.
- The organism is carried preferentially in the nasopharynx, occasionally on their skin and clothing and more rarely in the vagina, in the rectum and or perineal area.
- From these sites *S. aureus* can contaminate any site of the human body by intrapersonal transfer by aerosol and by direct contact.

Staphylococcus aureus

- The mucous membranes and the skin are very efficient barriers against local invasion.
- If the barrier is breached by trauma, surgery or other means (needles. Etc) the organisms gain access to the underlying tissue and creates a local abscess.
- The abscess is the hallmark of staphylococcal infection consisting of necrotic tissue, fibrin and a large number of live and dead PMN
- Toxin liberation o the skin and other organs can cause various types of rash, general symptoms as exemplified by TSS or diarrheal disease.

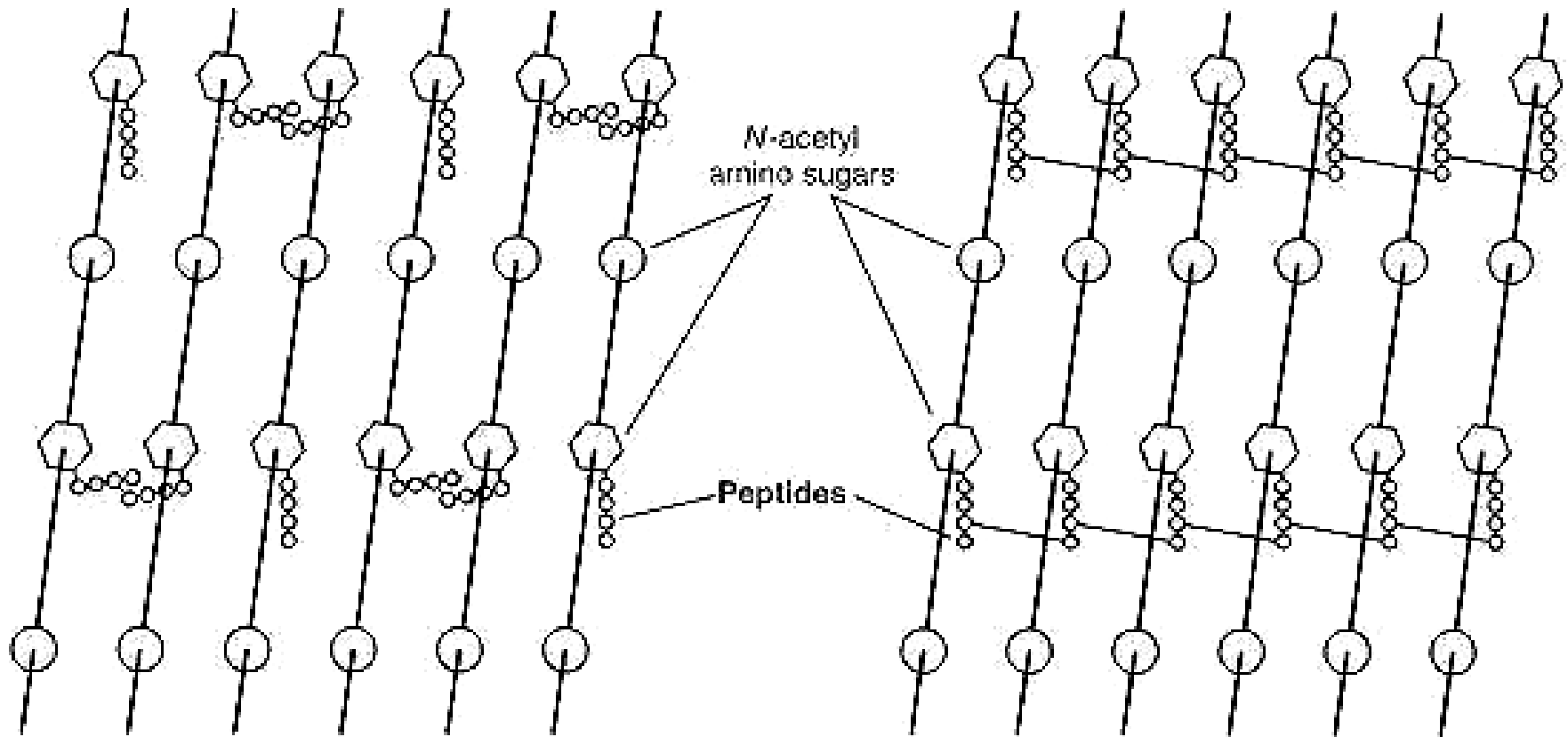
Staphylococcus aureus

- Bacteria in local abscesses or multiplying at any site, can sometimes overcome local phagocytic defenses and gain access to the lymph channels and blood stream.
- The resulting staphylococcal bacteria is a dreaded complication and can lead to deadly disease complications like pneumonia, endocarditis or osteomyelitis.
- Staphylococci are among the most robust microbes that infect humans. This and its propensity to develop antibiotic resistance establish this microbe as a major human pathogen.

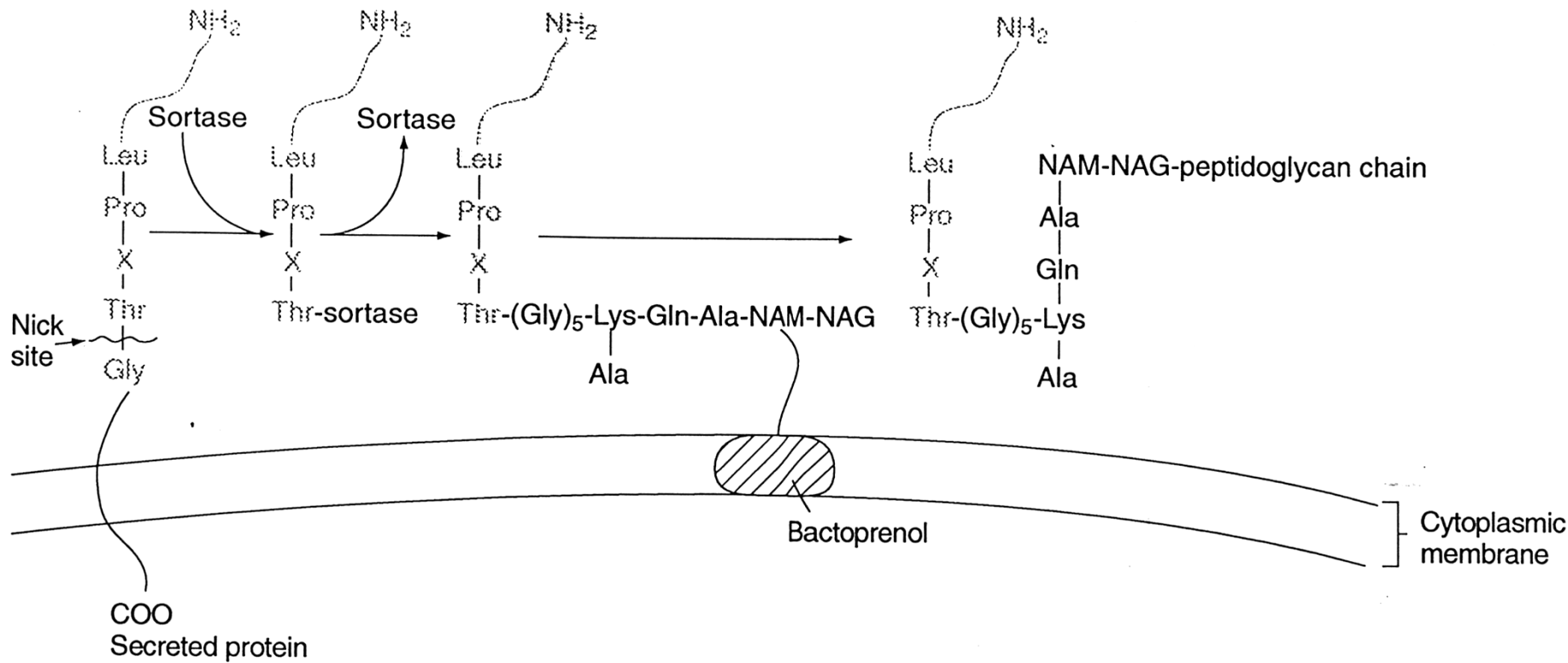


Gram-negative peptidoglycan

Gram-positive peptidoglycan



Diagrammatic representation of peptidoglycan structures with adjacent glycan strands cross-linked directly from the carboxyterminal D-alanine to the e-amino group of an adjacent tetrapeptide or through a peptide cross bridge, N-acetylmuramic acid; N-acetylglucosamine



Sortase

Sortase

NH₂

NH₂

NH₂

NH₂

Leu
Pro
X
Thr
Gly

Leu
Pro
X
Thr-sortase

Leu
Pro
X
Thr-(Gly)₅-Lys-Gln-Ala-NAM-NAG
Ala

Leu
Pro
X
Thr-(Gly)₅-Lys

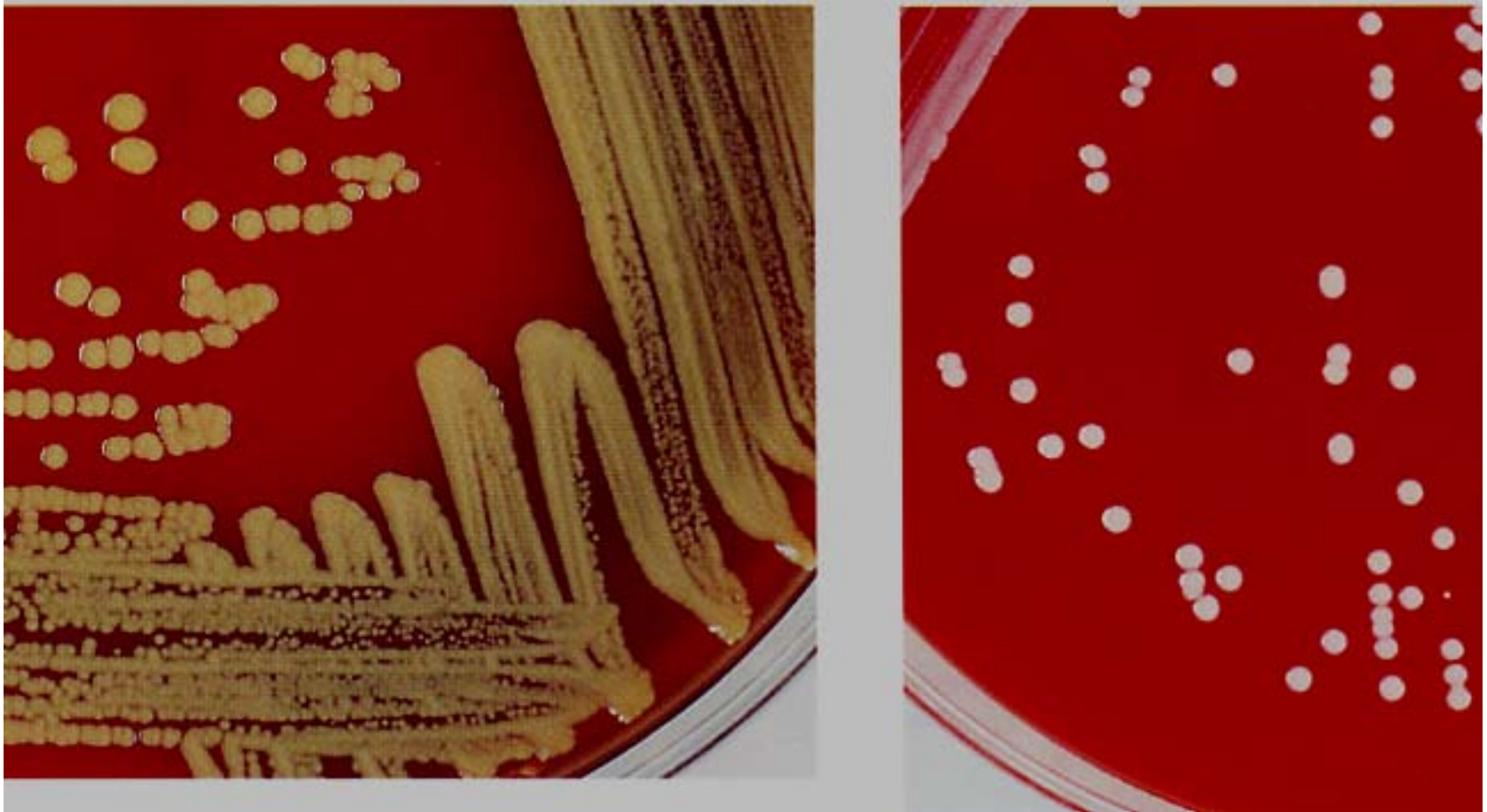
NAM-NAG-peptidoglycan chain
Ala
Gln
Ala

Nick site

COO
Secreted protein

Bactoprenol

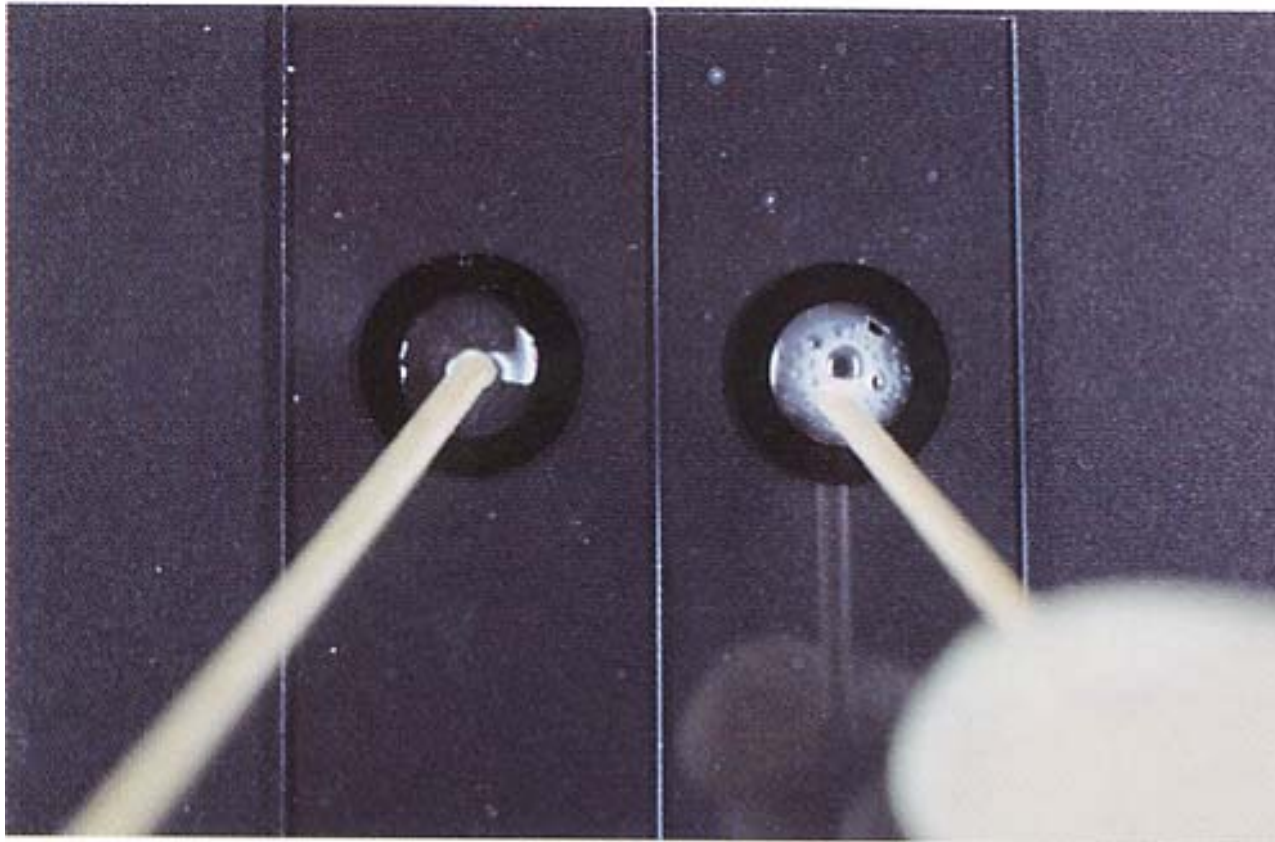
Cytoplasmic membrane



Typical colonies of *staphylococcus epidermidis* on right showing porcelain-white colonies as compared to *S. aureus* on the same medium (left) the golden appearance of the colonies. This clear distinction in colony color is not seen at all times.



Young colonies of *Staphylococcus aureus* showing beta hemolysis

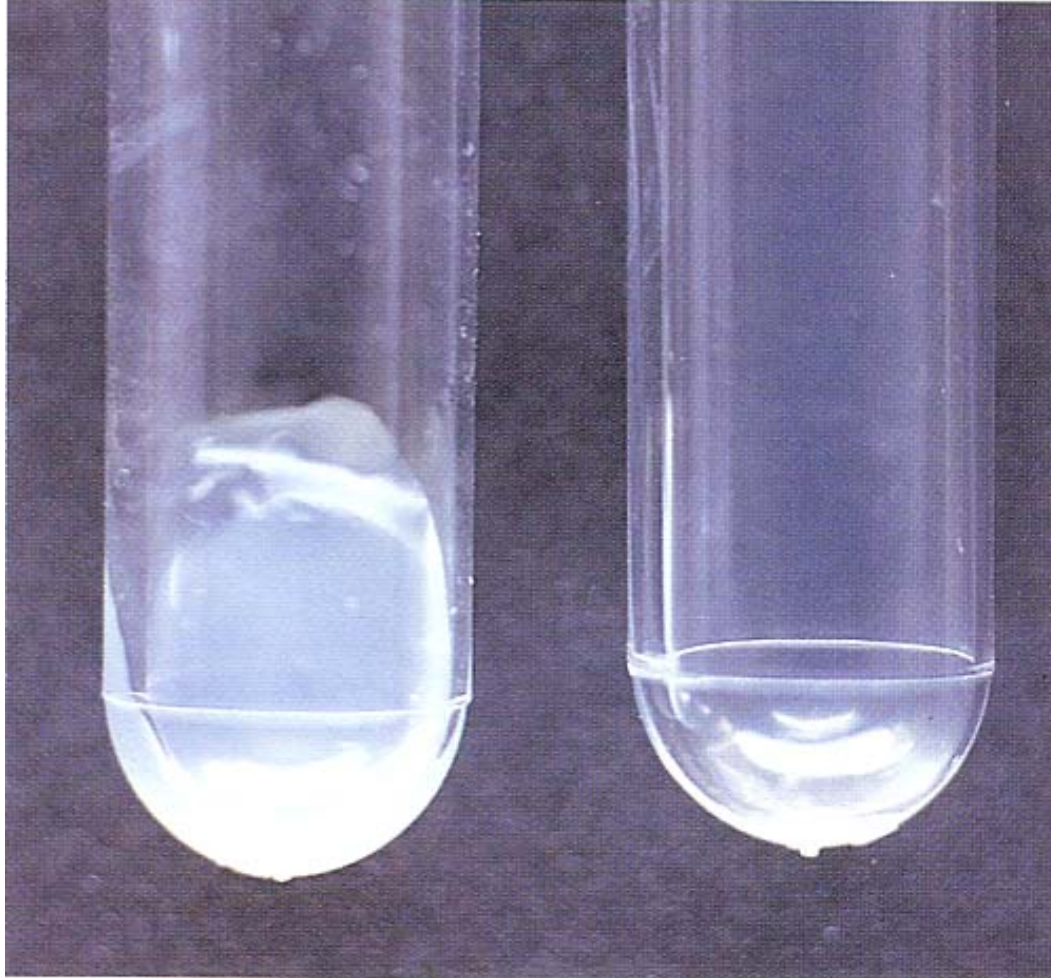


Staphylococci can be differentiated from other aerobic gram positive cocci by a positive catalase test. The test is performed by adding bacterial cells from a colony to a drop of 3% hydrogen peroxide. The appearance of bubbles (right) indicates the enzyme catalase while catalase negative bacteria give no reaction (left).



Slide Coagulase test.

The most important distinction among staphylococci is whether or not they produce the enzyme coagulase. *S. aureus* is the most common pathogen among the catalase positive gram positive cocci and is differentiated from other staphylococci by the coagulase test. Here the bacterial cells have been suspended in a drop of rabbit plasma. Coagulase bound to the cell wall acts on fibrinogen and causes the clumping of the bacteria (right). Coagulase is an important virulence factor of *S. aureus*.



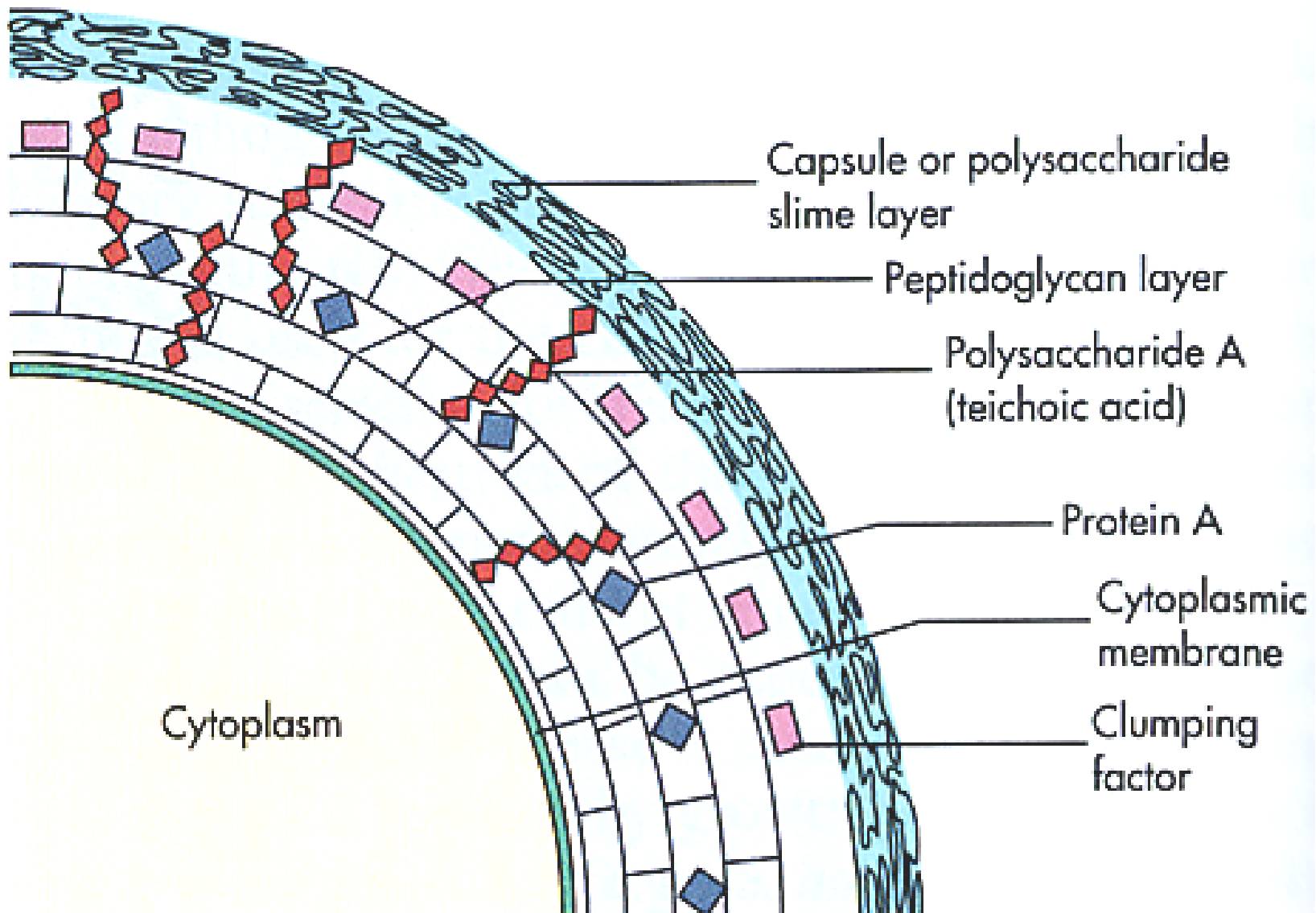
The tube coagulase test detects both free and cell bound coagulase of *S. aureus*. Bacteria are incubated in plasma for 2-4 hours and the tubes turned on their sides as illustrated. Free coagulase acts on prothrombin and fibrinogen in plasma and forms a fibrin clot (left). In many laboratories staphylococci are simply differentiated as coagulase positive or coagulase negative without speciation.



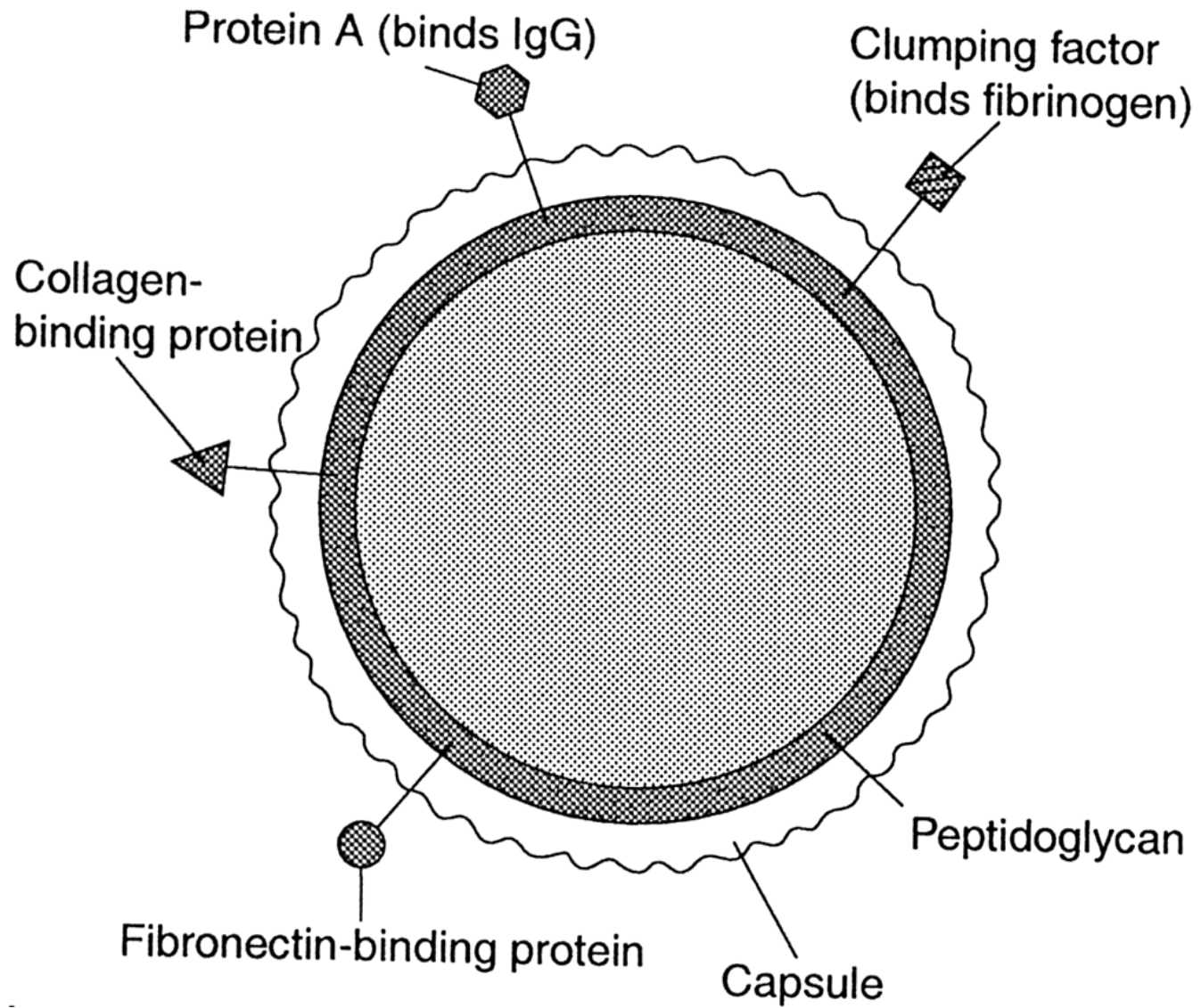
Growth on Mannitol-Salt agar differentiates *S. aureus* from other catalase positive gram positive cocci like *s. epidermidis*. *S. aureus* grows as shown here on an agar medium containing 7.5% NaCl which inhibits the growth of many other organisms. *S. aureus* also can ferment mannitol into acid detected here by the change in pH indicator from red to yellow. (right)

Staphylococcus

- Virulence Factors
 - ◆ Antigens
 - ☞ Capsule
 - ☞ Adhesins
 - ◆ Enzymes
 - ☞ Coagulase
 - ☞ Lipase
 - ☞ Hyaluronidase
 - ☞ Staphylokinase
 - ☞ Nuclease



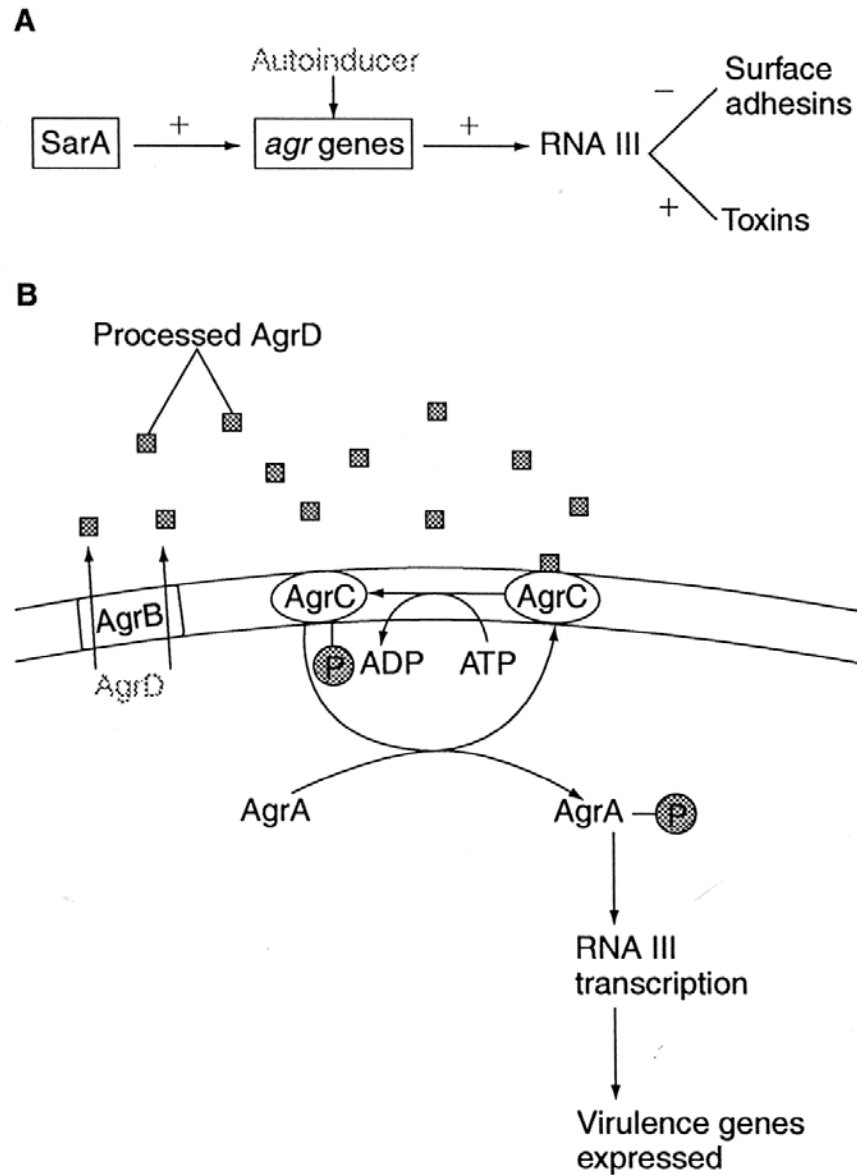
***Staphylococcus aureus* cell wall**



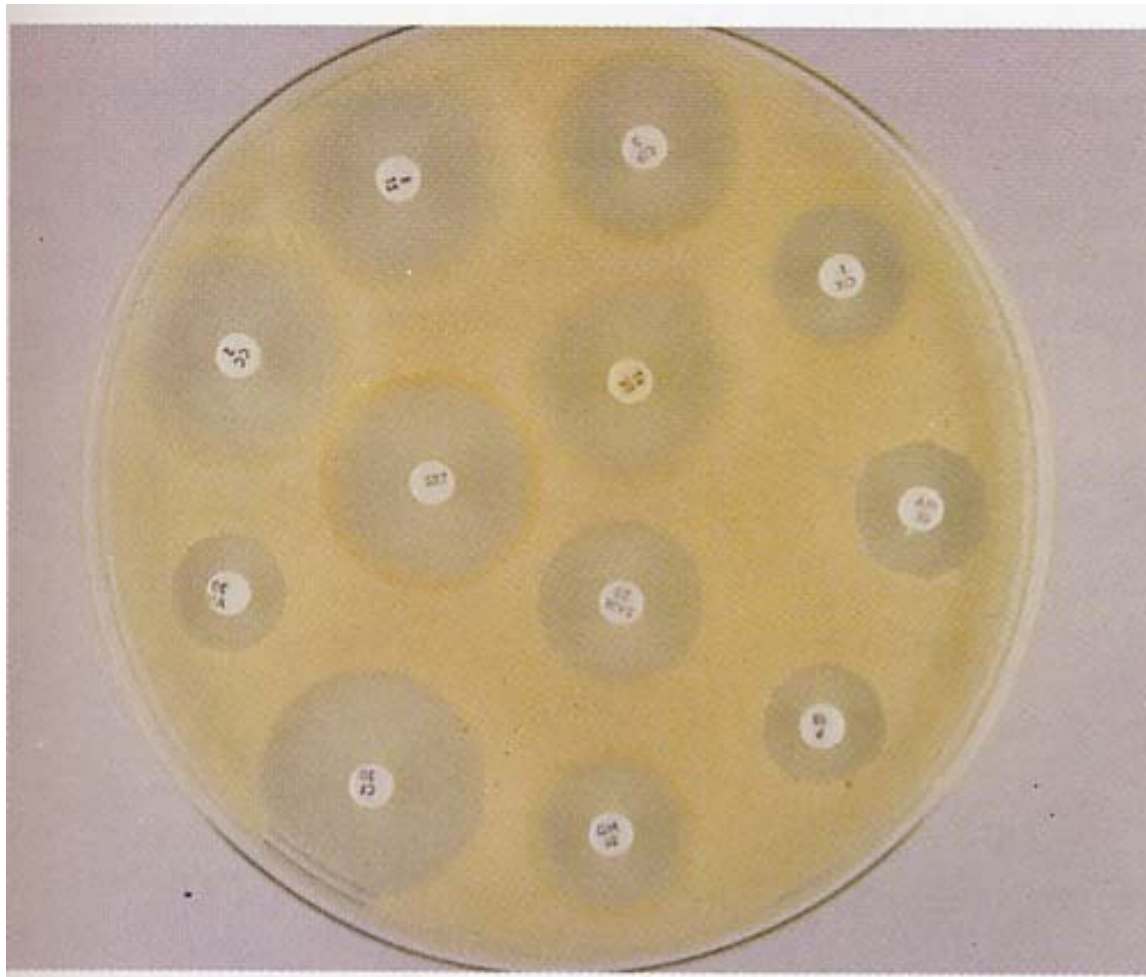
S. Aureus Surface Virulence Factors

Cell Wall Structure and Function

| Structure | Function |
|----------------------|--|
| Capsule | Inhibits opsonization and phagocytosis Protects from C'-mediated leukocyte destruction |
| Peptidoglycan | Osmotic stability Stimulates production of endogenous pyrogen Leukocyte chemoattractant |
| Protein A | Inhibits phagocytosis and chemotaxis Binds IgG1, IgG2, IgG4 Fc receptors Inhibits opsonization and phagocytosis Leukocyte chemoattractant |
| Teichoic acid | Anticomplementary Regulates cationic concentration at cell membrane Receptor for bacteriophages Attachment site for mucosal surface receptors |
| Cytoplasmic membrane | Osmotic barrier Regulates transport into and out of cell Site of biosynthetic and respiratory enzymes |



The AGR Pathway of Virulence Regulation in *S. aureus*



Antimicrobial susceptibility testing of *S. aureus*.

Staphylococcus

■ Characteristics

◆ Physiology and Genetics

☞ Facultative Anaerobe

☞ Antibiotic Resistance

- Beta lactamase (pen G, Amp, Ticarcillin)

- PBP - nafcillin

- Tolerance

- Tet, Ery, Aminoglycoside

- Susceptible to Vancomycin

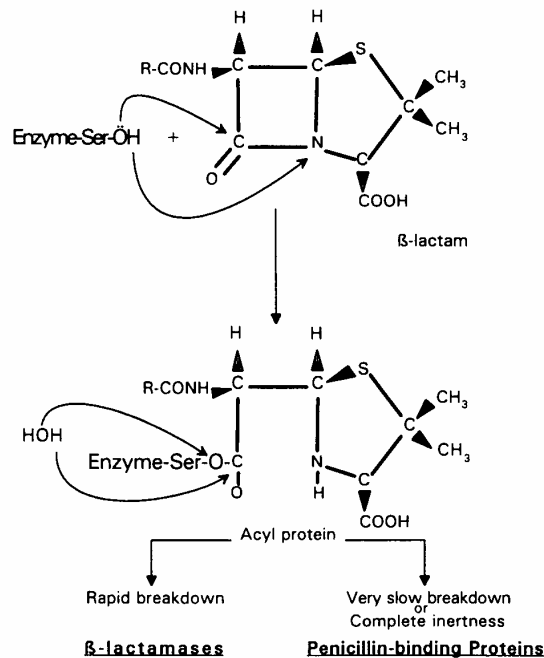


FIGURE 1 Penicillin-interactive, active-site serine peptidases and their reactions with β -lactam carbonyl donors. Modified with permission from reference 61.

Resistance to Antimicrobials particularly to β -lactam antibiotics is a major problem in the treatment of *S. aureus* and *S. epidermidis* disease.

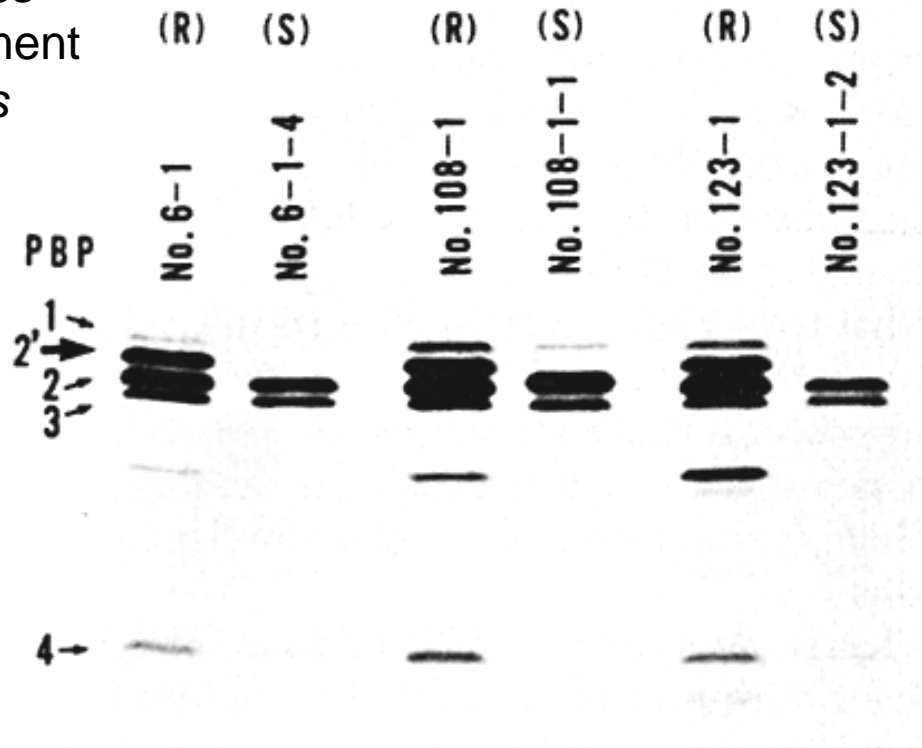


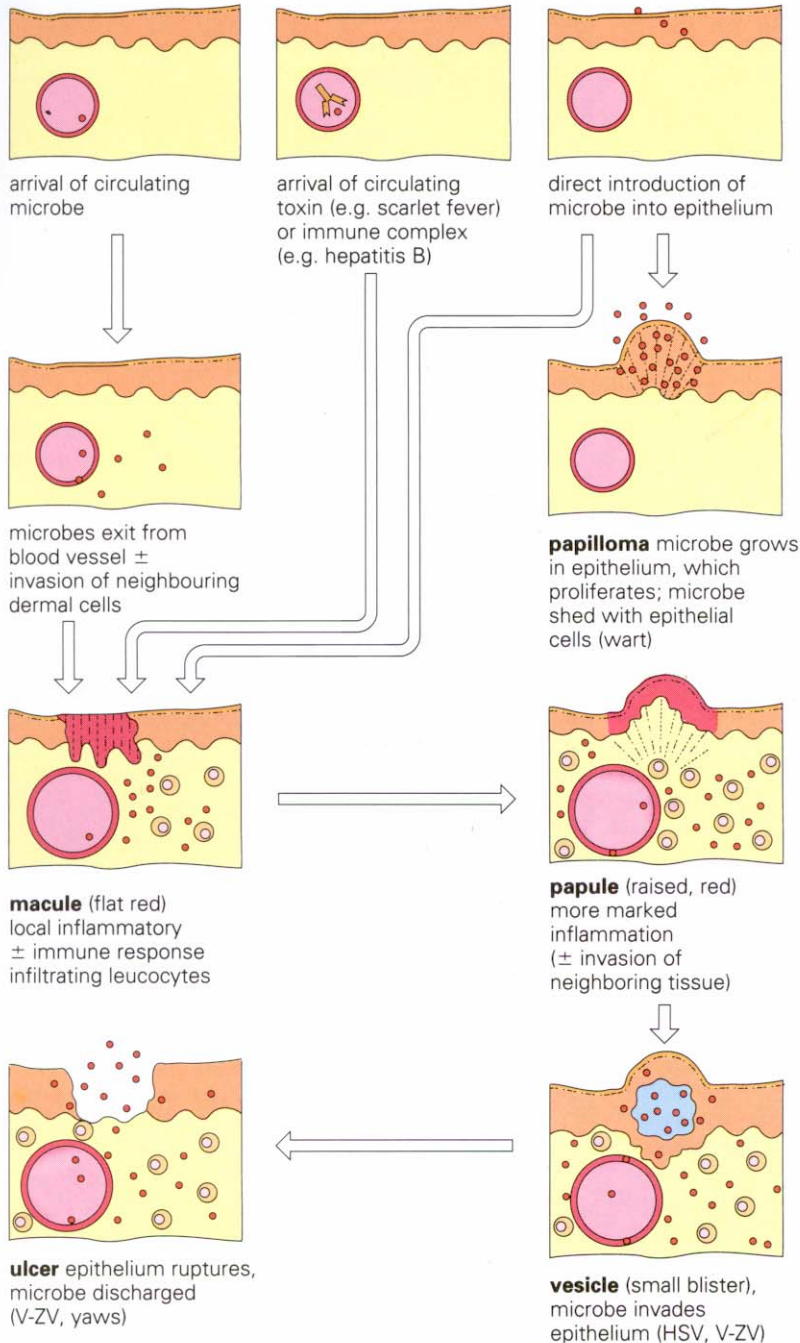
FIGURE 22-10 Penicillin-binding protein profiles of methicillin-resistant *Staphylococcus aureus* (6-1, 108-1, 123-1) and methicillin-susceptible revertant strains (6-1-4, 108-1-1, 123-1-2). Note that penicillin-binding protein 2' is present in all methicillin-resistant strains and absent in all methicillin-susceptible strains. (From Utsui Y, Yokota T: *Antimicrob Agents Chemother* 28:397-403, 1985.)



Methicillin resistant *S. aureus* (MRSA) are detected by their ability to grown on an agar medium containing 6ug/ml of oxacillin. Growth in 24hr incubation from a spot inoculum of MRSA is shown at the top and the lack of significant growth of a methicillin-susceptible *S. aureus* is shown at the bottom

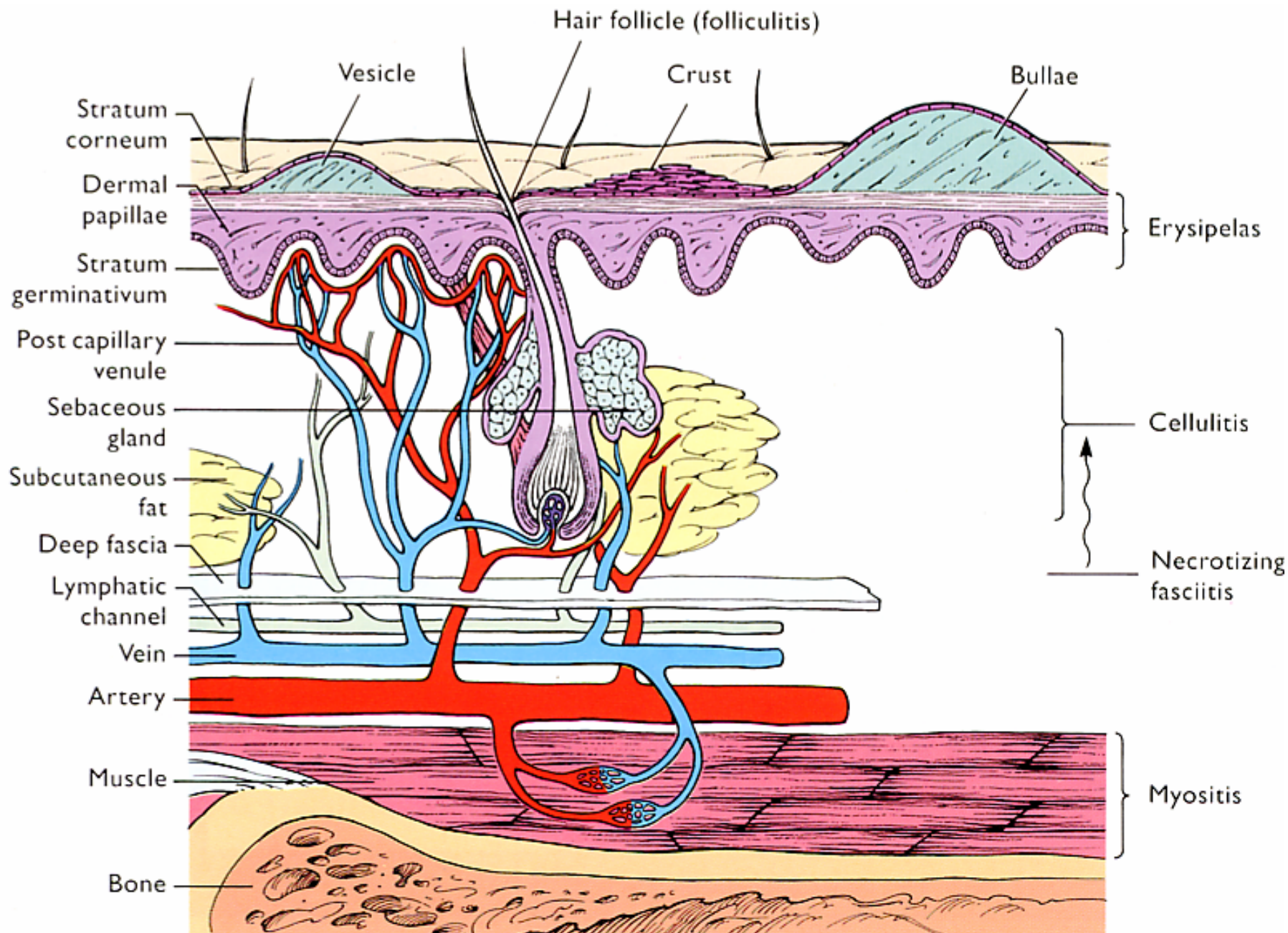
Clinical Manifestations

The basic anatomic lesion is the abscess. Toxins produced by the organism may predominate the clinical picture. Even the most benign localized infection can occasionally become the seeding site for a devastating systemic disease.



Types of Skin Lesions

- Macules - small (<10 mm) discolored spots
- Papules - small, solid raised elevations
- Vesicles - small, elevated lesions, containing serous fluid
- Bullae - larger vesicles
- Pustules - small, elevated lesions, containing pus
- Ulcers - circumscribed lesions, characterized by loss of epidermis and part of the dermis





Staphylococcal folliculitis

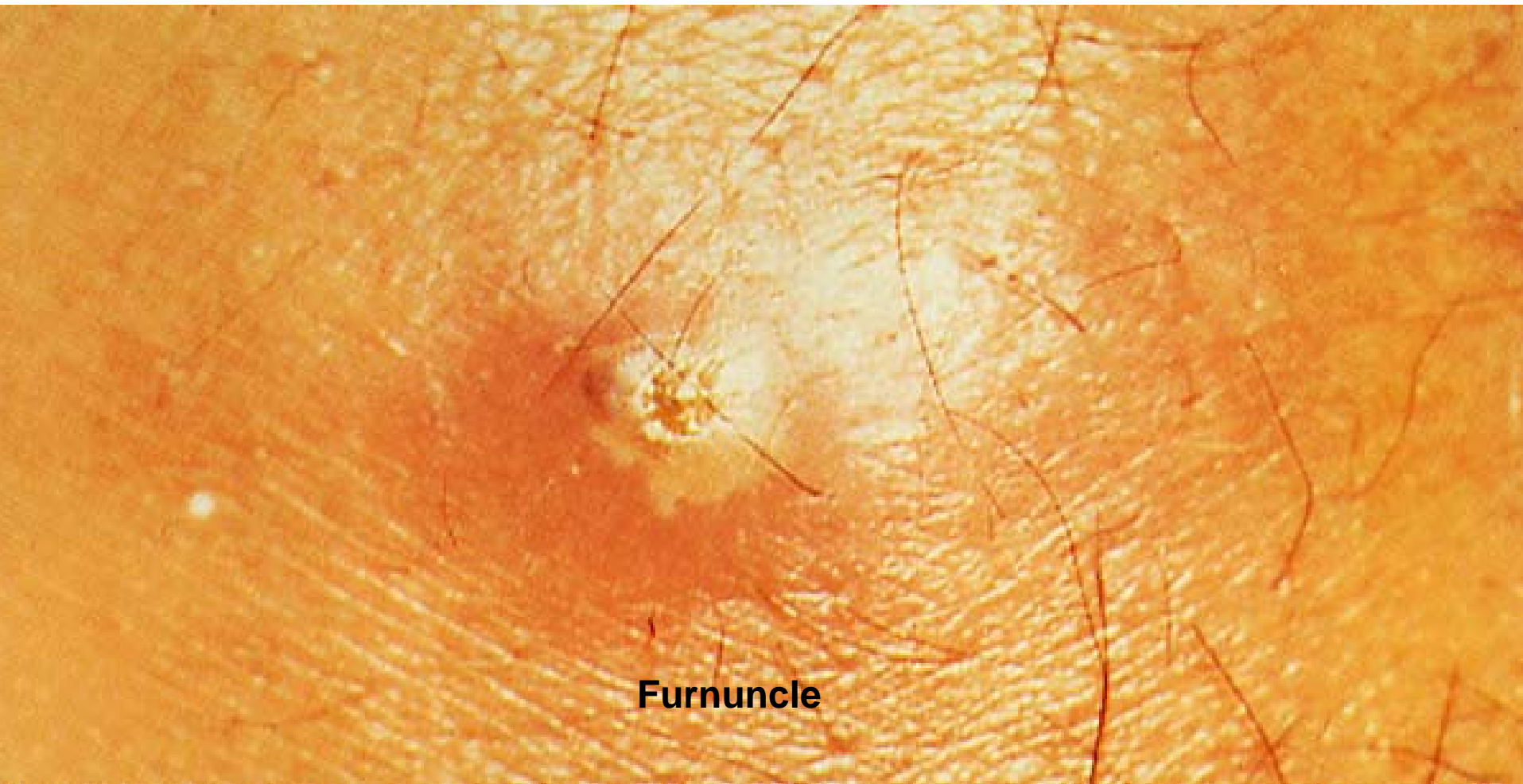




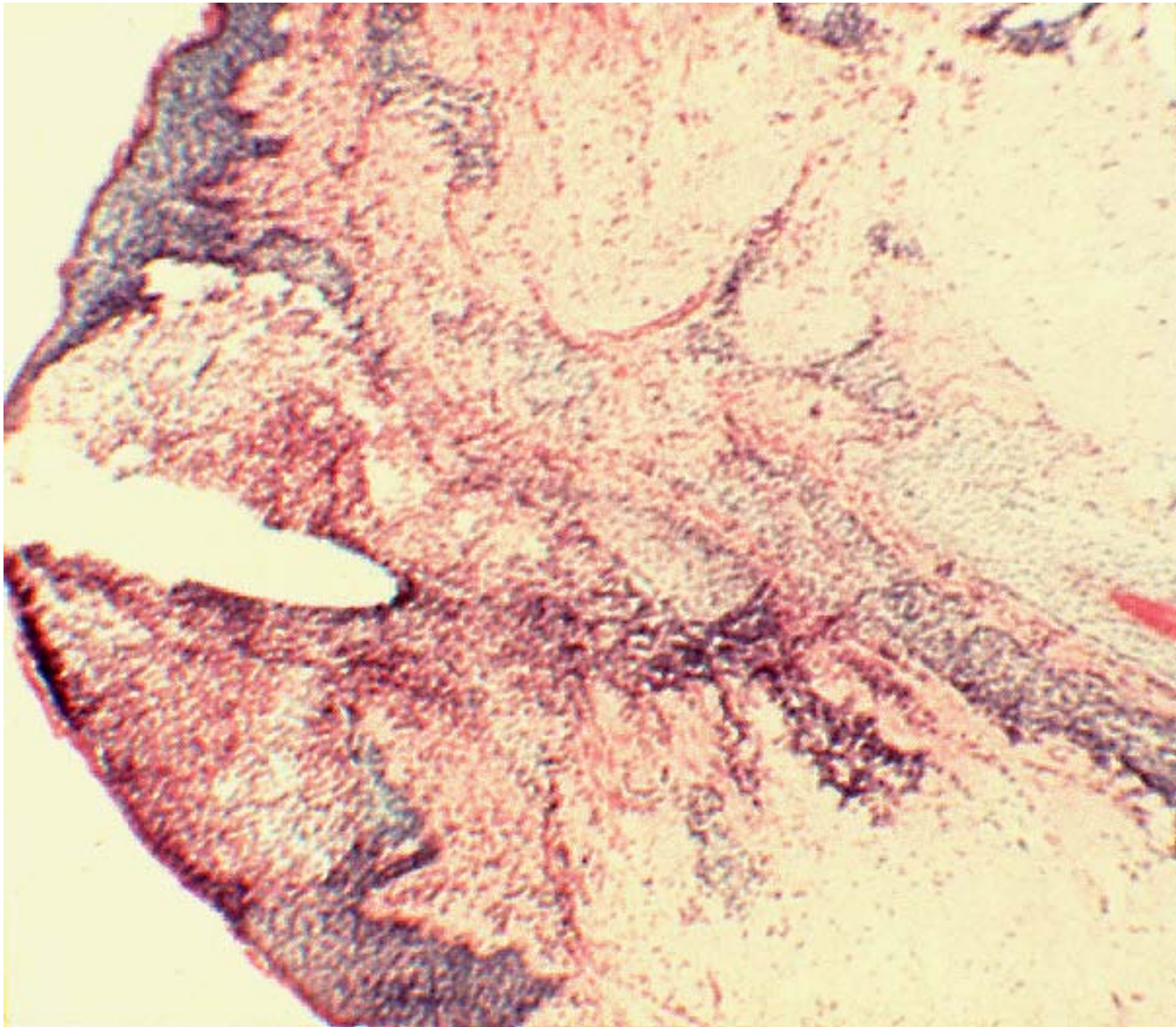
Staphylococcal Paronychia



Staphylococcal furuncle better known commonly as a “ Boil”



Furnuncle



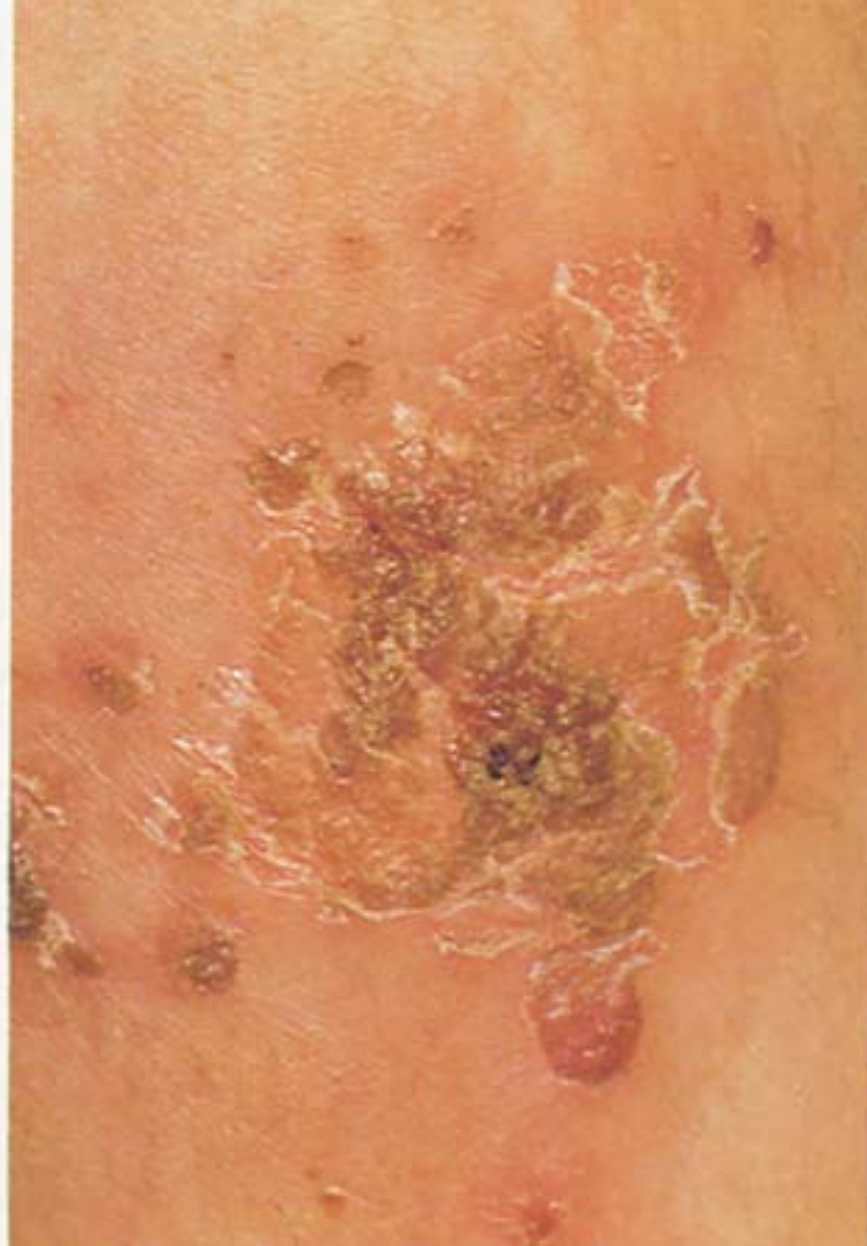
Resolving Staphylococcal abscess after drainage



Staphylococcal carbuncle



There is no end to the trouble that staphylococcal infection can cause





Staphylococcus

- Virulence Factors

- ◆ Toxins

- ☞ α -Toxin

- ☞ β -Toxin

- ☞ δ -Toxin

- ☞ P-V Leukocidin

- ☞ Enterotoxin

- ☞ Exfoliative Toxin

- ☞ Toxic Shock Syndrome Toxin

Staphylococcal Scalded Skin Syndrome. Erythema is prominent on the neck and around the eyes and mouth. Crusting is also apparent.

The usual sequence of this disease is:

1. Cutaneous erythema
2. Development of superficial vesicles and bullae.
3. Skin separation in sheets and ribbons leaving a moist red base that dries quickly.





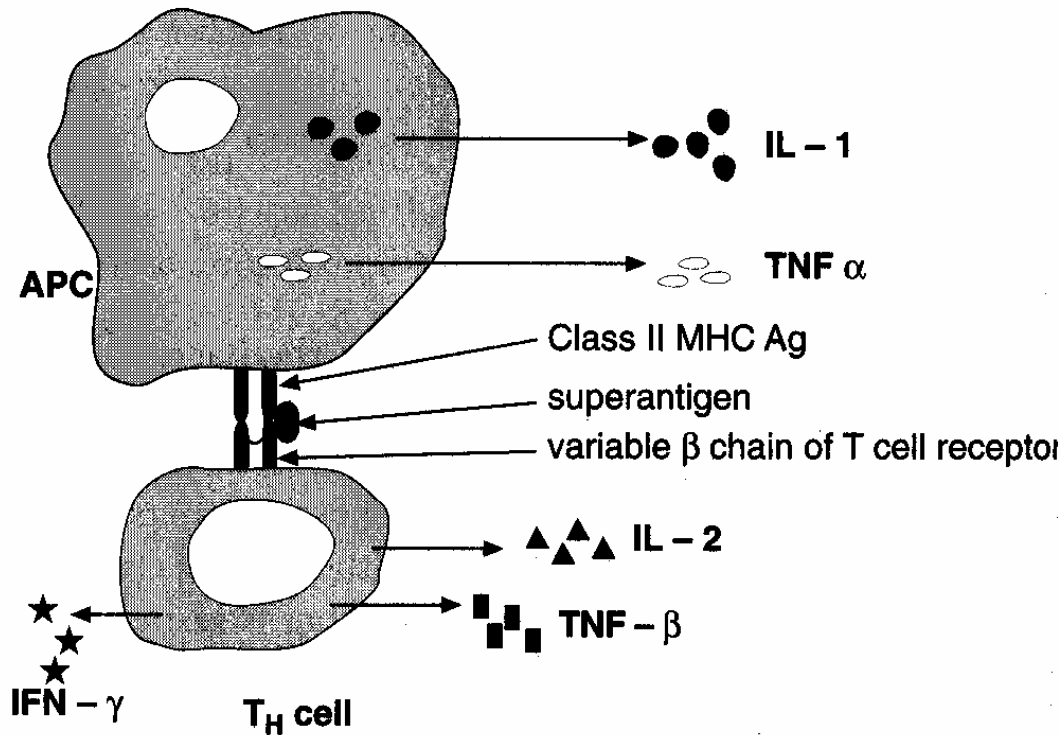
TABLE 22-4

Characteristics of Exfoliative Toxins

| PROPERTIES | EXFOLIATIVE TOXIN A | EXFOLIATIVE TOXIN B |
|-----------------------|----------------------------|----------------------------|
| Size | 24,000 daltons | 24,000 daltons |
| Temperature tolerance | Stable (100° C, 20 min) | Labile (60° C, 30 min) |
| EDTA treatment | Inactivated | No effect |
| DNA | Chromosomal | Plasmid |

Toxic shock syndrome toxin-1
Staphylococcal enterotoxin
Staphylococcal exfoliatin
Streptococcal pyrogenic exotoxins A-C

SUPERANTIGENS

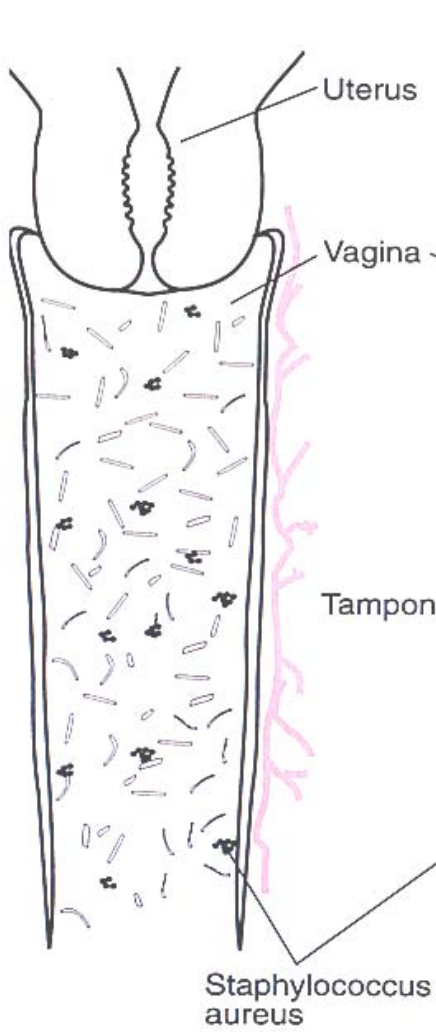


Binding of a T cell to a superantigen on the MHC of an antigen-presenting cell (APC), resulting in the synthesis and excretion of IL-1 and TNF- α by the APC and in the secretion of lymphokines by the T cell

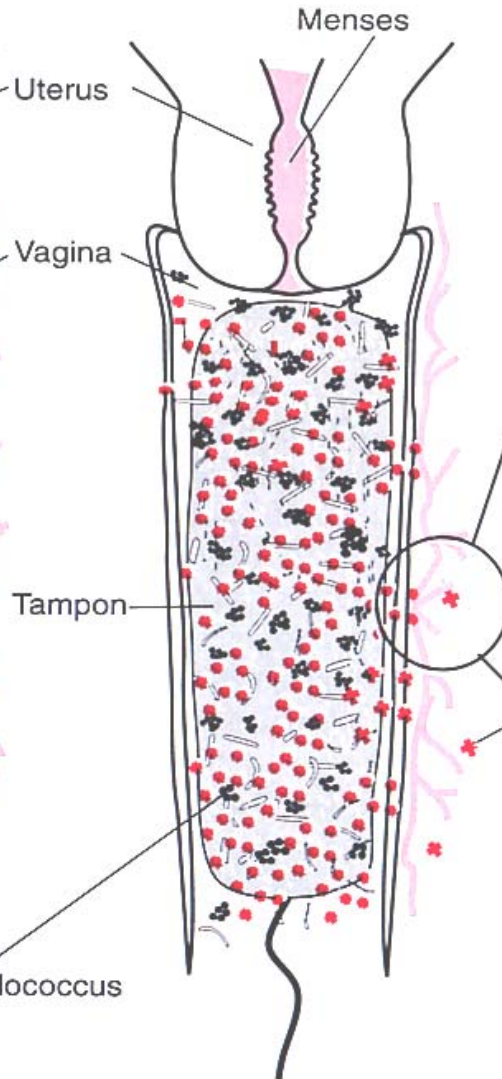


The Natural History of Staphylococcal Toxic Shock Syndrome

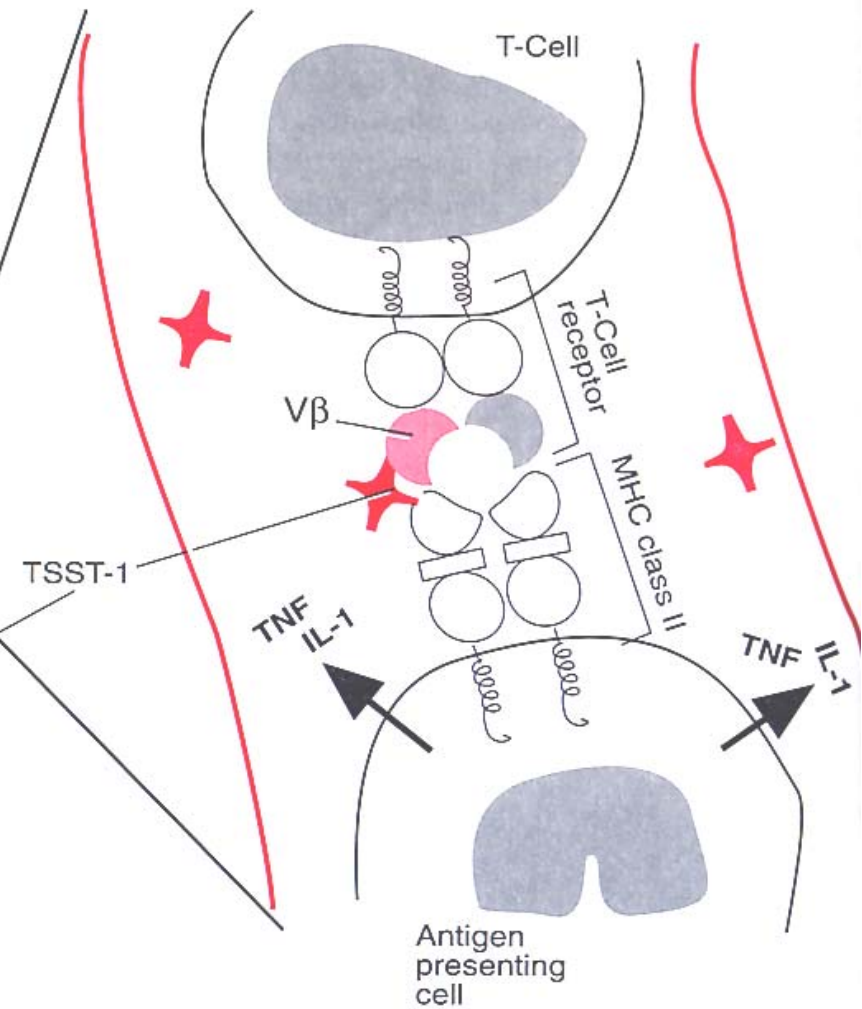
A. Vaginal colonization



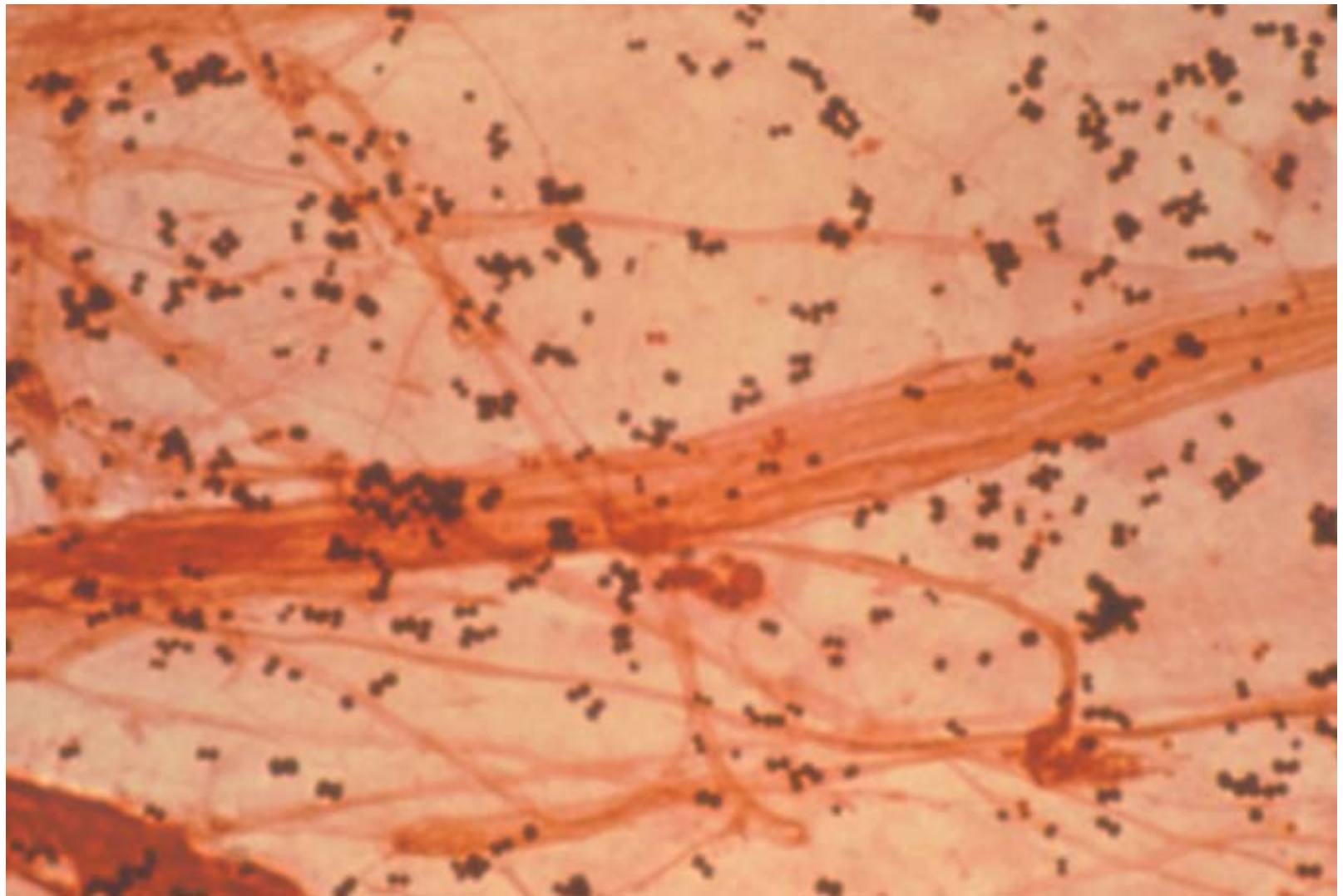
B. Toxin production during menses



C. Toxin absorption, and superantigen stimulation of cytokine production



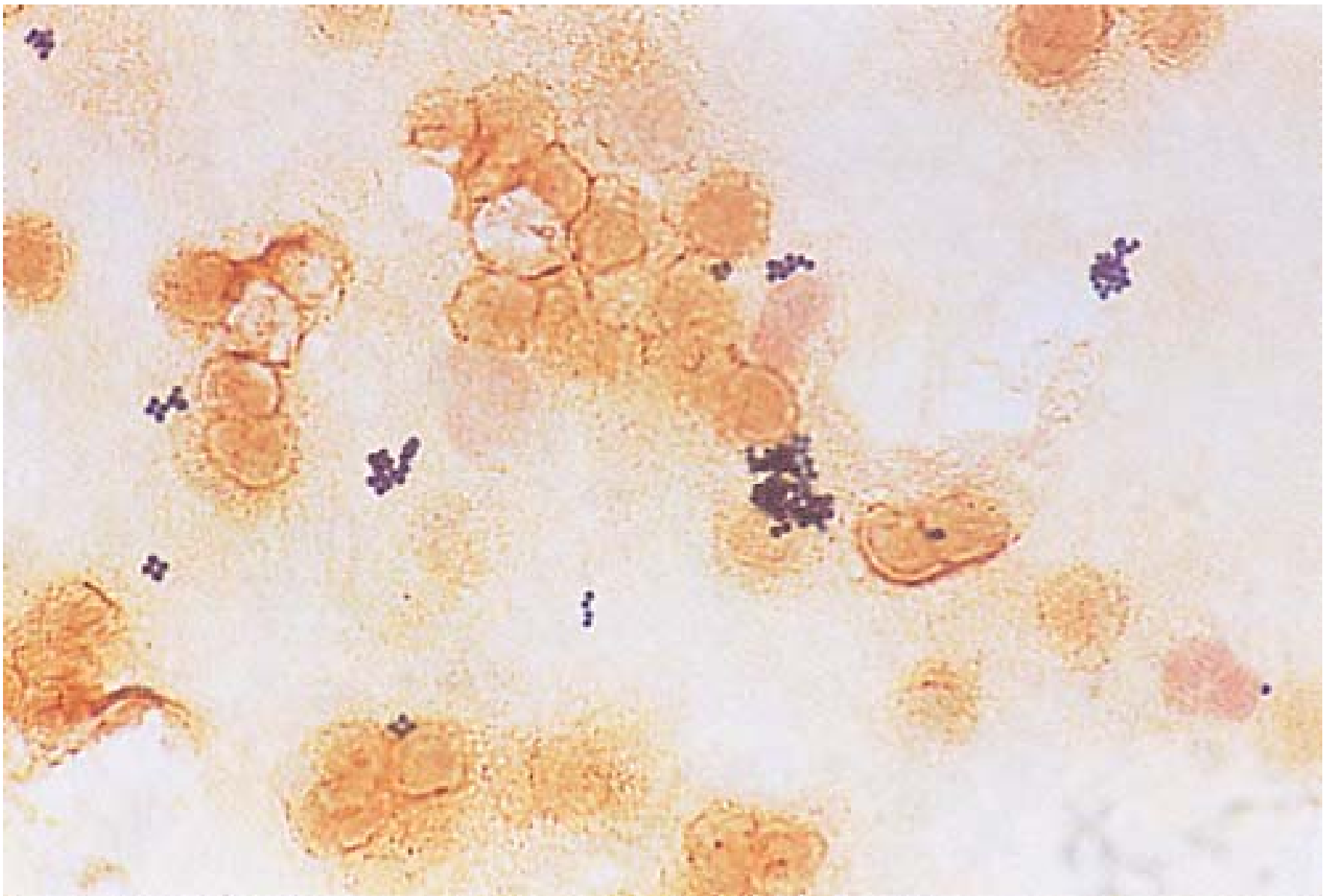
Staphylococcal Systemic Infections



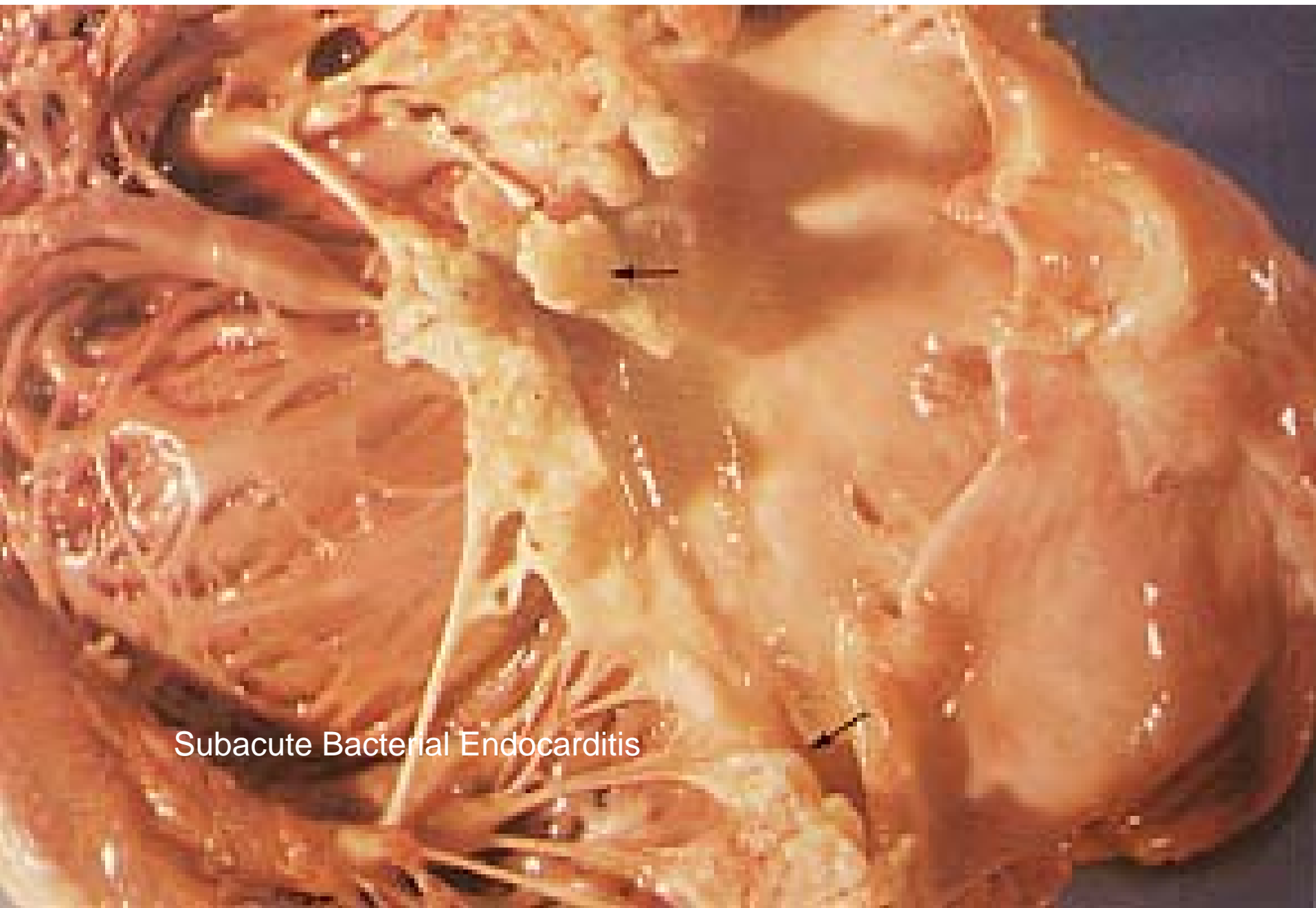




Septic arthritis. Following elective surgery complicated by a staphylococcal wound infection, this woman was re-admitted with fever, right shoulder pain and lumbar backpain. Needle aspiration of the right shoulder and an intravertebral disk revealed a coagulase positive *Staphylococcus aureus*.



Gram stain of *Staphylococcus aureus* from a positive blood culture bottle showing typical gram positive cocci in pairs , tetrads and grape-like clusters.



Subacute Bacterial Endocarditis

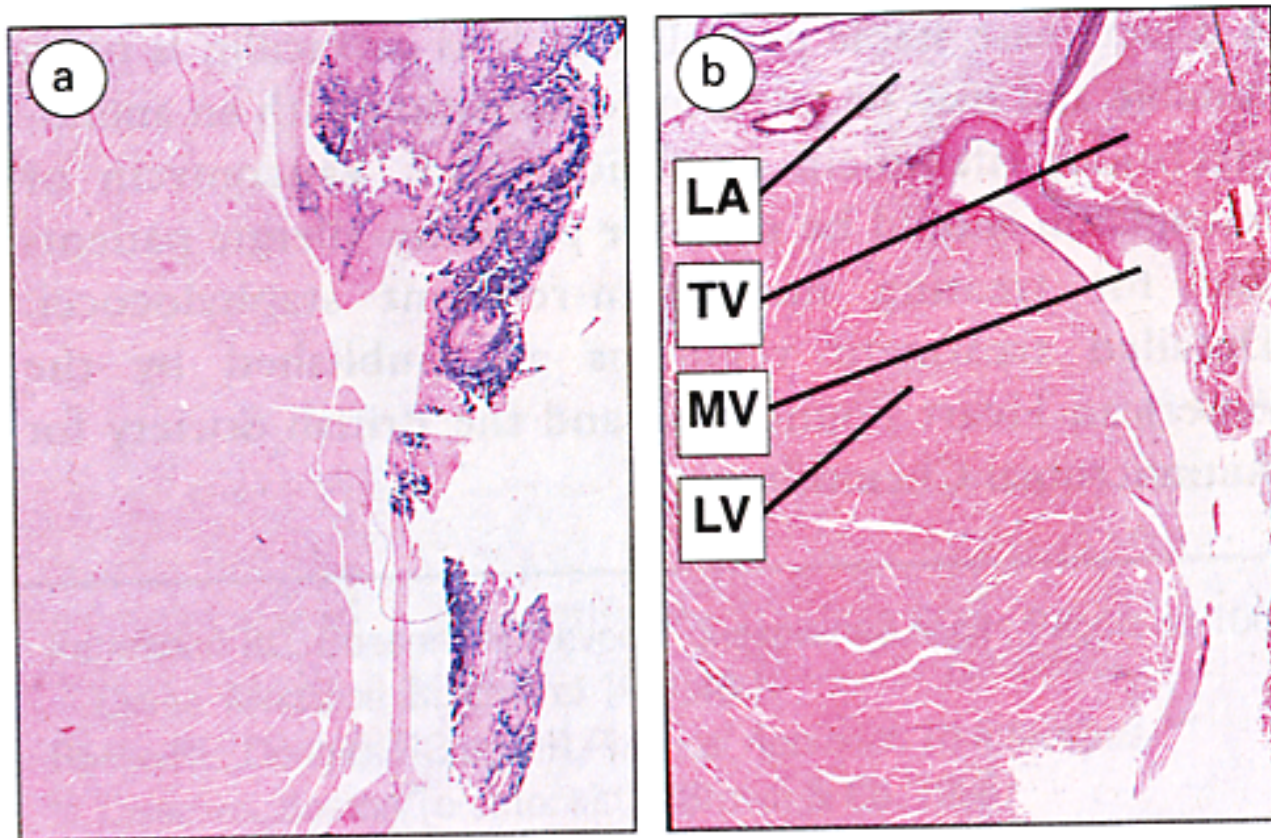


Fig. 27.10 Bacteria circulating in the bloodstream adhere to, and establish themselves on, the heart valves. Multiplication of the microbes is associated with destruction of valve tissue and the formation of vegetations, which interfere with, and may severely compromise, the normal function of the valve. These histologic sections show the virtual destruction of the leaflet at the mitral valve by staphylococci. (a) Gram stain. (b) Eosin-Van Geisen stain. (LA, left atrium; LV, left ventricle; MV, remnant of mitral valve; TV, thrombotic vegetation.) (Courtesy of RH Anderson.)

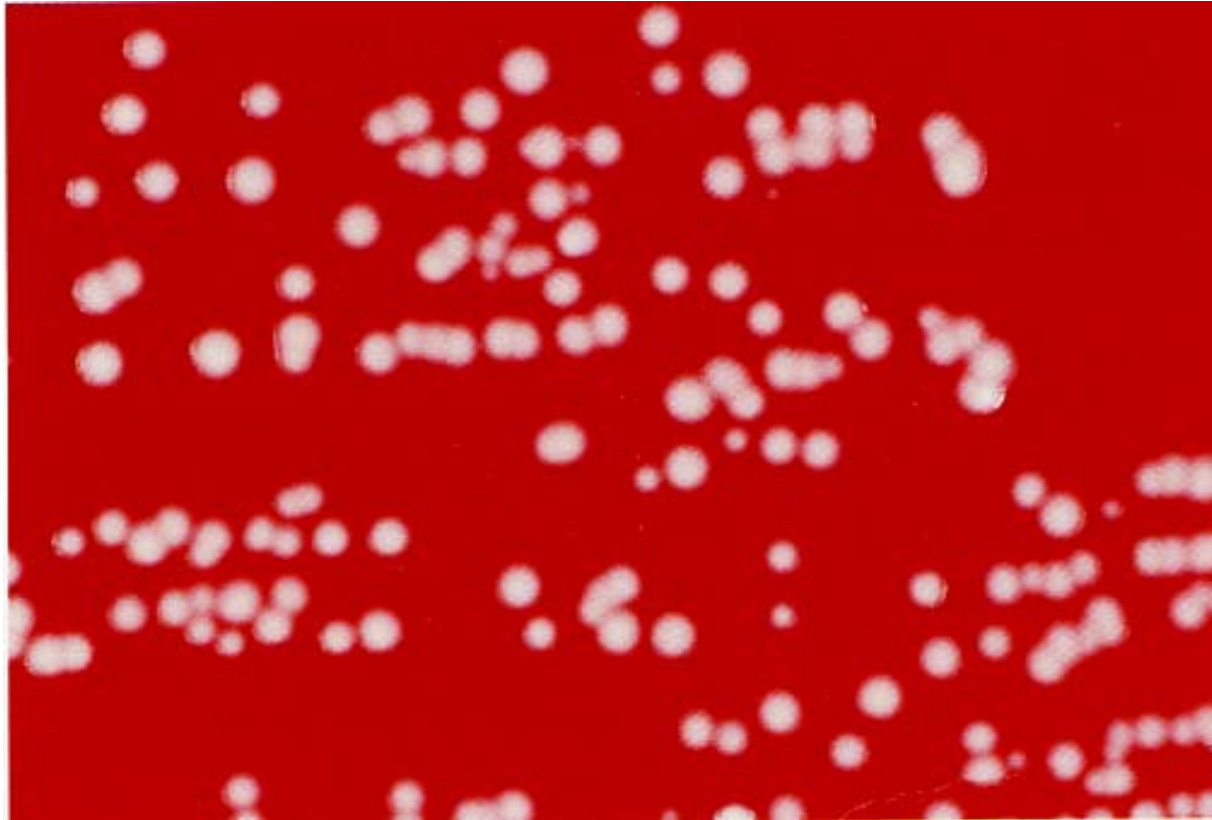




The left-hand figure shows Janeway lesions in a patient with *S. aureus* endocarditis. Janeway lesions are generally painless, flat and, as shown here occasionally hemorrhagic. Embolic in origin with microabscesses in the dermis they are considered to be pathognomonic of *S. aureus* endocarditis. On the right is another example from a case of *S. aureus* endocarditis secondary to intravenous drug use. This patient also has Osler's nodes, painful lesions in the tufts of the fingers and toes. Osler's nodes are likely an immunologic phenomenon. These lesions have become relatively uncommon in the antimicrobial era but if there is a significant delay in therapy they can be seen.

INFECTIONS INVOLVING STAPHYLOCOCCUS EPIDERMIDIS

| infection of: | % of infections caused by <i>Staph. epidermidis</i> |
|---|---|
| prosthetic heart valve early (<2 months postoperatively) late (>2 months postoperatively) | 30-70 20-30 |
| Prosthetic hip | 10-40 |
| Cerebrospinal fluid shunt | 30-65 |
| Vascular grafts | 5-20 |
| Peritoneal dialysis related | 30 |
| Intravascular catheters | 10-50 |



Growth of *Staphylococcus saprophyticus* on 5% sheep blood agar
These coagulase negative staphylococci are often found as the cause of first-time urinary tract infection in sexually-active women.

S. saprophyticus can be distinguished from other species of coagulase negative staphylococci by their resistance to novobiocin (right). The novobiocin susceptible staphylococcus is on the left.

