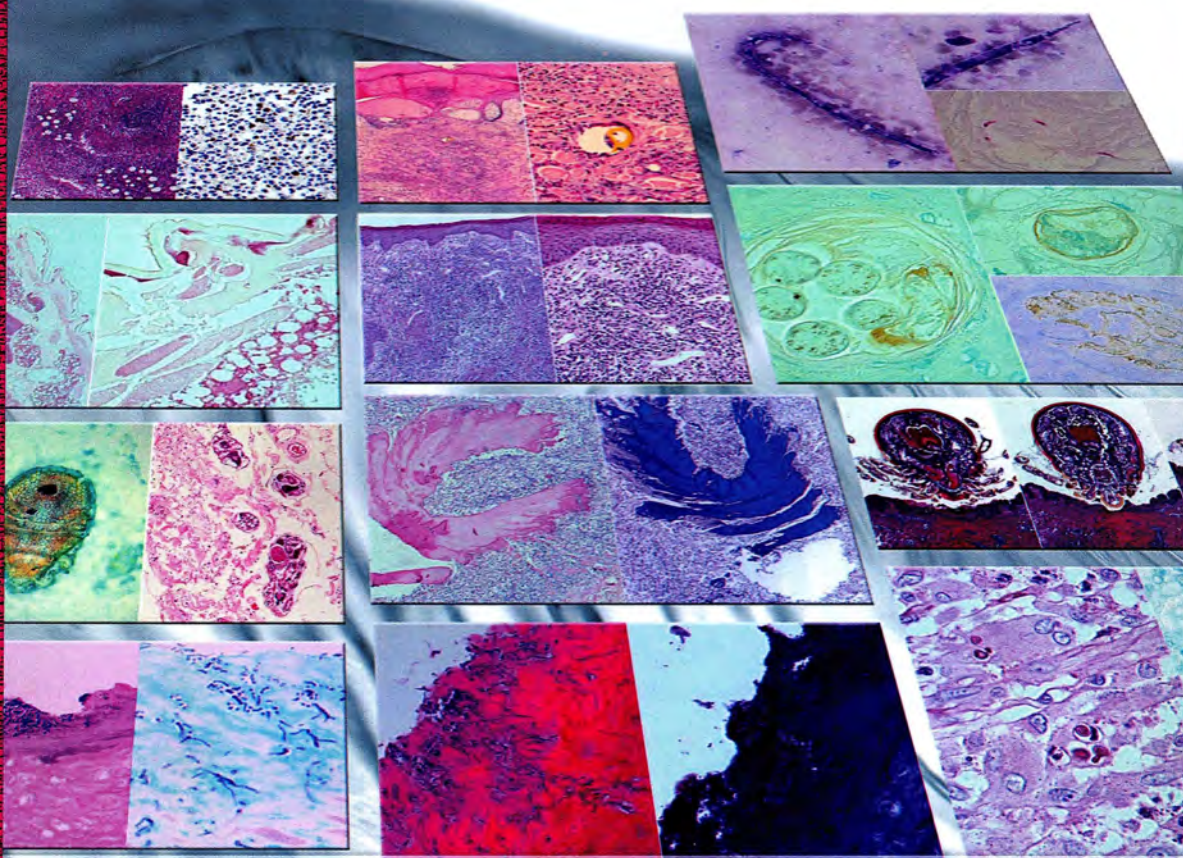
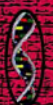


Pathology of Skin Infections

Yutaka Tsutsumi



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PATHOLOGY OF SKIN INFECTIONS

YUTAKA TSUTSUMI



New York

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Introduction

The skin, covering the whole surface of the body, is an organ of barrier, easily exposed to exogenous pathogens. Normal flora functions as a protector against colonization and invasion of unpleasant exogenous microorganisms. Skin infections are variegated, and a considerable number of pathogens cause infectious skin lesions. Any diagnostic pathologist has a chance to encounter any kind of infectious diseases of the skin. Unexpected infections may result from changes in life style and sexual life, popularization of gourmet diet, globalization accelerating persons going to any part of the world, spreading of drug-resistant microorganisms, advent of emerging and re-emerging infectious diseases, increasing prevalence of diabetes mellitus, and increase of immunosuppressive conditions such as administration of steroid and anti-cancer drugs and acquired immunodeficiency syndrome (AIDS).

The aims of this textbook are to overview and illustrate pathological features of skin infections caused by bacteria, fungi, viruses, protozoa, helminth and arthropods with a total of 365 figures. The author tried to use high-quality color photographs as much as he can. Histological features based on hematoxylin and eosin (H&E) staining and gross appearance comprise the major part of the illustrations. Immunostaining using the indirect immunoperoxidase or amino acid polymer method (Simple Stain Max, Nichirei, Tokyo, Japan) and non-isotopic *in situ* hybridization technique were employed when necessary. The indirect immunoperoxidase method using 1:500 to 1:1,000 diluted patients' serum as a primary antibody was also applied to localize pathogens within the lesion fixed in 10-20% formalin and embedded in paraffin. Papanicolaou- or Giemsa-stained cytology evaluation, as well as electron microscopic analysis and polymerase chain reaction (PCR), were also performed upon request.

In a routine practice of histopathological diagnosis, pathologists tend to give the maximal effort in making a diagnosis of neoplastic diseases as precise as possible. However, the same pathologists may use a too simple and ambiguous diagnostic term such as "chronic inflammation", "abscess", and "granuloma" for the lesion of infectious diseases. Instead of "granulomatous lymphadenitis", we pathologists are requested to clarify the etiology of the granuloma as much as possible. In the diagnostic pathology of the skin, for example, syphilitic infection should be given as the specific diagnostic term instead of chronic active inflammation.

Examples are demonstrated in Figures 0-1 and 0-2. The Japanese male aged 50's with a 2-year history of treated B-cell lymphoma now under complete remission complained of surface-eroded papules on the skin of the neck and scalp. Under the suspicion of skin

recurrence, biopsy was performed. The crusted skin nodule showed marked infiltration of small lymphocytes and numerous mature plasma cells. Endothelial cells were swollen. The pathologist (author) suspected a syphilitic lesion (syphilid), and immunostaining for *Treponema pallidum* antigens identified numerous spiral microbes among the involved epidermis. Serological confirmation followed clinically, and successful antibiotics therapy was given. In this case, clinicians did not consider the possibility of syphilis before biopsy.

The prompt and precise diagnosis of emerging and re-emerging infectious diseases, nosocomial infections and sexually transmitted infections has an important socio-public impact for the prevention of unnecessary transmission of the microorganisms. The skin may function as a mirror of internal organs.

It is of no doubt that the intimate communication and cooperation between pathologists and clinicians are essential. Diagnostic pathologists must not hesitate to ask clinicians to provide detailed clinical information and to have additional laboratory and molecular examinations. Phone calling and e-mailing are important and critical tools to make a high-quality histopathological diagnosis.

The author has tried to encompass the information something very Japanese in the text, and unique episodes which happened to be experienced in Japan are picked up as **Notes** for the readers' curiosity and intelligence. He presents a total of 57 **Notes**.

The author, as a diagnostic pathologist, sincerely hopes that this textbook helps pathologists, dermatologists and other clinicians make an appropriate diagnosis of skin infections. He strongly believes that the proper diagnosis may lead patients to proper treatment and cure, and may finally contribute to the human society by preventing infectious disorders.



Figure 0-1. Skin lesions in a malignant lymphoma case in remission. Red papules with surface erosions are presented on the skin of the neck and scalp of a Japanese male aged 50's with a history of malignant lymphoma. Clinicians suspected the possibility of skin recurrence, and biopsy was performed from the neck eruption.

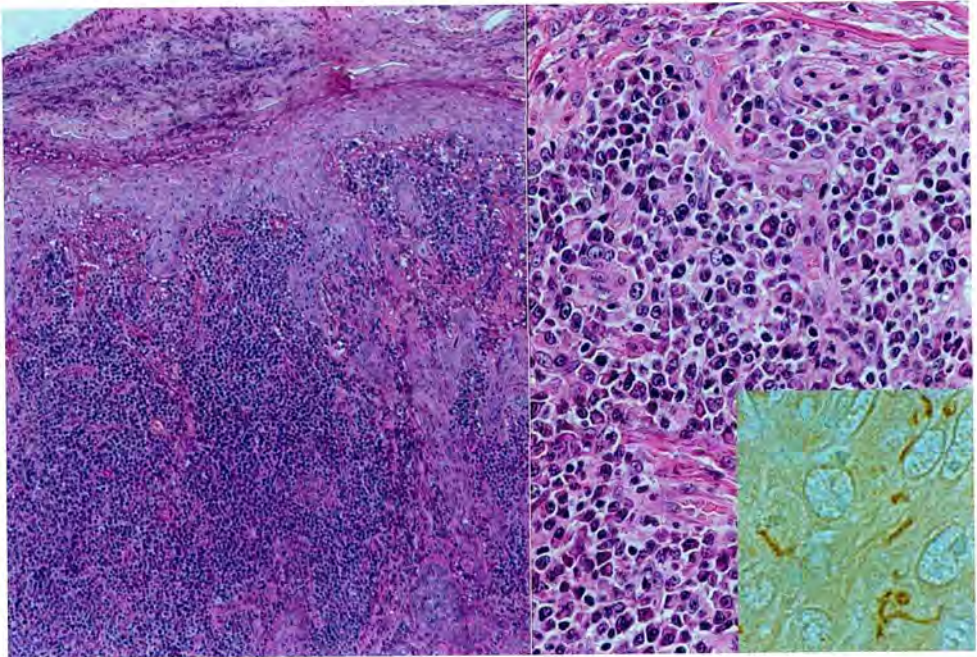


Figure 0-2. Biopsy diagnosis of syphilis. Microscopically, dense infiltration of mononuclear cells with plenty of mature plasma cells is seen in the dermis. Immunostaining for *Treponema pallidum* antigens clearly demonstrates the spiral pathogens among the involved epidermis (left and right upper: H&E, right lower: immunostaining).

Textbooks and Atlases

As well-written textbooks and atlases of infectious disease pathology and skin pathology, the followings are generally recommended.

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9. Garcia LS. Diagnostic Medical Parasitology (4th Ed). ASM Press, Washington DC, 2001
10. Tsutsumi Y. Pathology of Infectious diseases. <http://info.fujita-hu.ac.jp/~tsutsumi/>, 2004.
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12. Weedon D. Skin Pathology (3rd Ed), Churchill Livingstone, London, 2009.
13. Elder DE, Elemtas R, Johnson BL Jr, Murphy GF, Xu X. Lever's Histopathology of the Skin (10th Ed), Lippincott Williams & Wilkins, Philadelphia, 2009.
14. Barnhill RL, Crowson AN, Magro CM, Piepkorn MW. Dermatopathology (3rd Ed), McGraw Hill Medical, New York, 2010.
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Techniques for Visualization of Microbes within the Lesions

It is of no doubt that the microscopic detection of infectious agents within the lesion is essentially important for the histo- and cytopathologic diagnosis of infectious diseases. Demonstration of the microbe with immunohistochemistry and *in situ* hybridization is sensitive enough for this purpose. When one knows the cross-reactivity of the antibodies and probes to be applied, the results will lead the patients directly to the appropriate diagnosis and treatment.

As applications of immunoperoxidase staining and *in situ* hybridization technique for microbes to the diagnosis in surgical pathology and clinical cytology, the following four aspects are described. [1-3]

- A) Use of commercially available antibodies
- B) Use of diluted patients' serum as the primary antibody
- C) Immunostaining versus non-isotopic *in situ* hybridization
- D) Ultrastructural demonstration of microbes in routine material

A) Use of Commercially Available Antibodies

Mycobacterial infection (tuberculosis, non-tuberculous mycobacteriosis and leprosy) is demonstrated by using a commercially available rabbit antiserum against *Bacillus Calmette-Guérin* (BCG), with much higher sensitivity than Ziehl-Neelsen's acid-fast method [4]. Antisera against BCG, *Escherichia coli* and *Treponema pallidum* detect the corresponding bacterial antigens in paraffin sections (Figure 1-A), but those antisera are widely cross-reactive to a variety of related or unrelated pathogens [5-7]. Monoclonal antibodies generally show higher specificity, but the cross-reactivity should be checked to settle the specificity of immunostaining. It is extremely difficult to identify epitopes specific for *E. coli* common among the variety of serological types, but not cross-reactive with the other enterobacteriae. Not only one must know the specificity of the antibody, but also the wide cross-reactivity of the antiserum should be utilized for demonstrating pathogens in paraffin sections.

As nuclear counterstaining, we utilized either methylgreen or hematoxylin.

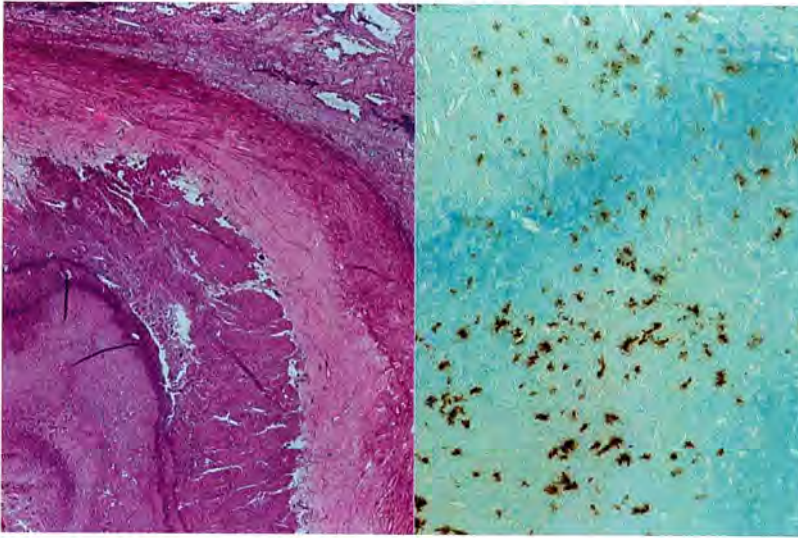


Figure I-A. BCG immunoreactivity in old tuberculosis of the lung. BCG antigens remain in the old calcified lesion, in which acid-fast stain gives a negative result (left: H&E, right: immunostaining for BCG antigens).

B) Use of Diluted Patients' Serum As the Primary Antibody

Patients' serum diluted at 1:500 to 1:1,000 becomes convenient probes for indirect immunoperoxidase localization of microbes in paraffin sections, particularly when inflammatory tissue reaction is evident histologically [8]. The microscopic recognition of abscess or granulomatous lesions definitely indicates that host immune system has reacted to the microbe in question to raise specific antibodies in the serum. Examples included staphylococcal pyoderma, cat scratch lymphadenitis, cryptococcosis, sporotrichosis, alternariosis, amebic and acanthoamebic infections, cutaneous and visceral leishmaniasis, isosporiasis, schistosomiasis, gnathostomiasis, ascariasis, etc. Regarding the specificity of immunoreaction, the immunostaining using known positive and negative controls is requested [1]. Generally speaking, the specificity is high enough in the lesion caused by protozoa or helminth. In contrast, the serum of patients with bacterial or fungal infection tends to cross-react with other bacteria or fungi. For example, the serum of a patient suffering from sporotrichosis functioned as a pan-fungal reagent (Figure I-B-1).

In a case of visceral leishmaniasis (kala azar), demonstration of patient's own serum-reactive protozoa-like organisms within microgranulomas in the liver biopsy specimen gave a definitive hint for the final diagnosis (Figure I-B-2) [2]. In this case, the sensitivity of the method is essential, since the specificity is completely unknown. Endogenous human IgG in paraffin sections is scarcely detected by the peroxidase-labeled secondary antibody. The staining method with high sensitivity must not be chosen, in order to suppress background staining of endogenous human plasma IgG. We must avoid using biohazardous sera, such as those with persistent infection of hepatitis B and C viruses and human immunodeficiency virus.

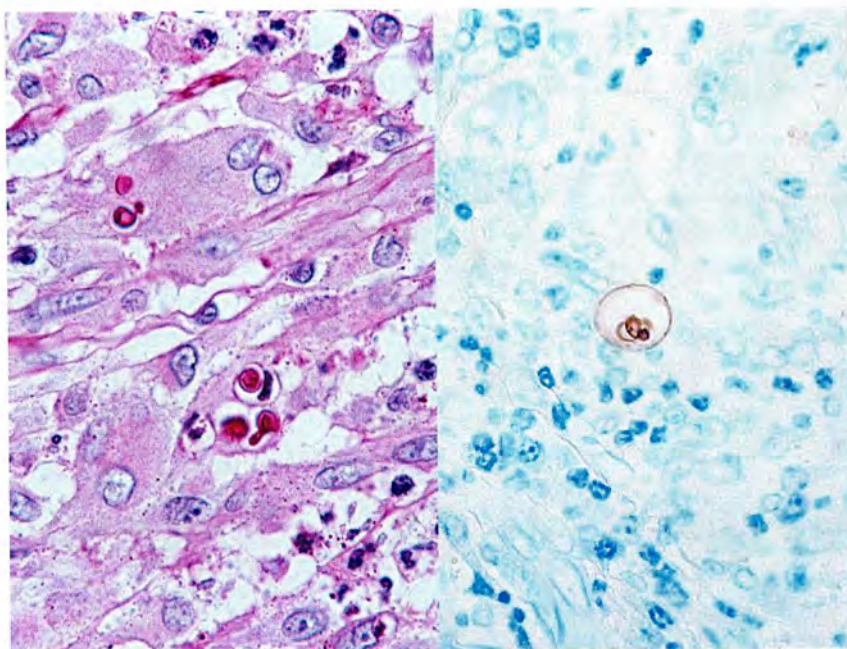


Figure I-B-1. Immunostaining using the patient's own serum at a 1:500 dilution in sporotrichosis. Yeast-form fungi are clearly demonstrated in routinely prepared paraffin sections. The sensitivity of the immunostaining using patient's serum is excellent, while the specificity is low (left: periodic acid-Schiff stain, right: indirect immunoperoxidase).

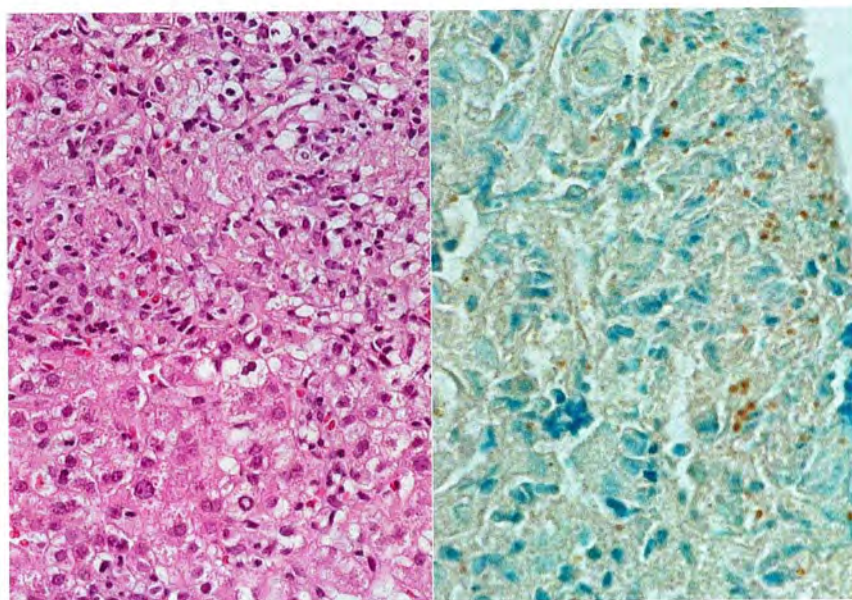


Figure I-B-2. Practical application of immunostaining using the patient's own serum. Oval-shaped positive signals of protozoan size were observed in the cytoplasm of epithelioid cells in microgranulomas in the liver biopsy specimen. This Japanese male businessman long stayed in Australia, Thailand and then India. His general condition was very poor. The diagnosis of visceral leishmaniasis was strongly suggested, and soon confirmed serologically. Eventually, the appropriate treatment saved his life (left: H&E, right: indirect immunoperoxidase).

C) Immunostaining versus Non-Isotopic *In Situ* Hybridization

Generally speaking, the results obtained with the histochemical techniques, immunostaining and *in situ* hybridization (ISH) method, are basically comparable: Examples included cytomegalovirus (CMV), *Chlamydia trachomatis*, *Aspergillus*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, and *Helicobacter pylori*. [1,9]. In Figure I-C, *Aspergillus* is comparatively signalized with both immunostaining and ISH. It should be noted that dead mycelia in aspergillosis are unstained with ISH using a probe for ribosomal RNA, but positively labeled with immunostaining. In the oncogenic viruses such as human papillomavirus (HPV) and Epstein-Barr virus (EBV), the viral antigens were less frequently detectable than the viral genomes in cervical condyloma/dysplasia, Hodgkin's lymphoma and chronic active EBV infection [10-14]. The oncogenic viruses do not form viral particles in the tumor cells, so that immunostaining for viral capsid antigens usually gives negative results.

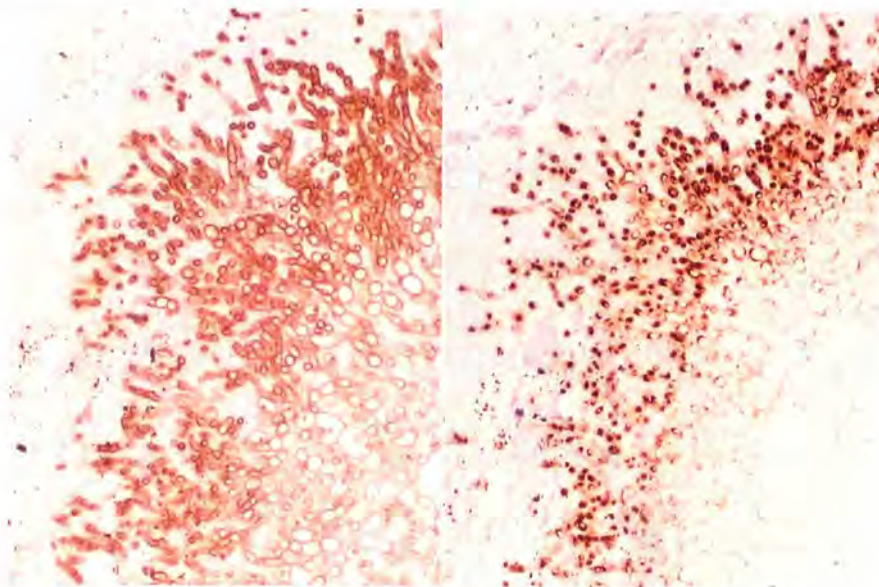


Figure I-C. Comparative localization of *Aspergillus* hyphae in the lung lesion using immunostaining using a commercial antiserum (left) and ISH technique for ribosomal RNA (right). Dead mycelia in the center of the colony are unstained with ISH, while the antibody is reactive with both live and dead fungal cells.

D) Ultrastructural Visualization of Microbes in Routine Material

The antigens of *Chlamydia trachomatis*, *E. coli*, CMV and HPV have been localized in paraffin sections by applying pre-embedding immunoelectron microscopy. In Figure I-D-1, light microscopic and immunohistochemical features of chlamydial epididymitis are shown.

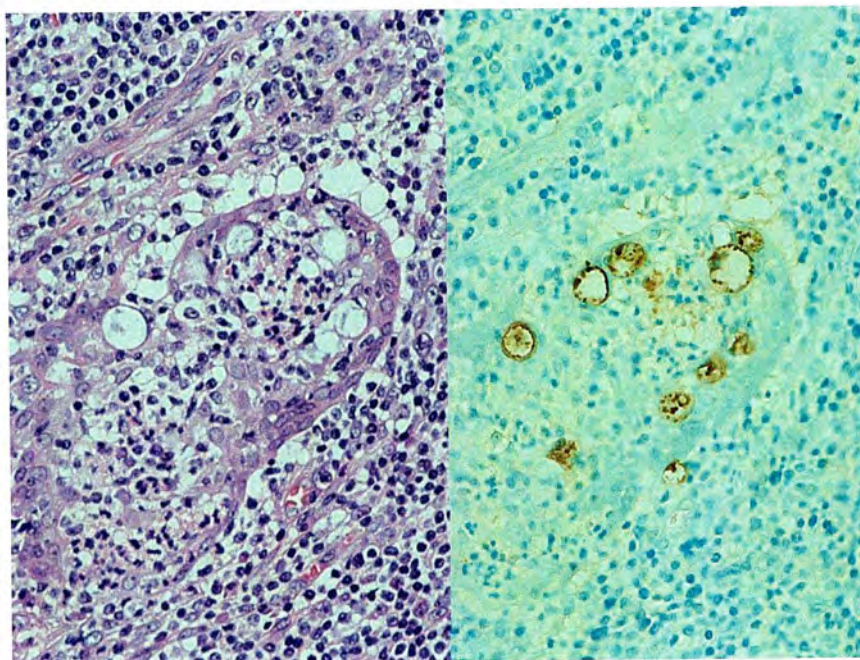


Figure I-D-1. Microscopic features of chlamydial epididymitis. The epididymal tissue is densely infiltrated by inflammatory cells, and large and round vacuoles are occasionally formed in the cytoplasm of the ductal epithelial cells. Chlamydial antigens demonstrated by commercial cocktail monoclonal antibodies are clustered in the vacuoles (left: H&E, right: immunostaining).

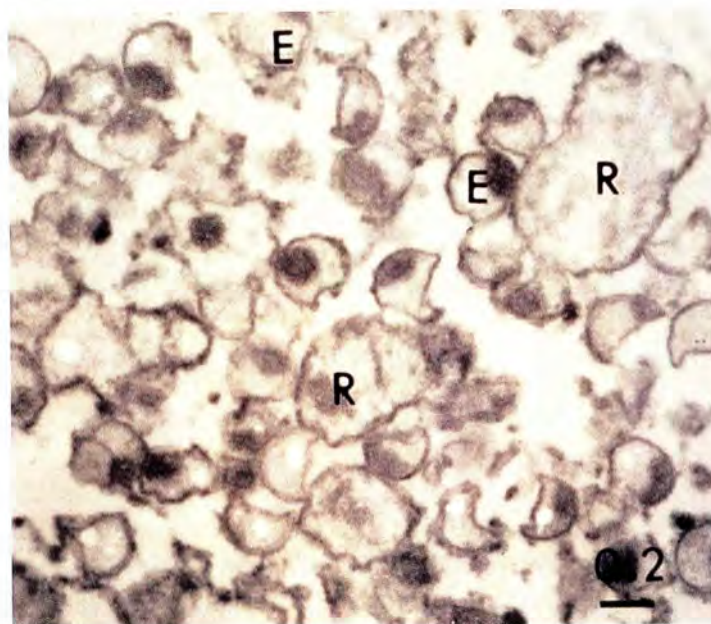


Figure I-D-2. Localization of chlamydial antigens with pre-embedding immunoelectron microscopy in a routinely prepared paraffin section of chlamydial epididymitis. The membranous structures of chlamydial particles are labeled with electron-dense reaction products. Both large-sized reticulate bodies (proliferative form: R) and small-sized elementary bodies (infective form: E) can be distinguished. Bar indicates 0.2 μm .

The immunoreaction in paraffin-embedded epididymitis tissue was further evaluated ultrastructurally, and positively labeled chlamydial particles are recognized (Figure I-D-2). Although the fine morphologic preservation is not good enough, this approach is useful to confirm the presence of microbes within the lesions and the specificity of the antibodies. The viral genomes are also identifiable at the ultrastructural level (see Figures IV-A-1-3 and IV-A-1-4) [15-17].

E) Summarizing Remarks

A wide variety of infectious agents have been detected with immunostaining and/or ISH in routine histo- and cytopathologic specimens. The unexpectedly powerful potentiality has been demonstrated in routinely processed paraffin sections. As in case of immunostaining using antisera against BCG, *E. coli* and *Treponema pallidum* and patients' sera, the sensitivity of detection is more important than the specificity. The reasons for this are as follows. 1) Principally, the histologic pattern of host reaction is the key aspect for presuming the causative agent. 2) Determination whether chlamydial, bacterial, fungal or viral should be a tentative goal of report in histologic examination. 3) Whether the lesion is caused by *E. coli*, *Pseudomonas aeruginosa* or *Mycobacterium tuberculosis*, for example, should primarily be determined by non-histologic methodology, such as microbial culture, serologic test and DNA analysis. It must be emphasized again that the appropriate histologic and cytologic diagnosis, not rarely aided by the appropriate immunostaining, is essentially beneficial for patients leading to the appropriate treatment.

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Bacterial Infections

A wide variety of bacterial species infect the skin. Abscess is caused by extracellularly growing microbes, while intracellularly growing microbes provoke granulomas. Some pathogens may cause “suppurative granuloma”, an intermediate form of abscess and granuloma. Many bacteria infect locally, but the other spread systematically. Production of exotoxins or superantigens is characteristic of certain bacteria. Skin lesions may be a secondary event followed by systemic infection in the internal organs. Some lesions are provoked by normal skin flora, while exogenous foreign pathogens may lead to the lesions in the others. A group of pathogens cause sexually transmitted infection (STI). When the host defense mechanisms are weakened, some pathogens with low pathogenicity become infected and many pathogens may show altered (aggressive) modes of infections.

It is easy to identify microbes microscopically in the pathological lesions in some lesions, while it is difficult in others. In addition to Gram and periodic acid-Schiff (PAS) stains, immunoperoxidase staining, *in situ* hybridization technique and polymerase chain reaction are useful in demonstrating pathogens within the lesion. However, it should be known that these techniques may be cross-reactive to the related (or even unrelated) pathogens (see Chapter I). Nonspecific reactions should not be confused with the real signals.

The histopathological features in the respective lesions are described below by using macroscopic and microscopic illustrations, as much as the author can.

A) Staphylococcal Infection

1) Normal Flora

Staphylococci and *Streptococci* are representative Gram-positive cocci infective to human bodies. Two types of *Staphylococci* colonize the surface of human body: *Staphylococcus epidermidis* and *Staphylococcus aureus* (“aureus” means “gold” in color in Latin). *S. epidermidis*, representing coagulase-negative species, is non-pathogenic, except for specific situations such as biofilm infection on the surface of ulceration, inserted tubes and cardiac valves. Most of the lesions caused by *Staphylococci* result from infection of *S. aureus*, a coagulase-positive species.

Within the hospital, colonization of methicillin-resistant *Staphylococcus aureus* (MRSA), particularly on the nostril (haired and moist skin, suitable for bacteria to colonize), is a serious and troublesome cause of nosocomial (hospital-acquired) infection via contact transmission from medical staff to inpatients. Recently, community-acquired MRSA became problematic, particularly in the western countries.

The human body surface is colonized by *S. epidermidis*, and the bacteria especially accumulate within the hair follicles. It has been documented that *Staphylococci* have a broad arsenal to combat antimicrobial peptides, which plays a central role in providing immunity to bacterial colonization on the human epithelial cells. Figure II-A-1 represents Gram-positive cocci (*S. epidermidis*) colonizing the hair follicle around the nose. *S. epidermidis* and *S. aureus* cannot be distinguished on the microscopic basis, and methicillin-sensitive *S. aureus* (MSSA), a common form of *S. aureus*, and MRSA are morphologically identical. It should be recognized that MSSA is resistant to Penicillin G because of the production of penicillinase.

In Note 1, biological features of *Staphylococcus* and *Streptococcus* are compared, explaining why *Staphylococci* colonize the skin and *Streptococci* colonize the oral cavity or vagina. Aerobic and anaerobic bacteria are subcategorized in Note 2.

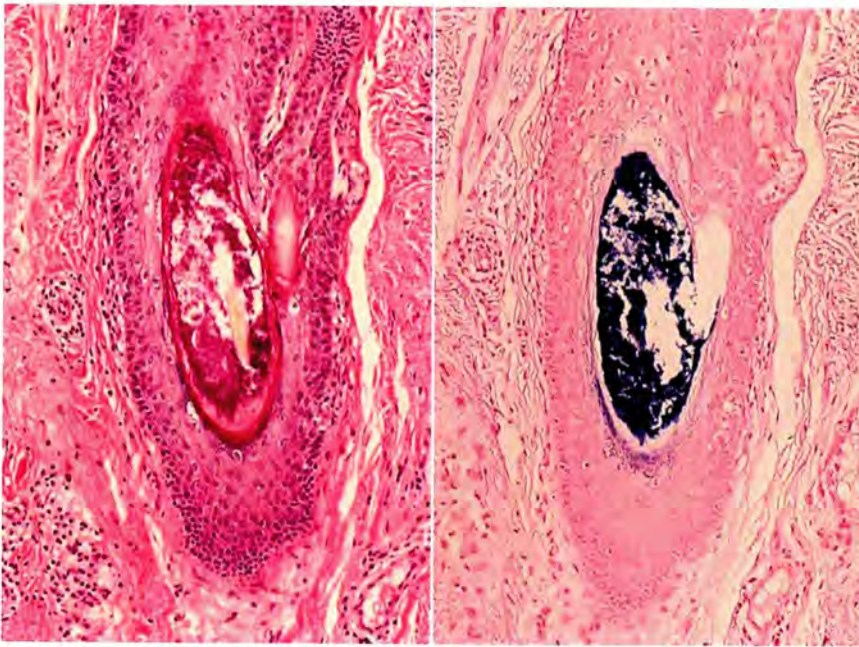


Figure II-A-1. Staphylococcal colonization as normal flora. Gram-positive cocci colonize the orifice of the hair follicle. No inflammatory reaction is seen. The phenomenon is frequently seen on the face, and the microbes are regarded as *Staphylococcus epidermidis* (left: H&E, right: Gram).

References

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Note 1. *Staphylococcus* versus *Streptococcus*

Staphylococcus and *Streptococcus* are common Gram-positive cocci, potentially pathogenic to the human being. Both are categorized as facultative anaerobes. *Staphylococcus* utilizes aerobic respiration under the oxygenated condition, and under the anaerobic condition, fermentation occurs. The growth is better under the oxygenated condition. In contrast, *Streptococcus* produces ATP molecules only by fermentation, irrespective of the oxygen status (*Streptococcus* does not utilize oxygen for energy production). It does not have a catalase activity. *Streptococcus* can survive in the aerated atmosphere through the production of superoxide dismutase (SOD) degrading activated oxygen molecules.

Staphylococcus likes aerated conditions and preferentially resides on the skin surface, while *Streptococcus* likes anaerobic or microaerophilic conditions, so that it prefers to distribute in the dental pits and tonsillar crypts or on the vaginal mucosa.

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Note 2. Aerobic bacteria vs. anaerobic bacteria

The bacteria are categorized into the 3 groups based upon oxygen utilization.

- i) **Aerobe** (It needs oxygen for growth, accompanying no fermentation-mediated ATP production)
 - ii) **Facultative anaerobe** (It can grow, irrespective of the presence of oxygen. Generally, the growth is better under the oxygenated condition)
 - iii) **Obligate anaerobe** (It grows under the anoxygenic condition. It does not grow and die under the oxygenated condition)
- i) The aerobes are divided into 3 subgroups.
 - a) **Obligate aerobe** (It can not survive without oxygen): *Pseudomonas putida*, *Mycobacterium*, *Bordetella*, *Francisella*, *Brucella*, part of *Bacillus*
 - b) **Common aerobe** (Anaerobic respiration is possible under the anaerobic condition.): *Pseudomonas aeruginosa*, *Alkaligenes*, *Flavobacterium*, *Legionella*, *Neisseria*, *Micrococcus*, *Leptospira*, *Nocardia*
 - c) **Microaerophile** (It grows well at a low oxygen concentration): *Helicobacter*, *Capnocytophaga*
 - ii) The facultative anaerobes are divided into 2 subgroups.
 - a) Aerobic respiration is used under the aerobic condition, and under the anaerobic condition, fermentation occurs: *Staphylococcus*, *Enterobacteriaceae*, *Vibrio*, *Pasteurella*, *Corynebacterium*, *Actinomyces*, part of *Bacillus*

- b) ATP is produced by fermentation irrespective of the oxygen status: *Streptococcus* (It expresses superoxide dismutase activity)
- iii) The obligate anaerobes are divided into 3 subgroups.
 - a) Both fermentation and anaerobic respiration (nitrate respiration, fumarate respiration, etc.) can be used: *Bacteroides*, *Propionibacterium*, *Veillonella*
 - b) Only fermentation can be used: *Peptostreptococcus*, *Fusobacterium*, *Clostridium*
 - c) *Aerotolerant anaerobe* (Fermentation can occur even under the oxygenated condition): *Lactobacillus*

References

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2) Secondary Staphylococcal Colonization

A variety of polypoid skin lesions show secondary colonization of *Staphylococci* on the top, particularly at the site of surface erosion/ulceration or among the hyperkeratotic squams. Staphylococcal colonization may exacerbate eczema and atopic dermatitis. The possibility of MRSA colonization should always be considered from the viewpoint of the prevention of nosocomial contact transmission, especially when the lesion is located on the face.

Figures II-A-2-1 and II-A-2-2 reveal secondary staphylococcal colonization on the surface of pyogenic granuloma, senile keratosis and the lip mucosa. An epidermo-adhesive pattern of colonization is evident on the lip, and the positivity with colloidal iron stain indicates biofilm formation (refer to Note 12). It should be noted that *Staphylococci* are prone to colonize the decubitus (see below).

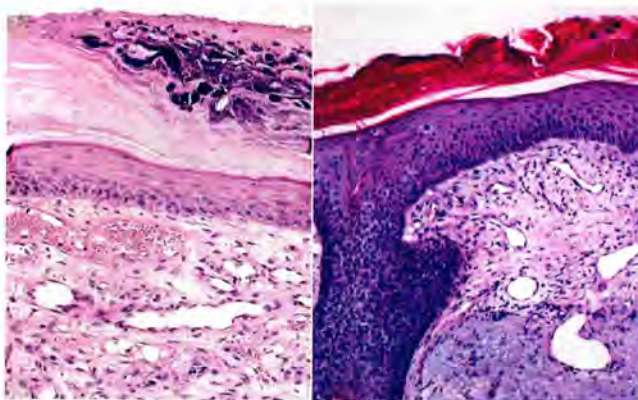


Figure II-A-2-1. Secondary colonization of *Staphylococci* on the surface of elevated skin lesions. Surface erosion and hyperparakeratosis especially accelerate the colonization (left: pyogenic granuloma, right: senile keratosis).

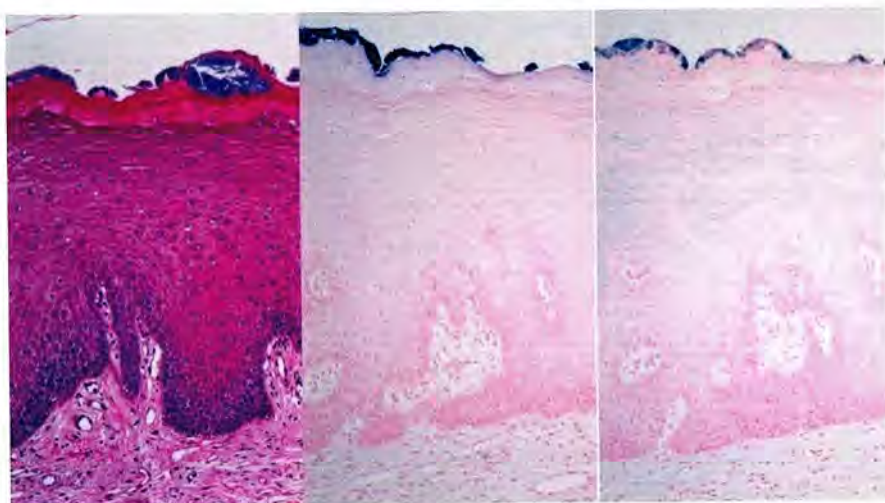


Figure II-A-2-2. Staphylococcal colonization on the lip mucosa. Epidermo-adhesive pattern of Gram-positive cocci is observed on the squamous mucosa. The positive colloidal iron stain indicates acidic biofilm formation (left: H&E, center: Gram, right: colloidal iron).

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3) Decubitus and Staphylococcal Infection

Colonization of *Staphylococcus aureus*, particularly MRSA, on the decubitus (pressure ulcer or bedsore) is also the important target of infection control. It should be noted that local eradication of MRSA can be achieved only after healing of the ulceration. Decubital ulcers usually develop over pressure areas such as the sacrum, the greater trochanter and the heel of the patient bed-rest for a long period. The ulcer often deeply reaches the periosteal tissue. There is a preceding erythematous stage. The late stage presents as a black eschar form. When a pocket is formed, secondary infection is inevitable (Figure II-A-3-1). Symptoms of infection in a pressure ulcer include slow or stalling healing, pale granulation tissue. Infected wounds may have a gangrenous odor. Biofilm formation leads to the delayed healing in decubitus ulcer.

Staging of decubitus ulcer, proposed by the National Pressure Ulcer Advisory Panel (NPUAP) in the United States, is as follows:

Stage I: Intact skin with non-blanchable redness of a localized area usually over a bony prominence.

Stage II: A shallow open ulcer with a red pink wound bed, without slough.

Stage III: Full thickness tissue loss. Subcutaneous fat may be visible but bone, tendon and muscle are not exposed. Slough may be present but does not obscure the depth of tissue loss.

Stage IV: Full thickness tissue loss with exposed bone, tendon or muscle. Slough or eschar may be present on some parts of the wound bed. Often include undermining and tunneling.

For the treatment purpose, the eschar stage decubitus may be totally removed by surgery for skin grafting (Figure II-A-3-2). Histologically, the advanced lesion shows full-thickness dermal necrosis with deep ulceration and abscess/gangrene formation. Patterns of bacterial infection are often unique. *Staphylococcus aureus*, including MRSA, mainly colonizes the superficial layer (Figure II-A-3-3), while Gram-negative rods, including *Pseudomonas aeruginosa* and *Escherichia coli*, are observed in the deep layer (Figures II-A-3-4 and II-A-3-5).



Figure II-A-3-1. A large and deep decubitus with pocket formation at the sacral region. A deep mining ulcer, 4 x 4 cm-sized pocket, is noted. Secondary infection is inevitable. The courtesy by Dr. Sandai Ohnishi at Nursing Home Hatta, Nagoya, Japan.



Figure II-A-3-2. Surgical removal of decubital ulcer covered with black eschar at the trochanter region. Surgical treatment was effective for cure in this intractable ulceration. The courtesy by Dr. Sandai Ohnishi, Nagoya, Japan.

The nature of MRSA can be demonstrated immunohistochemically in routinely formalin-fixed, paraffin-embedded lesions (Shimomura and Tsutsumi). *S. aureus* is immunoreactive not only for staphylococcal surface antigens but also for protein A, which is an immunoglobulin-binding protein, specifically expressed on the cell wall of *S. aureus*. The multidrug resistance of MRSA, determined by the expression of penicillin-binding protein 2' (PBP2'), can also be immunophenotyped with monoclonal antibodies. One typical example is demonstrated in Figure II-A-3-6.

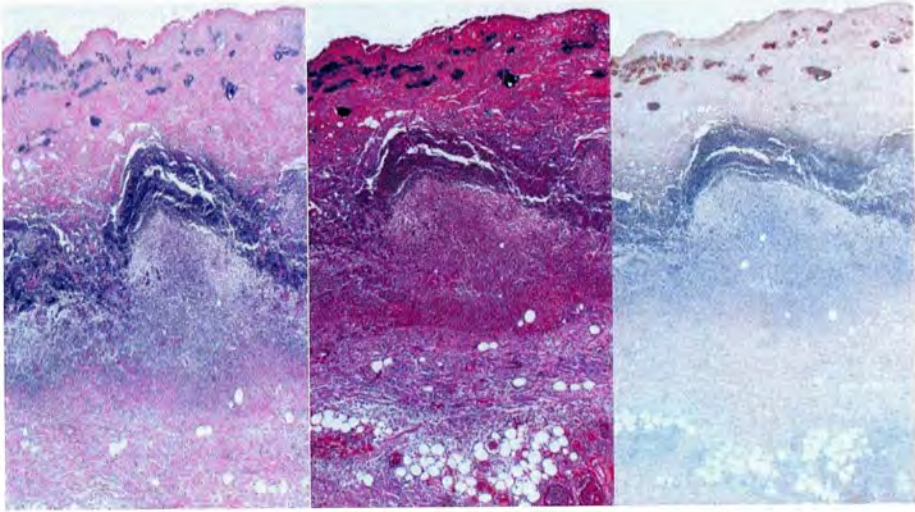


Figure II-A-3-3. Microscopic double-layered appearance of the resected decubital ulcer (H&E: left). Colonization of *Staphylococcus aureus*, probably MRSA, is observed on the eroded surface and clearly illustrated by Gram stain (center) and immunostaining for staphylococcal antigens (right). Abscess formation with ischemic gangrene is noted in the deep area.

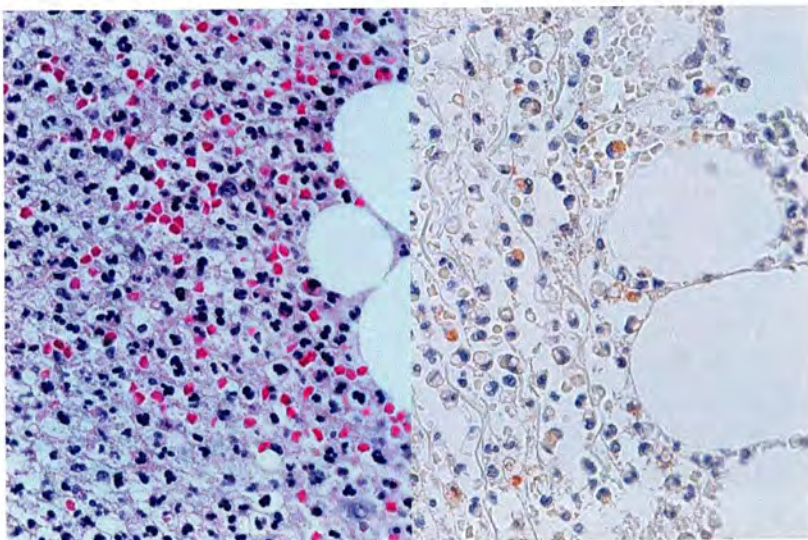


Figure II-A-3-4. *Pseudomonas* infection in the deep part of the decubitus. In the subcutaneous abscess (left: H&E), immunostaining using a monoclonal antibody against *Pseudomonas aeruginosa* demonstrates phagocytized microbes in some neutrophils (right).

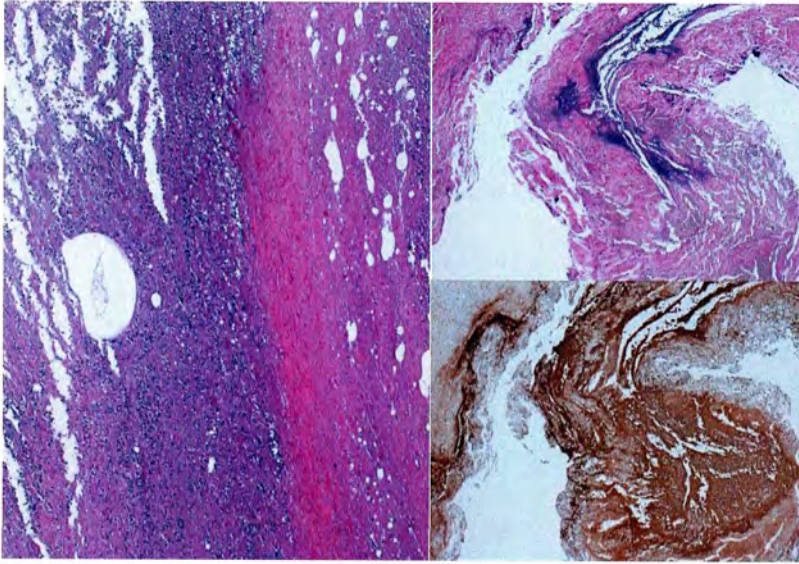


Figure II-A-3-5. Another decubital ulcer with massive colonization of Gram-negative rods in the deep gangrenous tissue. Gas formation is focally observed (left: H&E). A monoclonal antibody (J5) against lipopolysaccharide common in enterobacteriae illustrates an infective process in the decubitus (right top: H&E, right bottom; immunostaining).

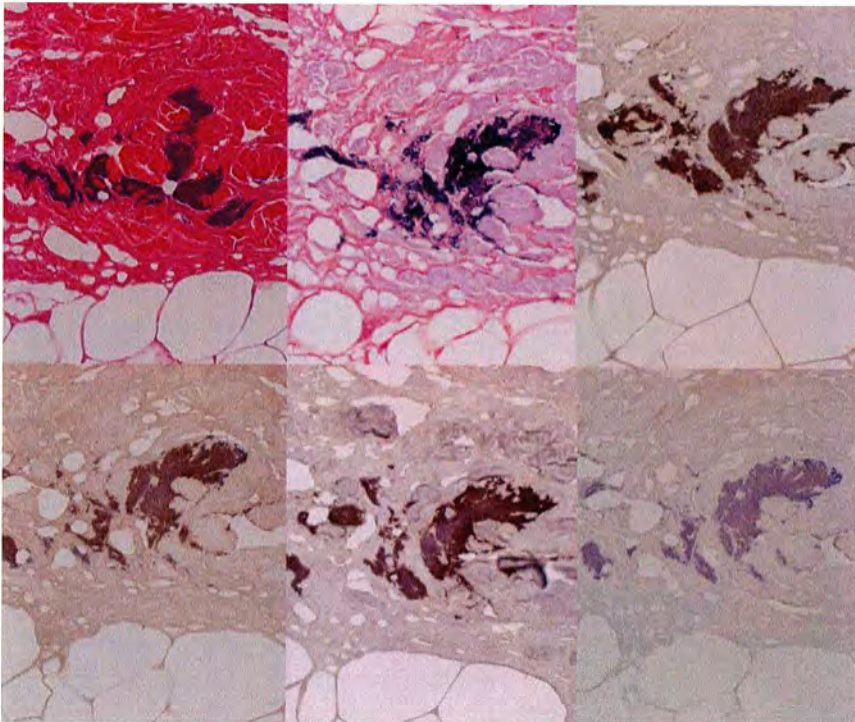


Figure II-A-3-6. Immunohistochemical identification of MRSA in formalin-fixed, paraffin-embedded sections. The Gram-positive coccal colonies (left top: H&E, center top: Gram stain) in the decubital lesion express staphylococcal antigens (right top), protein A (left bottom) and penicillin-binding protein 2' (PBP2') (center bottom), confirming the nature of MRSA. Streptococcal antigens (right bottom) are negative.

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4) Staphylococcal Conjunctivitis

Acute conjunctivitis is most frequently viral in origin, including Coxsackievirus, adenovirus and herpes simplex virus. Bacterial infection provokes acute purulent conjunctivitis, and *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae* comprise the major pathogens. These microbes preferentially infect eyes of infants below the age of 3 years. Regarding corynebacterial conjunctivitis, see Chapter II- F-2. The incidence of infective conjunctivitis caused by community-acquired MRSA (refer to Note 4) is increasing.

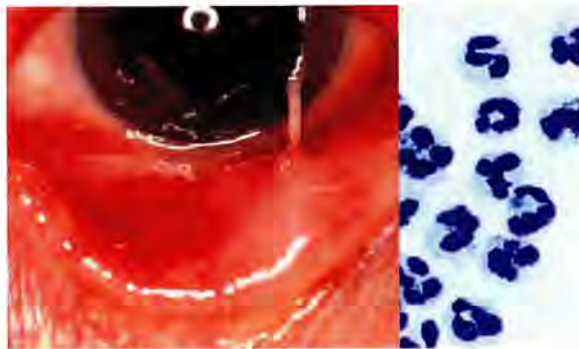


Figure II-A-4. MRSA conjunctivitis. Redness of the conjunctival mucosa with active purulent discharge is evident in the eye of the aged, long hospitalized for cerebral infarction (left). Smear preparation reveals clusters of cocci phagocytized by neutrophils (right: Diff-Quik Giemsa).

Here, nosocomial conjunctival infection of MRSA is briefly described. The victims include the aged inpatients suffering from chronic debilitating disorders such as dementia, cerebrovascular diseases, diabetes mellitus, etc. Because of active discharge, the diseased eyes can be the source of extended nosocomial infections. MRSA may cause corneal ulceration. Typical features are shown in Figure II-A-4.

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5) Acute Folliculitis and Subareolar Abscess

Acute folliculitis (boil or hot tub rash) is most frequently caused by *Staphylococcus aureus* infection. Preceding growth of normal flora of the hair follicle, *Propionibacterium acnes*, may accelerate local staphylococcal growth. In intractable cases, the infected lesion is surgically removed to be submitted for histological evaluation. In Figure II-A-5-1, the hair follicle was destroyed due to infection of Gram-positive cocci and abscess formation. Locally destructive infection of Gram-positive cocci around the hair follicle is called furuncle. The microbes causing the destructive folliculitis often produce a toxin, named Panton-Valentine leukocidin (PVL).

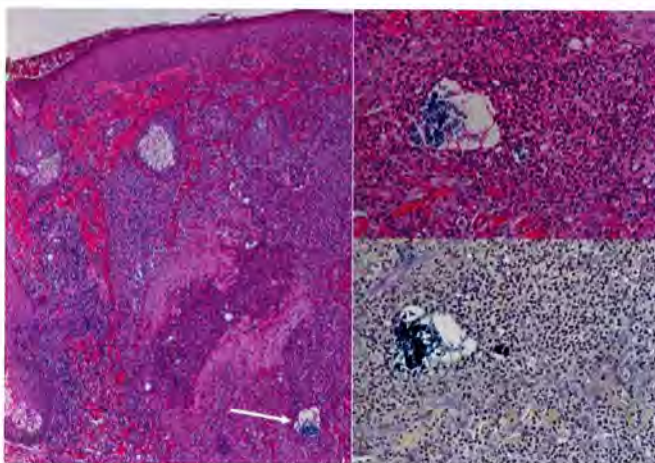


Figure II-A-5-1. Suppurative folliculitis on the back. Hair follicle-centered abscess formation is evident (left: H&E). At the arrowed area, colonization of Gram-positive cocci is observed (right top: H&E, right bottom: Gram). *Staphylococcus aureus* should be the causative microbe.

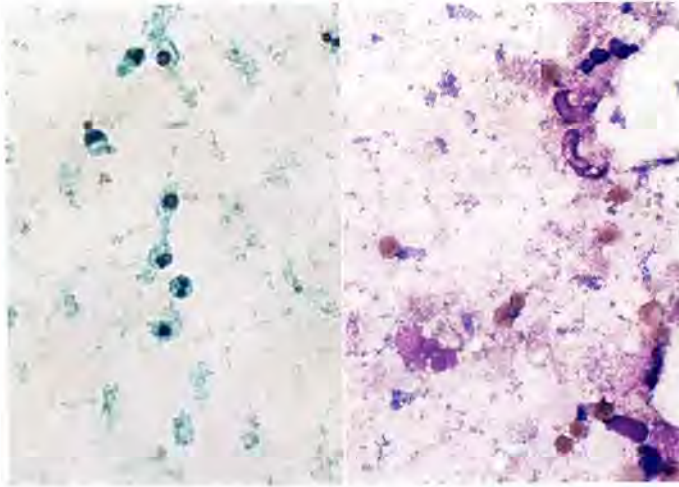


Figure II-A-5-2. Needle aspiration from subareolar abscess of the breast. Irregular (bunch-of-grapes-like) clusters of cocci are observed among neutrophilic infiltrates. Giemsa stain more clearly illustrates the bacteria (left: Papanicolaou, right: Giemsa).

Subareolar abscess is mediated by a blockage of the mammary ducts below the skin of the areola, resulting in localized staphylococcal infection of the duct. Not only lactating breasts but also non-lactating ones of younger or middle-aged women are affected. Risk factors include smoking, diabetes and nipple piercing. Cytologic needle aspiration can demonstrate coccal pathogens within the abscess lesion (Figure II-A-5-2).

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6) Staphylococcal Deep Pyoderma

Pyoderma means any pyogenic skin diseases (bacterial infections), such as impetigo, ecthyma, folliculitis, furuncle, carbuncle, etc. Bacterial pyoderma limited to the epidermis and hair follicles are referred to as superficial, whereas those that involve the dermis, deep dermis, or cause furunculosis are referred to as deep. Children are at a higher risk.

When the folliculitis lesions are fused locally to form intradermal and/or subcutaneous abscess of considerable size, we call it as deep pyoderma or carbuncle (Figure IIA-6-1). Carbuncle is a severe form of furuncle, a coalescence of multiple furuncles, frequently resulting in fistule formation onto the skin surface. When large verrucous plaques with

elevated borders and multiple pustular ulcerations are noted, the term pyoderma vegetans is used.

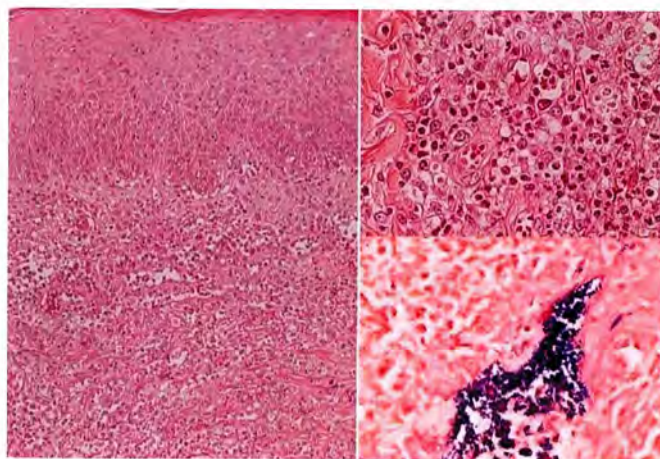


Figure II-A-6-1. Staphylococcal pyoderma (I). Acute and chronic inflammatory processes with multifocal abscess formation are seen extensively in the dermis through subcutis. Colonies of Gram-positive cocci are focally observed (left and right top: H&E, right bottom: Gram).

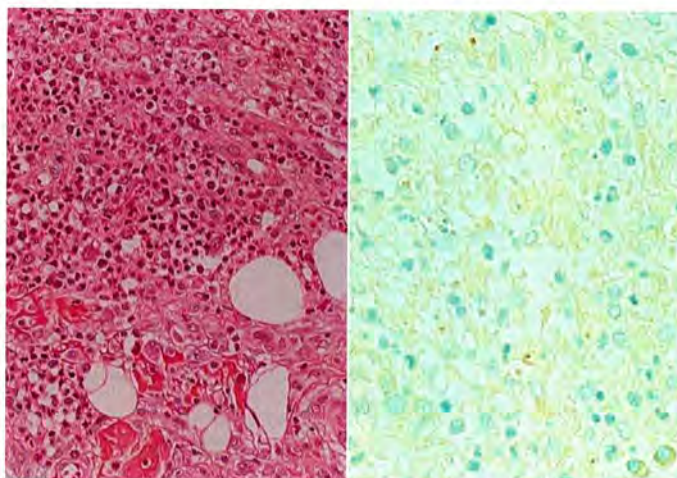


Figure II-A-6-2. Staphylococcal pyoderma (II). Coccal pathogens demonstrated with a 1:500 diluted patient's own serum. In the biopsy specimen, coccal microbes are scattered in the abscess lesion formed in the dermis (left: H&E, right: immunostaining).

The Gram-positive pathogenic cocci (*Staphylococcus aureus*) are dispersed within the lesion. Figure IIA-6-2 represents the coccoid bacteria in the dermal lesion (granulation tissue with abscess formation), visualized with the indirect immunoperoxidase staining using 1:500 diluted patient's serum (as a primary antibody) and horseradish peroxidase-labeled anti-human IgG (as a secondary reagent). Due to prolonged infection, specific antibodies have been raised in the patient's serum, and this can be used as a probe for visualization of pathogens in paraffin sections (see the Chapter I). The anti-bacterial antibodies raised in the

human body are usually widely cross-reactive to a variety of bacterial species. This approach is useful for visualizing the distribution, shape and size of the microbes within the lesion.

References

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Note 3. Hidradenitis suppurativa

Hidradenitis suppurativa, also called as acne inversa and apocrine acne, is a chronic, relapsing and painful inflammatory disorder involving apocrine gland-bearing areas, such as the axilla, breast, groin, pubic region and perineum. Recurrent, deep-seated inflammatory nodules are formed, resulting in draining sinus formation and scarring. The size of the lesion is as large as baseballs or as small as a pea. The flare-ups may be triggered by perspiration, friction from clothing and menstruation. The disease is mainly seen in post-pubertal and immunocompetent women. Histologically, mixed inflammatory infiltrates are seen in the deep dermis through the subcutis. Extensive fibrosis and the destruction of pilosebaceous follicles and eccrine and apocrine glands ensue (Figure II-A-6-3). Apocrinitis is commonly associated.

Reportedly, portal occlusion of the apocrine duct is androgen-dependent. Differing from the disease name, hidradenitis suppurativa is actually not an infectious disease. Microbial isolates from the lesion are to be non-pathogenic *Staphylococcus epidermidis*.

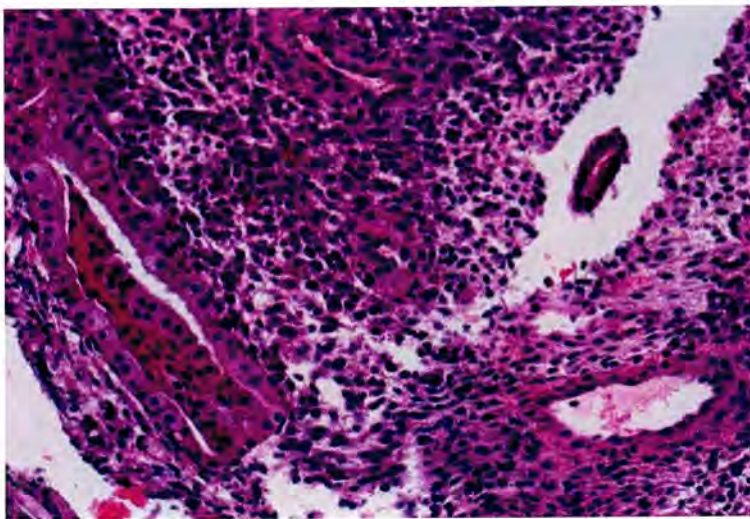


Figure II-A-6-3. Hidradenitis suppurativa. A biopsied axillary lesion in a 19-year-old female shows dense accumulation of lymphoid cells and fewer neutrophils among the eccrine sweat gland (H&E).

References

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- Farrell AM, Dawber RPR. Endocrine factors in pre- and postmenopausal women with hidradenitis suppurativa. *Br J Dermatol* 1997; 136: 802-803.
- von der Werth JM, Williams HC. The natural history of hidradenitis suppurativa. *J Eur Acad Dermatol Venereol* 2000; 14: 389-392.

7) Staphylococcal Botryomycosis

Botryomycosis is a chronic, suppurative infection characterized by an active inflammatory response to bacterial pathogens with fistule formation. The word root “botrys” means “bunch of grapes” in Greek. It occurs more commonly among patients with diabetes mellitus or alcoholism.

Staphylococcal botryomycosis (mycetoma) is a special form of deep pyoderma. Fistula tracts are formed from the deeply situated abscess cavity, and the bacteria colonize as grossly visible, white-colored grains (granules) within the abscess cavity. Bright red-colored grains may be encountered. The grains are composed of Gram-positive bacteria and the background matrix is called as Splendore-Hoepli material, which is eosinophilic, amorphous and periodic acid-Schiff (PAS)-reactive. Similar skin lesions containing grains are formed in actinomycosis, nocardiosis, and madura foot (pigmented fungal infection).

Figure II-A-7 shows gross and microscopic features of staphylococcal botryomycosis, representing a fistula-forming subcutaneous abscess and microscopic features of staphylococcal grains embedded in Splendore-Hoepli material.

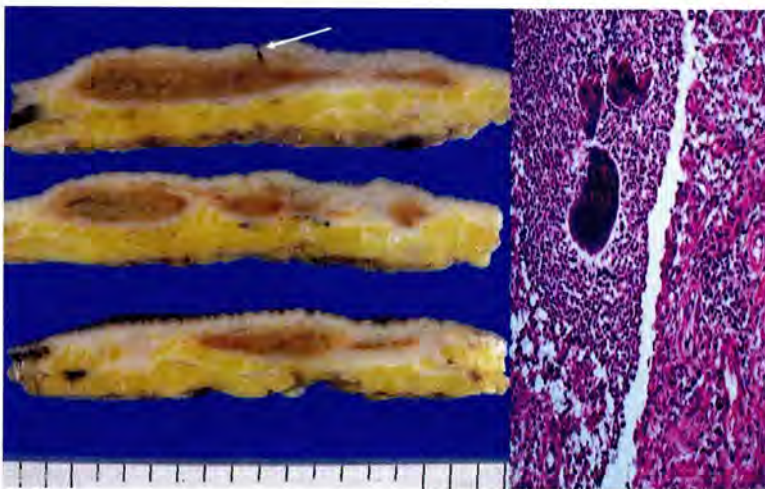


Figure II-A-7. Staphylococcal botryomycosis. Cut surfaces of the surgically removed skin of the hip (left) show brown-colored hemorrhagic abscess cavities at the junctional area between the deep dermis and subcutaneous fat tissue. Arrow indicates fistula tract. Microscopically, formation of grains is characteristic (right: H&E). The white-colored grains are composed of clustered Gram-positive cocci and homogeneous matrix called Splendore-Hoepli material.

References

- Katkar V, Mohommad F, Raut S, et al. Red grain botryomycosis due to *Staphylococcus aureus*. A novel case report. *Indian J Med Microbiol* 2009; 27: 370-372.
- Lalani T, Murray JC. Botryomycosis. 2011. <http://www.uptodate.com/contents/botryomycosis>
- Machado CR, Schubach AO, Conceição-Silva F, et al. Botryomycosis. *Dermatology* 2005; 211: 303-304.

8) Impetigo Contagiosa

Impetigo is characterized by acute and contagious superficial microabscess formation, principally caused by infection of *Staphylococcus aureus*. This is the most common bacterial skin infection in childhood, and multiple red papules with small pustules on the top are distributed on any part of the body. Contact transmission from child to child easily occurs. Adults such as classmates, athletes and military personnel may be affected. The nickname of impetigo is “school sores”. Two clinical forms are known: common type and bullous type. The common type is caused by not only *S. aureus* but also group A β -hemolytic *Streptococci* (*S. pyogenes*).

Figure II-A-8 illustrates gross and microscopic features of impetigo contagiosa. Biopsy trial is done usually in adult cases, because biopsy confirmation is unnecessary in typical childhood cases.

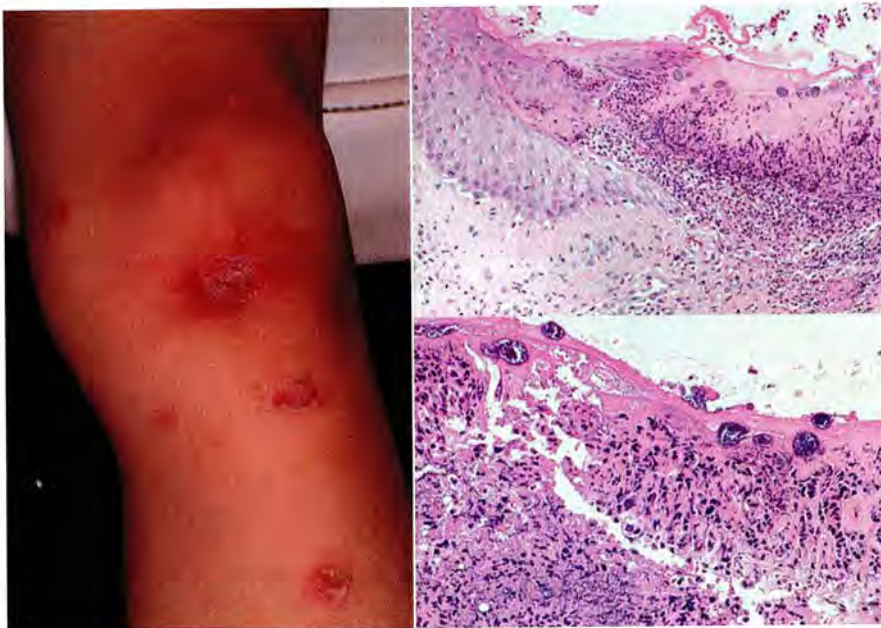


Figure II-A-8. Impetigo contagiosa. Multiple eroded pustules caused by highly contagious staphylococcal infection are observed in the arm of a child (left). Pustules easily rupture, thus to be a source of contact transmission. Biopsy histology of impetigo in an adult case shows an eroded subcorneal pustule densely colonized by Gram-positive cocci (right top and bottom, H&E).

References

- Darmstadt GL, Lane AT. Impetigo: an overview. *Pediatr Dermatol* 1994; 11: 293-303.
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- Melish ME. *Staphylococci, Streptococci and the skin*. Review of impetigo and the staphylococcal scalded skin syndrome. *Semin Dermatol* 1982; 1: 101-109.

Note 4. Community-acquired MRSA

MRSA, namely hospital-acquired MRSA (HA-MRSA), is a notorious pathogen causing nosocomial infection through contact transmission. In 1999 when young American adults suffering from furuncle or carbuncle contracted influenza, they eventually died of necrotizing pneumonia. The patients did not have a history of hospitalization. Causative bacteria were identified as a special type of MRSA or community-acquired MRSA (CA-MRSA), accompanying type IV staphylococcal cassette chromosome mec (SCCmec) and producing Panton-Valentine leukocidin (PVL) and staphylococcal enterotoxin, types A-Q (SEA-SEQ). The virulence of CA-MRSA is clonally related to active toxin production. In HA-MRSA, SCCmec commonly belongs to type I through type III, and lacks PVL production.

CA-MRSA tends to be tissue destructive, so that it easily causes destructive folliculitis (furuncle or carbuncle). The risk of CA-MRSA infection is high among school wrestling teams, day nurseries, army troops, prisons and male homosexuals. The suspicion of CA-MRSA infection is needed when severe folliculitis is endemic in such situations. CA-MRSA also provokes purulent conjunctivitis (refer to Chapter II-A-4).

References

- Baba T, Takeuchi F, Kuroda M, et al. Genome and virulence determinants of high virulence community-acquired MRSA. *Lancet* 2002; 359: 1819-1827.
- Koo I. Community-acquired Methicillin-resistant *Staph aureus* (MRSA). A superbug that found among athletes, students, and military recruits. 2009. <http://infectiousdiseases.about.com/od/diseasesbyname/a/CA-MRSA.htm>

9) Benign Embolic Pyemid

The "pyemid" is used to designate a cutaneous lesion resulting from pyemic metastasis of infection. Benign embolic pyemid, a historic idea of infectious dermatosis, is one type of acute staphylococcal infection, in which a primary banal suppurative focus is followed by repeated pyogenic metastasis. Staphylococcal emboli provoke localized semiglobular skin nodules, later with an umbilicated necrotic center (Figure II-A-9). The lesions occur preferentially on the forehead, but may be seen on any part of the upper body. The eruptions

appear suddenly in waves, and heal within 1-2 weeks. It should be distinguished from other septicemic diseases involving the skin by its absolute benignity. This entity must be distinguished from impetigo.

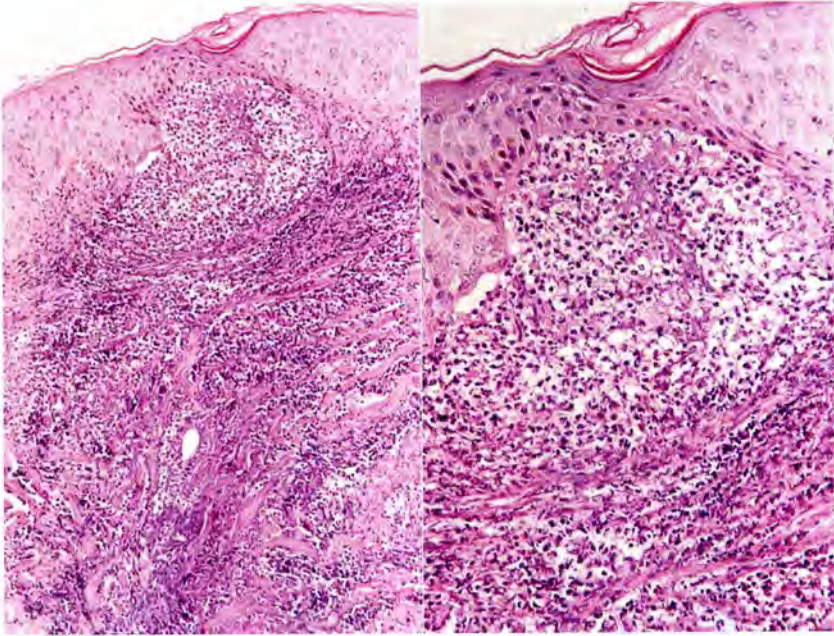


Figure II-A-9. Pyemid. A small necrotic nodule with neutrophilic infiltration is formed in the dermis (left and right: H&E). The lesion is supposed to result from staphylococcal embolization.

References

Balog P. Benign embolic pyemid. *J Invest Dermatol* 1942; 5: 107–115.

10) Staphylococcal Scalded Skin Syndrome

Staphylococcal scalded skin syndrome (SSSS) results from the secretion of exfoliative (epidermolytic) toxins from certain types (particularly type 71 of phage group II) of *S. aureus*, infected at the distant site. The causative low molecular weight exotoxins include exfoliative toxins A and B. SSSS predominantly affects previously healthy infants and children below the age 6. Figure II-A-10 shows a gross finding of the perioral skin of a newborn showing diffuse exfoliation. Involvement in adults rarely occurs in patients with immunosuppression or renal failure. Preferred sites of involvement include the face, neck, axilla and groin. The onset is sudden. Scarletiform eruptions are followed by large, easily ruptured bullae. Nikolsky sign is positive, and skin desquamation occurs in sheets and ribbons. Mucous membrane is spared. Spontaneous healing is expected, and the prognosis is excellent. Of note is that SSSS has erroneously been considered to be a variant of toxic epidermal necrolysis, and called as staphylococcal toxic epidermal necrolysis (S-TEN).

Histologically, subcorneal splitting with bulla formation is seen (**Figure II-A-10**, right panel). Acantholytic cells and neutrophils are sparse. No pathogens are identified in the exfoliative lesion.



Figure II-A-10. Staphylococcal scalded skin syndrome. The facial skin of an infant shows surface exfoliations (left). H&E-stained biopsy specimen shows splitting in the cornified layer (right). Inflammatory reaction is scarce. Pathogens are never seen within the lesion. Nuclear vacuolation is a biopsy artifact.

References

- Jeyakumari D, Gopal R, Eswaran M, et al. Staphylococcal scalded skin syndrome in a newborn. *J Glob Infect Dis* 2009; 1: 45–47.
- Ladhani S, Joannou CL, Lochrie DP, et al. Clinical microbial, and biochemical aspects of the exfoliative toxins causing staphylococcal scalded skin syndrome. *Clin Microbiol Rev* 1999; 12: 224–242.
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11) Staphylococcal Epidermolytic Toxin Syndrome and Staphylococcal Toxic Shock Syndrome

Local production of the exfoliatins lead to staphylococcal epidermolytic toxin syndrome (generalized bullous impetigo). In contrast to SSSS, the coccal organisms can be demonstrated within the bullous lesion. The involved epidermis is easily peeled off (with positive Nikolsky phenomenon). Infection of Gram-positive cocci is seen along the pustule formed within the epidermis. The histologic features are fundamentally identical to those of impetigo contagiosa. The condition should be distinguished from life-threatening toxic epidermal necrolysis (TEN), which is commonly caused by drug allergy, with no bacteria identified in the lesion (Figure II-A-11-1).

Fatal systemic infection of *S. aureus* with systemic skin rash (so-called toxic shock syndrome) may occur. Originally, healthy menstruating women who used tampons suffered from this serious condition: toxin-producing *S. aureus* proliferated in the vagina. The situation is similar to the disorder caused by streptococcal infection, but no gangrenous change of the extremity is observed (Figure II-A-11-2). Refer to Chapter III-C-1 “fulminant streptococcal infection”. As the cause of toxic shock syndrome, toxic shock syndrome toxin-1 (TSST-1), a superantigen, is secreted from *S. aureus*, provoking hypercytokinemia and hypergammaglobulinemia. Clinical manifestation of toxic shock syndrome includes fever, skin rash (resembling viral exanthema or erythema multiforme), pharyngitis, diarrhea, myalgia, liver and kidney dysfunctions, shock, disseminated intravascular coagulation and multiorgan failure. **Note 5** comments on the superantigen.

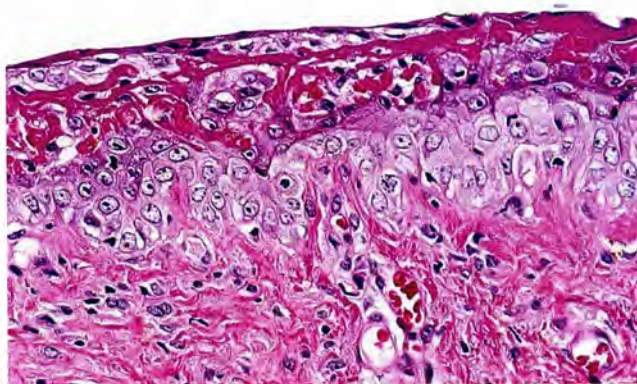


Figure II-A-11-1. Toxic epidermal necrolysis (TEN). Massive epidermal necrosis is commonly caused by drug allergy. No bacteria are identified histologically (H&E).

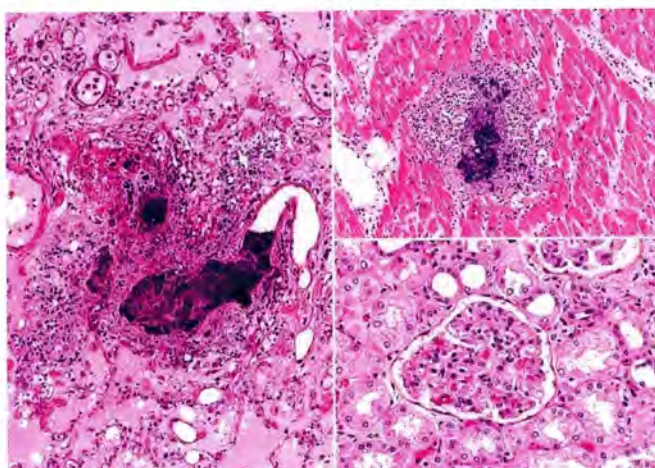


Figure II-A-11-2. Lethal MRSA septicemia accompanying staphylococcal toxic shock syndrome. A previously healthy adult suddenly suffered from MRSA septicemia to death. Autopsy revealed staphylococcal emboli in various organs, including the lung (left: H&E). Septic embolus is seen in the heart (right top). Multiple fibrin thrombi are noted in the renal glomeruli, indicating the association of DIC (right bottom). In this case, community-acquired MRSA (Note 4) was isolated. The entry of the microbes was unclear.

References

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- Sandhu K, Kanwar AJ. Generalized bullous impetigo in a neonate. *Pediatr Dermatol* 2004; 21: 667-669.
- Shands KN, Schmid GP, Dan BB. Toxic-shock syndrome in menstruating women: association with tampon use and *Staphylococcus aureus* and clinical features in 52 cases. *N Engl J Med* 1980; 303: 1436-1442.

Note 5. Superantigen

Superantigen is an exotoxin drastically activating T-cells polyclonally, through directly binding to the major histocompatibility complexes (MHC) class II antigen (DR, DQ and DP). As a result, cytokines such as interleukin-2 and -6 are overproduced (hypercytokinemia) and secondary B-cell activation leads to hypergammaglobulinemia. The representative superantigen is toxic shock syndrome toxin-1 (TSST-1), secreted by *Staphylococcus aureus*. TSST-1 provokes toxic shock syndrome (TSS). *S. aureus* produces different superantigens, enterotoxins A and C, which may cause intractable diarrhea in case of MRSA enteritis. *Streptococcus pyogenes* secretes superantigens termed streptococcal pyrogenic exotoxin (SPE) A, B, C and F and streptococcal superantigen (SSA). These may have roles in fulminant streptococcal myonecrosis or streptococcal toxic shock-like syndrome. Skin rash is commonly associated with superantigen-related disorders. Superantigens are secreted by *Yersinia pseudotuberculosis* (in Izumi fever, see Note 25), *Clostridium perfringens*, (enterotoxin) and EB virus (virus-associated hemophagocytic syndrome, see Chapter IV-G-1). Superantigens are also involved in the pathogenesis of scarlet fever, Kawasaki disease (Note 8) and neonatal toxic shock syndrome-like exanthematous disease (NTED) (Note 6).

References

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Note 6. Neonatal toxic shock syndrome-like exanthematous disease

Neonatal toxic shock syndrome-like exanthematous disease (NTED), is caused by the superantigen, toxic shock syndrome toxin-1 (TSST-1), produced by MRSA. All patients show colonization of MRSA. The pathogenesis is involved by the drastic activation of T-cells through TSST-1. The MRSA isolates show a single clone of coagulase type II, expressing TSST-1 and staphylococcal enterotoxin C. Systemic exanthema resembling measles or erythema subitum, fever and transient thrombocytopenia are seen until 4 days after birth. Atypical lymphocytes are seen in the peripheral blood. Rapid recovery from NTED without complications is expected within 5 days. No treatment is needed. Anti-TSST-1 IgG antibody of maternal origin may play a protective role in preventing the development of NTED.

References

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B) Streptococcal Infection

1) Secondary Streptococcal Colonization

As mentioned above in staphylococcal colonization and in **Note 1**, *Staphylococci* are skin inhabitants, while *Streptococci* mainly proliferate on the diseased mucosa under anaerobic or microaerophilic conditions, such as the oral cavity and vagina. Group B *Streptococcus* is a normal flora on the vagina, and may cause neonatal meningitis via transvaginal transmission. Refer to **Note 7**. Secondary streptococcal colonization preferentially occurs on ulcerative or polypoid lesions of the oral mucosa or vaginal cavity. One typical example is demonstrated in Figure II-B-1.

References

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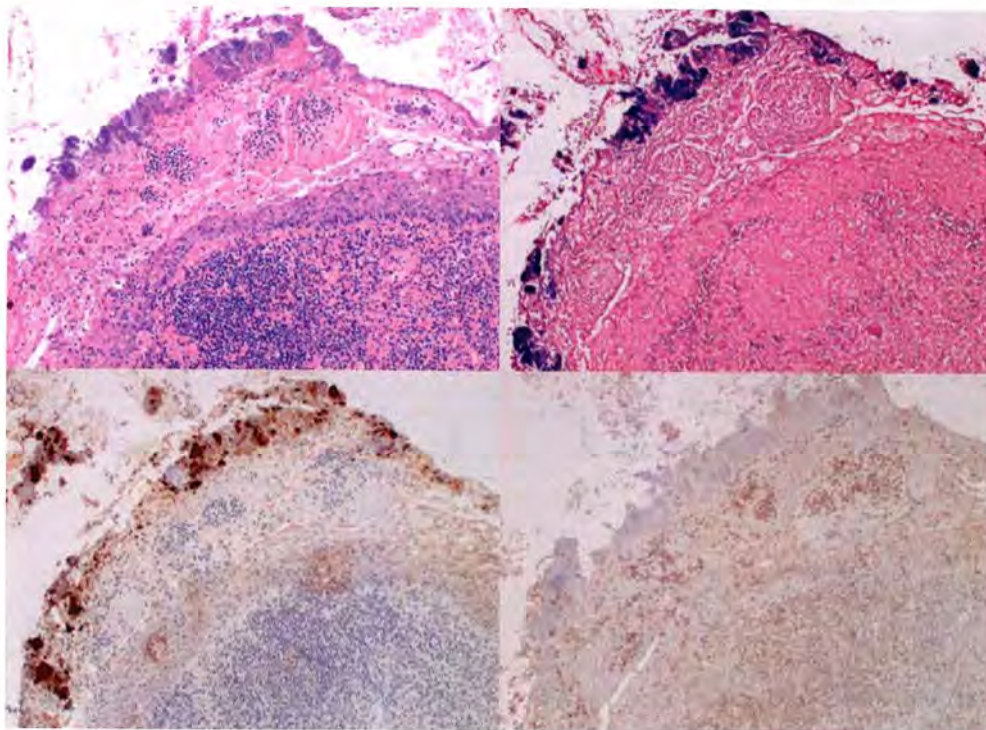


Figure II-B-1. Secondary streptococcal colonization on the tonsil. Gram-positive cocci densely cover the eroded surface. They are immunoreactive for streptococcal antigens but not for staphylococcal antigens (left top: H&E, right top: Gram, left bottom: immunostaining for streptococcal antigens, right bottom: immunostaining for staphylococcal antigens).

Note 7. Viridans group *Streptococcus*

Viridans group *Streptococcus* (virido- means green in Latin) is a general term used for α -hemolytic *Streptococcus* normally colonizing the oropharyngeal mucosa. It causes dental caries, infective endocarditis and aspiration pneumonia. The following species are known. 1) *S. salivarius* is the commonest flora in saliva and oral mucosal surface. 2) *S. sanguis* and *S. mitis* may cause infective endocarditis. 3) *S. milleri* group (*S. intermedius* and *S. constellatus*) is anaerobic and predominantly seen in the dental pit and thus called as aerotolerant or microaerophilic *Streptococcus*. This may cause sinusitis, lung abscess, aspiration pneumonia and brain abscess. Oral hygiene is very important for the prevention of aspiration pneumonia. 4) *S. mutans* is a causative agent of dental caries to form dental plaques as a result of biofilm infection. By consuming sucrose, it can produce glucose polymers (glucans). 5) *S. sobrinus* produces gelatinous material (zooglea) by using sucrose on the dental plaques.

Viridans *Streptococci* are occasionally identified in the blood of cancer patients with chemotherapy-related neutropenia. A toxic shock-like syndrome may be seen. Most frequently, *Streptococcus mitis* is the causative species, and the resistance to a variety of antimicrobial agents is clinically serious and problematic.

References

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- Bowden GH. Does assessment of microbial composition of plaque/saliva allow for diagnosis of disease activity of individuals? *Community Dent Oral Epidemiol* 1997; 25: 76-81.
- Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. *J Dent Educ* 2001; 65: 1028-1037.
- Tunkel AR, Sepkowitz KA. Infections caused by *Viridans Streptococci* in patients with neutropenia. *Clin Infect Dis* 2002; 34: 1524-1529.

2) Scarlet Fever

Scarlet fever (scarletina) is the secondary systemic manifestation of acute tonsillitis caused by group A β -hemolytic *Streptococcus* or *S. pyogenes*. Infants aged around 5 years are susceptible to droplet transmission of this microorganism. Clinical manifestations include fever, throat pain, tonsillar swelling with white-colored exudation, coughing, running nose and cervical lymph node enlargement. Two days after tonsillitis symptoms (sore throat), generalized exanthema develops, particularly on the neck, chest wall, back and extremities. The rash is fine, red and rough-textured, and blanches upon pressure. The rash tends to be accentuated along the skin folds (Pastia's lines). Perioral skin, palms and soles are spared. Strawberry tongue and red cheek are characteristic (Figure II-B-2).



Figure II-B-2-1. Scarlet fever. Fused but spotted fine exanthemas (left and right top) and strawberry tongue (right bottom) are characteristic. Red cheek and perioral haloe are noted.

In 4-8 days, scaling occurs, particularly on fingers and toes, and the disease subsides within 2-3 weeks. Active production of streptococcal pyogenic exotoxin (SPE) by the infected bacteria is the main cause of illness. SPE is categorized as a member of

superantigens (refer to Note 5). One should know late complications such as acute (post-streptococcal) glomerulonephritis and rheumatic fever (endocarditis) leading to chronic valvular heart diseases affecting mitral and/or aortic valves. In rare cases, scarlet-like fever is caused by *Staphylococcus aureus*.

References

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- CDC. Scarlet fever: a group A streptococcal infection. 2013. <http://www.cdc.gov/features/scarletfever/>

Note 8. Kawasaki disease

Kawasaki disease, or mucocutaneous lymph node syndrome, is common among Japanese infants below the age 4. The disease was first described in 1967 by a Japanese pediatrician, Dr. Tomisaku Kawasaki. The etiology remains unknown. Supposedly, superantigens released from *Staphylococcus aureus* or *Streptococcus pyogenes* may be causative factors. The skin lesion on the hand and foot, such as hard edema and membranous scaling, is quite characteristic of this acute febrile disorder. Polymorphous erythematous rash often involves the extremities, perineum, perianal region and the site of previous vaccination of BCG (Figure II-B-2-2). The lip and tongue are swollen red. Other clinical features include conjunctival hyperemia and cervical nodal swelling.

Coronary aneurysm, a sequela of coronary angiitis, is known to be a late, serious and often lethal complication. Neutrophilia is nominated as a risk factor for the coronary complication. Aspirin administration and a large scale immunoglobulin injection therapy are effective, and have considerably lowered the risk of the coronary complication.



Figure II-B-2-2. Kawasaki disease. Rash at the site of previous BCG vaccination (left), membranous scaling on the fingers (right top) and cervical nodal swelling (right bottom) are shown here. The courtesy by Prof. Tetsushi Yoshikawa, Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Japan.

References

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Harnden A, Takahashi M, Burgner D. Kawasaki disease. *Br Med J* 2009; 338: b1514.

3) Erysipelas and Streptococcal Pyoderma

Erysipelas is non-ulcerating, diffuse cellulitis (phlegmonous inflammation) of the facial skin and soft tissue, caused by group A β -hemolytic *Streptococcus*. The involved skin shows sharply demarcated raised edges, and is red-swollen, hard, warm and painful. High fever, shaking, headache and vomiting are associated. It is of note that *Streptococcus* causes either diffuse cellulitis or necrotizing inflammation, instead of abscess formation characteristic of staphylococcal infection. Gross findings of erysipelas are shown in Figure II-B-3-1.

Subclinical lymphatic dysfunction and preexisting lymphedema caused by mastectomy and axillary nodal dissection for breast cancer is a clear-cut risk factor for erysipelas on the arm. Chronic recurrent erysipelas may be encountered under such predisposing factors as alcoholism and diabetes mellitus.

Occasionally, localized pyoderma is caused by streptococcal infection, rather than staphylococcal infection (Figure II-B-3-2). Another rare form of localized and afebrile streptococcal infection is perianal streptococcal dermatitis, seen exclusively in children. It presents as perianal erythema with a defined border, followed by scaling and healing.

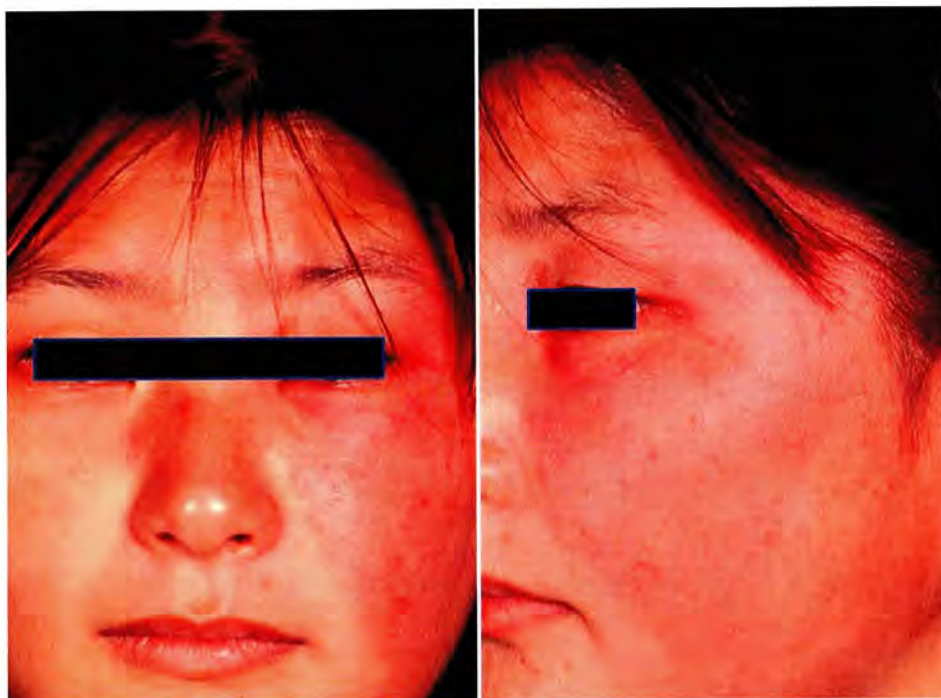


Figure II-B-3-1. Erysipelas. The involved facial skin shows demarcated red and hard swelling with pain and high fever.

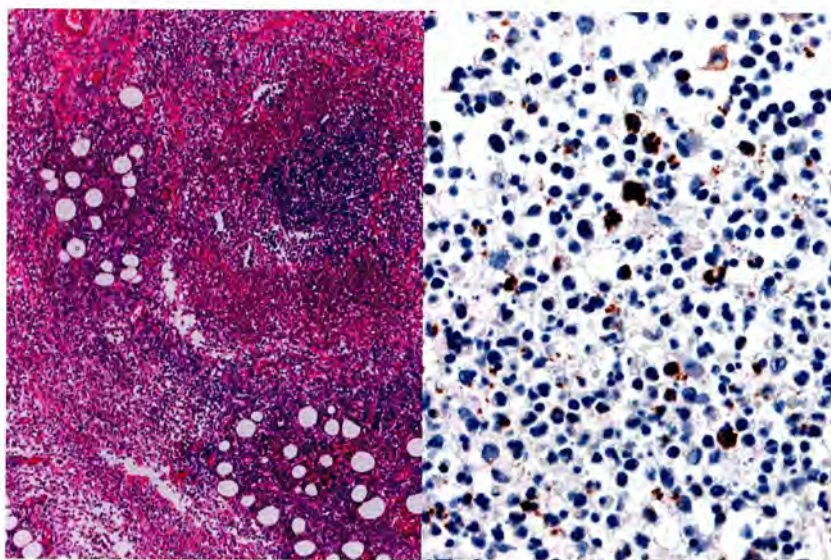


Figure II-B-3-2. Streptococcal pyoderma. In this case, subcutaneous abscess is formed by microbial culture-positive streptococcal infection, which is also confirmed immunohistochemically. Immunoreactive coccoid microbes are phagocytized by neutrophils. Staphylococcal antigens are undetectable (left: H&E, right: immunostaining for streptococcal antigens).

References

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Note 9. Erysipeloid

Erysipeloid is an acute zoonotic bacterial infection of traumatized skin caused by *Erysipelothrix rhusiopathiae*, a Gram-positive, facultative anaerobic bacillus. The disease is unrelated to streptococcal erysipelas. In animals, the organism causes swine erysipelas and several other diseases in poultry and sheep. Humans acquire erysipeloid after direct contact with infected animals, as an occupational disorder, so that the disease is seen among individuals who handle raw meat such as farmers, butchers, cooks, homemakers and anglers. Warm and tender lesions showing bright red to purple-colored and sharp-bordered plaques are formed on the hand, finger or forearm. They slowly expand over a few days, and small blisters are formed. Some patients may experience mild fever, chills and malaise. The infection is endemic during the summer to early fall.

Endocarditis with septicemia is uncommon but comprises a serious sequela.

References

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4) Necrotizing Fasciitis

Necrotizing fasciitis represents clinically severe, deep pyogenic infection (cellulitis) of the skin and underlying soft tissue. Deep, painful and intractable ulceration rapidly progress predominantly on the extremities (Figure II-B-4-1). Minor trauma may provide the entry of the pathogen. The condition uncommonly follows surgery. Immunosuppression, diabetes mellitus, alcoholism, drug abuse, atherosclerosis-related ischemia and malnutrition may be prodromal to this troublesome lesion. Occasionally, it is seen in healthy persons. Necrotizing fasciitis is categorized into two: type I (a polymicrobial infection) and type II (a monomicrobial infection).

In Figure II-B-4-2, a poorly controlled diabetic male case is presented. In the winter time, a fan heater gave the patient a severe burn on the sole, because he did not feel pain due to diabetic peripheral neuropathy. Severe atherosclerosis had provoked dry gangrene in his first and second toes. Neutrophil dysfunction led him to the vulnerability to infection. Polymicrobial (type I) necrotizing fasciitis resulted in septicemia. Emergency amputation finally saved his life.



Figure II-B-4-1. Necrotizing fasciitis. Deep and painful ulceration is caused by local bacterial infection. This male diabetic case had a history of arterial replacement therapy for atherosclerosis obliterans. In order to relieve pain and to avoid septicemic spread of infection, amputation surgery was performed.



Figure II-B-4-2. Localized severe burn on the sole of a diabetic male caused by a fan heater, resulting in necrotizing fasciitis. Because of diabetic neuropathy, deep ulcers occurred on the senseless foot. Dry gangrene on the first and second toes indicates the association of diabetes-related atherosclerosis obliterans. The importance of foot care should be emphasized in diabetic patients.

Classic pathogens of cellulitis are group A β -hemolytic *Streptococcus* and less frequently *Staphylococcus aureus*, but a diverse range of microorganisms, including *Pseudomonas aeruginosa*, cause cellulitis (see Chapter II-D-3). Erythematous nodular lesions formed on the leg of neutropenic or leukemic patients are caused by *Stenotrophomonas maltophilia*. Facial cellulitis may result from *Haemophilus influenzae* infection.

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C) Lethal Gangrene of Soft Tissue

A variety of microbes cause progressive and often lethal gangrenous lesions in the soft tissue, particularly on the extremities. In the mass media, this lethal condition is often referred to “flesh-eating bacteria infection”. Representative lesions are described below.

1) Fulminant Streptococcal Infection (Streptococcal Myonecrosis)

Streptococcal myonecrosis, a fulminant form of necrotizing fasciitis, presents a rapidly progressive gangrene of the extremities caused by infection of *Streptococcus pyogenes* (group A β -hemolytic *Streptococcus*), representing a prototype of "flesh-eating bacteria infection". Group G β -hemolytic *Streptococcus* can also cause a similar fulminant condition. Protein S deficiency may be responsible for the necrotizing inflammation in some cases. It has been reported that vimentin, an intracellular intermediate filament of non-epithelial cells, is up-regulated in the injured skeletal muscle cells and functions as the major skeletal-muscle protein binding to *Streptococci*. The life-threatening gangrene is seen either in the chronic form of necrotizing fasciitis or suddenly without pre-existing ulceration. Advanced deep decubitus in the sacral region may cause the progressive gangrenous lesion.

Clinically, high fever, pain at the site of infection and skin necrosis with bulla formation are associated. Scarlatiniform rash may be present. Grossly, massive gangrenous necrosis is noted in the involved extremity.

Microscopically, pronounced myonecrosis with foci of infection of Gram-positive cocci is observed. Gram-positive cocci can massively grow in the lesion of advancing gangrenous necrosis of soft tissue. Cellular reaction is minimal, because of the ischemic (anaerobic) state. In the cultured blood, short chains of Gram-positive cocci, morphologically typical of *Streptococcus*, are seen (Figure II-C-1-1). Streptococcal septicemia provokes streptococcal toxic shock-like syndrome. The bacteria are commonly sensitive to Penicillin and its derivatives, but the intravenous antibiotics administration is clinically ineffective because of the absence of blood flow. The drug can never reach the site of infection.

Groups A, B and G *Streptococci* may associate gas formation (Figure II-C-1-2), so as to be categorized in non-clostridial gas gangrene (see below).

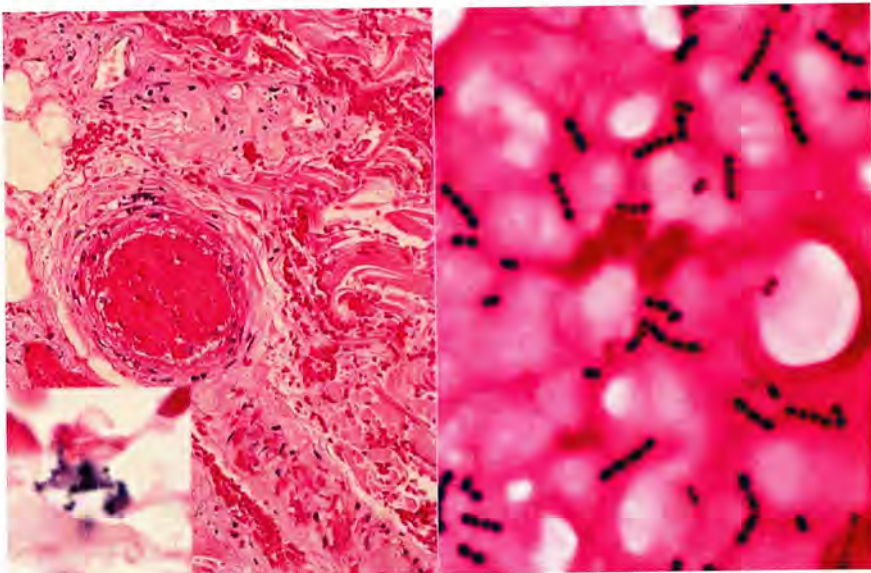


Figure II-C-1-1. Streptococcal myonecrosis. Vessels are thrombosed, and the striated muscle fibers show coagulation necrosis (left: H&E). Gram-positive cocci are identified within the ischemic tissue (inset). Numerous chained cocci are demonstrated in the cultured blood (right: Giemsa).

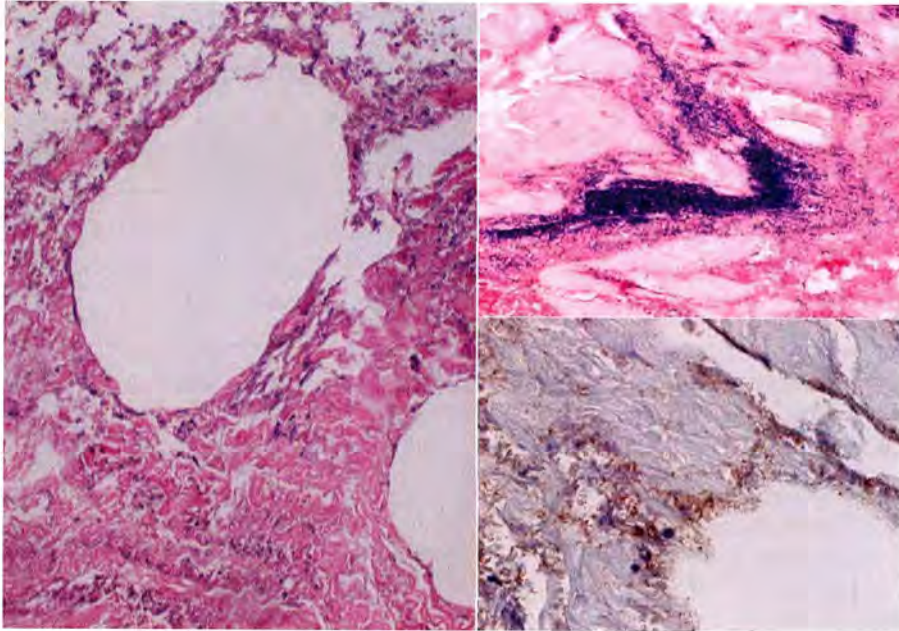


Figure II-C-1-2. Non-clostridial gas gangrene caused by Group G streptococcal infection. Massive ischemic necrosis is associated with Gram-positive cocci expressing streptococcal antigens. Gas formation is evident (left: H&E, right top: Gram, right bottom: immunostaining for streptococcal antigens).

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2) Fournier's Gangrene

Fournier's gangrene is a special form of fulminant cellulitis (fatal gangrene) involving the male scrotum and perineum. The necrotizing change rapidly progresses to the surrounding soft tissue, eventually resulting in septicemia (Figure II-C-2-1). Gas production and malodor may be associated. Histologically, massive necrosis of the skin tissue is evident (Figure II-C-2-2). Masturbation-related injury may cause the disease. Mixed bacterial infection, including *Streptococcus* and anaerobic bacteria, is proven. The prognosis is poor. Secondary surface infection of *Trichosporon* spp. may occur (refer to Figure III-J-2).



Figure II-C-2-1. Fournier's gangrene. Massive hemorrhagic necrosis started from the scrotum and extended to the left hip and leg. The rapidly progressive gangrene is often lethal. Emergency amputation was ineffective in this young male case in Nippon Medical School Hospital, Tokyo.

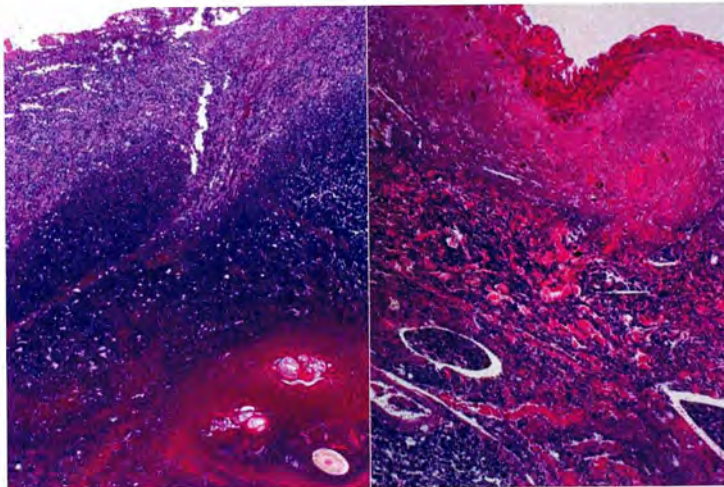


Figure II-C-2-2. Histology of Fournier's gangrene. Massive transmurial necrosis of the skin tissue is evident. Hair shafts remain in the necrotic deep dermis (left). Ischemic epidermis is shown in the right panel. No bacteria are observed here (H&E).

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3) *Vibrio Vulnificus* Infection

Progressive gangrene of the extremities caused by *Vibrio vulnificus* is characteristically seen in patients with liver cirrhosis or hemochromatosis. High iron concentration in the serum is essential for the bacteria to grow in the body. The “halophilic” bacteria also prefer to high salt concentration (at a sea water level: 3%) for growth on plates. *V. vulnificus* resides in the sea fish and oyster. The bacteria proliferate in the gut of the sea creature when the temperature is high. Two transmission pathways are known: transenteric infection and traumatic skin infection. The former septicemic condition is often fatal, initiating a painful skin lesion resembling honey bee bite. Gangrenous changes rapidly progress. Gas formation is absent. The traumatic infection of *V. vulnificus* is caused by accidental trauma of the hand or fingers during cooking raw fish (preparing sashimi). The prognosis is better than the former. The incidence of infection of the halophilic pathogens nicknamed “flesh-eating bacteria” is high in Japan.

Histologically, perivascular cuffing of Gram-negative bacteria is observed in the involved skin and soft tissue, and the cellular reaction is minimal (Figure II-C-3).

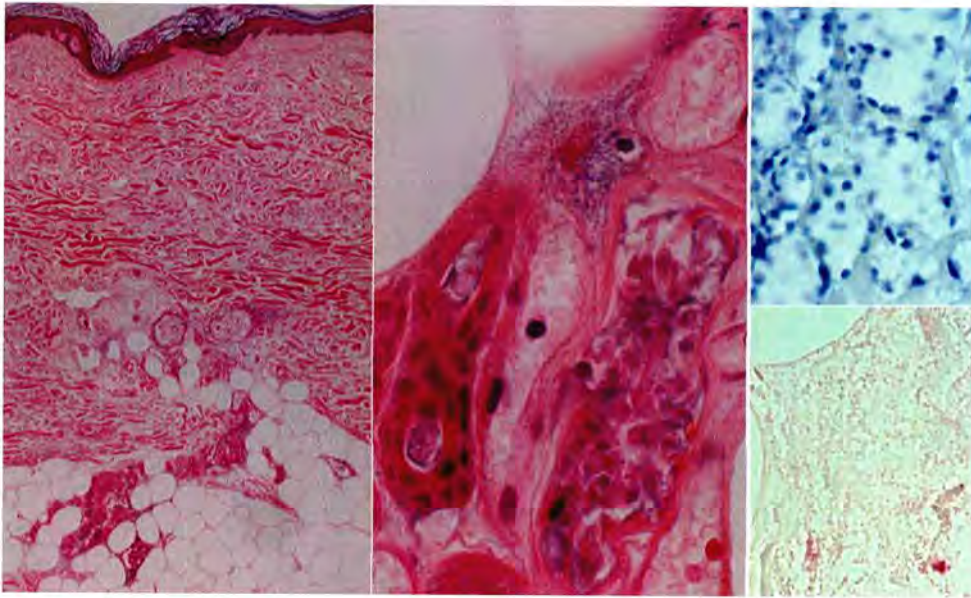


Figure II-C-3. *Vibrio vulnificus* infection in a cirrhotic patient. In a biopsy specimen taken in an emergency suite, perivascular cuffing by infected microbes is observed around small vessels and sweat glands in the deep dermis through subcutis (left: H&E). Under a high-powered view, vascular invasion of the microbe is evident. Coccoid transformation is recognized. Inflammatory reaction is scarcely observed (H&E, center). The coccoid-transformed bacteria in the scarcely inflamed subcutis are demonstrated with Giemsa stain (right top). Gram staining is negative (right bottom).

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4) *Aeromonas Hydrophila* Infection

Lethal gangrene of the extremities or face is also caused by *Aeromonas hydrophila* in immunocompromised patients as an opportunistic infection. Figure II-C-4-1 illustrates gross features of lethal necrotizing fasciitis of the right upper arm caused by *A. hydrophila*. Vesicles are formed in the necrotic skin. *A. hydrophila* belongs to the family *Vibrio*, and widely distributes in fresh water and soil. It can grow at low temperature to cause food poisoning. Histologically, the lesion shows clusters of Gram-negative rods around necrotic subcutaneous tissue. Cellular reaction is poor. Gas formation may be associated. In this case, similar necrotizing infective foci were seen in the rectum, epididymis, prostate, liver and kidney (Figure II-C-4-2).

A. hydrophila is a known pathogen of watery or bloody diarrhea caused by heat-labile enterotoxins. An outbreak of *A. hydrophila* wound infection has also been reported among participants for mud football games in Australia. There were many infected scratches and pustules distributed over the bodies.

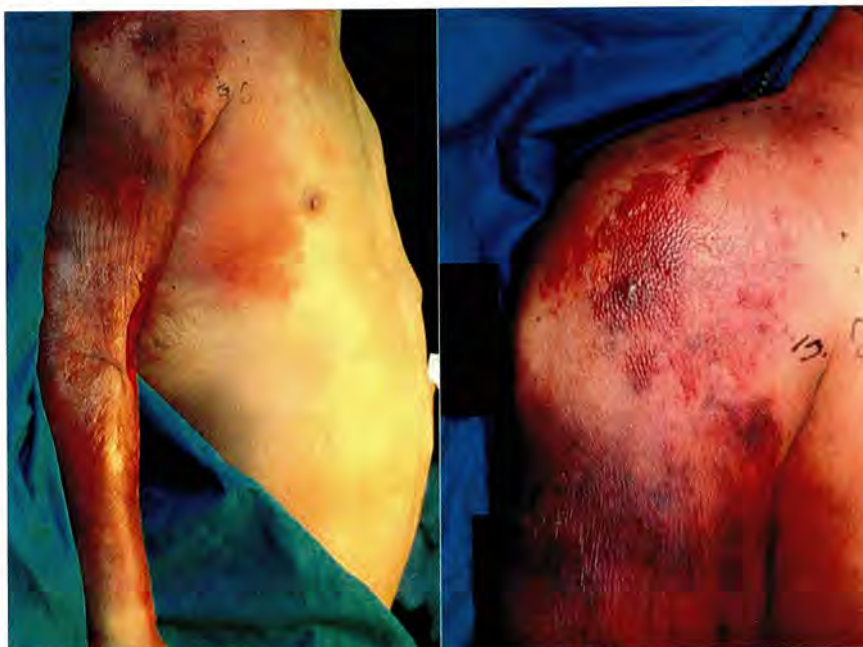


Figure II-C-4-1. *Aeromonas hydrophila* infection in a diabetic male patient. Lethal necrotizing fasciitis is observed on the right upper arm. Vesicular skin change is evident. Autopsy confirmed that septicemia caused multiorgan abscess formation.

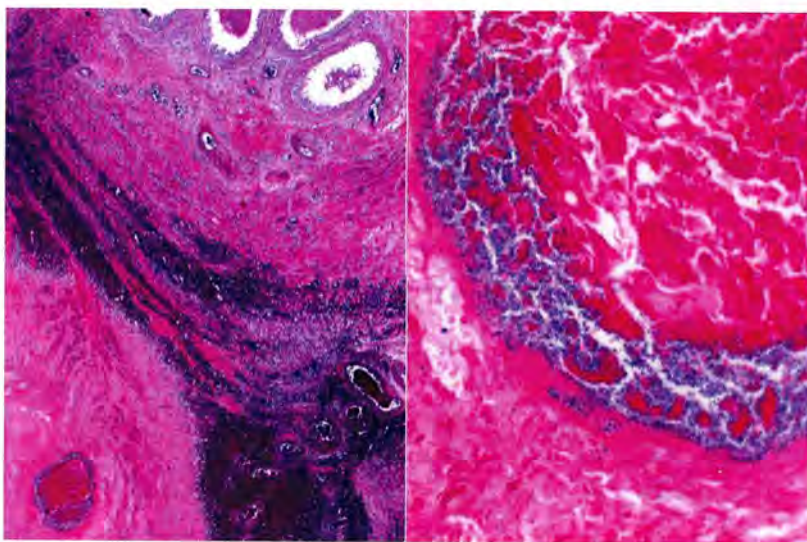


Figure II-C-4-2. Histology of *Aeromonas hydrophila* infection. In a septicemic and necrotic/hemorrhagic lesion of the epididymis, Gram-negative rods are clustered around the vascular structure. Inflammatory reaction is scarcely observed (H&E, left: low power, right: high power).

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5) Clostridial and Non-Clostridial Gas Gangrene

Gas gangrene caused by *Clostridium perfringens* (formerly called *C. welchii*) is a life-threatening emergency. *C. perfringens* is an obligate anaerobic Gram-positive bacillus forming spores. Traumatic skin invasion of the microbes results in massive ischemic necrosis (gangrene) of the soft tissue and striated muscles. Gas (hydrogen) production is quite characteristic, and the involved tissue reveals crepitation on touch (Figure II-C-5-1). Since the bacteria can grow under an anaerobic condition, the degree of ischemia is advanced in the

involved tissues and organs. Tissue necrosis is caused by α -toxin production of the microbe. Putrid odor is associated. Hemolysis is a common event due to bacterial production of hemolysin, δ -toxin. The prognosis is poor. Histologically, Gram-positive rods are seen adjacent to gas bubbles (Figure II-C-5-2).



Figure II-C-5-1. Gas gangrene of the thigh. Gas-forming gangrenous process of the soft tissue results in marked swelling of the thigh. Surgical debridement has been performed for the treatment purpose.

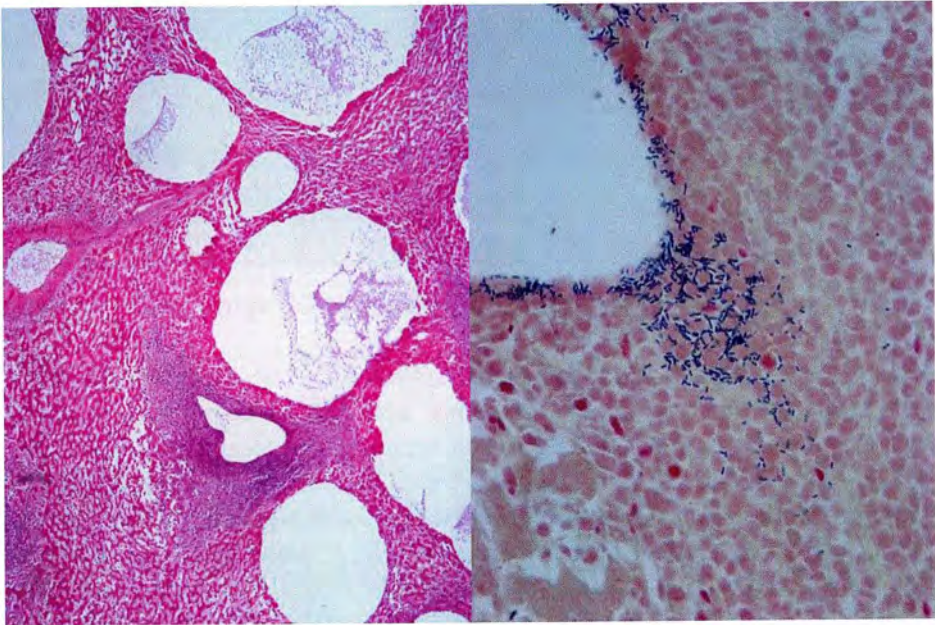


Figure II-C-5-2. Gas gangrene involving the liver. Multiple gas bubbles replace the parenchymal tissue, which shows massive ischemic changes (left: H&E). Gram-positive rods are clustered around the gas bubble (right: Gram stain). Note that the condition which allows the growth of obligate anaerobic *Clostridium perfringens* must be highly ischemic.

Not all of the bacteria are stained blue with Gram stain (some remain unstained), and the production of spores is abortive and partial in the living body. *C. perfringens* is commonly seen in the gut of healthy individuals, so that gas gangrene is encountered in the internal organs such as the gut, liver and pancreas.

Many cases of gas gangrene are caused by clostridial infection, but non-clostridial causes are also encountered, mostly seen in the extremities. Non-clostridial gas gangrene is most often caused by polymicrobial organisms and mainly seen in diabetic patients. The causative gas-producing bacteria include *Escherichia coli* (refer to Figure II-A-3-5), *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Bacteroides* and *Streptococcus milleri* group. Groups A, B and G *Streptococci* may also cause gas gangrene (refer to Figure II-C-1-2). Early diagnosis and therapy are required, since the disease may rapidly progress to fatal toxemia. A unique and true dermatologic emergency is the detection of non-traumatic subcutaneous emphysema of the leg with or without associated erythema, tenderness, or bullous lesions. Emphysematous inflammation with gas production may be observed in the perineum, vagina, gallbladder, stomach, urinary bladder and kidney.

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6) Vincent Angina, Ludwig's Angina and Noma (Gangrenous Stomatitis)

Vincent angina, named after the French physician, Jean H. Vincent (1862-1950), represents acute necrotizing ulcerative gingivitis. It is also called as "trench mouth". The patients complain of a progressive painful and hemorrhagic ulceration and swelling of the gingiva. The punched-out ulcer, 2-4 mm in size, is seen in the interdental papilla, and is covered with white pseudomembranes (films). Bad breath is associated. The infection can be treated with penicillin. Vincent angina may infrequently spread to involve the mouth and throat to be diagnosed as acute necrotizing periodontitis.

Ludwig's angina represents phlegmonous inflammation (cellulitis) of the oral floor caused by infection of oral flora from advanced dental caries of molar teeth. The disease was first described in 1836 by the German physician, Wilhelm F. von Ludwig (1790-1865). The infection extends to the submental, sublingual and submandibular spaces and even

mediastinum, presenting the redness, swelling and tenderness of the upper neck, under the chin. Breathing difficulty may be associated, and the disease is potentially lethal.

Noma, or cancrum oris, is a progressive necrosis of the soft and hard tissues of the oral cavity as the advanced clinical form of Vincent angina. It represents gangrenous stomatitis or necrotizing fasciitis of the oral cavity. The preferred age of the patients is below 10 years, and the disease mostly occurs in malnourished children of African poverty. The prognosis is poor. In developed countries, severely immunosuppressed patients (including AIDS) with poor oral hygiene may suffer from this serious condition. It begins in the form of Vincent angina, but rapidly followed by painless and extensive necrosis of the oral cavity. Eventually, the involvement of the cheek, nose, palate and maxillary bones results in serious facial destruction. Hence the name of “cancrum oris” (meaning: oral cancer). Gas formation may be associated. In noma neonatorum, the disease shows massive mucocutaneous gangrene in the neonate. A similar disorder may be encountered in the genitalia and is called noma pudendi.

The polymicrobial etiology is known in these conditions. Gram stain smeared from the ulcer easily identifies both fusiform bacteria and spirochetes (Figure II-C-6-1). The key players are anaerobic, Gram-negative fusiform pathogens, *Fusobacterium nucleatum* (older term: *Bacillus fusiformis*) and *Prevotella intermedia*. The spiral microbes are identified as *Borrelia vincentii*. Many other bacteria are also co-isolated, including *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, *Staphylococcus aureus* and nonhemolytic *Streptococcus*. *Pasteurella multocida* may cause Ludwig’s angina.

Figure II-C-6-2 demonstrates a Japanese diabetic male case aged 80 years, suffering from noma-like condition (progressive ulcerative gingivitis with massive maxillary necrosis). Numerous bacilli are infected, in association with gas formation.

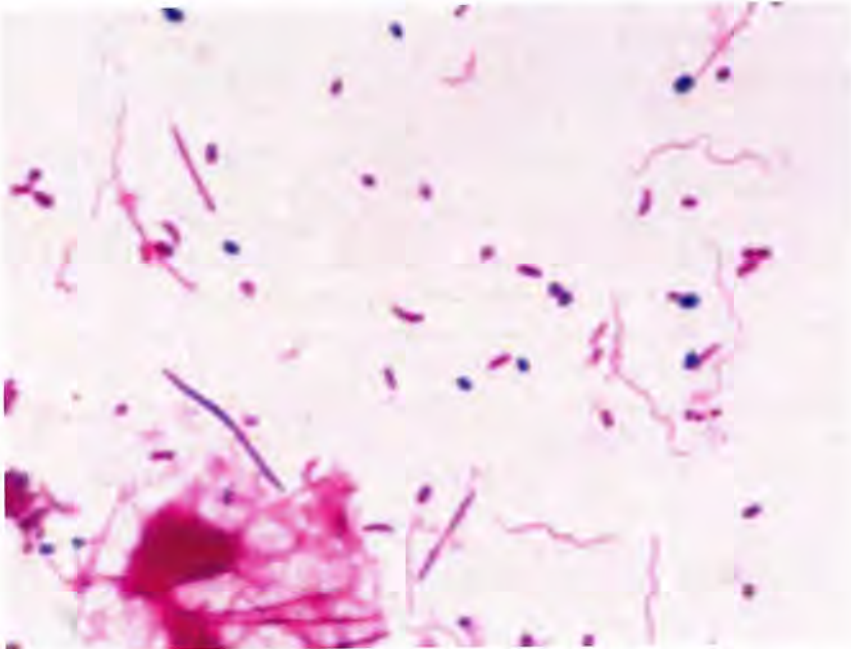


Figure II-C-6-1. Vincent angina. Gram-stained smear prepared from a painful gingival ulcer demonstrates a variety of bacteria, including Gram-negative fusiform bacilli and long spiral microbes. Gram-positive cocci and rods are also intermingled.

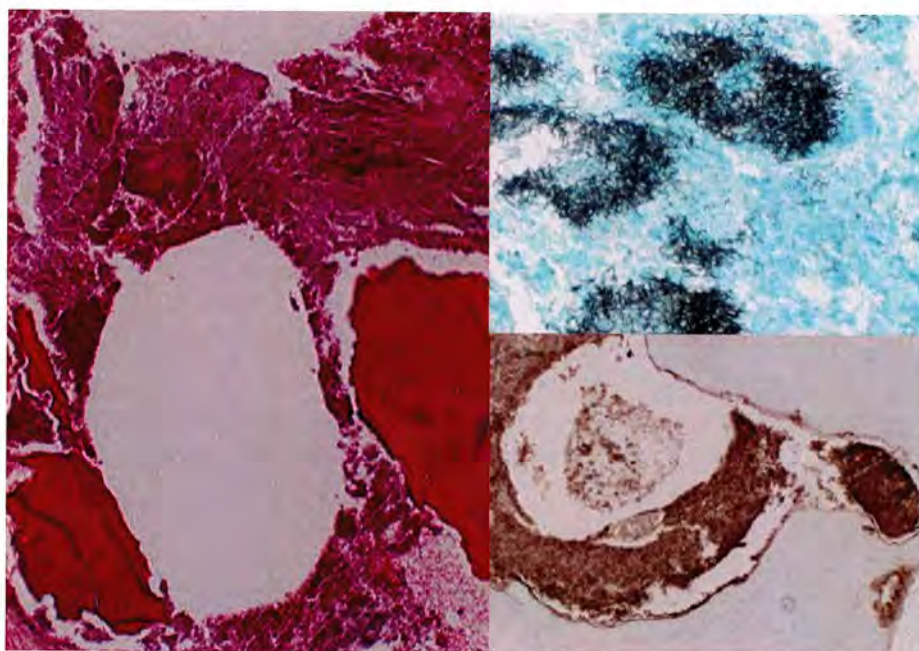


Figure II-C-6-2. Noma (progressive ulcerative gingivitis). A gas-forming, necrotizing lesion is observed in the biopsied maxillary sinus (left: H&E). Grocott methenamine silver stain identifies numerous colonies of rod-shaped bacteria in the lesion (right top). The Gram-negative bacteria around the gas bubble are visualized with a commercial antiserum against *Escherichia coli*, which shows wide cross-reactivity to Gram-negative bacteria (right bottom). The courtesy by Dr. Tatsuru Ikeda, Pathology Center, Hakodate Goryoukaku Hospital, Hakodate, Japan.

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Note 10. Tropical ulcer and the differential diagnosis

Tropical ulcer, an endemic skin disease of the “poor and hungry”, is seen among unhygienic and poorly nutritive people living in the tropics and subtropics, particularly in northern Papua New Guinea. Children and young adults are susceptible. The ulcer occurs below the knee, usually around the ankle. The lesion is initiated with minor trauma to form inflammatory papules and vesicles. Once developed, the ulcer may become chronic and stable, and it may run a destructive course with deep invasion.

Unlike Buruli ulcer (refer to Chapter II-X-6), tropical ulcer is very painful. It has a raised, slightly undermined border and a yellowish necrotic base. Human to human transmission may happen. Untreated chronic tropical ulcer results in scar formation with disability, and squamous cell carcinoma may develop later. With the improvement of the socioeconomic conditions and nutrition states, the incidence of tropical ulcer is declining.

Fusobacterium ulcerans, one of the non-oral species, is a unique pathogen of tropical ulcer. It is of note that the polymicrobial etiology of this disease principally shares with noma or gangrenous stomatitis, including *Fusobacterium*, other anaerobes and spirochetes. Secondary infection of *Staphylococci* and *Streptococci* is common.

Differential diagnosis of ulcerating skin lesions in tropics includes pyoderma, diphtheria, anthrax, Buruli ulcer, *Mycobacterium marinum* infection, cutaneous tuberculosis, sporotrichosis, chromomycosis, mycetoma and cutaneous leishmaniasis. Refer to the recent review article by Zeegelaar and Faber.

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D) *Pseudomonas* Infection

Pseudomonas aeruginosa, an aerophilic, glucose non-fermentative Gram-negative bacillus widely distributed in the environmental water, is a representative opportunistic microbe, and may cause nosocomial (hospital-acquired) infection in immunocompromised

patients. Both the chemoresistance and the resistance to disinfectants are clinically troublesome features of this pathogen. Refer to Note 12.

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Note 11. *Pseudomonas* folliculitis

It is known that *Pseudomonas* folliculitis accompanying papulopustular rash on the lower trunk and buttock of children results from bathing in heated pools, hot tubs or spa baths. An outdoor games event for children may be at a high risk of this infection. Adult employees at a US cardboard manufacturing facility suffered from skin rash primarily affecting the upper and lower extremities. The outbreak was mediated through water heavily contaminated with *Pseudomonas aeruginosa*, which invaded the hair follicles to provoke acute folliculitis.

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1) Ecthyma Gangrenosum

Ecthyma is a special term for deep pyoderma occurring on the extremities of children. Ecthyma gangrenosum represents a severe variant of ecthyma seen in immunosuppressed individuals with septicemia of *Pseudomonas aeruginosa*. An erythematous macule appears on the trunk or limbs, rapidly becomes pustular and finally develops into a gangrenous ulcer with a dark eschar (Figure II-D-1). Bacterial thrombosis provokes the infarct-like necrosis. When the lesion is multifocal, the prognosis is poor. Similar necrotic ulceration may be associated with infection of *Aspergillus*, *Candida* or *Morganella morganii*.



Figure II-D-1. Ecthyma gangrenosum associated with *Pseudomonas* septicemia. Localized necrotic skin lesion is formed on the extremity in a diabetic patient. Necrotic center is surrounded by erythematous edges. In this case, the skin lesion is solitary.

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2) Malignant Otitis Externa

The external ear canal guards against infection by producing a protective layer of cerumen, which creates an acidic and lysozyme-rich environment. Malignant otitis externa is a life-threatening infection in the aged, poorly controlled diabetic patients or those immunocompromised patients who suffer from AIDS, undergo chemotherapy, and take immunosuppressant medications such as glucocorticoids. Once infection in the external meatus becomes established in the susceptible patients, the bacteria invade the deeper underlying structures of the soft tissue and destroy the underlying temporal bone, and finally resulting in septicemia. This is called malignant otitis externa, and this serious complication should be suspected if tenderness, erythema or edema of the external ear or adjacent tissues is present on physical examination. *Pseudomonas aeruginosa* is the inciting organism in the vast majority of cases. Features of biofilm infection are observed (Figure II-D-2). Much less

frequently it is caused by *Staphylococcus aureus* and group A β -hemolytic *Streptococcus*. Fungal etiology is also known and *Aspergillus* and *Candida* are the causative microbes. When untreated, the mortality rate is around 50%.

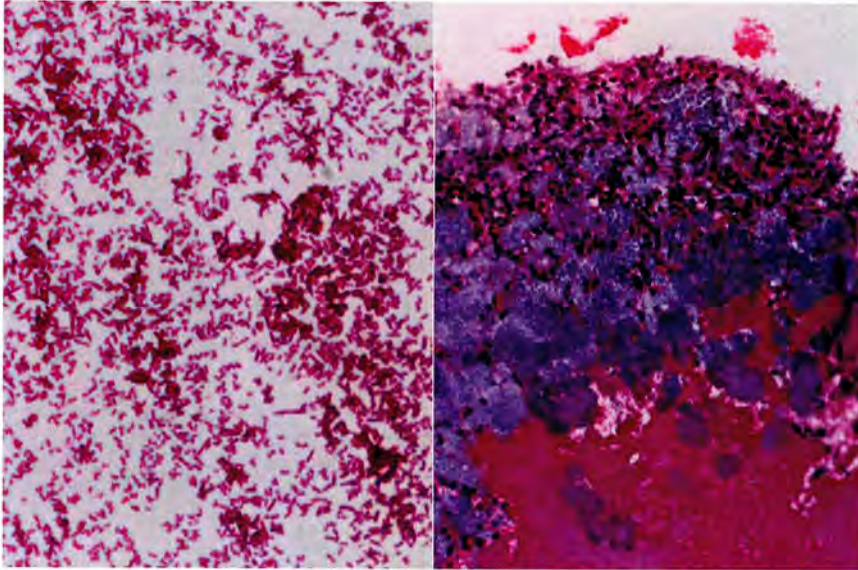


Figure II-D-2. Malignant otitis externa. In this lethal diabetic case accompanying pseudomonal septicemia, Gram-negative rods densely colonize the necrotic debris in necrotizing petrositis. Myxoid matrix of the colony indicates biofilm infection (left: Gram stain on a smear preparation, right: H&E).

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3) *Pseudomonas*-Related Necrotizing Fasciitis

Necrotizing fasciitis is a severe, insidiously advancing soft tissue infection of the extremities, characterized by widespread myonecrosis. As described in Chapter II-C-1, the pathogen is β -hemolytic *Streptococcus* in most cases. Infrequently, it is caused by

Pseudomonas aeruginosa. Reportedly, the mortality rate is 30%, and most infections occurred in the immunocompromised patients. Clinicians should consider empiric pseudomonal antibiotic coverage in the severely immunocompromised with potentially necrotizing infections.

An 18-year-old female presented here had suffered from anorexia nervosa for 6 years. She developed phlegmonous inflammation on the left lower leg, rapidly progressing to multifocal ulceration and gangrene. In 3 days, she underwent surgical amputation. *Pseudomonas aeruginosa* was cultured from blood and the leg lesion of necrotizing fasciitis. Immunohistochemical identification of the microbes was achieved with a commercial monoclonal antibody. Representative features are illustrated in Figure II-D-3.

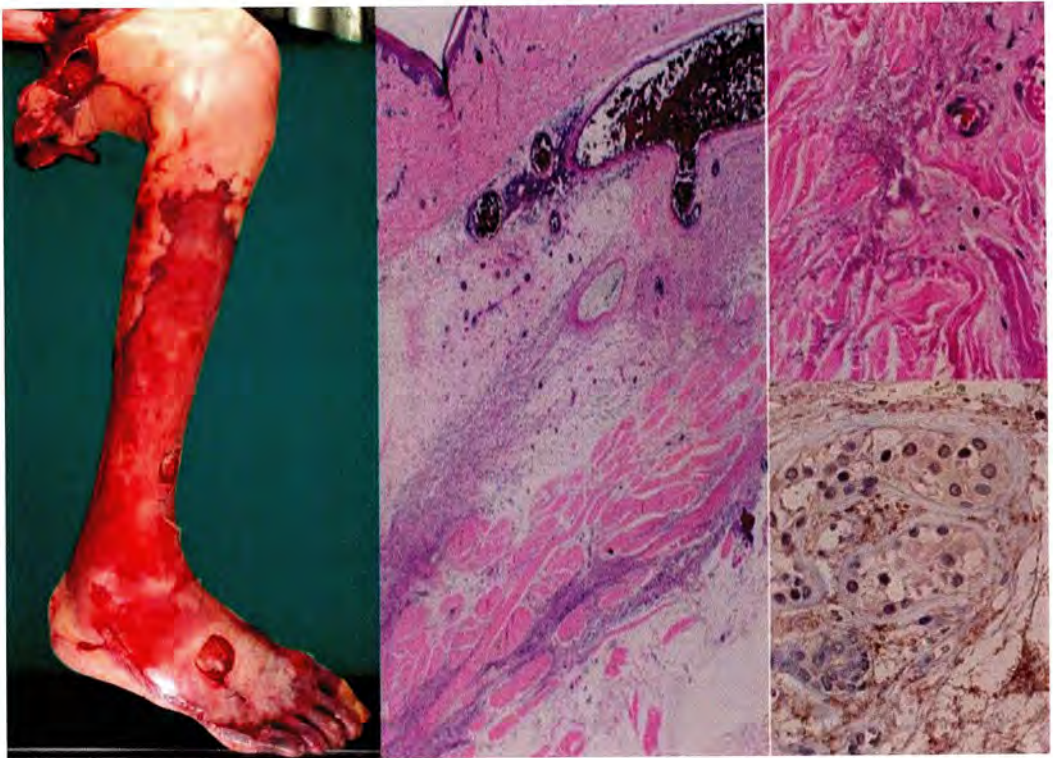


Figure II-D-3. *Pseudomonas*-related necrotizing fasciitis in a young female suffering from anorexia nervosa. Her leg with massive necrotic/gangrenous lesions was amputated (left). Soft tissue microscopically reveals massive bacterial growth without inflammatory reactions (center and right top: H&E). The bacteria are immunoreactive for *Pseudomonas* antigen detected by a monoclonal antibody (right bottom). The courtesy by Dr. Takashi Tsuchida, a pathologist in Hamamatsu Medical University Hospital, Hamamatsu, Japan.

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4) Secondary *Pseudomonas* Infection on Burnt Skin

Skin barrier dysfunctions resulting from skin trauma, decubitus and burn accelerate *Pseudomonas* colonization. In severe burn, *P. aeruginosa* often causes green-colored purulent exudation, and eventually leads to lethal septicemia (Figure II-D-4). Antibiotics resistance is common to this glucose non-fermentative Gram-negative bacillus, and formation of biofilm (Note 12) in the lesion by *P. aeruginosa* accelerates therapeutic difficulty. It should be noticed that *Pseudomonas* is the detestable enemy of patients with severe burn.

Pseudomonas aeruginosa and related glucose non-fermentative Gram-negative rods (Note 13) are notorious pathogens causing nosocomial (hospital-acquired) infection. In neutropenic patients, they often cause septicemia, and infection in the decubital ulcer is a common complication (refer to Figure II-A-3-4).



Figure II-D-4. Generalized burn complicated with *Pseudomonas* septicemia, photographed on the autopsy table. Intractable infection of *Pseudomonas aeruginosa* persisted on the burnt skin. Note green-colored ulcerative lesions. Debridement surgery had been performed to relieve inflammatory edema.

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Note 12. Biofilm infections

The bacteria may secrete mucoid material termed “exopolysaccharide”, and the colonies formed within the exopolysaccharide are protected from phagocytes and bacteriolytic substances such as immunoglobulins, complements, lactoferrin and lysozyme. These peculiar structures are called as the biofilm, which is often seen on the surface of the mucosa and foreign material (catheter). The biofilm causes prolonged, therapy-resistant infection, and acute exacerbation may occur easily.

Examples of biofilm infection include chronic airway infection by mucoid-type *Pseudomonas aeruginosa*, *Staphylococcus aureus* infection on the surface of decubitus or cutaneous fistula, and infection of *Staphylococcus epidermidis* and also *S. aureus* on the surface of catheters, and *Streptococcus mutans*-infection within the dental plaques causing dental caries.

The biofilm-forming bacterial colonies which show acidic charges are positively labeled with Alcian blue and colloidal iron stains (refer to Figure II-A-2-2). In the H&E preparation, the colonies accompany myxoid matrix (refer to Figure II-D-2).

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Note 13. Glucose non-fermentative Gram-negative rods

The bacteria of this group are aerobic Gram-negative rods growing on the regular agar plate, but not metabolizing glucose by fermentation under anaerobic condition. General features of glucose non-fermentative Gram-negative rods are as follows. They are widely distributed in the environment, seen everywhere in the water, and prone to resistant to antibiotics and disinfectants. It is of note that they can grow on the alcohol-containing cotton and disinfectant-soaked gauze. They often form “hospital flora” in the air-conditioning system, sink and water tap. Nosocomial infection among the immunocompromised patients, including the patients suffering from generalized burn, is especially problematic.

The following bacilli are included. a) *Pseudomonas aeruginosa*, b) *Burkholderia cepacia*, c) *Stenotrophomonas maltophilia*, d) *Acinetobacter*, e) *Achromobacter*, f) *Alkaligenes*, g) *Xanthomonas*, h) *Flavobacterium meningosepticum*.

P. aeruginosa is the champion of this group microbe. It produces pyocyanin pigment, fluorescein, and, under iron-deficient state, pyoverdine. Mupirocin (antibacterial nasal ointment agent against MRSA infection) is a product of *P. fluorescens*. Similar to *P. aeruginosa*, *B. cepacia* causes pneumonia and septicemia in immunocompromised patients.

It shows the resistance to chlorohexidine, a widely used disinfectant. *S. maltophilia*, the second most common microbe in this group, is often multidrug-resistant and occasionally shows the microbial substitution phenomenon to cause pneumonia, septicemia, meningitis or urinary tract infection.

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E) Infection of *Escherichia Coli* or Other Enterobacteriae

1) Perianal Abscess and Anal Fistula

Perianal abscess (also called as anorectal abscess) represents an abscess adjacent to the anus. It is caused by an acute suppurative inflammation of the anal gland. When untreated, infection may spread to the groin and rectal wall, to cause serious generalized infection. Patients with diabetes mellitus and hematopoietic disorders have an elevated risk for developing the disease. In addition to *Escherichia coli* and other enterobacteriae, *Staphylococcus aureus* (including MRSA) may become a causative agent.

Anal fistula, an abnormal connection between the anal canal and perianal skin, represents chronic persistent infection of the anal gland. The male predominance may be related to the androgen-dependent differentiation of prostate-specific antigen-expressing acinar cells in male anal glands (Kamoshida and Tsutsumi). Common pathogens are *E. coli* and other enterobacteriae. *Enterococcus* and *Bacteroides* can also be the isolates.

A total of 6-11 anal glands are located between the two layers of the anal sphincters, and drain to the transitional mucosa at the level of the dentate line of the anus. When the orifice of the anal gland is obliterated, abscess is formed in the perianal tissue, typically in the intersphincteric space (intersphincteric type). A chronic and relapsing process of inflammation eventually results in fistula tract formation. Superficial (subcutaneous/submucosal) type, transsphincteric type, suprasphincteric type and extrasphincteric type are also observed.

Diabetes mellitus, Crohn's disease, pregnancy, foreign body placement in the anus and immunosuppression are the underlying conditions for developing anal fistula. Pus-draining anal fistula is painful, and may become a nidus for systemic spread of infection, particularly

when the patient is immunosuppressed. Surgical draining is essential for the decompression of acute perianal abscess, and surgical repair of the fistula draining tract should be considered when the patients complain of hateful discomfort or inconvenience for daily living.

Histologically, along the fistula tract, pyogenic inflammatory reactions are surrounded by fibrosing granulation tissue. Foreign body giant cell reactions against material derived from the feces are also frequently associated. Lymphoid follicles are scattered in the surrounding fibrotic tissue. Inflammatory fibrosis extends to the anal sphincter muscles.

When vaseline-impregnated gauze is used for the treatment, oleogranuloma may be formed. Part of anal gland ducts can be identified along the tract (Figure II-E-1). Squamous metaplasia is often associated.

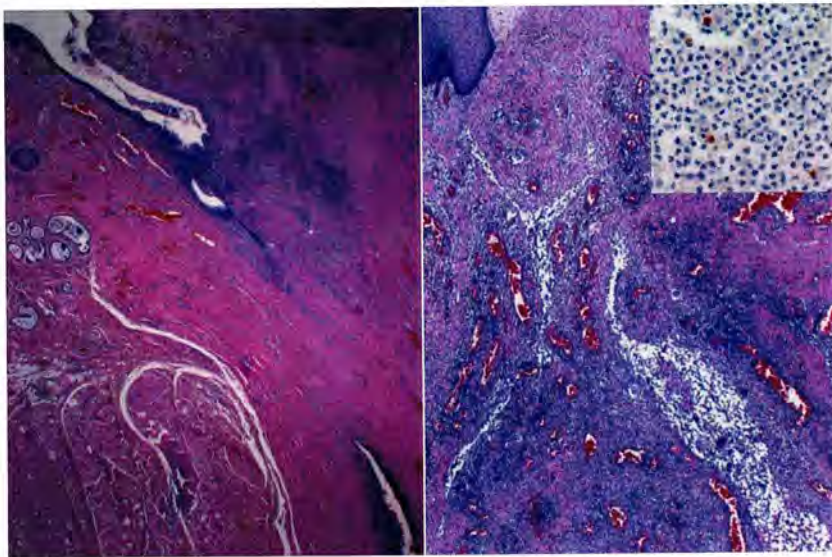


Figure II-E-1. Anal fistula. Formation of abscess and foreign body granuloma is observed along the fistula tract. Anal gland duct focally remains within the fistula tract. Anal fistula is caused by infection of enterobacteriae, especially *E. coli*, in the anal gland, and immunostaining demonstrates the bacterial antigens in the cytoplasm of phagocytes located along the fistula tract (left: H&E, right: immunostaining for *E. coli* antigens).

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2) Malacoplakia

Malacoplakia (malakoplakia) is an inflammatory (granulomatous) disorder, which makes its presence known as papules, soft plaques or ulceration. Urinary tract involvement is most common, but the other organ systems may also be affected, including the gastrointestinal tract, bone, lung, lymph node and skin. The disease occurs primarily in immunocompromised individuals. Cutaneous malacoplakia is very rare, usually seen in the vulva and perianal region. Yellow-pink indurated or polypoid masses are observed. The axilla, forehead and injection site may also be involved.

Malacoplakia is thought to result from the insufficient killing of bacteria by phagocytes, so that partially digested bacteria are observed within the macrophages. Microscopically, the malacoplakia lesion is characterized by dense accumulation of phagocytic foamy macrophages occasionally with intracytoplasmic Michaelis-Gutmann bodies, which represent round, concentric, laminar inclusion bodies, positively stained with PAS, Berlin blue and von Kossa stains because of deposition of iron and calcium. Causative bacteria belong to *Escherichia coli*, *Klebsiella pneumoniae* and other enterobacteriae, and immunostaining for *E. coli* or BCG antigens reveals granular positivity in the cytoplasm of macrophages. Michaelis-Gutmann bodies are also immunoreactive for the bacterial antigens (Figure II-E-2). When Michaelis-Gutmann bodies are not found, it is simply called xanthogranuloma. Ultrastructurally, phagocytized rods can be identified in lysosomes of macrophages.

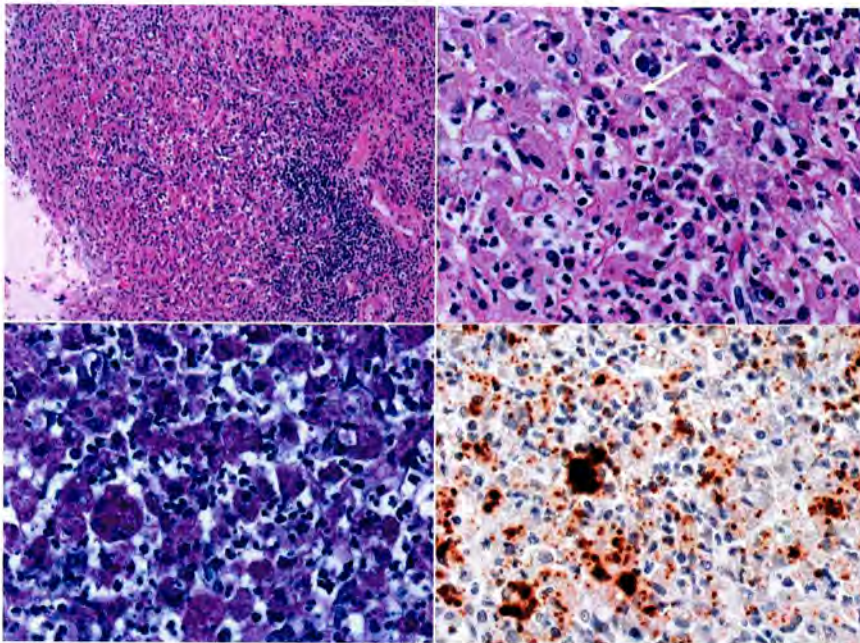


Figure II-E-2. Malacoplakia of the urinary bladder. Malacoplakia is a special type of polypoid xanthogranuloma (top panels: H&E). A concentric round and calcified cytoplasmic inclusion, Michaelis-Gutmann body, is indicated by an arrow. The xanthomatous macrophages are positive with PAS stain (left bottom), and strongly immunoreactive for lipopolysaccharide antigens of *E. coli*, demonstrated with a J5 monoclonal (right bottom).

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F) Corynebacterial Infection

1) Diphtheria

Diphtheria is caused by acute infection of *Corynebacterium diphtheriae*, a club-shaped and aflagellated Gram-positive rod (Coryne means a club in Greek). The bacilli proliferate on the pharynx, larynx, trachea and nasal mucosa to form thick and white pseudomembranes (Figure II-F-1). Diphtheria toxin production may lead to motor paralysis of the heart and striated muscles. Rarely, particularly in tropical areas, cutaneous diphtheria may cause punched-out ulceration with a defined but irregular margin. Ulcer base is covered with a gray slough. The bacilli tend to colonize the pre-existing skin lesion such as impetiginized eczema. Microscopic identification of bacteria in ulcer base necrotic debris is often difficult. Genital ulceration caused by *C. diphtheriae* may be seen.

Vaccination as a form of DPT (diphtheria, pertussis and tetanus) combination is effective to prevent this lethal infection. Droplet transmission should be avoided. Russian endemic of diphtheria after the recent evolutionary political change was a serious public health problem.

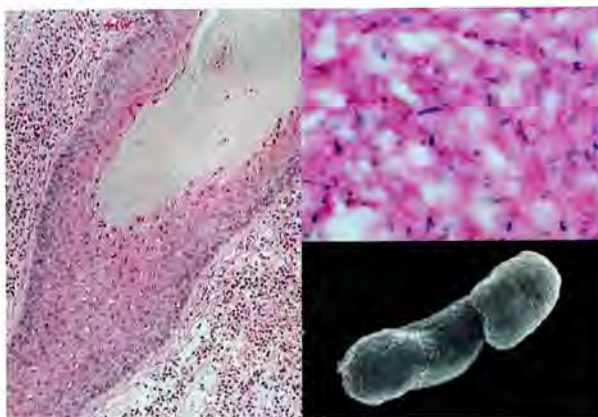


Figure II-F-1. Diphtheria on the throat of the aged. A thick pseudomembrane composed of finely basophilic filamentous material is formed on the pharyngeal squamous mucosa in this lethal male case (left: H&E). Within the pseudomembrane, cut surfaces of Gram-positive rods are identified (right top). Scanning electron microscopy shows a rod-shaped, non-flagellated bacterium, being consistent with *Corynebacterium* species (right bottom). The courtesy by Dr. Masafumi Ito, a pathologist at Japanese Red Cross Nagoya Daiichi Hospital, Nagoya, Japan.

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2) Conjunctivitis Caused by Diphtheroid Bacilli

Non-diphtherial *Corynebacterium* spp., a Gram-positive non-flagellated, club-shaped bacillus akin to *Mycobacterium* spp., is ubiquitous in nature and commonly colonize the skin, oral, pharyngeal, nasal and conjunctival mucosa and the gut of the human. *C. pseudodiphtheriticum* is nonpathogenic. *C. jeikeium* rarely causes septicemia and peritonitis. Recently, Jousen et al. reported unilateral conjunctivitis caused by diphtheroid bacillus, *Corynebacterium macginleyi*, which is exclusively isolated from the ocular surface. *C. macginleyi*-induced corneal ulcer has also been described. *C. macginleyi* is uniformly susceptible to topical antibiotics.

Figure II-F-2 illustrates normal flora on the conjunctiva and the histology of localized corynebacterial conjunctivitis.

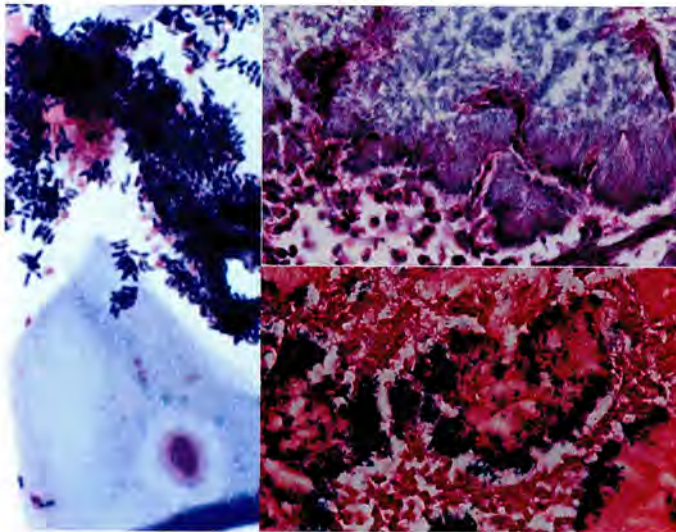


Figure II-F-2. *Corynebacterium* as a normal flora on the conjunctiva and diphtheroid bacillus-induced conjunctivitis. Non-pathogenic *Corynebacterium* is widely distributed as a normal flora on the skin and mucosa. Giemsa stain demonstrates rod-shaped bacteria in the swab specimen from the conjunctiva (left). Infrequently, diphtheroid bacilli colonize the conjunctiva to provoke localized conjunctivitis (right top: H&E, right bottom: Gram).

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3) Skin Lesions Caused by Diphtheroid Bacilli

Diphtheroid corynebacterial skin infection is called “corynebacterial triad”, as follows. It is of note that the ultraviolet light of a Wood's lamp (see Note 34) causes the organism to fluoresce a coral red color.

Pitted keratolysis presents multiple asymptomatic “moth-eaten” or crateriform pits and superficial erosions on the plantar surface of the foot, particularly in the pressure area (Figure II-F-3-1). Hot climate and hyperhidrosis are prodromal to this disorder. The pits are caused by keratolytic activity of *Corynebacterium* spp. (diphtheroid bacillus) such as *C. keratolyticum*. *Dermatophilus congolensis* or *Micrococcus sedentarius* may also be involved as the cause. Histologically, multiple crater-like defects are seen in the stratum corneum, and numerous Gram-positive rod-like, filamentous or coccoid organisms are observed (Figure II-F-3-2).

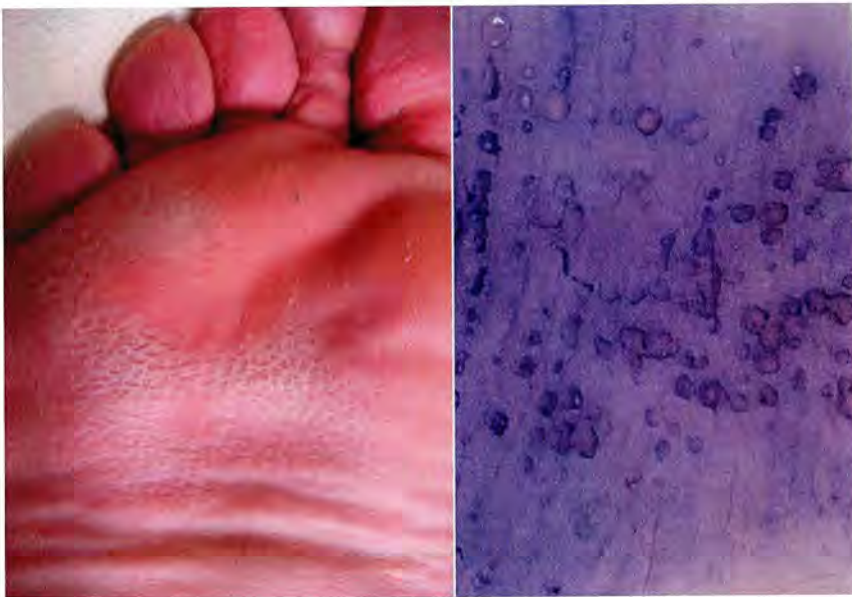


Figure II-F-3-1. Pitted keratolysis. Multiple “moth-eaten” or crateriform pits (superficial erosions) are seen on the pressure area on the sole (left). Dermatographic observation after Parker ink staining clearly demonstrates the demarcated surface defects (right). The courtesy of Dr. Haruo Ito at Ito Dermatology Clinic, Tokyo, Japan.

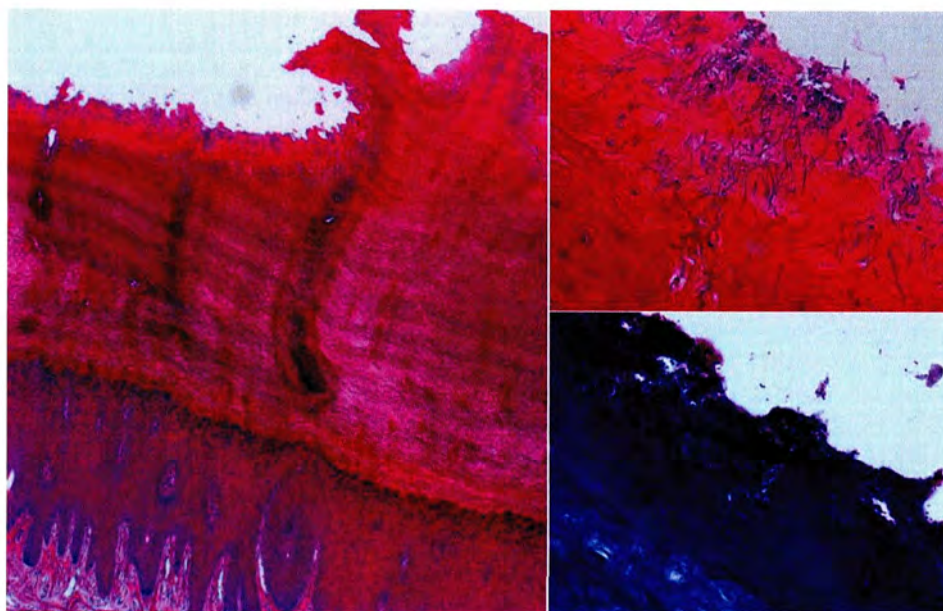


Figure II-F-3-2. Histology of pitted keratolysis. On the base of crater-like defect on the surface of the cornified layer, numerous rods are clustered (left and right top: H&E). In Gram-stained picture (right bottom), not only the rods but also the keratotic layer are stained blue. The courtesy of Dr. Junko Ishizaki, Department of Dermatology, Tokyo Women's Medical University East Medical Center, Tokyo, Japan.

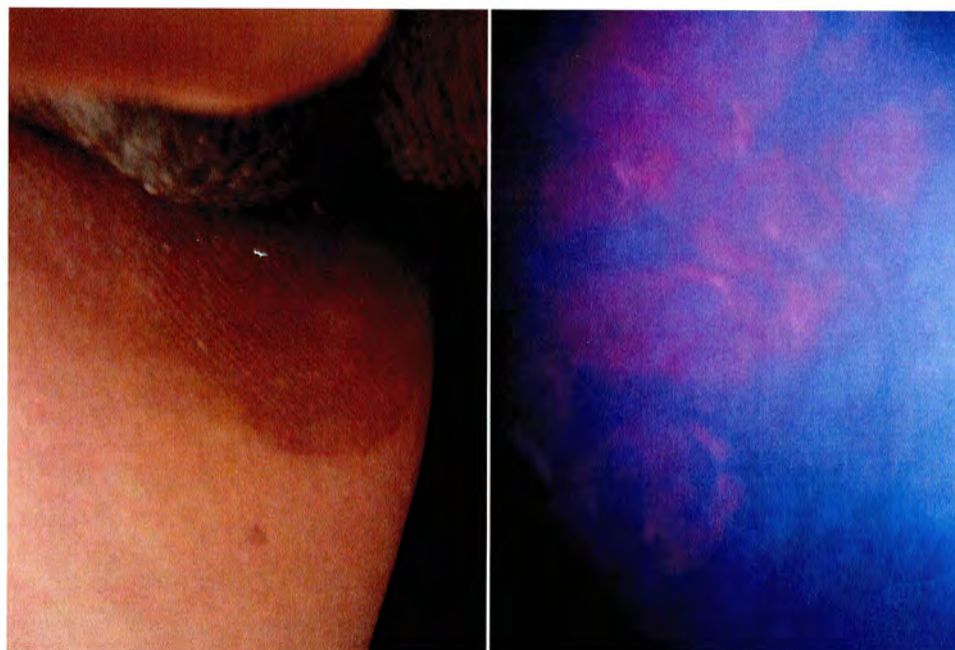


Figure II-F-3-3. Erythrasma. Tinea cruris-like but asymptomatic, demarcated reddish-brown scaling patch is presented (left). Wood lamp test shows a coral-red fluorescence (right). The courtesy of Dr. Hiroshi Tanabe at Department of Dermatology, Hyogo Prefectural Tsukaguchi Hospital, Amagasaki, Hyogo, Japan.

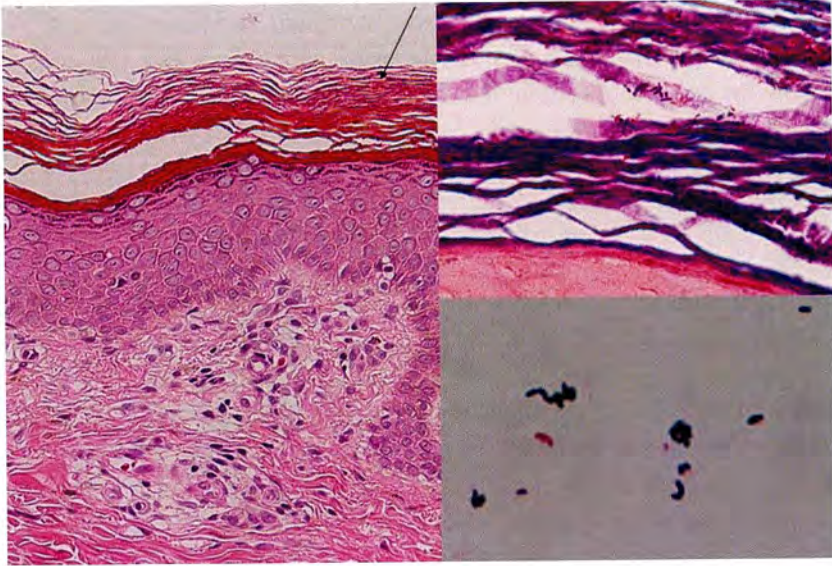


Figure II-F-3-4. Histology of erythrasma. The cornified layer focally appears to be compact (arrow), and dermal inflammation is minimal (left: H&E). Gram stain demonstrates clustered Gram-positive rods in the involved cornified layer (right top). The Gram-positive rods are also identified in a surface-scraped specimen (right bottom). The courtesy of Dr. Hiroshi, Tanabe, Amagasaki.

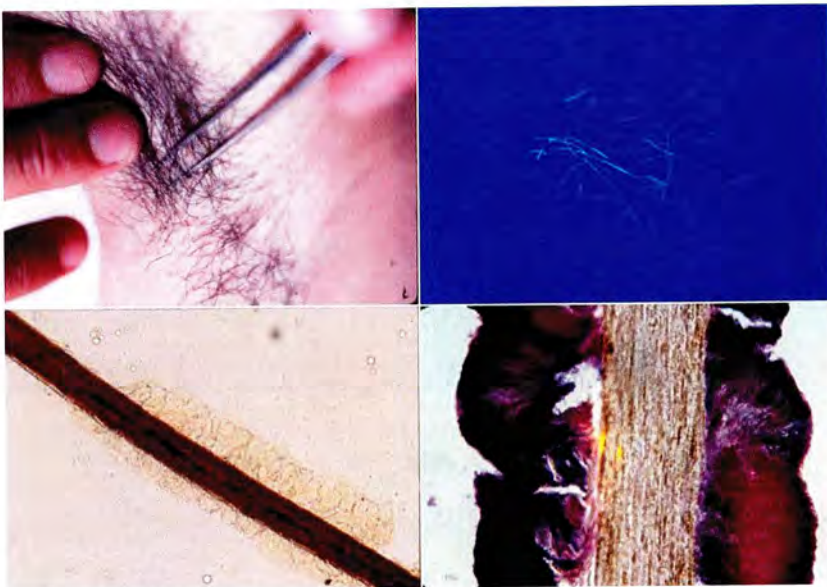


Figure II-F-3-5. Trichomycosis. The axillary hair is involved (left top). Wood lamp test gives fluorescence (right top). Pale yellow bacterial colonies, 1-2 mm in size, attach to the hair shaft (left bottom). Gram stain reveals clusters of Gram-positive rods on the hair shaft (right bottom). The courtesy of Dr. Hiromichi Takama at Takama Dermatology Clinic, Kasugai, Aichi, Japan.

Pitted keratolysis one of the causes of foot malodor, presumed to be due to production of sulfur-compound by-products, such as thiols, sulfides, and thioesters. Foot malodor without pitted skin change is caused by overgrowth of *Bacillus subtilis* and specifically an isovaleric acid produced by *Staphylococcus epidermidis* overgrowth.

Erythrasma, caused by infection of *Corynebacterium minutissimum*, presents as well-defined, reddish-brown scaling patches on the skin fold, particularly the inner side of the thigh just below the crural fold (Figure II-F-3-3). It clinically resembles fungus-negative tinea cruris (ring worm), but is asymptomatic. Erythrasma is often seen in obese or diabetic patients. Wood's ultraviolet lamp examination reveals a characteristic coral-red fluorescence. Histological evaluation often reveals minimal inflammation in the dermis, hence erythrasma being categorized as an "invisible dermatosis". Gram and PAS stains demonstrate small coccobacilli clustered in the superficial part of the stratum corneum (Figure II-F-3-4).

Trichomycosis is a bacterial infection of axillary hair (trichomycosis axillaris). Pubic hair may also be involved. The main causative microorganism is Gram-positive diphtheroid *Corynebacterium* spp., particularly *Corynebacterium tenuis*. Large bacterial colonies, 1-2 mm in size, attach to the hair shaft to form pale yellow, red or black-colored concretions (Figure II-F-3-5). Malodor is common. The bacteria are embedded in concentrated matrix, probably derived from apocrine sweat substances.

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4) Granulomatous Mastitis

Idiopathic granulomatous mastitis is defined as granulomatous inflammation of the breast without any other attributable cause such as tuberculosis, sarcoidosis and Wegener's granulomatosis. Females up to 6 years after pregnancy, age range: 17-42 years, suffer from this disorder. Patients present with a hard lump in unilateral breast, and nipple retraction, pain, inflammation of the overlying skin, nipple discharge and fistula formation may be associated. Distinction from breast cancer is needed. Histologically, epithelioid histiocytes with multinucleated giant cells forming non-caseating granulomas are seen around the lobules. Autoimmune etiology as an IgG4-related disorder has been described.

Recently, infectious etiology for granulomatous mastitis has been proposed. Normal lipophilic skin flora, *Corynebacterium kroppenstedtii*, has been isolated from the lesion. Microscopically, Gram-positive bacilli are identified in small empty spaces, consistent with dissolved lipid, and are surrounded by suppurative granuloma (Figure II-F-4). Subcutaneous fat tissue may also be involved. The infective cases tend to manifest fever and neutrophilia and often form fistula tract, while these clinical features and suppurative granulomas are uncommon in autoimmune granulomatous mastitis. Renshaw et al. have proposed the term “cystic neutrophilic granulomatous mastitis” for the corynebacterial lesions.

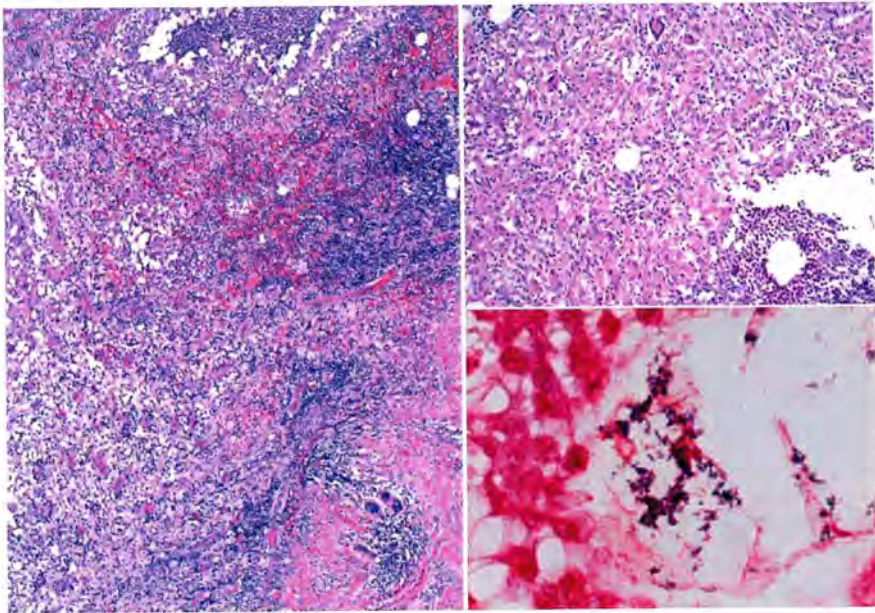


Figure II-F-4. Granulomatous mastitis in a Japanese female aged 30's after delivery. Formation of suppurative granulomas (epithelioid granuloma surrounding abscess cavity) with lymphocytic cuffing is evident in the breast tissue. Langhans-type giant cells are scattered (left and right top: H&E). In an empty space, probably representing dissolved lipid, Gram-positive rods are clustered (right bottom: Gram). Neutrophils surround the bacteria.

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G) Dermatophilosis

Dermatophilus congolensis is a facultative anaerobic Gram-positive bacterium akin to *Actinomyces*, causing a zoonotic skin disease, dermatophilosis. The affected skin shows exudative dermatitis, followed by the formation of crusty scabs rich in organisms. Cattle, horses, pigs, sheep and goats are commonly affected. Orangutan, turtle and lizard are also infected. Humans get this skin disorder after a close contact with the infected animals in poorly hygienic countries, though cosmopolitan in distribution. Horseback riding is one of the risk factors for papule/pustule formation in the medial thigh. *D. congolensis* may cause pitted keratolysis on the sole. In animals, hair is matted by the exudate, and after spontaneous healing, patches of alopecia remain. Epidemics occur during the rainy season.

Microscopically, branching filamentous hyphae ultimately fragment into packets of coccoid cells (chains of flattened organisms), and the coccoid cells mature into flagellated ovoid zoospores, 0.6-1 μm in size. An animal skin lesion is shown in Figure II-G-1, and *D. congolensis*-induced pitted keratolysis in the human is seen in Figure II-G-2. Characteristically, the division of the filaments occurs in both transverse and longitudinal planes. The microscopic features resemble fungal infection, so that this infection has erroneously been categorized in superficial mycosis.

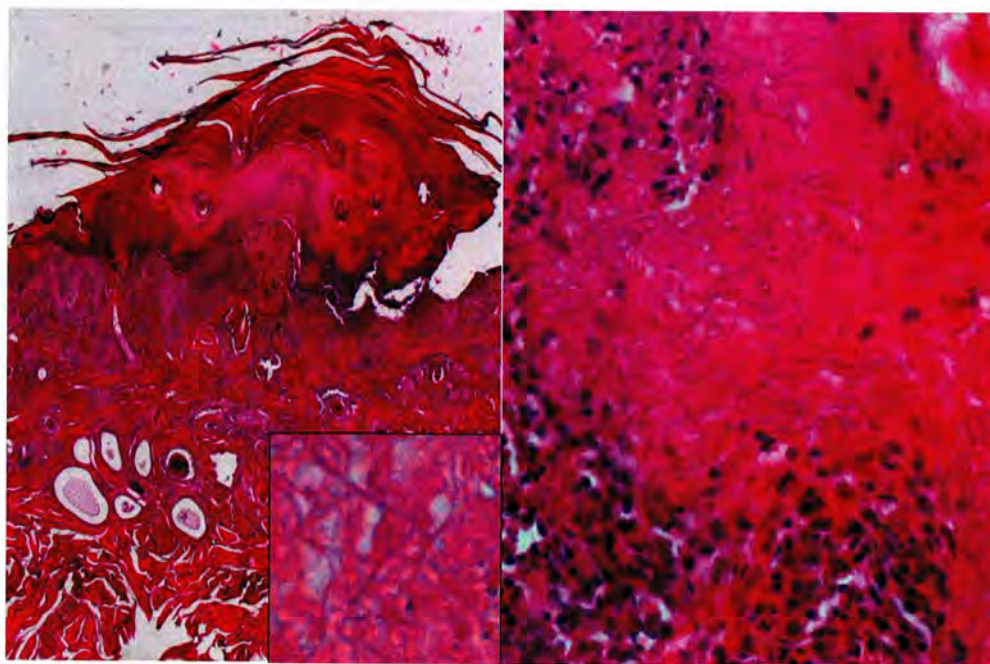


Figure II-G-1. Dermatophilosis in cattle. Extensive verrucous skin lesions are observed (left: H&E). In the hyperkeratotic layer, long filamentous branched chains of the bacteria are densely infected (right: H&E). Oil immersion picture is shown in the inset. The courtesy by Ms. Makiko Goto at National Institute of Animal Health, North Iwate, Japan.

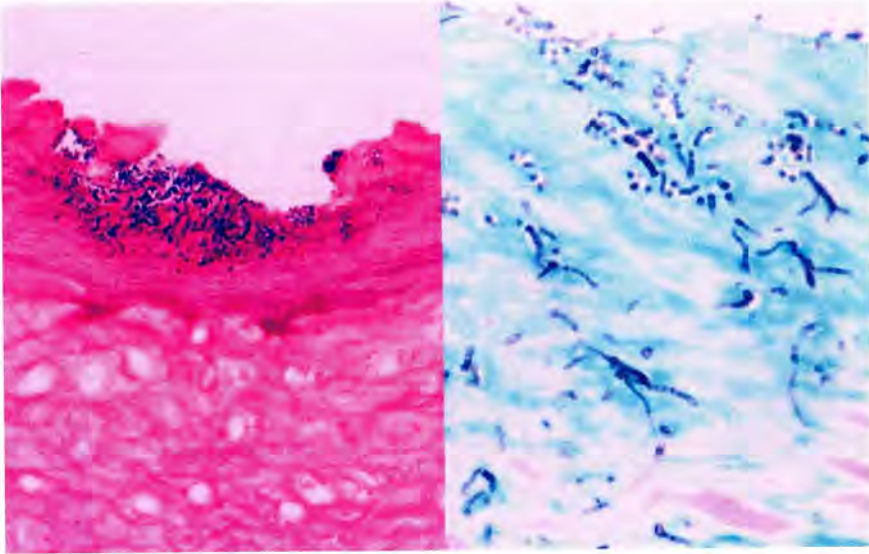


Figure II-G-2. Pitted keratolysis caused by *Dermatophilus congolensis* infection. In this case, the pit on the sole is infected by Gram-positive microbes characteristically showing branching filamentous hyphae composed of chains and packets of coccoid cells (left: H&E, right: Grocott). The courtesy of Dr. Hiromichi Takama at Takama Dermatology Clinic, Kasugai, Aichi, Japan.

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H) Neisserial Infection

Neisseria gonorrhoeae commonly causes sexually transmitted infection, purulent urethritis (gonorrhea, the clap), in the male (Figure II-H-1), while approximately 80% of infected women are asymptomatic. Smear preparation of the pus discloses Gram-negative diplococci phagocytized by neutrophils. Oral sex-mediated gonococcal pharyngitis in the female is also frequently asymptomatic, and becomes a potential source of sexual transmission. Infection is repetitive, because no immunity is obtained. Recently, plasmid-mediated β -lactamase production, as well as chromosome-mediated penicillin and tetracycline resistance, by *N. gonorrhoeae* became problematic in the treatment of gonorrhea.

Primary gonococcal infection on the extragenital skin is rarely seen as a pustular lesion of the finger. Though very rare, disseminated gonococcal infection (chronic gonococcal septicemia) with female preponderance may be seen in two forms: arthritis-dermatitis syndrome and localized septic (suppurative) arthritis. The former presents classic trias:

dermatitis, multiple tenosynovitis on the hands and migrating polyarthrits of the large joints. The “dermatitis” represents septic vasculitis, resulting in painless, non-pruritic, vesicopustular or hemorrhagic lesions.



Figure II-H-1. Gonorrhea. Purulent exudates are observed at the urethral orifice. The glans penis is red-swollen (left). Microscopically, diplococci are phagocytized by neutrophils (right: Giemsa). In the urology outpatient clinics, Giemsa or methylene blue stains are commonly performed, instead of Gram stain.

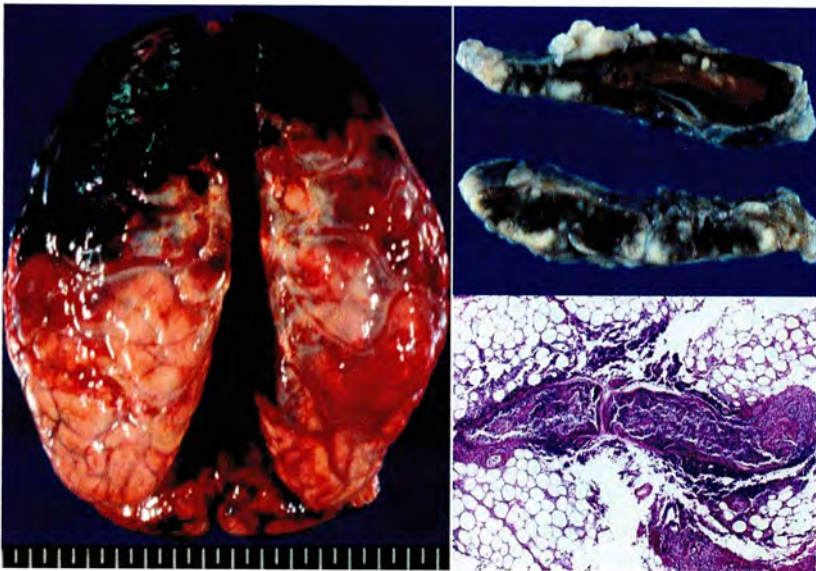


Figure II-H-2. Waterhouse-Friederichsen syndrome in a young Japanese female. Fulminant meningococcal septicemia with disseminated intravascular coagulopathy is featured by purulent and hemorrhagic meningitis (left) and bilateral adrenal hemorrhage (right top). Arterioles in the subcutaneous tissue microscopically show septic embolization (right bottom, H&E).

Arterioles and venules in both the deep and upper dermis show neutrophilic vasculitis with fibrin thrombosis. Localized septic arthritis involves a large joint, particularly the knee. Interestingly enough, the Greek word root “gono” means both the sexual organs (gonad) and the knee.

Similarly, acute septicemia caused by *Neisseria meningitidis* commonly accompanies hemorrhagic skin/subcutaneous lesions (septic vasculitis), with a predilection for the extremities and pressure sites. There are erythematous macules, nodules, plaques and petechiae, surrounded by small pustules. Fulminant meningococcal septicemia is featured by Waterhouse-Friederichsen syndrome, in which acute purulent and hemorrhagic meningitis, bilateral adrenal hemorrhage (acute adrenal insufficiency) and disseminated intravascular coagulation are noted (Figure II-H-2).

In Waterhouse-Friederichsen syndrome, petechial hemorrhage is seen on the skin and mucosa. The chronic septicemic form, “dermatitis-arthritis syndrome”, is also encountered in *N. meningitidis* infection, in addition to infective endocarditis.

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Note 14. Gonorrhea as a seasonal disorder

In Japan, urologists say that gonorrhea is a seasonal disease. In many Japanese companies, bonus salary is given to the employees twice a year in July and December. The incidence of oral sex-mediated gonorrhea is peaked in August and January, since many young guys with temporary money in the pocket visit the special places where professional ladies, probable carriers of *Gonococcus* on the throat, give them special oral sex services.

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I) Rhinoscleroma

Rhinoscleroma is caused by infection of *Klebsiella pneumoniae*, type C or *Klebsiella rhinoscleromatis*, endemic in Central America, Africa and Asia. In addition to the nasal mucosa, pharynx, larynx and perinasal/perioral skin are also involved. Infiltrative and nodular lesions may result in nasal obstruction and deformity. Sclerosing granuloma is infiltrated by both foamy macrophages and Russell body-forming plasma cells. PAS-positive and Gram-negative eosinophilic globules, 2-3 μm in size, are seen in the cytoplasm of the foamy macrophages, and these cells are nicknamed as “Mikulicz cells” (Figure II-I). Warthin-Starry silver is suitable for visualizing the intracellular microbes.

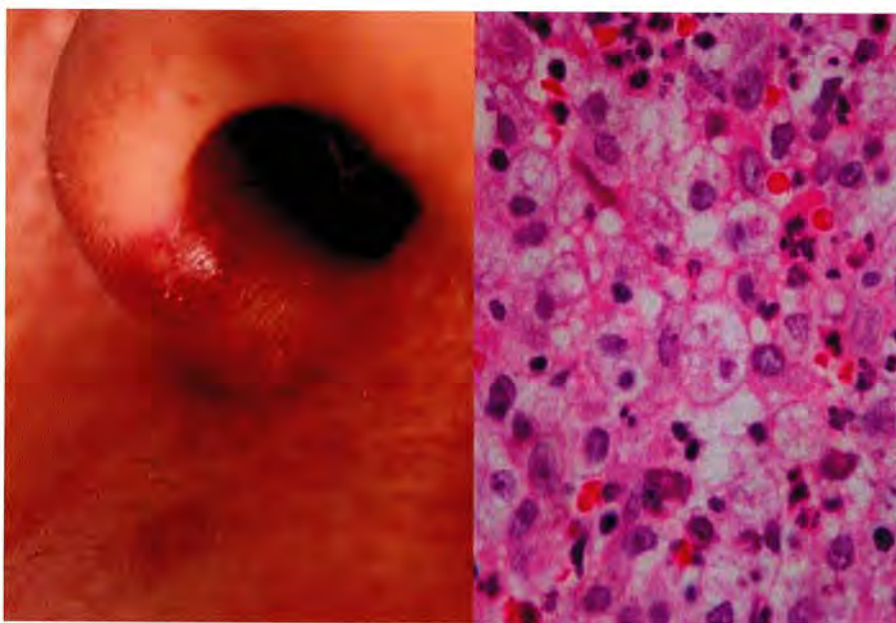


Figure II-I. Rhinoscleroma. The skin of nostril shows a red-colored nodular lesion (left). Biopsy reveals xanthogranulomatous reaction with infiltration of foamy macrophages, lymphocytes, plasma cells and neutrophils. Eosinophilic globules, 2-3 μm in size, are observed in the cytoplasm of the macrophages (so-called Mikulicz cells) (right: H&E). The courtesy by Dr. Symez Lucinda Tan, National Skin Centre, Singapore.

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J) Granuloma Inguinale (Donovanosis)

Granuloma inguinale, or donovanosis, is a sexually transmitted infection caused by *Calymmatobacterium granulomatis*, a Gram-negative bacillus resembling *Klebsiella*. The disease is different from lymphogranuloma venereum caused by *Chlamydia trachomatis* (serotypes L1-L3). Painless hemorrhagic ulcer is formed in the external genitalia (Figure II-J-1), groin, and perianal or perioral skin, and spreads gradually to involve adjacent parts (thigh and lower abdomen). Penis or vulva may be destroyed extensively. Histologically, foamy macrophages and plasma cells are distributed adjacent to microabscesses.



Figure II-J-1. Granuloma inguinale. In Papua New Guinea people, painless hard ulcers are seen on the penis (left) and the vulva (right). The courtesy by Late Prof. Robin A. Cooke, Mayne Medical School, University of Queensland and Pathology Queensland, Brisbane, Australia.

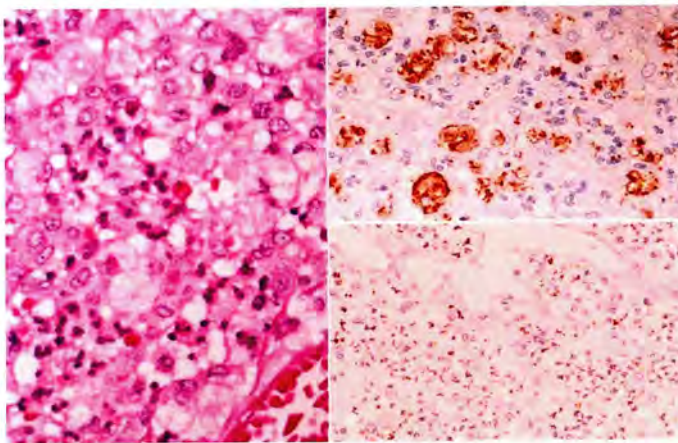


Figure II-J-2. Histology of granuloma inguinale. Foamy macrophages are clustered together with plasma cells and neutrophils. Donovan bodies, representing 1-2 μm -sized globular inclusions, are seen in the cytoplasm of foamy macrophages (H&E). With immunostaining using monoclonal antibodies, the cytoplasm of the foamy cells is positively stained for lipopolysaccharide antigen (J5) common to the Gram-negative bacteria (right top) and *Klebsiella pneumoniae* antigen (right bottom). The courtesy by Late Prof. Robin A. Cooke, Australia (providing us with unstained slides).

Donovan bodies, representing 1-2 μm -sized small globular inclusions, are seen in the cytoplasm of foamy macrophages (Figure II-J-2). Giemsa stain on imprint cytology preparations is helpful to visualize the microbes. Warthin-Starry's silver is used for demonstrating the microbes in paraffin sections. More specifically, lipopolysaccharide common to the Gram-negative bacteria and *Klebsiella*-related antigens are immunostained in their cytoplasm.

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K) Chancroid

Chancroid (soft chancre) is one of the classical sexually transmitted infections caused by Gram-negative small-sized bacilli, *Haemophilus ducreyi*. Painful, irregular-shaped and non-indurated ulceration is formed in the genital area (Figure II-K). The ulcer is single or multifocal. Groin nodal swelling may be associated. Gram-negative rods forming parallel chains (school of fish appearance) are seen on smear preparations. Chancroid is a major cause of genital ulceration in Africa. The incidence is low in western countries, including Japan. Biopsy specimen reveals nonspecific changes such as vascular-rich granulation tissue reaction at the base of ulcer and reactive acanthosis of the surrounding epidermis.

A non-STI form *H. ducreyi* infection on the skin has also been reported from New Zealand and Australia. Chronic cutaneous ulcers are seen on the lower limbs of children.

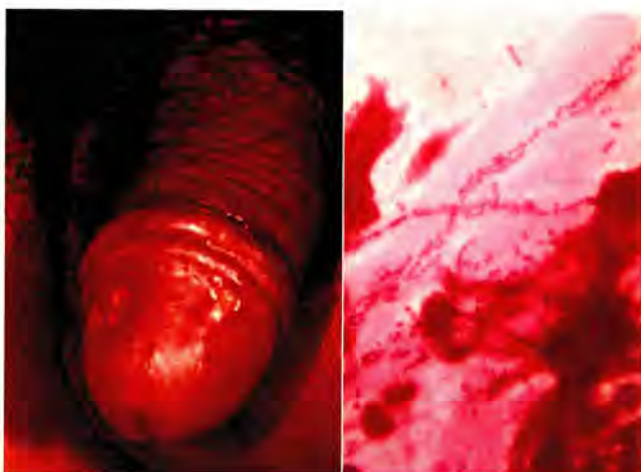


Figure II-K. Chancroid. The glans penis shows multiple and painful shallow ulceration (left). Gram-stained smear preparation reveals school of fish appearance by Gram-negative coccobacilli in the neutrophilic background (right).

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L) Chlamydial Infection and Lymphogranuloma Venereum

Three kinds of *Chlamydia*, an obligatory intracellular microorganism, are known to be pathogenic to the human, including *C. trachomatis*, *C. psittaci* and *C. pneumoniae*. The latter two accompany pulmonary infection. *C. psittaci* is known to be transmitted from the feces of psittacines (parrots) by an oral route and replicates in the liver and spleen to reach the lung by hematogenous dissemination. Psittacosis may accompany morbilliform rash or skin lesions resembling the rose spots of typhoid fever. The association of erythema multiforme or erythema nodosum has also been reported.

C. trachomatis causes chronic conjunctivitis ("trachoma") (serotypes A-C), sexually transmitted infection (STI) of genital organs (serotypes D-K), and lymphogranuloma venereum (serotypes L1-L3). In the cytoplasm of host epithelial cells, both reticulate bodies (the replicative form) and elementary bodies (the infective form) are observed (Figure II-L-1). Chlamydiasis is one of the most common STIs, occasionally resulting in female infertility and abortion. The fallopian tube is markedly swollen, resulting in "sausage-like appearance". In males, persistent chlamydial infection causes prostatitis and epididymitis (refer to Figures I-D-1 and I-D-2). The infection, including chlamydial oropharyngitis, is often asymptomatic in the female, and this makes the reason for its high prevalence. The importance of cytological diagnosis should be emphasized for detecting and preventing chlamydial infection. The cytoplasm of the affected epithelial cells contains pathognomonic "nebular inclusion bodies", in which chlamydial antigens are localized immunocytochemically (Figure II-L-2). When only one cytological preparation is available, a re-staining method can be applied to the same specimen after bleaching the dyes for Papanicolaou staining in acid alcohol overnight. Recently, acute conjunctivitis as a form of STI (serotypes D-K) is increasing among young male. Chlamydial cytoplasmic inclusions are demonstrated in a Giemsa-stained preparation sampled in ophthalmology outpatient clinics (Figure II-L-3). In both sexes, chlamydial proctitis with active lymphoid follicle formation is provoked by anal sex.

Lymphogranuloma venereum, also known as the fourth venereal disease, is an STI caused by *C. trachomatis*, serotypes L1-L3. The disease is mainly seen in tropical and subtropical areas, being rare in the developed countries, including Japan, but may cause

troublesome proctitis in homosexual men. Three clinical stages are known. The first stage, showing small ulceration on the external genitalia, is transient and often imperceptible. In the second stage several weeks after initial exposure, groin lymphadenopathy (bubo) is often accompanied by draining sinuses (Figure II-L-4). Histologically, formation of suppurative granuloma (stellate abscesses surrounded by epithelioid cells) is characteristic. At the tertiary stage, the patient complains of rectal stricture, fistule formation and genital lymphedema.

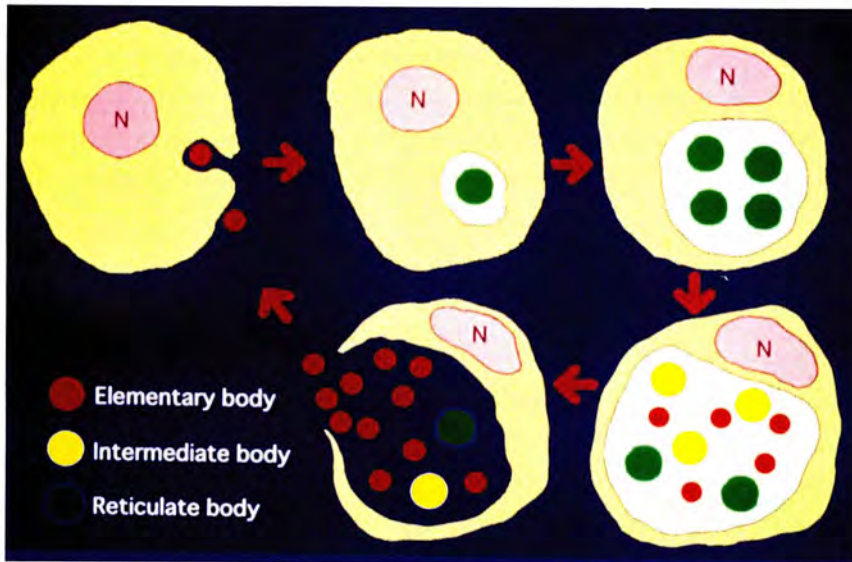


Figure II-L-1. Schematic illustration of the life cycle of *Chlamydia*. The infective form (the elementary body) infects the target cells to form a cytoplasmic inclusion, in which the proliferative form (the reticulate body) increases to transform into the infective form. See also Figure I-D-2.

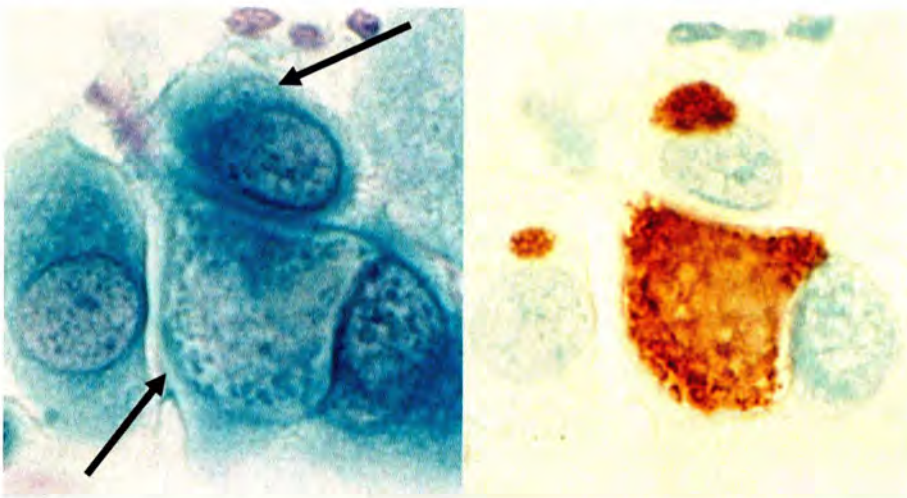


Figure II-L-2. Cytopathology of chlamydiasis on a uterine cervical smear. Papanicolaou-stained preparation demonstrates “nebulous inclusion bodies” (arrows) in the cytoplasm of the infected columnar or metaplastic epithelial cells (left). The re-staining method clearly visualizes chlamydial antigens in the same inclusion bodies. It is difficult for cytopathologists to recognize small inclusions on the pap smear.

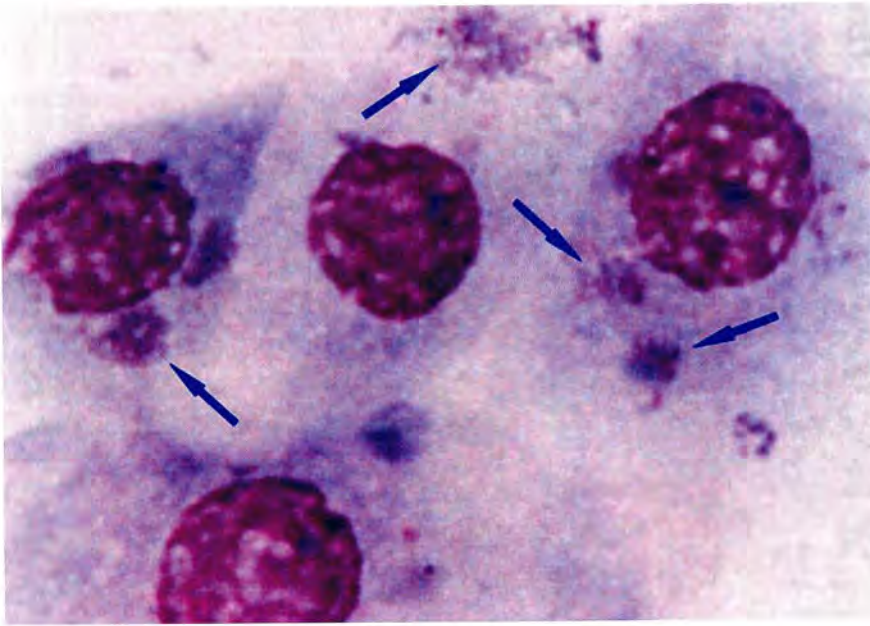


Figure II-L-3. Cytodiagnosis of chlamydial conjunctivitis. Cytoplasmic inclusions (arrows) are easily observed on the conjunctival smear preparation (Giemsa). This acute form, representing sexually transmitted infection, should be distinguished from a chronic and classic epidemic form, trachoma.

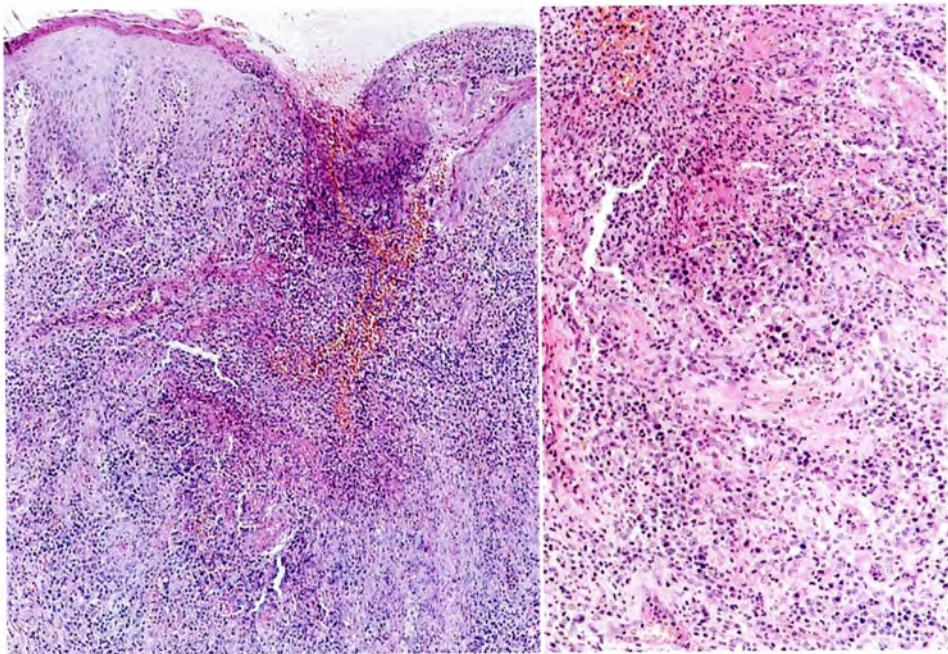


Figure II-L-4. Lymphogranuloma venereum. Draining sinus formation on the swollen groin node is noted in a skin biopsy specimen. Nonspecific active inflammation with vascular-rich granulation tissue is observed. Here, epithelioid granulomatous reaction is scarcely discerned in the dermis (H&E, left: low power, right: high power).

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M) Pasteurellosis

Pasteurellosis is a zoonotic infection of *Pasteurella multocida*, a small facultative anaerobic Gram-negative bacillus. In 1880, Louis Pasteur isolated *P. aviseptica*, causing chicken cholera. *P. multocida* causes lethal infection in various animals, including cow and chicken, and often colonizes the oral cavity and the upper airway of the dog, cat, rodents and birds. It should thus be emphasized that this microorganism is transmissible from the pet animals. Human pasteurellosis is categorized into three forms: a) cutaneous infection at the site of animal (dog or cat) scratch or bite, b) respiratory (nasal, paranasal and lung) infection, and c) systemic infection (septicemia, meningitis or peritonitis). *Pasteurella* infection may cause Ludwig's angina (refer to Chapter II-C-6). Reportedly, acute pasteurellosis of the paranasal cavity occurred in a male patient, who daily gave food with his mouth for his lovely pet dog. In the case presented here, a cat bite soon caused painful local ulceration on the forearm, in 3 days developing to phlegmonous inflammation in the entire forearm (Figure II-M-1). Leukocytosis was associated, and axillary lymph nodes were swollen. The bacteria were cultured from the cat claw. Skin biopsy revealed that in the intradermal abscess lesion, small rods were demonstrated with Warthin-Starry's silver (Figure II-M-2).

Of note is that in cat scratch disease (bartonellosis), no purulent skin infection occurs, and the clinical course is much milder and prolonged without leukocytosis. In two weeks, axillary nodes are enlarged to form suppurative granuloma microscopically (refer to Chapter II, DD-1).



Figure II-M-1. Pasteurellosis. Red swelling due to phlegmonous inflammation is observed in the entire forearm (left). In the house, the patient happened to have a cat bite 3 days earlier. Cat bitemarks on the forearm are painful and ulcerated (right). The courtesy by Prof. Kayoko Matsunaga, Department of Dermatology, Fujita Health University School of Medicine, Toyoake, Japan.

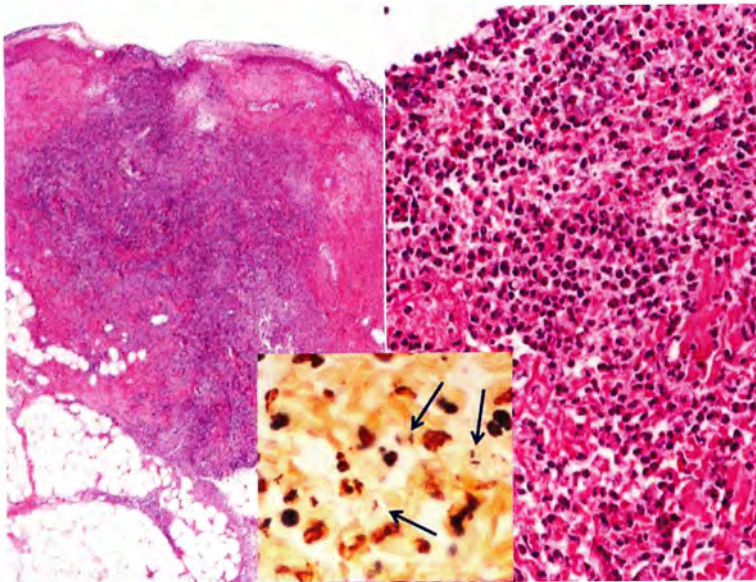


Figure II-M-2. Histology of pasteurellosis. At the site of ulceration, intradermal abscess formation is observed (H&E, left: low power, right: high power). Warthin-Starry's silver demonstrates black-stained rod-shaped pathogens in the cytoplasm of neutrophils (inset).

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Note 15. Dr. Shibasaburo Kitasato and *Yersinia pestis*

The plague resulted in frequent pandemics in Europe in the medieval period, and was called as "black death disease". Up to one third of the population was killed by the miasma-like pathogens, actually transmitted by rats. Flea was the reservoir of the microbes. Nodal plague involving the lymph nodes is most common, and highly lethal. Lung plague is the severest form. Skin plague is presented as an abscess or ulcer at the site of flea bite. The name of the pathogen was once called as *Pasteurella pestis*. Now it is classified as *Yersinia pestis*.

In June, 1894, Dr. Shibasaburo Kitasato, one of the most famous bacteriologists in Japan, stayed in Hong Kong. As a member of the Japanese medical team for clarifying the etiological agent of locally endemic plague, he tried to culture and separate the pathogen of black death disease. In 2 weeks, a total of 12 autopsies were performed by the clinician/pathologist Dr. Sanemichi Aoyama, Imperial Tokyo University. Dr. Aoyama contracted plague for 3 weeks eventually to recover, whereas his assistants died of plague as an occupational infection. Meanwhile, Dr. Kitasato for the first time succeeded to identify the bacteria now termed as *Yersinia pestis*. Dr. Alexandre Emil Jean Yersin, a French bacteriologist, was also there in this small island in the southern part of China to be the second person cultivating the microbe. The August issue of the journal *Lancet* reported a rapid communication on Dr Kitasato's success. To his and our regret, he erroneously described the pathogen was Gram-positive.

During his 79-years life, Dr. Kitasato lost two big internationally honorable chances. One is the name of the plague bacillus "*Kitasatella pestis*". Another is the very first Nobel prize of medicine and physiology in 1901, when Dr. Emil Adolf von Behring, his research colleague, got it by his discovery of diphtheria toxoid in Dr. Robert Koch's laboratory. The real originality belonged to Dr. Kitasato who had already succeeded to make a toxoid of tetanus in the same laboratory. At least, both Kitasato and Behring must have got the prize!

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N) *Capnocytophaga* Septicemia

Capnocytophaga canimorsus, a slow-growing commensal Gram-negative bacillus, is found in the oral cavity of normal healthy dogs and cats. Dog bites or cat bites may cause fatal septicemia of this microbe. No suppurative lesion is seen in the bite wound on the extremities. The infection mainly occurs in immunocompromised individuals, alcoholics, patients with chronic respiratory disease or those who have a history of splenectomy, but normal individuals may also be involved. The incubation period is as long as 8 days. Early signs include lethargy and restlessness, rapidly progressing to fever, rigors, embolic events and eventual multi-organ failure. Inflammatory response in the internal organs is poor.

Rods are identified in the cytoplasm of neutrophils on peripheral blood smear preparations (Figure II-N-1). An important message is that domestic pets carry potentially lethal bacteria. Representative features of the kidney at autopsy are shown in Figure II-N-2.

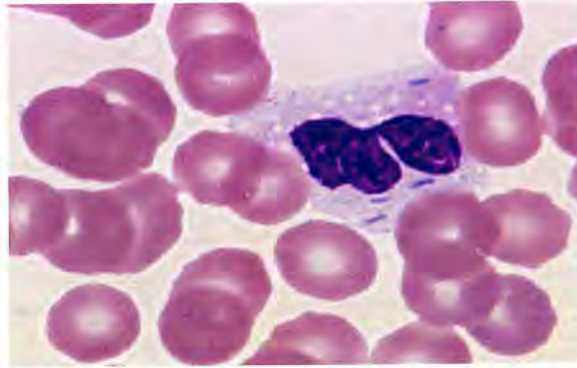


Figure II-N-1. Peripheral blood in *Capnocytophaga canimorsus* septicemia. The neutrophil contains bacillary bacteria in the cytoplasm. The courtesy by Dr. Takeshi Oya, a pathologist at Niigata Prefectural Central Hospital, Joetsu, Japan.

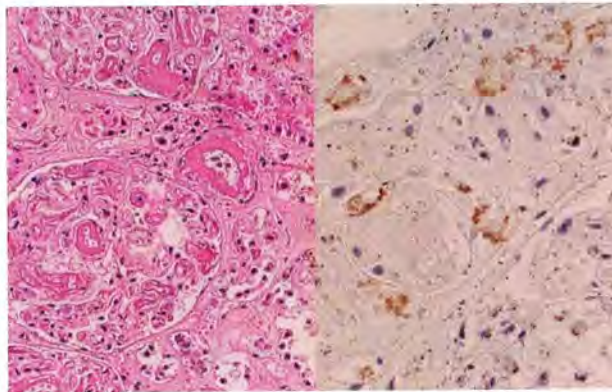


Figure II-N-2. Autopsied kidney in *Capnocytophaga canimorsus* septicemia. The kidney shows bilateral cortical necrosis with fibrinous thrombosis in glomerular capillaries, indicating the association of disseminated intravascular coagulopathy (left: H&E). Immunostaining using a rabbit antiserum against *Escherichia coli* antigens (right) demonstrates the stasis of numerous pathogens in the capillary vessels. The antiserum, widely cross-reactive with Gram-negative bacteria, is useful to show the distribution of Gram-negative pathogens in paraffin sections. The courtesy by Dr. Takeshi Oya, Joetsu.

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Note 16. Animal bites

The vast majority of animal bites involve dogs, followed by cats. Infectious complications occur in 15-20% of dog bites and more than 50% of cat bites. Dog bites are associated with younger animals. Cat bites, usually provoked by female cats, occur on the upper extremities or face. Sharp and long teeth of cats easily penetrate the human skin and create a deep puncture wound. Approximately 10% of animal bites need medical cares, and 1-2% require hospitalization.

Representative infections by animal bites, particularly by dogs and cats, include pasteurellosis, *Capnocytophaga* infection and cat scratch disease. See Chapter II-M, N and DD, respectively. Fungal diseases such as sporotrichosis, chromomycosis, mucormycosis and North American blastomycosis may be transmitted by animal bites. Transmission of rabies should be avoided. Bites from nonimmunized domestic animals and wild animals have the risk of rabies. Rabies is more common in bats, raccoons, skunks and foxes than in cats and dogs. Rabbits, squirrels and other rodents rarely carry rabies. Another risk of animal bites includes tetanus.

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O) Human Bite

Approximately 10-15% of human bite wounds become infected. Patients frequently come to the clinic days to weeks after injury, when infection is well established and medical attention is unavoidable. Young males tend to be the victim. The injuries most frequently occur on the hands and arms (Figure II-O). Soft tissue infection is easily established when the finger is bitten, where the tendons are in close proximity to the skin. The other sites of injury include head and neck region (particularly the ear, nose and lip), breast, genitals and thigh. Bite infections of the ear are difficult to be treated, since ear cartilage is poorly vascularized.

Serious complications include infectious tenosynovitis and arthritis, osteomyelitis and even necrotizing fasciitis.

The saliva contains as many as 100 million organisms per milliliter. Inoculated pathogens in human bite wound belong to the oral flora. These include *Streptococci* and *Staphylococcus aureus*, as well as obligate anaerobic bacteria such as *Eikenella*, *Fusobacterium*, *Prevotella*, *Peptostreptococcus* and *Porphyromonas* spp. Talan et al. analyzed 50 patients with infected human bites, all the lesions showed mixed infection with the median number of isolates per wound culture being 4. Note that *Fusobacterium* and *Prevotella* cause noma (gangrenous stomatitis: refer to Chapter II-C-6) and tropical ulcer (refer to Note 10).

Viral pathogens, including hepatitis B and C, human immunodeficiency virus and herpes simplex virus, are transmissible by human bites. Human bite transmission of syphilis, actinomycosis and tetanus has also been described.



Figure II-O. Bitemark on the arm caused by a human bite. In this forensic pathology case, the degree of injury was mild, and no infection followed. The courtesy by Dr. Fumiko Satoh, Department of Forensic Medicine, Tokai University School of Medicine, Isehara, Japan.

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P) Tetanus

Tetanus (meaning: to stretch in Greek) is an infection of *Clostridium tetani*, a spore-forming Gram-positive obligate anaerobic bacillus. A neurotoxin, termed tetanospasmin, provokes prolonged contraction of skeletal muscles. The bacteria live in soil and animal feces. Tetanus occurs worldwide, but is common in tropical areas with soil rich in organic matter. Tetanus clinically begins with mild spasms in the jaw muscles (lockjaw). The spasms extend to generalized muscles, and back muscle spasms cause arching (opisthotonos). Other symptoms include drooling, excessive sweating, irritability, swallowing difficulty and uncontrolled urination or defecation. The incubation period is usually about 8 days, but it may be up to several months. The shorter the incubation period, the more severe the symptoms. In neonatal tetanus infected via the contaminated umbilical cord, symptoms usually appear from 4 to 14 days after birth. Infection can be prevented by proper immunization (DPT vaccination) and also by post-exposure prophylaxis.

Infection generally occurs through skin wound contamination from a deep puncture wound on the extremities. To achieve a low-oxygen (anaerobic) environment, the puncture wound delivers the bacterial endospores to a suitable environment for growth. Figure II-P illustrates the original deep wound on the foot seen at autopsy case of tetanus. Predominant *Candida* infection aided to provide an anaerobic tissue condition. Group atrophy with regenerative change of the striated muscles and central chromatolysis in the spinal cord motor neurons represent the persistence of systemic convulsion.

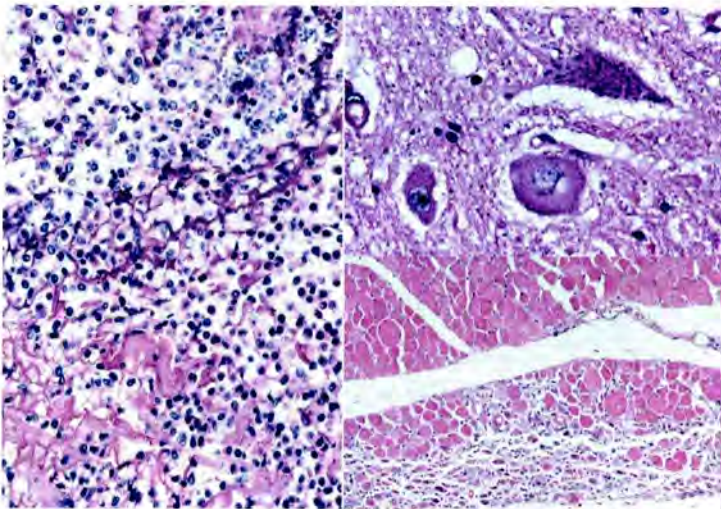


Figure II-P. Tetanus. A deep puncture wound on the foot shows predominant growth of *Candida* at autopsy (left: H&E). Motor neurons of the spinal cord reveal central chromatolysis (right top: H&E), and striated muscles show clusters of regenerative small-sized fibers at the periphery of the muscle fascicle (right bottom: H&E), representing persistent systemic convulsion.

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Q) Tularemia

Tularemia is caused by infection of a rickettsia-like small Gram-negative non-motile coccobacillus *Francisella tularensis*. Fever and swelling of lymph nodes of the axilla and supraclavicular region are the main clinical presentations. Ulcerative skin lesion may be seen in the upper extremities, 3-5 days after the contact with rodents, including the hare, squirrel and rat. The lymph nodal lesion may be complicated with a cutaneous fistula. Occasionally, typhoid fever-like septicemic dissemination is associated. Infection may also be transmitted by tick and mosquito. In Japan, the disease has been endemic in the northern part of Honshu mainland. Of note is that *F. tularensis* is transmissible through the normal (intact) skin, and aerosol-mediated, occupation-related and intralaboratory infection is plausible, so that it is nominated as one of the bioterrorists (Table).

Table. Bioterrorists

<i>Francisella tularensis</i>	tularaemia
<i>Yersinia pestis</i>	plague
<i>Bacillus anthracis</i>	anthrax
<i>Brucella abortus</i> , <i>B. melitensis</i>	brucella
<i>Burkholderia mallei</i>	glanders
<i>Burkholderia pseudomallei</i>	meliodosis
<i>Coxiella burnetti</i>	Q fever

Histologically, formation of suppurative granuloma is characteristic. Cutaneous fistula formation is commonly observed (Figure II-Q). Central abscess-type necrosis is more prominent and irregularly fused and Langhans giant cells are more easily observed than the lesion of cat scratch disease. It is difficult to identify pathogens in the lesion microscopically. Serologic testing is important for making a definite diagnosis.

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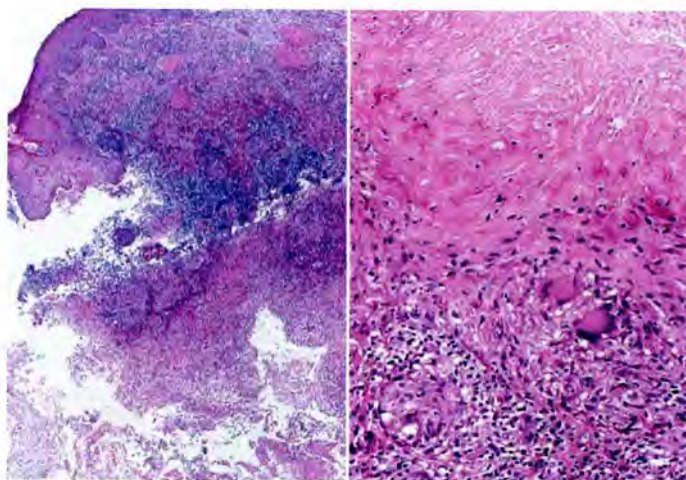


Figure II-Q. Tularemia. Actively inflamed fistula formation is seen in the neck skin (left, H&E). Suppurative granuloma with extensive central abscess formation is noted in the cervical lymph nodal lesion (right, H&E).

Note 17. Ohara disease and tularemia

The febrile infectious disease was first found in Tulare County in California in 1911, the term tularemia was given by Dr. Edward Francis, a microbiologist in the United States, in 1921, and the microbe was named *Francisella tularensis* for his honor. In 1925, Japanese physician Hachiro Ohara, living in Fukushima, wrote of a disease that affected rabbits and those who ate rabbits. In Japanese, the disease was called Yato-Byo (rabbit fever) or Ohara disease. Dr. Ohara took the blood from an infected rabbit and rubbed it on his wife's skin. A large ulcer appeared and Dr. Ohara's wife developed the characteristic symptoms of Yato-Byo. Further, Dr. Ohara saw the connections between Yato-Byo in Japan and tularemia described by physicians in the United States. In 1926, Dr. Francis reported Dr. Ohara's findings in the JAMA article. Ohara Hospital was established in Fukushima city already in 1896, and Dr. Hachiro Ohara was the second president of the hospital.

Since 1973, Ohara Research Laboratory in Ohara General Hospital has long functioned as a research and lab-testing center of tularemia and rickettsial diseases in Japan. Regrettably enough, the laboratory was closed in 2011.

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R) Glanders and Melioidosis

Glanders and melioidosis are related diseases caused by obligate aerobic, Gram-negative bacilli, *Burkholderia* species (formerly categorized in *Pseudomonas*). Both infectious disorders manifest similar symptoms and pathophysiologic consequences, but their epidemiology significantly differs. Glanders and melioidosis are notorious for potential weaponization as biological warfare weapon. It is believed that, during the World War II, the Japanese army tried to use glanders for infecting horses and prisoners. The United States and Russia also studied glanders as the possible bioweapon.

Glanders (also called “malleus”), systemic infection of *Burkholderia mallei*, is primarily a disease of animals such as horses, mules and donkeys. Human cases are rare and sporadic, typically seen in such individuals with a close and frequent contact with infected animals, as veterinarians, animal caretakers, abattoir workers and laboratory personnel. Infection is mediated via a direct contact with broken skin or mucous membranes or less frequently via inhalation of droplets from the infected equid. No cases happen in Japan. The bacteria produce toxins, such as pyocyanin (blue-green pigment interfering with the terminal electron transfer system), lecithinase (causing cell lysis by degrading lecithin in cell membranes), collagenase, lipase, and hemolysin.

Melioidosis, also known as Whitmore disease, is caused by *Burkholderia pseudomallei*. The bacteria thrive in tropical climates, and the disease is endemic in Southeast Asia and Australia, but it is also distributed in the Middle East, India and China. In Japan, Europe and the United States, melioidosis is encountered as an imported infection. Both humans and other susceptible animals (goats, pigs, cattle, etc) may contract the disease through direct contact with a contaminated soil and water, especially during the rainy season. Those who have such chronic disorders as diabetes mellitus, alcoholism, immunosuppression and renal failure are susceptible.

The symptoms (common in both infections) are nonspecific, including fever, rigors, night sweat, myalgia, anorexia and headache. Chest pain, cough, photophobia, lacrimation and diarrhea may be observed. The following 4 clinical forms are recognized.

- a) In the primary cutaneous form, the bacteria enter the skin through a laceration or abrasion, and a local infection with abscess and ulceration develop. The incubation period is 1-5 days. Regional lymph node swelling may be associated.
- b) Pulmonary form may develop when the bacteria are aerosolized and enter the respiratory tract via inhalation. Pneumonia, lung abscess and suppurative pleuritis occur. The incubation period is 10-14 days. With inhalational melioidosis, cutaneous abscess may develop but take months to appear.
- c) The septicemic form is usually fatal if untreated. In glanders, the septicemic abscesses affect multiple organs, including the skin, liver and spleen. In melioidosis, bacteremia is observed in patients with chronic debilitating disorders. The patients develop respiratory distress, diarrhea, and abscesses throughout the body, including the skin.
- d) The chronic form (symptoms lasting longer than 2 months) accompanies multiple abscesses in the liver, spleen, lung, muscles, heart, salivary gland and skin (with

multiple pustules, ulcers and scars). The lung lesion clinically resembles tuberculosis.

Microscopically, formation of suppurative granuloma, abscess surrounded by epithelioid granuloma, is characteristic of the chronic form (Figure II-R). The chronic cutaneous form of glanders is known as “farcy”. In melioidosis, the infection may become active many years after the primary infection, namely, the incubation period can vary from days to years.

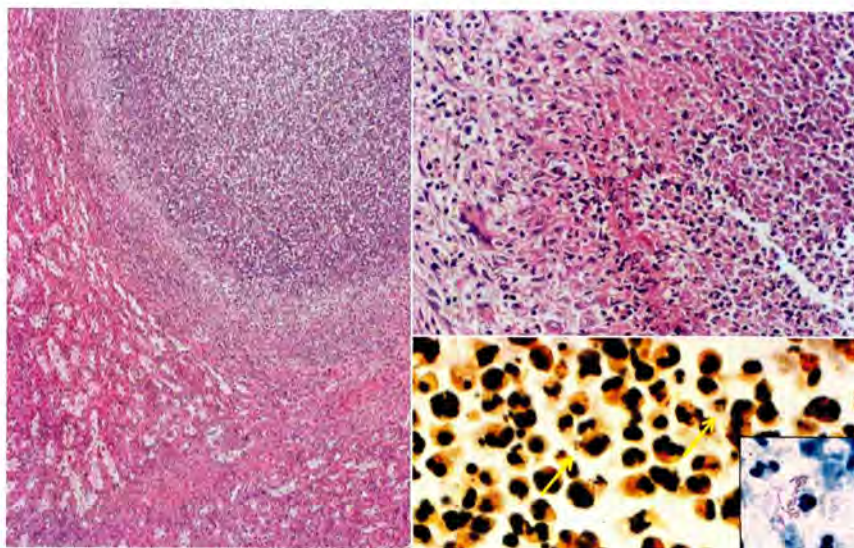


Figure II-R. Melioidosis. Multifocal splenic abscess in melioidosis is microscopically indistinguishable from the lesion in bartonellosis (cat scratch disease) (left, H&E). Granulomatous reaction is observed at the periphery of the abscess lesion (right top, H&E). Warthin-Starry's silver demonstrates a few short rod-shaped pathogens (arrows) in the cytoplasm of phagocytes (right bottom). Inset shows Gimenez stain visualizing red-stained bacilli in the macrophages in a stamp preparation. The courtesy by Dr. Toshio Oyama, a pathologist at Yamanashi Prefectural Central Hospital, Kofu, Japan.

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Note 18. Zoonotic skin infections

Animal diseases may also affect humans. The infectious diseases affecting both animals and human are called zoonosis. A variety of pathogens belonging to bacteria, fungi, viruses, protozoa, helminth and arthropods cause zoonotic skin infections. Generally speaking, only a few viruses are zoonotic. Individuals who have close contact with large numbers of animals, such as farmers, abattoir workers, shearers, knackery workers and veterinarians are at a higher risk of contracting zoonotic disorders. Healthy pets can be the source of infection. Those who are susceptible to contracting a zoonotic disease include immunosuppressed individuals, pregnant women, alcoholics and diabetics. It is important to realize that zoonoses may be contracted from both ill and apparently healthy animals.

Representative zoonotic skin infections are listed as follows.

1. **Bacteria:** *Dermatophilus*, *Chlamydia psittaci*, *Pasteurella*, *Capnocytophaga*, *Francisella*, *Burkholderia*, *Bacillus anthracis*, *Erysipelothrix*, *Actinomyces*, *Nocardia*, non-tuberculous *Mycobacteria*, *Borrelia*, *Bartonella*, *Rickettsia*, *Listeria*, *Brucella*, *Mycoplasma*
2. **Fungi:** *Dermatophytes*, *Sporotrichum*, pigmented fungi group, *Candida*, *Aspergillus*, *Zygomycetes* (*Mucor* group), *Fusarium*, *Cryptococcus*, *Histoplasma*, *Paracoccidioides*, *Blastomyces*, *Coccidioides*, *Prototheca*
3. **Virus:** Parapoxvirus
4. **Protozoa:** *Leishmania*, *Trypanosoma*, *Entamoeba*, *Acanthamoeba*
5. **Helminth:** *Onchocerca*, *Gnathostoma*, spiruroid nematoda, *Dirofilaria*, *Ancylostoma*, *Paragonimus*, *Sparganum*, *Cysticercus*
6. **Arthropod:** Scabies, ticks, flea

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S) Anthrax and *Bacillus Cereus* Infection

Anthrax is a zoonotic infection of a large-sized Gram-positive bacillus, *Bacillus anthracis*. Formation of spores and capsules is closely related to the pathogenicity of this microbe. Three clinical forms are known, involving the skin, lung and intestine. The latter two are often lethal. Skin anthrax, mainly involving the arm, is an occupation-related infection of veterinarians and those who treat animal hair, skin or carcass. The latent period is within 4 days. The skin lesion is necrotic and ulcerated to form hemorrhagic crust (eschar or black necrosis) (Figure II-S-1). The ulcer is painless. Microorganisms are easily found in the exudate.

B. anthracis is the best known bioterrorist (refer to Chapter II-Q), because the spores are tolerant to dry conditions for a long period of time, and inhalation of the spored microorganisms provokes lethal necrotizing pneumonia. Ulcer-forming skin infection is also caused by other *Bacillus* species, such as *B. megaterium* and *B. pumilus*.



Figure II-S-1. Cutaneous anthrax. Occupation-related infection in a Japanese veterinarian is shown. The skin lesion on the left forearm is necrotic and ulcerated to form hemorrhagic crust (eschar). The courtesy by Dr. Keiko Oka, a dermatologist at Tokyo Hospital of Health Insurance Association of Nippon Express, Tokyo, Japan.

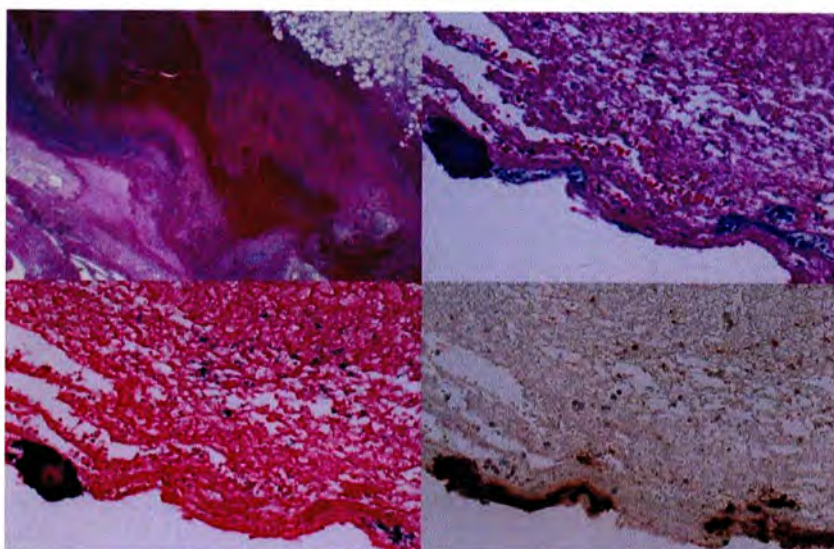


Figure II-S-2. *Bacillus cereus*-induced necrotizing fasciitis. Trauma-related soft tissue gangrene on the hip, caused by a spore-forming Gram-positive bacillus, *Bacillus cereus*, led this diabetic patient to death. Gram-positive bacteria colonize the necrohemorrhagic tissue. Top panels: H&E, left bottom: Gram, right bottom: immunostaining for *Bacillus cereus* antigens. The antiserum mainly detects spores. The courtesy by Dr. Etsuko Nakamura, a pathologist at Toyohashi Medical Center, Toyohashi, Japan.

Bacillus cereus is associated mainly with food poisoning, but it may cause potentially fatal non-gastrointestinal infections. The pathogenicity of *B. cereus* is related to the

production of tissue-destructive exoenzymes. Clinically, anthrax-like progressive pneumonia, fulminant sepsis and devastating central nervous system infections may be seen in immunosuppressed individuals, intravenous drug abusers and neonates. Primary cutaneous infections, mimicking necrotizing fasciitis or non-clostridial gas gangrene induced subsequent to trauma, have also been documented (Figure II-S-2). *B. cereus* produces a potent β -lactamase conferring marked resistance to β -lactam antibiotics.

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Note 19. Capsule-forming microbes

The following three types of microbial capsules are known: 1) Macrocapsule, 2) microcapsule (shown only by electron micrography or serological test) and 3) slime layer (mucoid or polysaccharides secreted to the surrounding fluid and loosely attached to the cells). The capsule consists of either homopolysaccharide, heteropolysaccharide or poly-D-glutamic acid (*Bacillus anthracis*). The capsule functions as a permeability barrier, an iron-exchange system to protect the bacteria from dryness and phagocytosis, and the attachment to the surface of the tissue or foreign material.

Capsule-forming Gram-positive bacteria represent *Staphylococcus*, *Streptococcus* (groups A and B), *Streptococcus pneumoniae*, *Enterococcus* and *Bacillus anthracis*. Gram-negative bacilli forming the capsule encompass *Neisseria meningitidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa*. *P. aeruginosa* is notorious for producing the slime layer (mucoid type).

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Note 20. "Sago palm" disease

"Sago palm" disease, also known as Sepik granuloma, is a rare condition endemic in the Sepik district of New Guinea. Injury with the sago palm tree (*Metroxylon* species) allows the entry of the causative organism, a Gram-positive bacillus, which has not been cultured.

The affected individuals usually present with multiple cutaneous nodules on the extremities or on the face. Histologically, a diffuse dermal infiltrate mainly consists of large foamy histiocytes, admixed with plasma cells, lymphocytes and fibroblasts. A syncytium of amorphous pink, PAS-positive material extends between the histiocytes. The material contains numerous gray, round to oval dots, representing Gram-positive organisms surrounded by a ground substance.

Sago palm disease generally means a worldwide plant illness, in which the sago palm (*Cycas revoluta* or *Sotetsu* in Japanese) is susceptible to the scale insects or to manganese deficiency.

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T) Actinomycosis

Actinomycosis is a bacterial infection caused by *Actinomyces* species such as *Actinomyces israelii* and *A. gerencseriae*. A similar condition is also provoked by *Propionibacterium propionicus*. Actinomycosis is common in cattle as a disease called *lumpy jaw*, forming a large abscess lesion on the head and neck of the infected animal. Swine, horses, sheep and dogs may also be involved.

A. israelii, an anaerobic filamentous Gram-positive bacterium, forms painful abscesses in the mouth, lung or gut. The lesion may penetrate the surrounding bone and muscle, resulting in fistula formation onto the skin. The purulent leakage via the sinus cavity contains "sulfur granules".

Actinomycosis is common in immunocompetent individuals, and is clinically divided into three forms:

- 1) head and neck type, involving the paranasal cavity, gingiva and tonsils
- 2) thoracic type, involving the lung
- 3) intestinal type, involving the appendix and terminal ileum

Liver actinomycosis is rare and usually extends from the intestinal lesion.

The white-colored grains are composed of fine filamentous bacteria and Splendore-Hoeppli material (homogeneous eosinophilic matrix of the grain), and positively stained with PAS, Grocott and Warthin-Starry's silver. Acid-fast stain is negative.

The skin lesion is often called as actinomycetoma. Dermal and subcutaneous abscess lesions contain actinomycotic grains or sulfur granules. The size of the white grains is often very large, up to 1-2 mm (Figure II-T-1). The perianal skin is the common site of actinomycetoma (Figure II-T-2). The lacrimal duct is another preferred site of actinomycetoma formation.

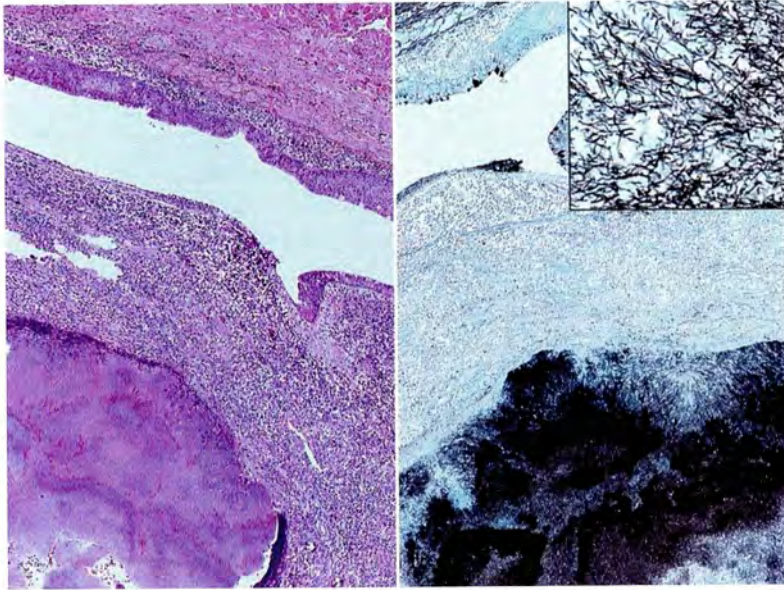


Figure II-T-1. Actinomycosis of the eye lid. A large grain composed of Gram-positive and Grocott-stained filamentous bacilli is observed beneath the conjunctiva. Neutrophilic and lymphocytic reactions are associated. (left: H&E, right: Grocott, inset: high power).

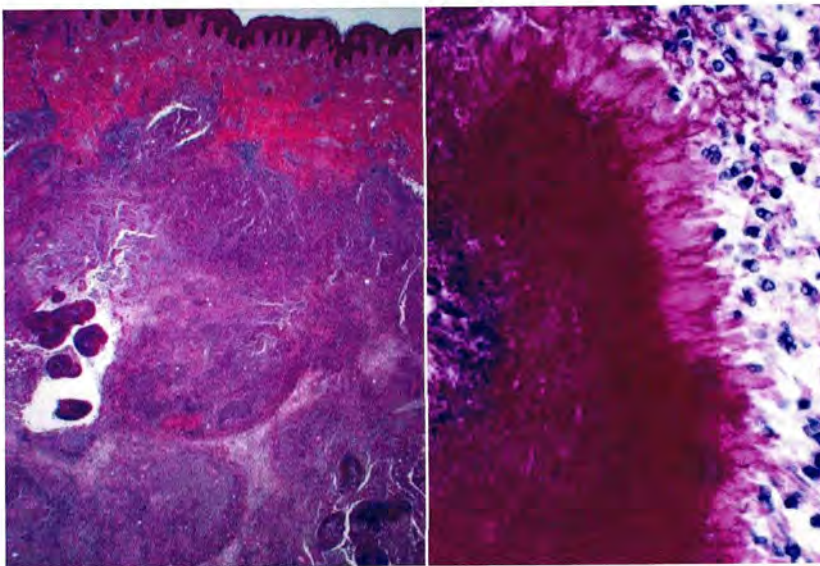


Figure II-T-2. Perianal actinomycetoma. Deep cutaneous abscess is formed in the perianal skin. Multiple actinomycotic grains are clustered in the abscess cavity (left: H&E). Splendore-Hoeppli material of the grain is PAS-reactive (right). Neutrophils surround the grains.

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U) Nocardiosis

Nocardia asteroides is a filamentous Gram-positive bacterium of genus *Actinomyces*. *N. asteroides* is an aerobe and weakly acid-fast, and usually does not form grains. Grocott and Gram stains demonstrate branched filamentous bacteria in suppurative lesions (Figure II-U-1). In contrast to actinomycosis, nocardiosis is seen mainly as a form of opportunistic infection.

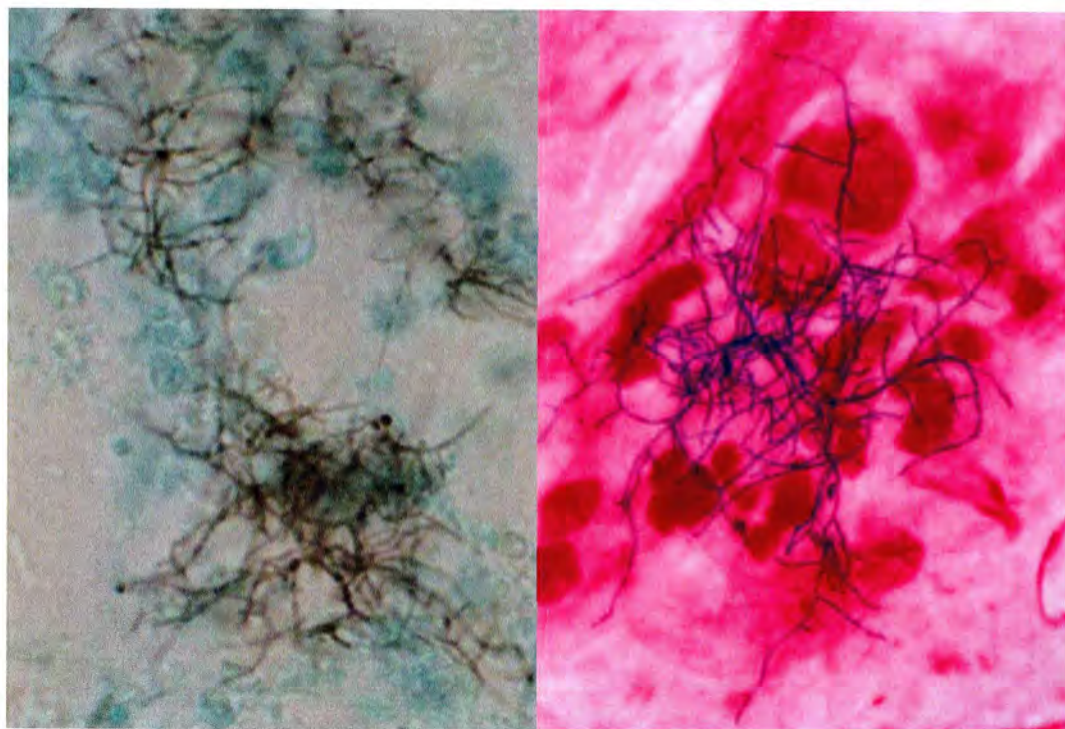


Figure II-U-1. Cytologic appearance of *Nocardia asteroides* in the sputum cytology. Branched filamentous bacteria are observed with Grocott (left) and Gram (right) stains. It is difficult to see the bacteria with Papanicolaou stain. In this case, cytologic diagnosis of nocardiosis led the patient who showed lung cavitation to appropriate treatment and cure.

Primary cutaneous nocardiosis is extremely rare (Figure II-U-2). In most cases, a history of trauma is recognized. Abscess is formed in the dermis through subcutis. A lymphangitic form resembles sporotrichosis. Bacteria are shown with Grocott and Gram stains. Similar to actinomycosis, small sulfur grains embedded in Splendore-Hoepli material may be formed in the skin lesion (Figure II-U-3). In the case shown here, infection of *Nocardia thailandica* was confirmed by PCR analysis. This makes a sharp contrast to visceral nocardiosis, where filamentous bacteria are dispersed, not forming grains. The size of the grains in cutaneous nocardiosis is much smaller than that seen in actinomycetoma.



Figure II-U-2. Cutaneous nocardiosis. A crusted reddish nodule is formed in the skin of the hip. The courtesy of Dr. Chikako Suzuki at Department of Dermatology, Teikyo University Mizonokuchi Hospital, Kawasaki, Japan.

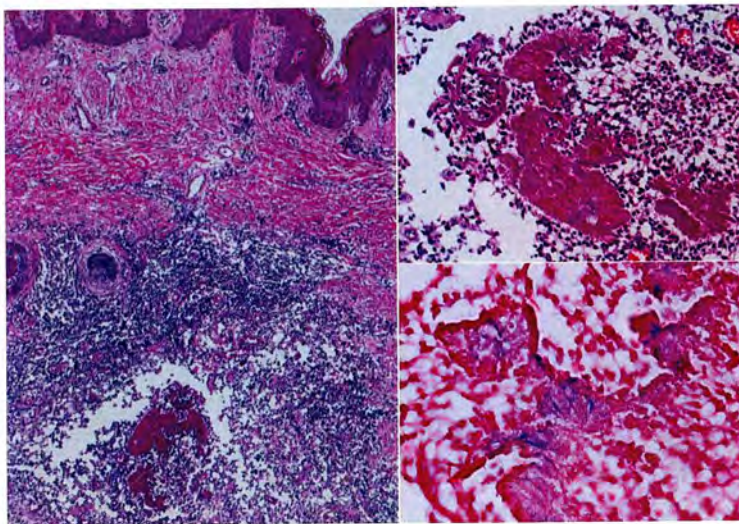


Figure II-U-3. Histology of cutaneous nocardiosis. Abscess is formed in the deep dermis, and an irregular-shaped, small-sized grain with eosinophilic matrix is observed in the center of the abscess cavity (H&E, left: low power, right top: high power). Gram stain demonstrates fine Gram-positive filaments in the grain matrix (right bottom). The courtesy of Dr. Chikako Suzuki, Kawasaki.

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V) *Propionibacterium Acnes*, Folliculitis and Sarcoidosis

Propionibacterium acnes, named after its ability to synthesize propionic acid, is a relatively slow-growing, aerotolerant anaerobic Gram-positive rod. This bacterium, as a normal flora, colonizes the skin of healthy adults, but is barely detectable in preadolescents. It lives deep within hair follicles, primarily depending on fatty acids in the sebum secreted by sebaceous glands (Figure II-V-1). Metabolic byproducts and bacterial debris produced by *P. acnes* can trigger inflammation in acute folliculitis and acne vulgaris. Reportedly, the microorganism can generate enzymes for degrading skin components and proteins that activate the host immune system.

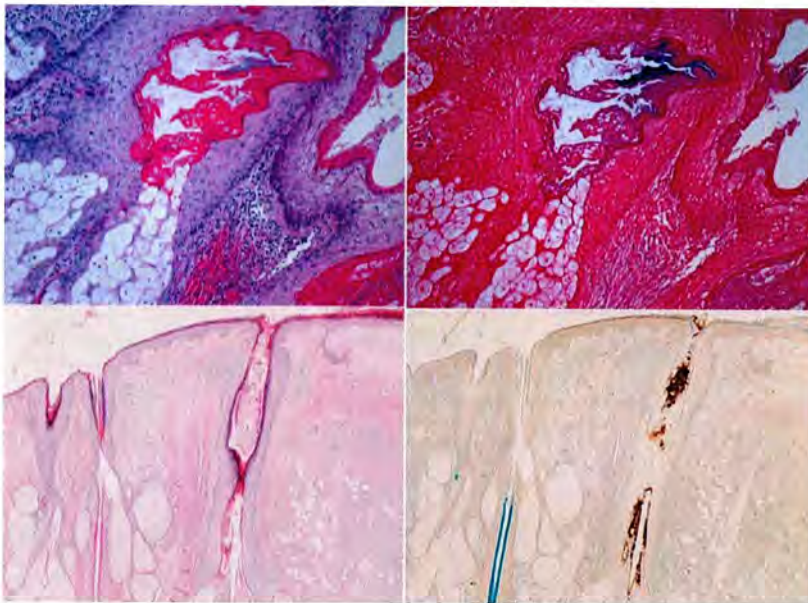


Figure II-V-1. *Propionibacterium acnes* as normal skin flora. As a normal flora, the Gram-positive bacilli are clustered in the hair follicle, particularly at the outlet of the sebaceous gland (left, top and bottom: H&E, right top: Gram, right bottom: immunostaining for *P. acnes*-specific cell-membrane-bound lipoteichoic acid). The courtesy by Prof. Yoshinobu Eishi, Department of Pathology, Tokyo Medical and Dental University, Tokyo, Japan for the bottom case.

Scalp folliculitis is characterized by the appearance of small, white or yellowish pustules resembling acne vulgaris. The pustules are itchy and painful at times. In severe cases, the development of large and hard bumps on the scalp leads to scarring and hair loss. Excessive production of sebum increases the chance of provoking a bacterial infection. The bacteria most frequently found in scalp folliculitis are *P. acnes* (Figure II-V-2) and, in severe cases, *Staphylococcus aureus*. Malassezian fungi and *Demodex folliculorum* may also play a role in this lesion.

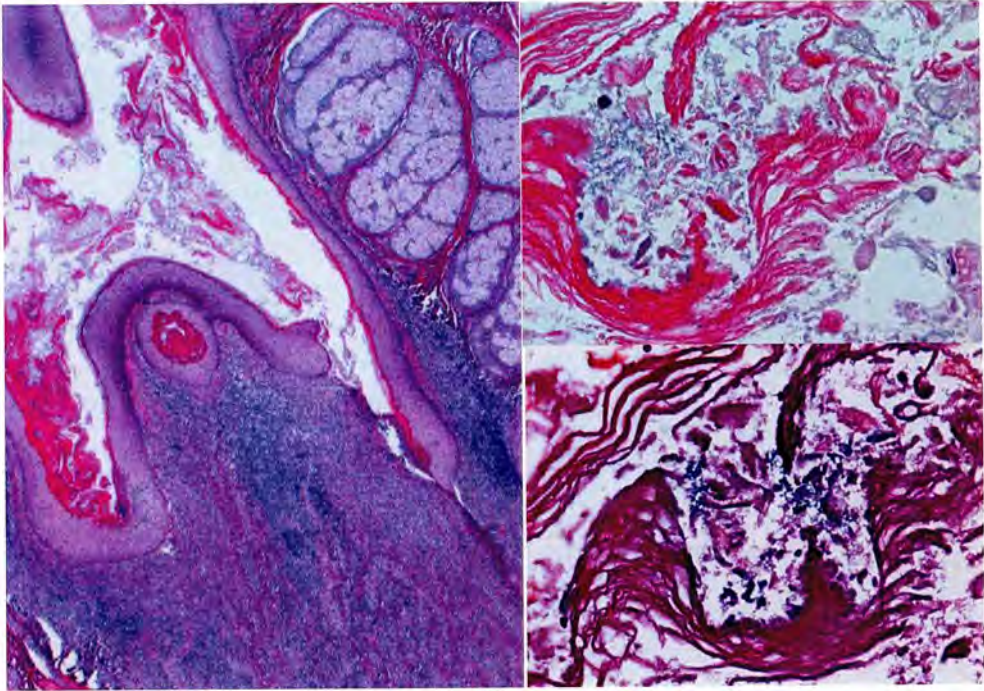


Figure II-V-2. Scalp folliculitis. Acute and chronic folliculitis with lymphoid follicle formation has been provoked by short rods, *P. acnes*, which locally proliferate among the keratinized squams in the dilated hair follicle lumen (left and right top: H&E, right bottom; Gram).

Prof. Yoshinobu Eishi and his group of pathology at Tokyo Medical and Dental University, Tokyo, Japan have described that components of *P. acnes* are identified in the granulomatous lesions of sarcoidosis. Immunostaining using monoclonal antibodies against *P. acnes*-specific cell-membrane-bound lipoteichoic acid and ribosome-bound trigger-factor protein has visualized positive granular signals in the cytoplasm of epithelioid cells and Langhans-type multinucleated giant cells (Figure II-V-3). Immunoreactivity is also noted in large-spheroidal, yellowish brown-colored and acid-fast bodies, Hamazaki-Wesenberg bodies (yellow bodies) in sinus histiocytes of the lymph node involved with sarcoidosis. Normal flora in the hair follicle is also stained positively with these antibodies. Electron microscopic study revealed bacterial structures in the Hamazaki-Wesenberg bodies. Reverse-transcriptase polymerase chain reaction also detected bacterial RNA within the lesion. Antibiotics (Minomycin/doxycycline) therapy is effective in some cases of sarcoidosis. They propose that this indigenous bacterium might be the cause of granuloma formation in sarcoidosis.

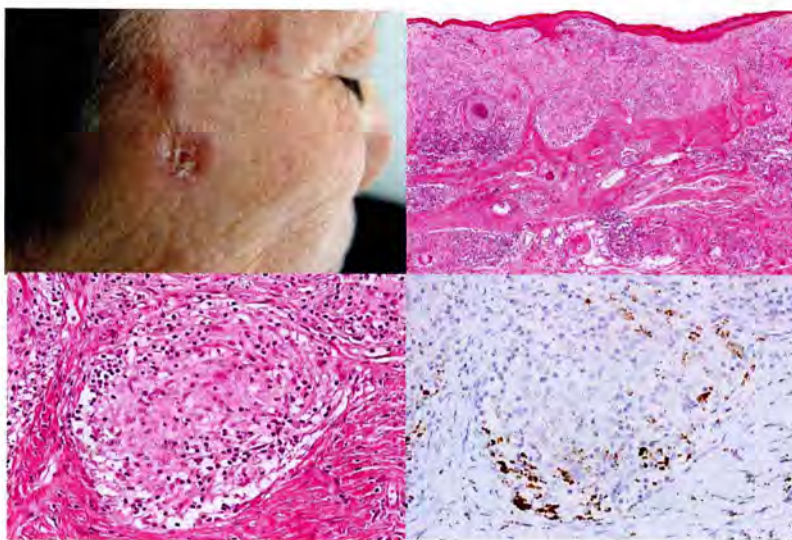


Figure II-V-3. *P. acnes* etiology of sarcoidosis. Cutaneous sarcoidosis seen on the facial skin of an aged female is presented (left top). Multiple non-caseous epithelioid granulomas are formed in the dermis (right top and left bottom: H&E). Lymphocytic reaction is relatively mild. Positive granular signals immunoreactive for *P. acnes*-specific cell-membrane-bound lipoteichoic acid are recognized, particularly at the periphery of the granuloma (right bottom). The courtesy by Prof. Yoshinobu Eishi, Tokyo, Japan.

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W) Tuberculosis

Tuberculosis of the skin has been declining in incidence all over the world, though it is still prevailing in India and part of Africa. Skin tuberculosis is divided into two: primary and secondary. Primary inoculation of *Mycobacterium tuberculosis* into the skin is extremely rare. Reinfection (secondary) skin tuberculosis is further classically subdivided on the basis of clinical features into lupus vulgaris, tuberculosis verrucosa, scrofuloderma, orificial

tuberculosis and disseminated cutaneous tuberculosis. Skin reactions to tuberculous infection elsewhere in the body are called tuberculids, where no tuberculous bacilli are detectable.

Tuberculosis is generally characterized by formation of caseating granulomas. Grossly, the caseous granuloma makes a localized nodular lesion, with central caseous (cheese-like) necrosis yellowish white in color, where pre-existing normal structures such as vessels and skin adnexae remain in the lesion, indicating a form of coagulation necrosis. In old tuberculosis, the caseous foci tend to be encapsulated, and dystrophic calcification follows (refer to Figure I-A). Histologically, the caseous granuloma typically consists of central coagulation necrosis, the surrounding epithelioid cell layer with Langhans-type giant cells and the outermost lymphocytic cuffing with fibrosis.

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Note 21. Detection of mycobacterial antigens

Rabbit antiserum to BCG (Dako) diluted at 1:3,000 is a very sensitive probe for demonstrating mycobacterial antigens in paraffin sections. Not only exudative (active) tuberculous lesions but also a considerable percentage (19/32 = 59%) of the encapsulated caseous and fibrocalcified lesions show positivity, while Ziehl-Neelsen's acid-fast staining rarely gives positive findings in such old tuberculous lesions (refer to Figure I-A). In addition, the judgment whether positive or negative is easily reached with the BCG immunostaining.

Hence, the observation time can be saved considerably. Foamy positive signals are seen in the cytoplasm of live or dead macrophages. Bacilliform positivity is seen only occasionally. The reason for this is that the mycobacterial antigens are kept intact in lysosomes of the macrophages even after phagocytosis and digestion of the bacilli. Normal human tissues, sarcoidosis or related non-caseous granulomas remain unstained. Staining with the normal rabbit serum serves as a negative control.

The BCG antiserum, allegedly detecting more than 100 antigenic substances expressed on the BCG (*Mycobacterium bovis*) cell body, is reactive with non-tuberculous mycobacteria and *Mycobacterium leprae*. In dermal or visceral lesions of lepromatous leprosy, the cytoplasm of foamy macrophages is diffusely and strongly immunoreactive (refer to Figure II-Y-2-5). This antiserum is known to be further cross-reactive with a wide variety of pathogens including Gram-positive and -negative bacteria and fungi. Absorption by formalin-fixed *Candida albicans* results in significant abolishment of the cross-reactions with the non-mycobacterial species without any change in positive staining in the mycobacterial lesions. However, the absorption procedure seems to be unnecessary for the practical diagnostic application, since such bacterial and fungal lesions can be distinguished in H&E preparations.

We have applied this diagnostically valuable method, BCG immunostaining, for nearly 30 years on a routine basis. Occasionally, we experience an exceptional case, in which an endoscopic lung biopsy specimen containing small non-caseating granulomas failed to reveal the BCG antigens, but the acid-fast staining demonstrated a few red-stained bacilli. Therefore, we recommend you to employ a combination of BCG immunostaining and Ziehl-Neelsen's staining for the histopathological diagnosis of mycobacterial diseases.

It should be reminded that some antisera against unrelated antigens give a false positive reaction in mycobacterial lesions, since they were raised by immunizing the animal with Freund's complete adjuvant, which contains dead tuberculous bacilli.

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1) Lupus Vulgaris, Tuberculosis Verrucosa and Scrofuloderma

Lupus vulgaris is the most common form of reinfection skin tuberculosis, predominantly seen in the head and neck regions of young adults. Extremities and penis are also involved. Multiple erythematous papules form a plaque. Histologically, tuberculoid granulomas are seen in the dermis with a tendency to confluence (Figure II-W-1-1). Caseation is a relatively rare event. Bacilli are usually sparse. PCR is now used for confirming the diagnosis.

Tuberculosis verrucosa is uncommon, and shows verrucous plaques on the back of the hand and fingers (Figure II-W-1-2). It may occur as an occupational hazard in the autopsy room. Histologically, caseous granulomas are covered with the papillomatous (verrucous) epidermis showing pseudoepitheliomatous hyperplasia.

Scrofuloderma is a skin involvement from underlying tuberculous lesion in the lymph node or bone, and undermined ulcer and discharging sinus are associated (Figure II-W-1-3). The neck and submandibular regions are the most common sites of involvement. Acid-fast bacilli can be commonly demonstrated in abscess and/or caseous necrosis involving the dermis and subcutis. This type may also be caused by non-tuberculous mycobacteria.

Orificial tuberculosis, also known as acute tuberculous ulcer, occurs at the mucocutaneous borders of the nose, mouth, anus, urinary meatus and vagina, and on the mucosa of the mouth and tongue.

Disseminated cutaneous tuberculosis shows widespread pustules on AIDS patients' skin.

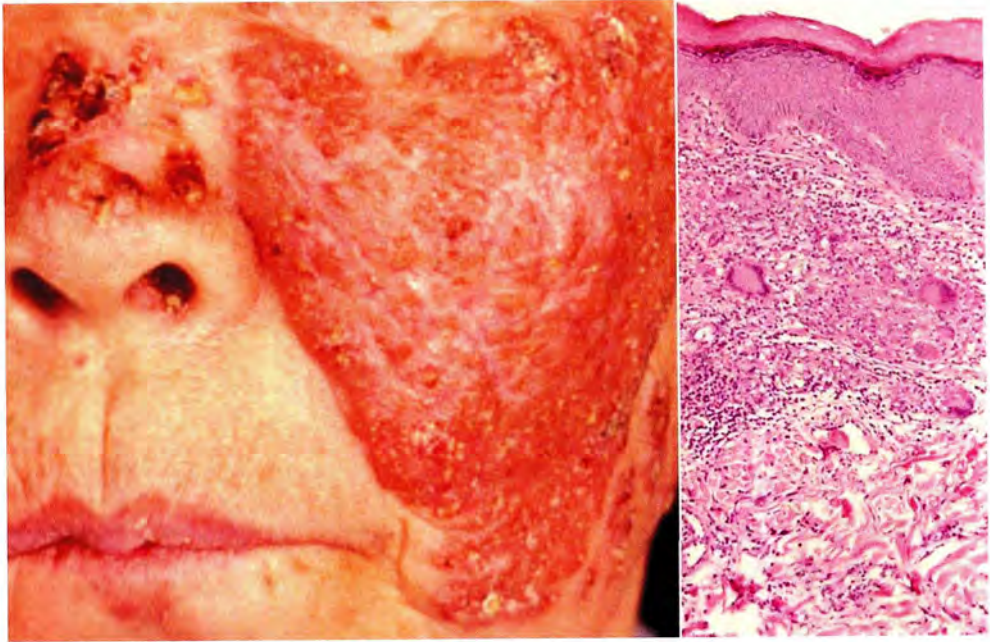


Figure II-W-1-1. Lupus vulgaris. On the facial skin of the aged Japanese female, multiple and fused erythematous papules form a plaque (left). Biopsy histology reveals tuberculoid granulomas in the dermis (right: H&E). Caseation is minimal, so that acid-fast bacilli are barely identified histochemically. The courtesy by Prof. Akira Ozawa, Department of Dermatology, Tokai University School of Medicine, Isehara, Japan.

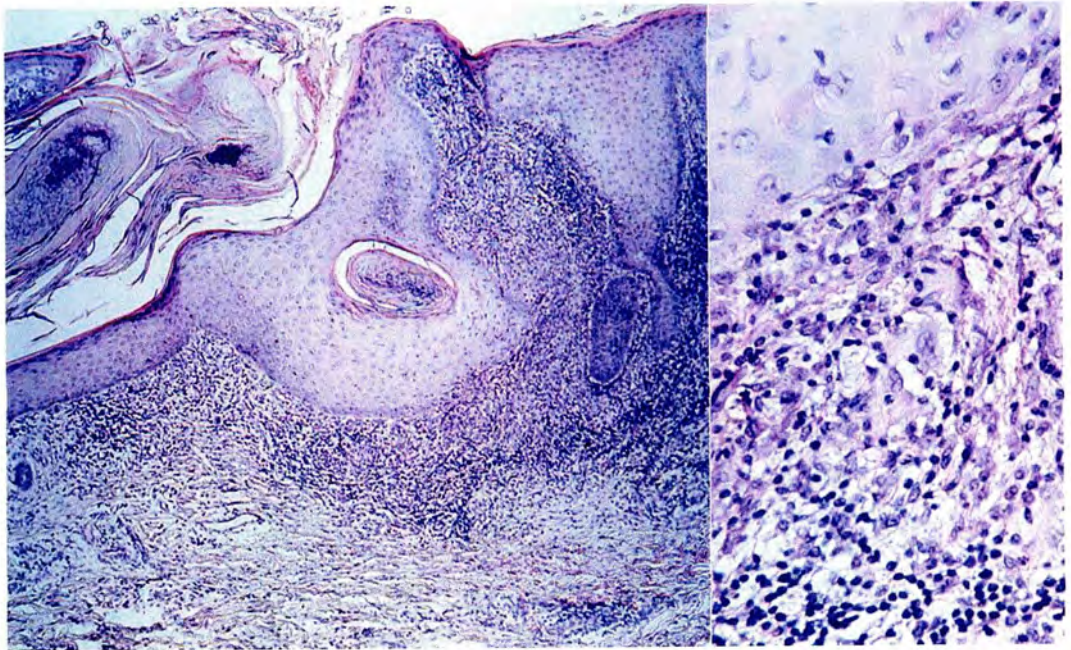


Figure II-W-1-2. Tuberculosis verrucosa. The verrucous lesion on the back of the hand microscopically shows reactive papillomatosis of the epidermis, provoking pseudoepitheliomatous hyperplasia (left: H&E). Granulomatous reaction is seen in the dermis (right: H&E).

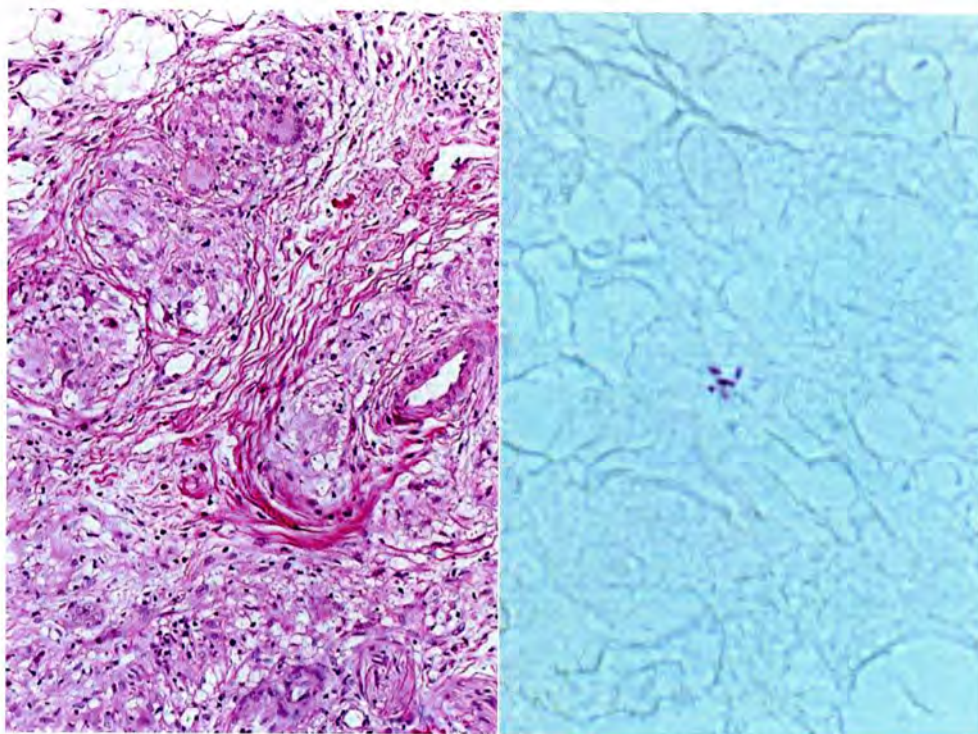


Figure II-W-1-3. Scrofuloderma. The neck skin is involved by caseous granulomas, which represent a direct drainage from the cervical nodal lesion (left: H&E). Ziehl-Neelsen's stain identifies a few acid-fast bacilli in the necrotic lesion (right: H&E).

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Note 22. Occupational skin infections

The most serious biohazard to medical staff includes tuberculosis and blood-borne viral infections such as hepatitis B and C and HIV. Intrahospital transmission of prion (the pathogen of Creutzfeldt-Jakob's disease) has been recorded. Hemorrhagic fever viruses and coronavirus provoking severe acute respiratory syndrome (SARS) have killed many devoted medical staff in the hospital. Highly contagious viral infections such as measles, varicella, rubella, mumps, influenza may provoke endemics in the hospital. Contact with patients may result in infections such as impetigo, tinea corporis, scabies and head lice. Nosocomial infection of scabies in the ward of neurosurgery and in the care homes/stations for the aged is especially problematic (Chapter VII-A-1).

Unique occupational hazards for special situations include contact transmission of *Mycobacterium tuberculosis* to the finger of pathologists (tuberculosis verrucosa) and primary syphilis at the tip of finger of gynecologists. Dentists and dental assistants are often exposed to herpes simplex virus and HIV.

Laboratory infections are also serious. Historically, Dr. Hideyo Noguchi was killed by yellow fever virus, and Dr. Stanislaus von Prowazek and Dr. Howard Ricketts were defeated by rickettsial pathogens (Note 29). In Japan, a pathologist died of laboratory infection of *Brucella abortus*. Of course, bioterrorists are highly biohazardous (Chapter II-Q).

Contact with animals may result in infections such as orf (from sheep and goats), milker's nodules (from cows) (Chapter IV-C), anthrax (from sheep and cows) (Chapter II-S), erysipeloid (from pigs) (Note 9) and zoonotic tinea corporis (from horses, cattle, pigs, cats and dogs) (Chapter III-A). Farmers, florists and tree trimmers are susceptible to cutaneous sporotrichosis (Chapter III-C), chromomycosis (Chapter III-D) and protothecosis (Chapter III-U).

Dishwashers and poultry processors working with wet hands are susceptible to candidal paronychia (Chapter III-I). Contact with aquatic life may result in marine wounds and stings from jelly fish and sea urchins (Chapter VII-M), as well as *Mycobacterium marinum* infection (Chapter II-X-4) and *Vibrio vulnificus* infection (Chapter II-C-3) from fish.

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2) Tuberculids

The tuberculids represent three types of dermatosis: papulonecrotic tuberculid, lichen scrofulosorum and erythema induratum Bazin. Tuberculids are skin lesions in patients with tuberculosis elsewhere in the body (tuberculin skin test is strongly positive), but acid-fast staining and mycobacterial culture are negative. Anti-tuberculous therapy is effective. Tuberculids are considered to be immunologic reactions against dead bacilli or antigenic fragments deposited in the skin. Mycobacterial DNA may be identified in the lesion.

Papulonecrotic tuberculids are symmetrical, erythematous and ulcerated papules on the limbs or ears. Depressed scars remain after healing. Histologically, wedge-shaped skin infarction is formed (Figure II-W-2-1), and at the base of the lesion, necrotizing vasculitis often with mild epithelioid cell reaction may be observed.

Lichen scrofulosorum consists of yellow or brown follicular papules, 0.5-3 mm in size, mainly seen on the trunk. No scar remains after healing. Histologically, epithelioid granulomas with Langhans giant cells are noted in the upper dermis along the hair follicle or sweat gland ducts. No caseation is observed.

Erythema induratum Bazin is a form of panniculitis on the back of the calves. The disease is categorized as one the three tuberculids, but a non-tuberculous type is also known and called “nodular vasculitis”. Recurrent crops of tender erythematous nodules are formed. They break down to form small and multiple ulcers. The ankle skin becomes sensitive to temperature changes. When weather is cold, ankle is cold, blue and often tender. Chilblains may be associated. In hot weather, ankle becomes hot, edematous, swollen and painful. Histologically, lobular panniculitis is associated with caseating granulomas, vasculitis (phlebitis and arteritis) and septal fibrosis in the adipose tissue (Figure II-W-2-2).

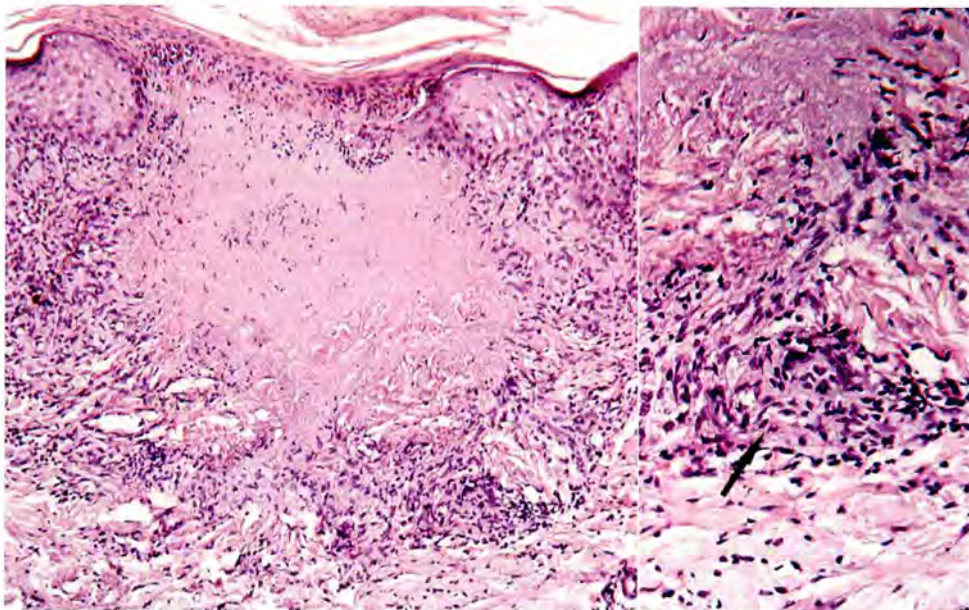


Figure II-W-2-1. Papulonecrotic tuberculid. Erythematous and ulcerated papules are formed on the extremities. In the dermis, wedge-shaped skin infarction with neutrophilic reactions is formed (left: H&E). Vasculitis is seen at the base of the ischemic lesion (arrow) (right: H&E).

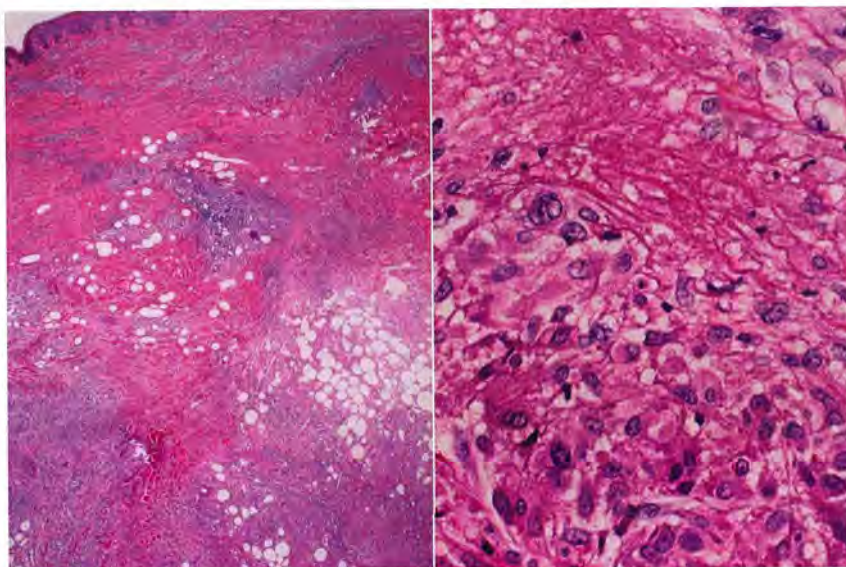


Figure II-W-2-2. Erythema induratum Bazin. Active inflammatory reactions are visible mainly in the subcutaneous tissue (left: H&E). Higher powered view (right) demonstrates epithelioid granulomas with central necrosis. Acid-fast bacilli are undetectable.

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3) Tuberculin Reaction

Tuberculin reaction represents delayed (type IV) hypersensitivity against *Mycobacterium tuberculosis*. Purified protein derivative (PPD), 0.1 ml containing 0.05 µg, is injected into the skin, and allergic reaction against the PPD is judged after 48 to 72 hours. When positive, itchy nodular erythematous changes are observed. Histologically, perivascular lymphocytic infiltration is observed, but no granuloma (Figure II-W-3).

The 100 years-old tuberculin skin test does not distinguish cellular immune reactions against *M. tuberculosis* from those obtained by BCG vaccination. As a modern alternative, QuantiFERON-TB Gold test (QFT-3G, Cellestis) and T-spot TB (Oxford Immunotec) offer clinicians a simpler and more accurate, reliable and convenient diagnostic tool. QFT-3G specifically detects a cell-mediated immune response against three kinds of *M. tuberculosis* proteins such as ESAT-6, CFP-10 and TB-7.7, and a positive test result is strongly

predictive of true infection of *M. tuberculosis*. The QFT does not cross-react with most non-tuberculous mycobacteria, except for the cross-reactivity to *M. kansasii*, *M. szulgai* and *M. marinum*. The whole blood is incubated with the mycobacterial antigens, and then interferon- γ secreted from sensitized T-lymphocytes is measured via an enzyme-linked immunosorbent assay (ELISA). Both active tuberculosis and latent *M. tuberculosis* infection show positivity. T-spot TB similarly detects activated interferon- γ -producing T-cells after incubation with two kinds of *M. tuberculosis*-specific antigens (ESAT-6 and CFP-10). The testing system is simpler in T-spot TB than in QFT-3G.

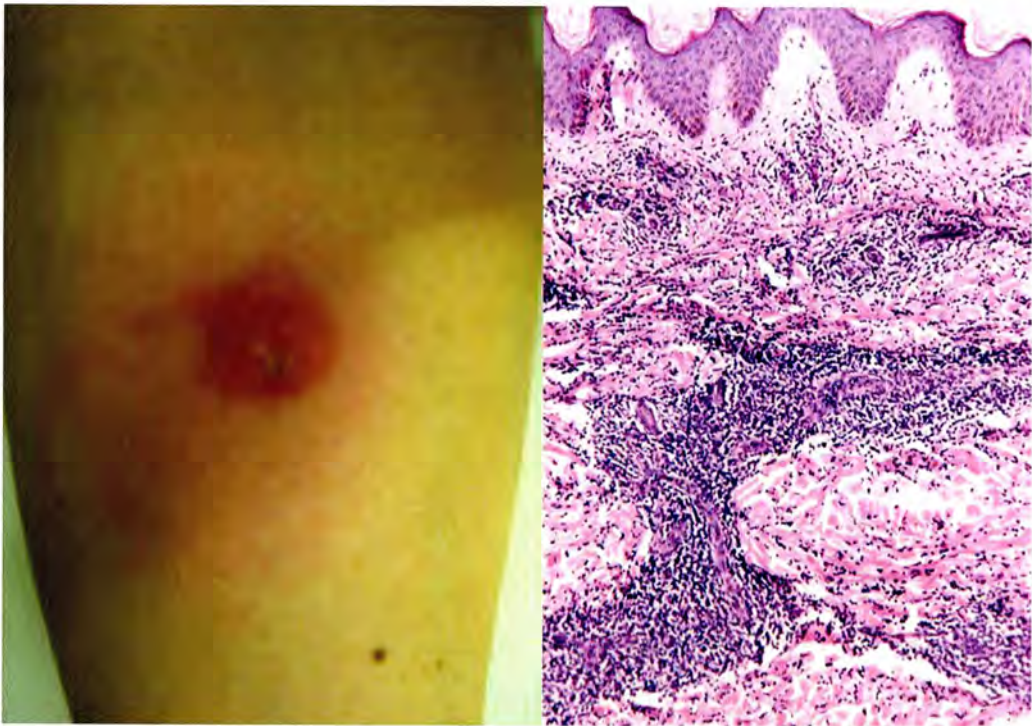


Figure II-W-3. Tuberculin skin reaction. An itchy erythematous nodule is formed in the forearm at the site of intradermal PPD injection (left). This represents a delayed hypersensitivity reaction to mycobacterial antigens, but no granuloma is observed. Perivascular lymphocytic infiltration is histologically nonspecific (right: H&E).

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X) Non-Tuberculous *Mycobacterium* Infection

Runyon classified non-tuberculous (atypical) mycobacteria into four groups (Table). Runyon's groups 1-3 correspond to slow growers (more than a week needed to grow: usually 3-5 days). Group 4 *Mycobacteria* are called as fast growers (less than a week needed to grow). The most common non-tuberculous mycobacteria causing pulmonary granulomatous infection are *M. avium-intracellulare* (MAI) and *M. kansasii*. The disease is common in older patients, and males predominate over females. Non-tuberculous mycobacteria are less pathogenic to human and less chemosensitive than tuberculous bacilli. It should be noted that human to human transmission does not occur in non-tuberculous mycobacteriosis.

We occasionally encounter skin lesions infected by a variety of non-tuberculous mycobacteria, including *Mycobacterium kansasii*, *M. marinum* (group 1), *M. scrofulaceum*, *M. szulgai*, *M. goodii*, (group 2), MAI and *M. xenopi* (group 3), and *M. fortuitum*, *M. chelonae* and *M. abscessus* (group 4). Mycobacterial inoculation may occur after skin trauma, cosmetic treatment (mesotherapy), breast implant surgery and liposuction. Histologic features are fundamentally indistinguishable from tuberculosis. Occasionally, features of plasma cell granuloma are observed (Figure II-X-0).

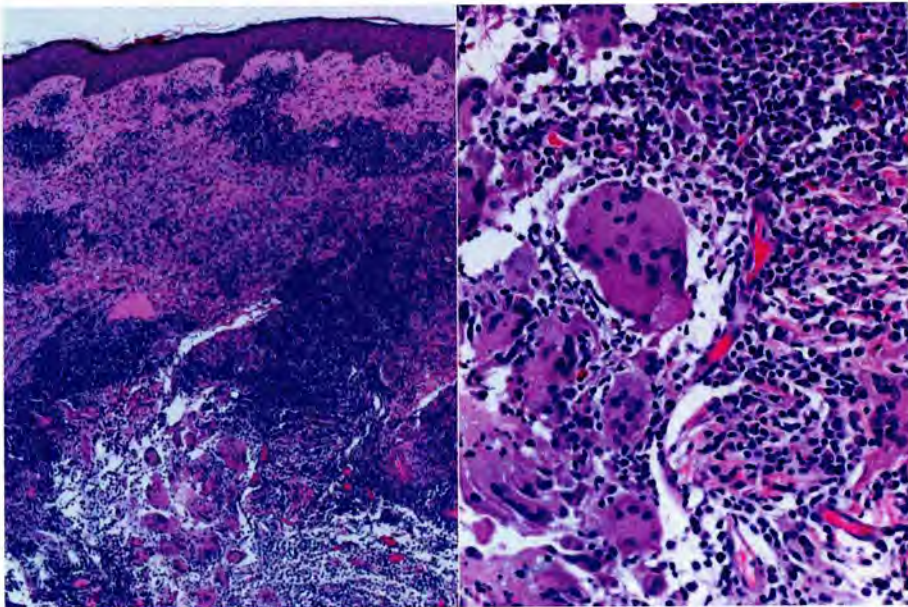


Figure II-X-0. Plasma cell granuloma caused by non-tuberculous mycobacterial infection. In this case, mononuclear lymphoid cells densely infiltrate in the dermis (left: H&E). Plasma cells are predominant, and granulomatous reaction is focal, with foreign body-type multinucleated giant cells seen (right).

Acid-fast bacilli-rich xanthogranulomatous reactions somewhat resembling lepromatous leprosy may be encountered. Some lesions show predominant necrosis with mild granulomatous reaction.

Table. Runyon's classification of non-tuberculous mycobacteria

Group 1 - Photochromogens (<i>Mycobacterium kansasii</i> , <i>M. marinum</i> , <i>M. simiae</i>)
Group 2 - Scotochromogens (<i>M. scrofulaceum</i> , <i>M. szulgai</i> , <i>M. goodii</i>)
Group 3 - Nonphotochromogens (<i>M. avium-intracellulare</i> , <i>M. ulcerans</i> , <i>M. haemophilum</i> , <i>M. malmoense</i> , <i>M. xenopi</i> , <i>M. paratuberculosis</i>)
Group 4 - Fast growers (<i>M. fortuitum</i> , <i>M. chelonae</i> , <i>M. abscessus</i>)

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1) Opportunistic Infection of Non-Tuberculous Mycobacteria in AIDS

Non-tuberculous mycobacteria are important opportunistic pathogens in AIDS patients. Infection is often seen in the lung and lymph nodes, and infrequently in the bowel mucosa and skin. Granulomatous reaction is usually abortive in AIDS patients, and the infected macrophages are presented as "striated histiocytes", resembling Gaucher cell-like morphology (Figure II-X-1). The striated histiocytes show periodic acid-Schiff (PAS) reactivity and contain numerous acid-fast bacilli in the cytoplasm.

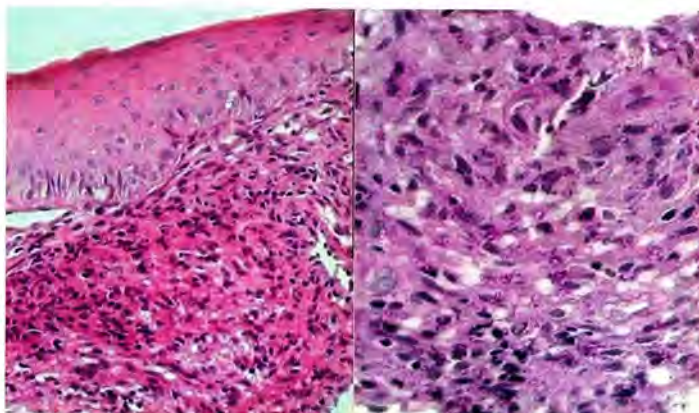


Figure II-X-1. Cutaneous non-tuberculous mycobacterial infection in AIDS. This Cambodian male presented multiple skin nodules. Biopsy reveals clusters of striated histiocytes in the dermis (left: H&E). PAS stain shows numerous large-sized rods in the cytoplasm of the macrophages (right). The courtesy by Dr. Chhut Vathana, a pathologist at Sihanouk Hospital Center of HOPE, Phnom Penh, Cambodia.

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2) BCG Inoculation

Formation of granuloma caused by live acid-fast *Bacillus Calmette-Guérin* (BCG), or *Mycobacterium bovis*, is a rare complication after BCG inoculation. BCG vaccine should be given intradermally. If given subcutaneously, it may induce local infection and spread to the regional lymph nodes, causing either suppurative or nonsuppurative lymphadenitis. When BCG is accidentally given to an immunocompromised patient, disseminated and life-threatening infection may happen. It becomes occasionally problematic when BCG is inoculated in infants within 3 months after birth. Regional lymph nodal involvement is more prominent in the skin lesion at the site of injection (Figure II-X-2-1). Acid-fast bacilli are observed in caseous necrosis of the granulomatous lesion with Ziehl-Neelsen's stain. The same situation is seen in the BCG inoculation therapy against superficial urinary bladder cancer (Note 23).

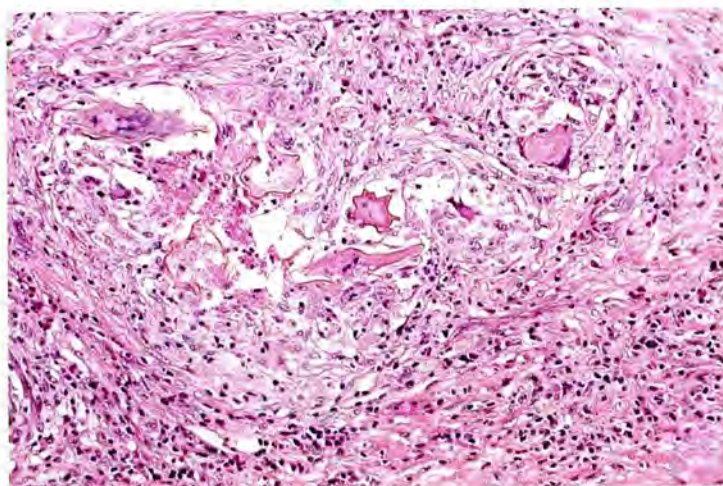


Figure II-X-2-1. BCG-induced lymphadenitis in infancy. Live acid-fast bacteria cause caseous granulomas in the cervical lymph nodal tissue (H&E).

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Note 23. *Mycobacterium bovis*-induced cystitis after intravesical BCG therapy

Intravesical therapy with BCG has proven effective in the prophylaxis and treatment of superficial urothelial carcinoma and carcinoma *in situ* of the urinary bladder. Instillations with BCG may provoke granulomatous inflammatory changes in the bladder (Figure II-X-2-2). This therapy may also cause systemic side effects varying from mild malaise and fever to, in rare instances, life-threatening systemic dissemination. In general, 95% of the patients have no serious side effects. Traumatic catheterization and concurrent cystitis are the risk factors for systemic BCG spread.

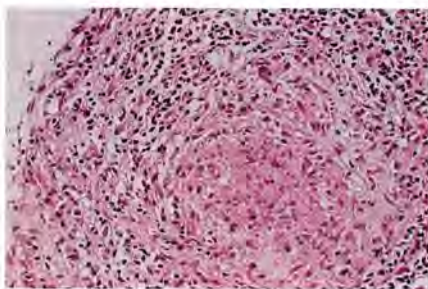


Figure II-X-2-2. BCG-induced cystitis. Intravesical inoculation of BCG (live *M. bovis*) against superficial urothelial carcinoma of the urinary bladder occasionally provokes intractable granulomatous cystitis. Biopsy reveals multifocal epithelioid granulomas with lymphocytic cuffing beneath the eroded urothelial mucosa.

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3) *Mycobacterium Avium* Infection

Mycobacterium avium and *M. intracellulare* comprise a group of genetically related saprotrophic mycobacteria, and collectively called as *M. avium-intracellulare* complex (MAI or MAC). *M. avium* subspecies *paratuberculosis*, commonly found in mammals, causes Jahne's disease (paratuberculosis), serious ulcerative bowel infection in cows, somewhat resembling Crohn's disease in the human. Numerous acid-fast bacilli proliferate in the cytoplasm of macrophages/epithelioid cells.

M. avium, widely distributed in the environment (soil and animal feces, especially excretions of the bird and deer), often causes human non-tuberculous mycobacteriosis, particularly in the lung. In immunocompromised patients, systemic infection may happen. In childhood, cervical lymphadenitis and skin infection caused by *M. avium* are encountered occasionally. Friedman, et al. reported that 9 of 11 cases of primary skin infection were in an immunocompromised state or after steroid administration.

Recently, the incidence of *M. avium* infection of the skin is increasing among Japanese children. The reason for this is related to the fact that "the 24-hour bathing system" becomes popular in many Japanese houses. Because of the high body temperature of the birds, *M. avium* can grow even at 45°C. In contrast, the optimal temperature of *M. marinum*, the causative agent of swimming pool granuloma or fish tank granuloma, is 30-32°C, and it cannot grow at 40°C. When one sees antibiotics-resistant multifocal skin ulcer or nodules in Japanese children, the possibility of *M. avium* infection should be suspected. Children suffering from atopic dermatitis are especially susceptible to this infection. Microscopically, multifocal necrotizing granulomas are observed in the dermis (Figure II-X-3). Multibacillary infection may be encountered. Infection via the hair follicle has been suspected. The point of diagnosis is to ask a history of "the 24-hour bath" taking.

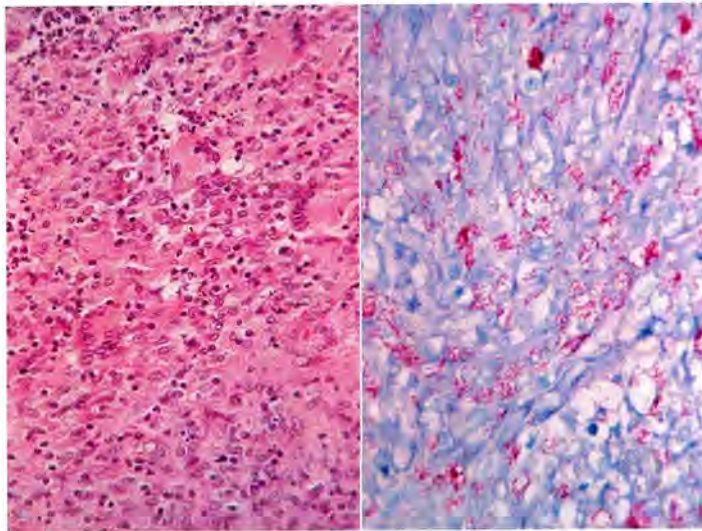


Figure II-X-3. *Mycobacterium avium* infection seen in the chest skin of a 7-year-old male. Xanthogranulomatous reactions with Langhans-type giant cells somewhat resemble the histology of lepromatous leprosy (left: H&E). Ziehl-Neelsen stain (right) reveals numerous acid-fast bacilli in the cytoplasm of foamy histiocytes.

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4) *Mycobacterium Marinum* Infection

The infection occurs when the exposed skin, particularly hand, is exposed to *Mycobacterium marinum* (marinum means “sea”) by superficial trauma against sea rock or the wall of the swimming pool. This lesion has a nickname of swimming pool granuloma. Dark red-colored, irregularly elevated and ulcerated infiltrative lesions are histologically composed of non-caseous epithelioid granulomas in the dermis. Scarring is associated. Delay of diagnosis is common, and invasion into deeper structures such as the synovia, bursae and bone occurs in approximately one third of the reported cases. Infection of *M. marinum* is usually confirmed by PCR analysis.

Representative features of swimming pool granuloma and invasive bursitis are demonstrated in Figures II-X-4-1 and II-X-4-2, respectively.

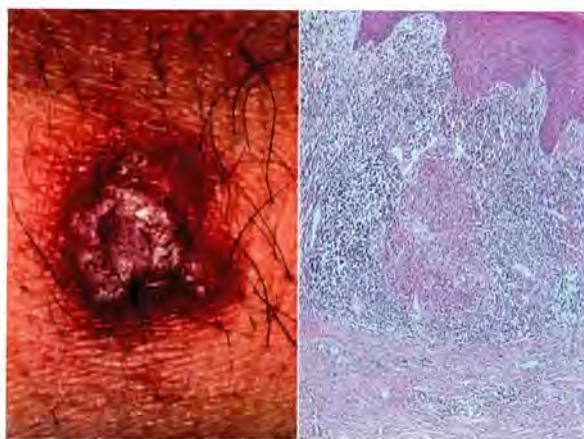


Figure II-X-4-1. Cutaneous *Mycobacterium marinum* infection. A dark red-colored, irregularly elevated infiltrative lesion is seen on the dorsum of the hand (left). Histologically, non-caseous epithelioid granuloma is formed in the dermis (right: H&E). Fibrotic scarring is associated. Infection of *Mycobacterium marinum* was confirmed by PCR analysis.

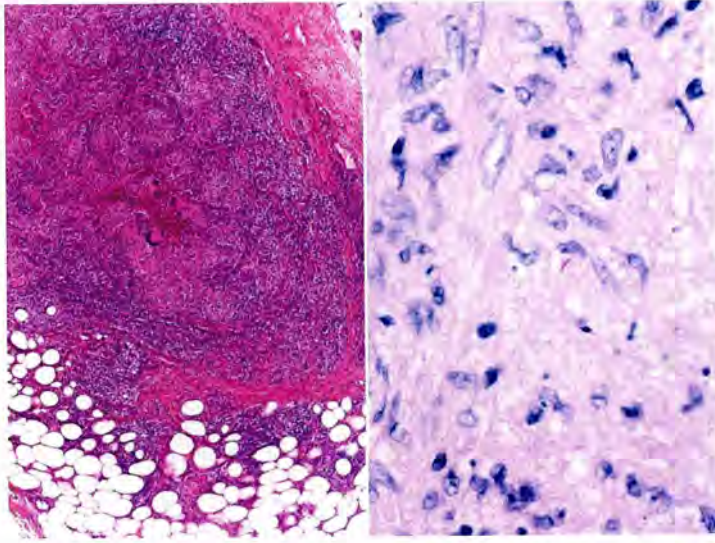


Figure II-X-4-2. Deep involvement by *Mycobacterium marinum*. The subcutaneous tissue and bursa are involved in this case (left: H&E). A few acid-fast bacilli are observed with Ziehl-Neelsen stain (right).

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5) *Mycobacterium Abscessus* Infection

Mycobacterium abscessus is a rapidly growing *Mycobacterium*, a common water contaminant. *M. abscessus* grows at 28°C and 37°C, but not at 43°C, and causes chronic lung disease, otitis media, post-traumatic wound infection and disseminated cutaneous diseases, often in patients with suppressed immune systems. Iatrogenic infection due to contamination of the organisms in xylocaine solution for local anesthesia has been reported by Rodriguez et al. The infection may follow tattooing.

Skin infected with *M. abscessus* is usually red, warm, tender to the touch, swollen, and/or painful. Boils or pus-filled vesicles may be seen. Fever, malaise, chills and muscle aches are commonly associated. For the definite diagnosis, the organism has to be cultured from the infection site. PCR analysis is also available.

Histologically, formation of suppurative granuloma (abscess surrounded by epithelioid granuloma) is most common (Figure II-X-5-1), but predominantly neutrophilic abscess with scanty granulomatous reaction may be encountered, hence the bacterial name given (Figure II-X-5-2).

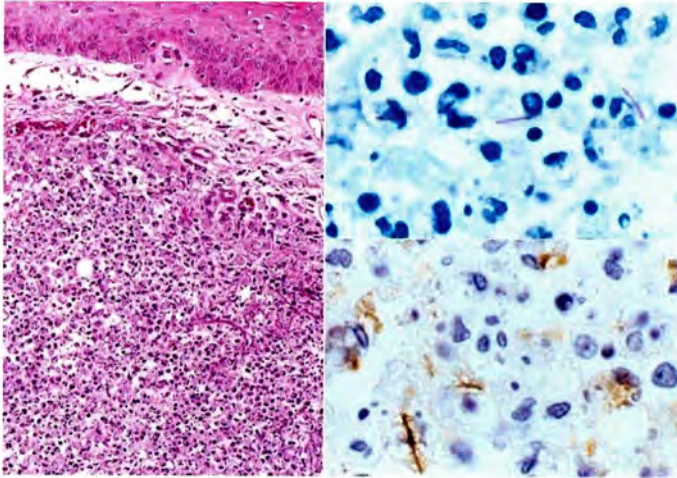


Figure II-X-5-1. Cutaneous *Mycobacterium abscessus* infection (I). Well-developed suppurative granuloma is formed in the dermis (left: H&E). Ziehl-Neelsen stain (right top) and immunostaining for BCG antigens (right bottom) identify intracellular growth of large-sized acid-fast rods.

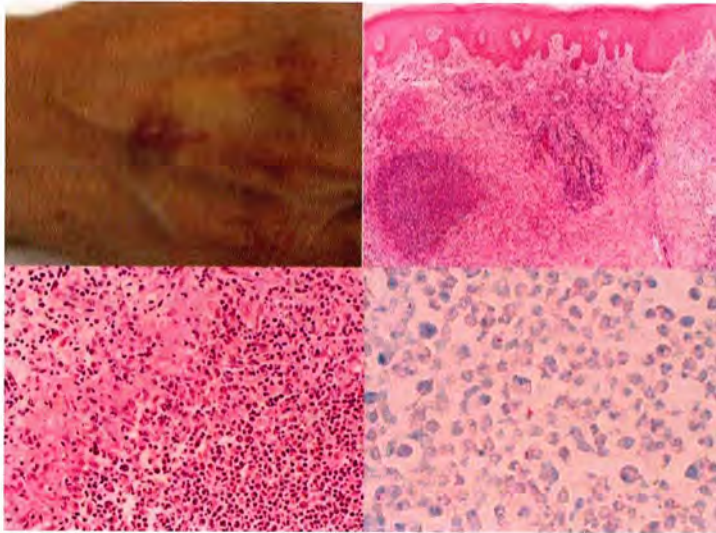


Figure II-X-5-2. Cutaneous *Mycobacterium abscessus* infection (II). Red nodules are clustered on the back of the hand (left top). Biopsy reveals multifocal intradermal abscess lesions (right top: H&E), and higher powered view clarifies epithelioid granulomatous reactions around the abscess cavity (left bottom). Acid-fast bacilli are phagocytized by neutrophils (Ziehl-Neelsen's stain: right bottom). The courtesy by Dr. Takashi Tsuchida, a pathologist at Hamamatsu Medical University Hospital, Hamamatsu, Japan.

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6) *Mycobacterium Ulcerans* Infection (Buruli Ulcer)

Buruli ulcer (large painless ulcer) caused by *Mycobacterium ulcerans*, is seen in a total of 32 central and west African countries. Buruli is the name of an endemic area in Uganda. Each West African country has established the National Buruli Ulcer Control Programme. The incidence is relatively high among people living in marshy places or along the river. The leg skin is affected. Recently, infection of this type is observed also in Japan, and *M. ulcerans* subsp. *shinshuense* was identified. It has been reported that a wide variety of animals, fish, water insect and river shrimps become a carrier or reservoir of *M. ulcerans*. Diffuse coagulative necrosis with little cellular reaction is formed in the dermis though subcutaneous tissue (to form so-called “fat cell ghost”). Undermining ulceration is characteristic. Granulomatous reaction is scarcely seen. *M. ulcerans* secretes toxic lipid substance named mycolactone, which induces tissue necrosis and suppresses host immune reaction. Ziehl-Neelsen acid-fast stain reveals numerous clumps of acid-fast bacilli within the lesion. The acid-fast bacilli often form globular structures in the necrotic tissue. They are extracellular in location. Representative features are shown in Figure II-X-6.

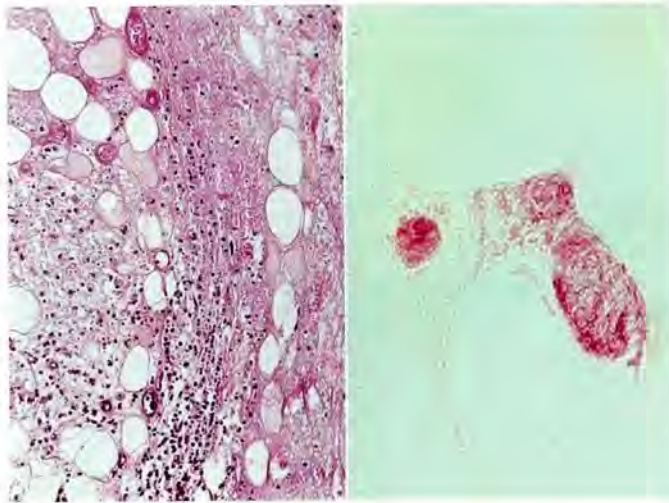


Figure II-X-6. Buruli ulcer. Buruli ulcer, *Mycobacterium ulcerans* infection, on the leg of a Kenyan adult. Diffuse coagulative necrosis without granulomatous reaction is formed in the dermis though subcutaneous tissue (left: H&E). Ziehl-Neelsen stain (right) reveals numerous extracellular clumps of acid-fast bacilli among the subcutaneous fat cells. The acid-fast bacilli often form globular structures in the necrotic tissue.

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Y) Hansen's Disease (Leprosy)

Hansen's disease or leprosy, caused by *Mycobacterium leprae*, predominantly affects the skin, nasal cavity, larynx, iris, testis, and superficial peripheral nerves. *M. leprae* is an obligate aerophilic intracellular acid-fast bacillus, categorized in the Gram-positive microbe. Gram stain is, however, useless for detecting acid-fast bacilli in the specimen. It is non-culturable *in vitro*, but grows in nude mice and armadillo. This chronic debilitating disease is endemic in tropical and subtropical countries, particularly in the Indian subcontinent, Southeast Asia, sub-Saharan Africa and Brazil. In Japan, only a few new cases appear in the southwestern islands, including Okinawa, and certain numbers of cases are yearly imported from South America. The mode of transmission is supposed to be airborne. Because of low infectivity, direct person-to-person transmission does not occur. The incubation period is thought to average 5 years.

Leprosy shows a spectrum of clinical characteristics, which are closely correlated with histopathological and immunological features. At both ends, there are tuberculoid leprosy (paucibacillary leprosy) and lepromatous leprosy (multibacillary leprosy). Cellular immune reaction is active in the tuberculoid type, resulting in granulomatous tissue reactions. The lepromatous type is associated with defective cellular immune response, so that numerous acid-fast microbes are demonstrated in the cytoplasm of foamy macrophages. Clinical forms between these poles are as follows: borderline-tuberculoid (BT), borderline (BB) and borderline-lepromatous (BL) types.

Extracutaneous involvements in cases of lepromatous leprosy include mucosal lesions of the head and neck and visceral lesions. The mucosal lesions include nasal septal defect, laryngeal stenosis/deformity and iridocyclitis. The rate of blindness is very high in leprosy patients, because of anterior ophthalmitis, exogenous infection secondary to trauma caused by loss of sensation, and nerve palsy resulting in failure of eye closing. Visceral lesions include leproma formation in the liver, spleen, lymph nodes, adrenal glands and testis. Superficial peripheral nerves, particularly the sensory component, are the target of infection, while visceral nerve involvement is infrequent. The lepromatous lesions preferentially occur in the organs of low temperature exposure, such as the skin and mucosal (nose and throat) lesions, peripheral nerves of body surfaces, and the testicular and epididymal parenchyme. In the skin, acral regions of the extremities, eye lids, nose and ear lobes are especially vulnerable to be involved. The thyroid gland, ovary and placenta are usually spared.

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1) Tuberculoid Leprosy

Tuberculoid leprosy is clinically characterized by erythematous swelling and decreased sensation (numbness) of the involved skin. There are one or several sharply defined reddish brown anesthetic plaques on the trunk and limbs. The distribution of the skin lesions is asymmetrical. Superficial nerve swelling is characteristic (Figure II-Y-1-1). Skin biopsy shows intradermal formation of epithelioid granulomas without caseous necrosis (Figure II-Y-1-2).

The papillary dermis is involved. Acid-fast staining is negative or scarcely positive, thus called as paucibacillary type. Hints for histopathological diagnosis include the identification of dermal peripheral nerve involvement by the granulomatous lesion. Host immune cells show strong reactivity against *Mycobacterium leprae*, to provoke granulomatous reaction, thus resulting in paucity of the microbes.



Figure II-Y-1-1. Tuberculoid leprosy. Asymmetrical erythematous swelling of the 3rd finger is seen (left). Thickening of the superficial nerve fascicles is characteristic of tuberculoid leprosy (right).

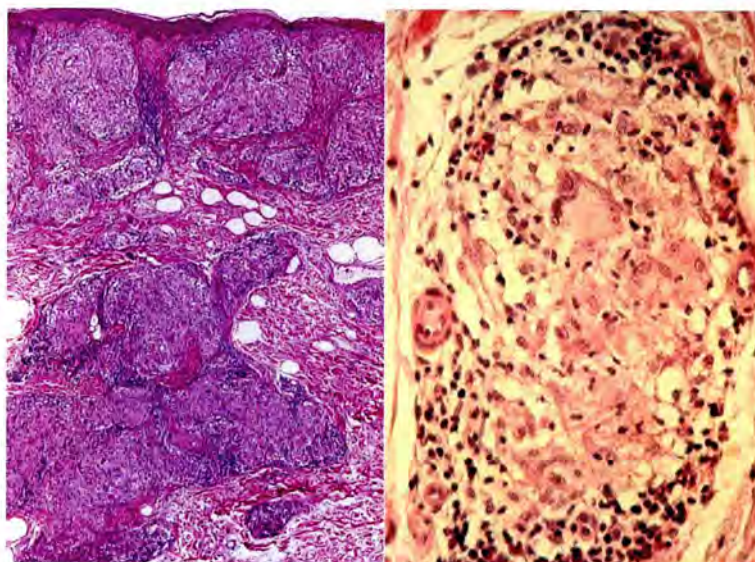


Figure II-Y-1-2. Histology of tuberculoid leprosy. Non-caseating epithelioid granulomas are formed in the dermis, including the papillary dermis (left: H&E). In the tuberculoid granuloma, foamy cells are scarcely noted (right: H&E). Acid-fast staining is negative. Diagnostic hint is the granulomatous involvement of the peripheral nerve fibers.

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2) Lepromatous Leprosy

The skin lesions of lepromatous leprosy, commonly symmetrical, include multiple small macules, infiltrative plaques and poorly defined nodules (Figure II-Y-2-1). Vitiligo is frequently accompanied. Multiple facial nodules give a bovine or lion-like appearance. Eyebrows become sparse. Mucosal involvement may lead to ulceration of the nasal septum. Laryngeal involvement may result in suffocation to death. Peripheral nerve lesions are the cause of acral anesthesia, claw hand and foot drip. Neuralgia often embarrasses the patients.

Incision at the senseless skin lesion gives a tissue exudate, and the multibacillary lesion shows acid-fast bacilli on the smear preparation (Figure II-Y-2-2). This makes a quick diagnostic test.

Histologically, infiltration of foamy histiocytes (xanthogranuloma) is seen in the dermis. In sharp contrast to tuberculoid leprosy, the papillary dermis is spared. The xanthogranulomatous lesion is formed around peripheral nerve fascicles in the deep dermis, an important hint for the diagnosis of lepromatous leprosy (Figure II-Y-2-3). The acid-fast bacilli are densely clustered within the cytoplasmic vacuoles of foamy histiocytes. This

unique vacuolated structures called "globi" are clearly demonstrated by the Fite method of Ziehl-Neelsen stain. The "globi" are positively stained with periodic acid-Schiff (PAS) sequence. Colloidal iron stain demonstrates rich hyaluronic acid in the globi formed by *M. leprae* which utilizes this acidic substance as a source of proliferation (Figure II-Y-2-4).



Figure II-Y-2-1. Lepromatous leprosy. Symmetrical skin lesions are commonly presented, such as multiple small macules, infiltrative plaques and poorly defined nodules. Facial skin is frequently involved, and hair is lost.



Figure II-Y-2-2. Skin incision testing. Because of the numbness, the skin lesion can be incised by a knife (left). The smear preparation reveals acid-fast bacilli in the cytoplasm of macrophages (right: Ziehl-Neelsen).

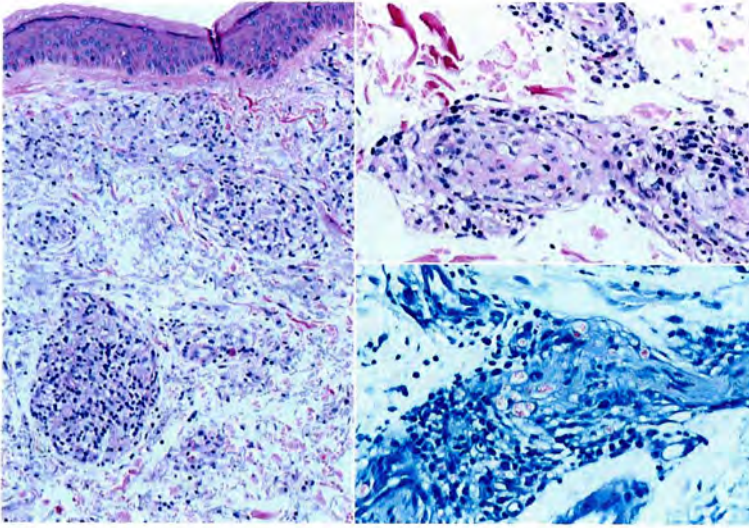


Figure II-Y-2-3. Histology of lepromatous leprosy. Xanthogranulomatous lesions consisting of foamy macrophages are scattered in the dermis, but the papillary dermis is spared (left: H&E). The dermal nerve fascicles are involved by the granulomatous lesion, which characteristically contain globi, large cytoplasmic vacuoles (right top: H&E). Numerous acid-fast bacilli are detected in the globi (right bottom: the Fite method).

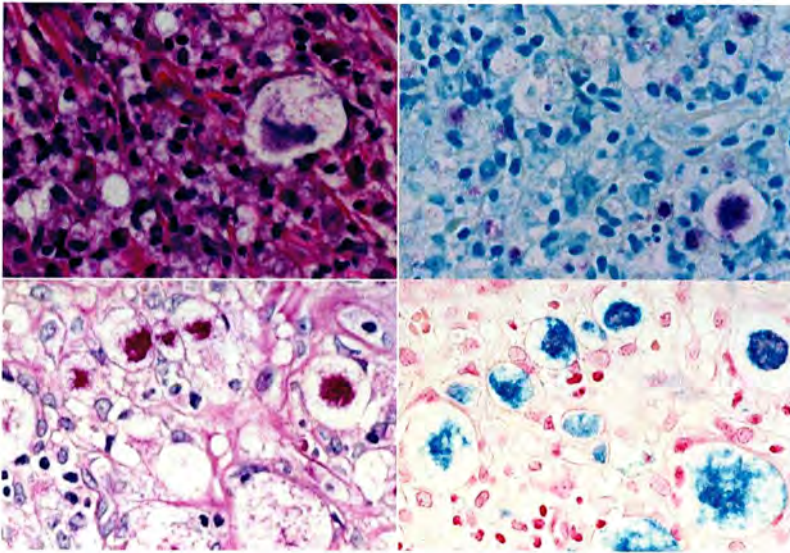


Figure II-Y-2-4. Histochemical features of globi. The globi represent large cytoplasmic vacuoles in the foamy macrophages. Clusters of long rods are visible even with H&E staining (left top). The clustered bacilli are positively stained with the Fite method (right top), periodic acid-Schiff (left bottom) and colloidal iron stain (right bottom).

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Note 24. Special stains for *Mycobacterium leprae*

In order to identify *Mycobacterium leprae* reproducibly, Fite's method should be used for acid-fast (Ziehl-Neelsen) staining. Regular procedure results in much less intense staining and fewer stained bacilli (Figure II-Y-2-5). In the Fite's method, deparaffinization should be done by using oil-xylene (a 1:1 mixture of peanut oil and xylene). After adsorption of oil-xylene by filtration paper and without soaking in ethanol, the sections are stained in carbol-fuchsin solution. The reason for this is because *M. leprae*-infected leprosy cells are rich in neutral fat and phospholipid. Kinyoun method has also been used for demonstrating weak acid-fast nature.

The lepra cells containing large cytoplasmic vacuoles (globi) accumulate a large amount of hyaluronic acid, so that colloidal iron staining is positive in lepromatous leprosy cases. *M. leprae* utilizes sugar moieties for growth by cleaving hyaluronic acid.

As described above (Note 21), immunostaining with BCG rabbit antiserum (Dako) is useful to identify acid-fast bacilli, including *M. leprae*, in routinely prepared sections.

We happened to have a chance to examine more than 800 autopsy cases of a leprosy sanatorium Oku-Komyoen, Okayama, Japan, and the tissue were kept fixed in formalin for years (up to 70 years). The director, Dr. Masanao Makino, kindly allowed us to evaluate the precious samples. *M. leprae* and *M. tuberculosis* were reproducibly identified in paraffin sections with the BCG immunostaining. The antigenicity of mycobacterial proteins was found to be very stable (Figure II-Y-2-6). The cause of death of the leprosy patients in pre-Promin (glucosulfone sodium) era was tuberculosis and less frequently lobar pneumonia.

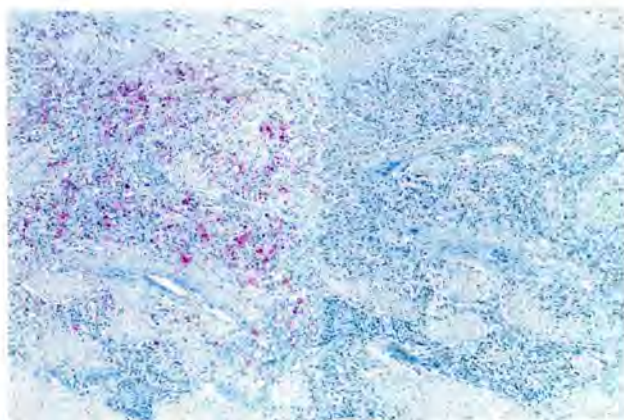


Figure II-Y-2-5. Fite's method for demonstrating *M. leprae*. The Fite's method strongly stain the bacilli in the globi (left), while the conventional Ziehl-Neelsen stain shows weak and focal reactivity (right).

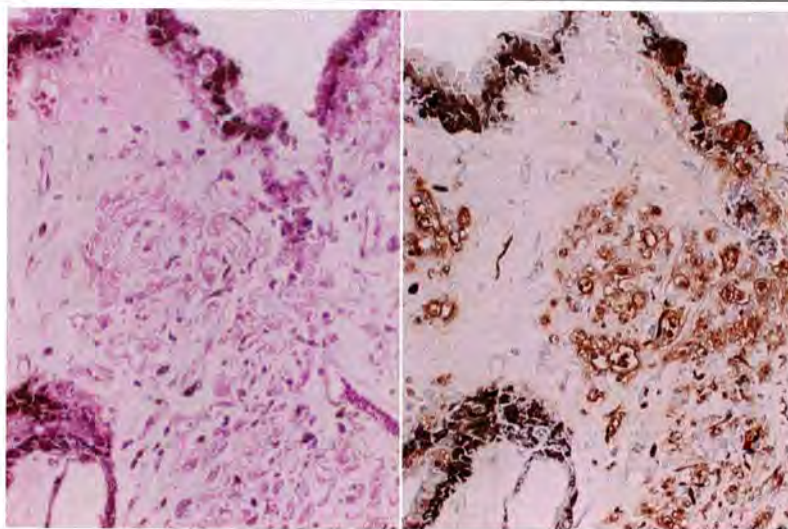


Figure II-Y-2-6. Immunostaining for BCG for identifying *M. leprae* in tissues fixed in a long period of time. Lepromatous iridocyclitis often leads the patient to loss of vision. With immunostaining for BCG antigens, *M. leprae* can stably be demonstrated in macrophages infiltrating in the iris tissue fixed in formalin for more than 60 years (left: H&E, right: immunostaining). Ciliary epithelial cells densely contain black-colored melanin pigment.

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Z) Infection-Related Erythema Nodosum-Like Lesions

Erythema nodosum presents painful erythematous nodular eruptions on the extremities, particularly on the shin bilaterally. Numbers of inflammatory disorders associate erythema nodosum as a remote skin manifestation. Allergic response to infection or systemic disease is a possible pathogenesis. Histologically, septal panniculitis with lymphocytic infiltration and foreign body granulomatous reaction is seen. Vasculitis (phlebitis) is frequently associated (Figure II-Z).

Erythema nodosum leprosum, a type (type II) of reactional states in leprosy, commonly occurs in lepromatous leprosy patients, particularly after the bactericidal treatment. Crops of painful, erythematous and violaceous nodules are seen on the extremities, and last for 1-3

weeks. Japanese patients call them as “hot lumps”. This may result from immune complex-mediated cutaneous vasculitis.

Erythema nodosum is also accompanied by a variety of infections caused by *Francisella tularensis*, *Brucella*, *Streptococcus*, *Neisseria meningitidis*, *Campylobacter*, *Salmonella*, *Tropheryma whipplei*, *Histoplasma*, *Coccidioides*, *Mycoplasma pneumoniae*, *Chlamydia psittaci*, *Mycobacterium tuberculosis*, *Dermatophytes* (*Kerion celsi*) and HIV. Erythema nodosum-like lesions may also be seen in cases of yersiniosis and legionellosis.



Figure II-Z. Erythema nodosum. Painful erythematous nodules are bilaterally seen on the extremities, particularly on the shin (left). Histologically, vasculitis (phlebitis) is observed in addition to septal panniculitis with lymphocytic infiltration and foreign body granulomatous reaction is seen (right: H&E).

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Note 25. Izumi fever (Far East scarlet-like fever)

Yersinia pseudotuberculosis, a low temperature-tolerant Gram-negative bacterium, causes Far East scarlet-like fever or Izumi fever in Japanese children, in addition to classic food poisoning illness. Prof. Sensuke Izumi, a pediatrician in Kanazawa, Japan, described this infectious disorder in 1929. The patients get infected through water and food contaminated with rats urine and feces. Izumi fever usually becomes apparent 5–10 days after exposure to the microbes and typically lasts 1–3 weeks if without treatment. The main clinical manifestations closely resemble scarlet fever, including fever, skin rash, desquamation, strawberry tongue, red and cracked lips, vomiting, diarrhea, abdominal pain, arthralgia, hepatomegaly and conjunctivitis. The clinical manifestations and courses of illness also resemble Kawasaki disease (mucocutaneous lymph node syndrome), which is endemic among Japanese children (refer to Note 8).

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AA) Syphilis

Long spiral Gram-negative bacteria such as *Treponema*, *Leptospira* and *Borrelia* cause spirochaetosis in the human. Leptospirosis, a systemic and zoonotic infection caused by *Leptospira interrogans* (Weil's disease), mainly affects the liver, kidney and meninges, causing jaundice and hemorrhagic spots. *Leptospira* can infect through the intact skin, but no skin lesion remains. *Borrelia* is a pathogen of Lyme disease and relapsing fever (refer to Chapter II-CC). Syphilis (lues) is sexually transmitted infection caused by *Treponema pallidum*. Typical microscopic features of *T. pallidum* are shown in Figure II-AA-0.

Syphilis is clinically divided into three stages. Hard chancre (painless ulcer on the external genitalia) and bubo indolenta (painless swelling of the groin lymph nodes) are noted in stage I. The latent period is three weeks on average, and the lesions soon disappear spontaneously. After around 8 weeks, stage II exanthemas appear at the mucocutaneous locations. These lesions also disappear spontaneously within weeks. After an indolent period for several years, stage III visceral involvement follows. These include syphilitic aortitis, neurosyphilis and gumma formation in various internal organs.

Histological features of the stages I and II lesions are similar. Massive mononuclear infiltration with numerous plasma cells and swollen endothelial cells is characteristic. Plasma cell infiltration is especially prominent in stage I. No granulomatous reaction is seen at stages I and II. Non-caseous granuloma (gumma) is observed in the stage III lesions. To confirm the diagnosis, special staining such as Warthin-Starry's silver stain or immunostaining is requested.

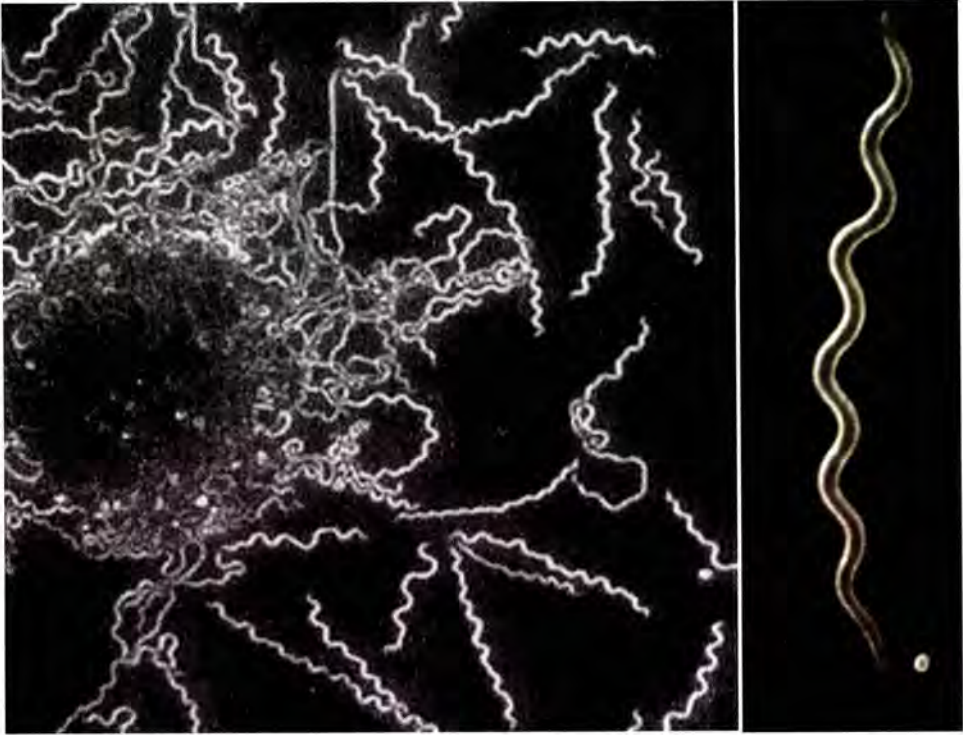


Figure II-AA-0. Microscopic features of *Treponema pallidum*. Dark field observation clearly illustrates long spiral-shaped bacteria forming colonies around the target cell (left). Scanning electron microscopic appearance is demonstrated in the right panel. The courtesy by Prof. Emeritus Yasutake Yanagihara, Shizuoka Prefectural University, Shizuoka, Japan.

The silver method is technically difficult and not so reproducible, so that immunostaining using specific monoclonal or polyclonal antibodies to *Treponema pallidum* is strongly recommended. Dr. Hideyo Noguchi, a well-known Japanese bacteriologist, made deep effort to identify spirochaetes in the brain of neurosyphilis by using silver impregnation techniques.

The key point of the correct histopathological diagnosis of clinically unsuspected syphilis for diagnostic pathologists by using immunostaining is to strike upon the possibility of syphilis in H&E-stained preparations.

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Note 26. Pathologists' roles in the diagnosis of sexually transmitted infection

Prompt and appropriate diagnosis of sexually transmitted infection (STI) is important not only for the patient and his/her sex partner(s) but also for the human society. Pathologists must realize the importance of the histologic and cytologic diagnosis, just as clinicians are requested so. In other words, pathologists must contribute to preventing transmission of STI. Changes in sexual behavior lead to the changes of the modality of STI. Many of the male patients suffering from gonorrhea or chlamydiasis contract the disease not through the direct contact of sexual organs but through the oral sex. It is of note that gonorrheal or chlamydial oropharyngitis in females is often asymptomatic.

Chlamydial infection of the cervix, oviduct and epididymis should be suspected by characteristic histological or cytological features (Hori and Tsutsumi). The diagnosis of syphilitic lesions is very difficult unless pathologists and/or clinicians suspect the possibility of syphilis (see Figures 0-1 and 0-2). Immunohistochemical confirmation is of essential importance here.

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1) Primary Syphilis

A deep but painless ulcer of the external genitalia (hard chancre) and painless swelling of the groin nodes (bubo indolenta) are the features of primary syphilis. Histologically, reactive squamous epithelial thickening with downward elongation is noted. In the dermis with marked mononuclear infiltration, plasma cells predominate, and endothelial cells are swollen.

Coiled spiral microbes are identified among the squamous epithelium with Warthin-Starry's silver, but more easily by immunostaining using a specific antiserum against *T. pallidum*. The spirochaetes are mainly localized in the intercellular space of the involved epidermis. Capillary walls in the inflamed dermis are also involved by the microbes. The lesion is highly contagious.

Representative features are shown in Figure II-AA-1.

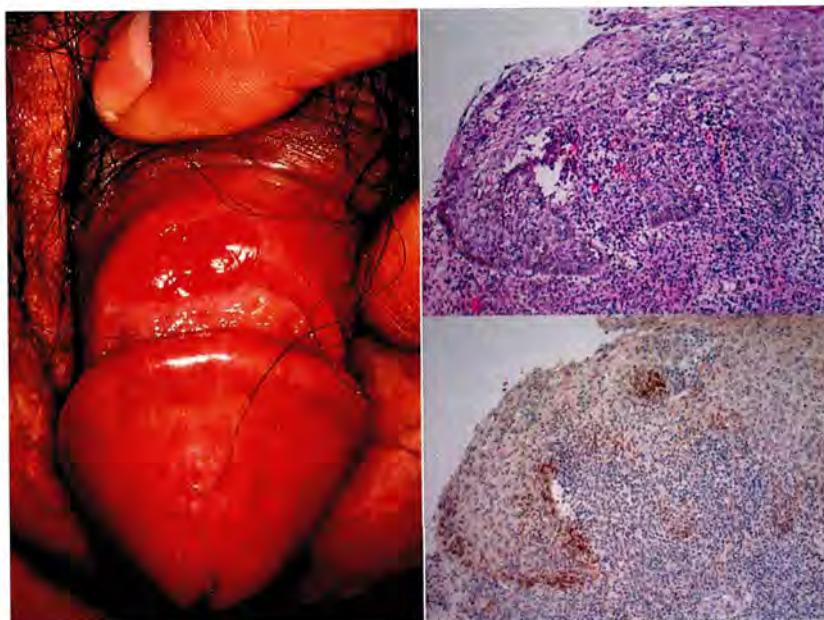


Figure II-AA-1. Primary syphilis. A painless deep ulcer (hard chancre) is observed at the corona glandis (left). Histologically, reactive acanthosis of the squamous mucosa and dense dermal infiltration of mononuclear cells are noted (right top: H&E). Coiled spiral microbes are clustered mainly among the squamous epithelium (right bottom: immunostaining for *Treponema pallidum* antigens).

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2) Secondary Syphilis

Several forms of skin lesions (syphilids) are clinically encountered in the secondary syphilis, including roseola spots on the trunk, syphilitic pustules and syphilitic frambesia on the palm, syphilitic psoriasis on the palm and sole, oral mucosal eruptions, and condyloma lata on the external genitalia (Figures II-AA-2-1 and II-AA-2-2). The palms and soles are the prerequisite site of the secondary syphilid. The secondary syphilid is seen 6-8 weeks after formation of the primary syphilitic lesion in the genitalia. Systemic manifestations include iritis, alopecia, mucous patches and severe pain in the head, joints and periosteum.

Skin biopsy shows irregular acanthosis and marked exocytosis with inflammatory edema in the dermis. The histological features seem to be nonspecific, except for the predominance of plasma cell infiltration. Without clinical information, it is often difficult for pathologists to go to the next diagnostic step using the histochemical approach. With the immunostaining for *T. pallidum* antigens, numerous long spiral microbes are observed among the squamous epithelial cells and around dermal capillary vessels, confirming the diagnosis of syphilis

(Figure II-AA-2-3). A case of 2nd stage syphilis in which histopathological diagnosis was quite effective is already shown in the Introduction (Figures 0-1 and 0-2). In Figure II-AA-2-4, a tumorous lesion on the hard palate is shown. The polypoid, vascularized and thus red-colored lesion with surface erosions resembled pyogenic granuloma, but numerous spiral pathogens were immunohistochemically identified among the vessels.

Syphilis may be co-infected with HIV. According to immunosuppression in AIDS, clinical and histological manifestations may be altered. Clinically, syphilis in HIV disease manifests as “malignant secondary syphilis (lues maligna)”, an aggressive ulcerating form of secondary syphilis. Widespread noduloulcerative or vesiculonecrotic lesions are formed. Serum anti-*Treponema* antibody titers tend to be low. In Figures II-AA-2-5 and II-AA-2-6, an HIV-seropositive Japanese male case manifesting fever and surface-eroded papular eruptions on the face, trunk and extremities is presented.



Figure II-AA-2-1. Syphilids in secondary syphilis (panel I). Roseola spots on the trunk (left), syphilitic papules on the arm (center) and hyperkeratotic eruptions on the palm (right) are shown.



Figure II-AA-2-2. Syphilids in secondary syphilis (panel II). Syphilitic pustules on the palm (left top), syphilitic frambesia on the palm (right top), psoriatic syphilids on the sole (left bottom) and condyloma lata around the anus (right bottom) are demonstrated.



Figure II-AA-2-3. A penile lesion in secondary syphilis. A penile induration in the secondary syphilis is shown (left). Biopsy confirms active mononuclear infiltration in the dermis and epidermotropic infection of *T. pallidum* (right top: H&E, right bottom: immunostaining).

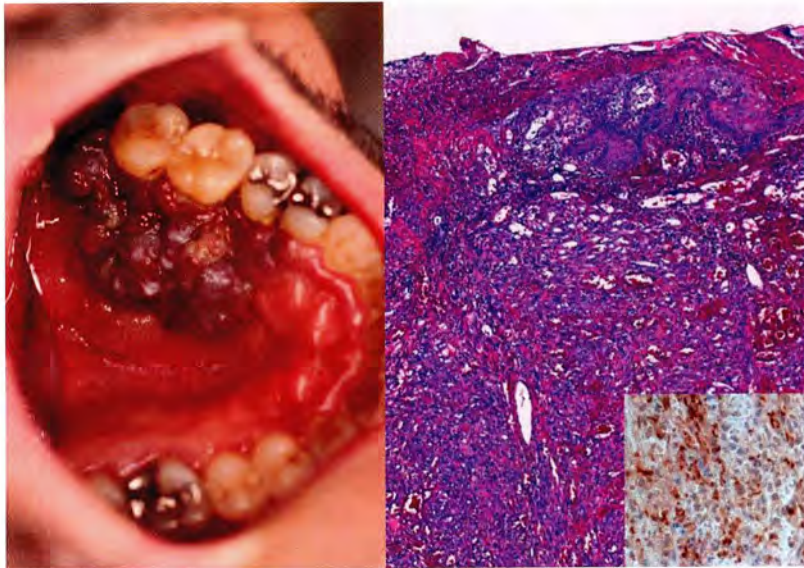


Figure II-AA-2-4. A syphilitic polypoid lesion formed in the oral cavity. Red-colored, multinodular and surface-eroded polypoid projections are seen on the hard palate (left). Highly vascularized histology somewhat resembles pyogenic granuloma (right: H&E). Numbers of spiral pathogens are identified among the vasculature (inset: immunostaining for *T. pallidum* antigens). The courtesy by Dr. Yuri Muroi, Department of Oral Surgery, Osaka Dental University, Osaka, Japan.

Histological features resemble “HIV-related CD8+ cutaneous pseudolymphoma”, showing dense infiltration of CD8+ small lymphocytes in both the dermis and epidermis. Plasma cells are hardly noted in the lesion. The vascular endothelial cells are swollen.

Infection of *T. pallidum* is proven immunohistochemically around dermal vessels and within the epidermis. Antibiotic therapy completely cleared the skin lesions.

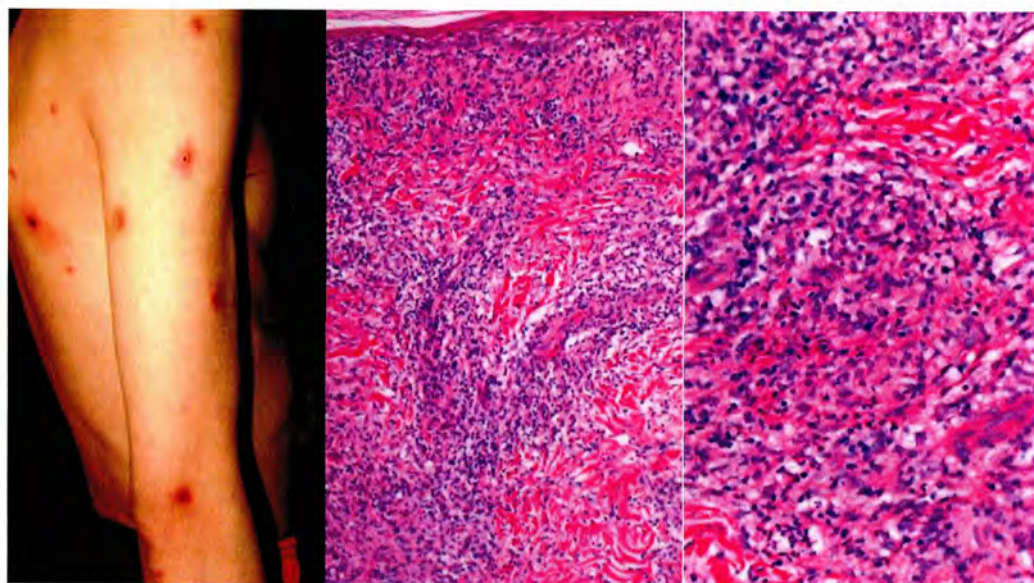


Figure II-AA-2-5. Secondary syphilid in a Japanese AIDS patient (I). Multiple eroded papules are distributed on the arm and back (left). Histologically, dense infiltration of small lymphocytes is observed around the dermal vessels accompanying endothelial swelling. Activated macrophages are also observed. Epidermotropism is also associated (H&E, center: low power, right: higher power). The courtesy by Dr. Michiko Yamashita and Dr. Yoshio Urano, a pathologist and dermatologist at Tokushima Red Cross Hospital, Komatsushima, Japan.

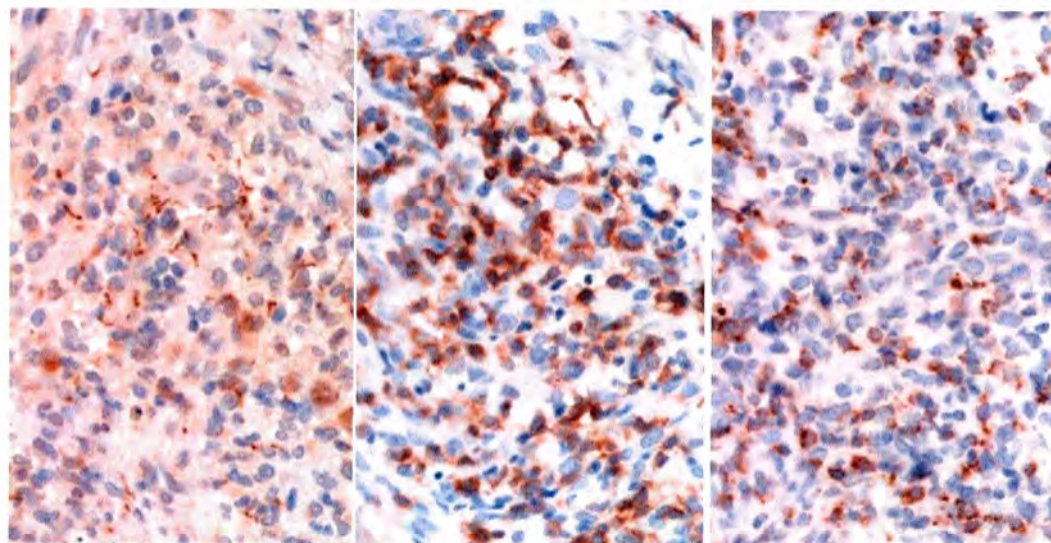


Figure II-AA-2-6. Secondary syphilid in a Japanese AIDS patient (II): malignant secondary syphilis. Immunohistochemically, numbers of *Treponema* are clustered among the lymphoid infiltrate, confirming the diagnosis of syphilis (left). Most of the lymphocytes express CD8 (center) and granzyme B (right), an enzyme of killer NK/T cell granules, and therefore, the histological features resemble those of HIV-related CD8⁺ cutaneous pseudolymphoma.

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3) Tertiary Syphilis

The tertiary syphilis is microscopically features by epithelioid granuloma with extensive necrosis, so-called gumma. Palatal or nasal involvements result in perforation and deformity. Cutaneous tertiary syphilis is also a frequent occasion (Figure II-AA-3-1). The number of pathogens is sparse, due to cellular immune reaction against *Treponema*. Figure II-AA-3-2 shows microscopic features of granulomatous lymphadenitis in the tertiary syphilis.



Figure II-AA-3-1. Skin lesions in tertiary syphilis. Large ulcerative and cratered lesions are multifocally formed on the back. The courtesy by Prof. Emeritus Yasutake Yanagihara, Shizuoka Prefectural University, Shizuoka, Japan.

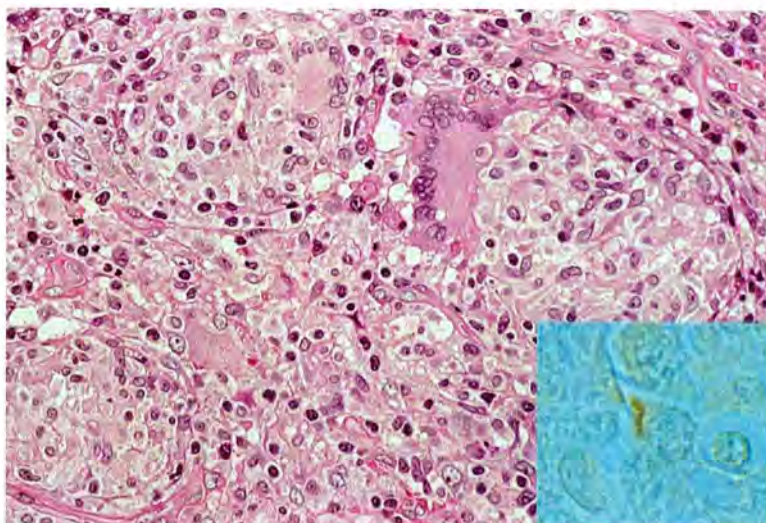


Figure II-AA-3-2. Gummatous lymphadenitis. The biopsied swollen lymph node shows epithelioid granulomatous reaction (left: H&E). Immunostaining for *Treponema pallidum* identifies a few spiral-shaped microbes in the granulomatous lesion (right), confirming the histopathological diagnosis of clinically unsuspected tertiary syphilis with gumma formation.

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Note 27. Medical students' activity to change the alphabetical spelling of the name of a hotel in their own city

In Maebashi, the capital city of “Gunma” prefecture, there is a national university named Gunma University. A group of medical students of Gunma University once made a deep effort to change the alphabetical spelling of the name of an international hotel located in the center of Maebashi city. There was a big sign board in front of the hotel, describing something like “Gumma XXX Hotel”. Phonetically, the pronunciation of “Gunma” is very close to “Gumma” in Japanese, but literally it should be written as “Gunma”, as is so in the names of the prefecture and university. The medical students persuaded and warned the hotel manager repeatedly to change the alphabetical spelling from tertiary syphilis to the healthy one. Finally, they succeeded! Now, you can stay in these international hotels very safely, I believe.

Note 28. History of syphilis in Japan

In 1494, one year after the Christopher Columbus' return from the North American Continent, a highly contagious and often lethal disorder, syphilis, prevailed in Spain, France, Italy and other European countries. It is believed that *Treponema pallidum* was transformed from *Treponema pertenue*, a pathogen causing frambesia (yaws), and the transmission form was simply changed from contact skin transmission to sexual transmission. In 1497, Vasco da Gama, a Portuguese explorer (1460-1524), led his ships to the East to reach the Southeast Asian countries. The first description of syphilis in Okinawa and Nagasaki, Japan, appeared in 1512. The sexually transmitted infection ran throughout Japan from south to north within one year.

In 1603, Ieyasu Tokugawa, the first Shogun (General) of the Edo Era, established stable, peaceful and culture-rich Japanese society in the Edo Era (Edo is the ancient name of Tokyo). The Japanese society was soon shut out from the outside countries, except for the continuing business with the Netherlands (Holland), China, Korea and Ryukyu (Okinawa). Ryukyu was an independent kingdom at that time. Edo city contained one million population (the biggest city in the world at that time), and the roads connecting Edo and other cities were well developed. Water and sewer services were provided.

Public prostitute houses were established in 1626, and the prevalence rate of syphilis was extremely high (up to 80%) among Edo prostitutes. Alopecia was a well known symptom of syphilis (at the 2nd stage). In the 3rd stage, the gummatous lesions extensively involved the nose (the lack of nose), skin (rash and nodules), joints (fulminant arthralgia), ear (deafness) and eyes (blindness), and finally the patients could not walk and swallow (central nervous system involvement).

At the end of the Edo Era after re-opening of the country in 1854, William Willis, a British medical doctor, pointed out that one third of men in the Edo area suffered from syphilis. Many European medical doctors documented with surprise that most Japanese citizens accepted this sexually transmitted infection as a destiny ("Sadame" in Japanese) in the daily life and living. The health check system for the prostitute was first introduced in Yokohama, a new city with the main port open to foreign countries, in 1868 by George B. Newton, a British military doctor. However, there was no effective treatment to kill the microbes at that time. Mercuric chloride was given to the patients as one of the medicines, which were believed to be effective, but terrible side effects were inevitable.

Treponema was identified by Fritz Shaudinn and Eric Hoffman in 1905, Salvarsan 606 was synthesized by Sahachiro Hata and Paul Ehrlich in 1910, and penicillin, the first antibiotics, was found by Alexander Flemming in 1929 and clinically applied by Howard W. Florey and Ernst B. Chain in 1940.

References

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BB) Frambesia (yaws) and Pinta

Frambesia, or yaws, is non-venereal treponematoses endemic in the tropical woodland in West Africa, Indonesia, Papua New Guinea and the Oceanian islands. *Treponema pertenue* mainly affects children. Flies transmit infection. *T. pertenue* is indistinguishable from *T. pallidum* both morphologically and serologically.

Similar to syphilis, three clinical stages are seen. In the first stage, ulcerating papules are formed on the leg. In the second stage, multiple yaws appear widespread on the skin (Figure II-BB-1).

Well-demarcated nodular and papillomatous lesions grossly resemble wild raspberry (or framboises in Scotland). Histologically, dense dermal infiltration of lymphocytes and plasma cells is noted, together with pseudoepitheliomatous hyperplasia of the epidermis. Warthin-Starry's silver demonstrates spiral pathogens among the epidermis and in the exudate (Figure II-BB-2). After long latent periods for years, the third stage gummatous ulcers form on the central face and extremities with underlying bone involvement. Neither brain nor aortic lesions are seen.

Another endemic treponematoses, named pinta, is seen in the Caribbean islands, Central America and tropical South America. Transmission is caused by skin-to-skin contact. The causative microorganism is *Treponema carateum*. The skin lesions of pinta become extensive, showing peripheral extension and often coalesce. Widespread scaly plaques are long-lasting and show a striking variety of colors: red, pink, slate blue and purple. In a late stage, depigmentation and epidermal atrophy follow (Figure II-BB-3).



Figure II-BB-1. Frambesia (yaws) in Papua New Guinean children. Well-demarcated nodular and papillomatous lesions are multifocally observed on the face (left) and the lower extremities (right). Yellowish color is characteristic. The courtesy by Prof. Emeritus Yasutake Yanagihara, Shizuoka Prefectural University, Shizuoka, Japan.

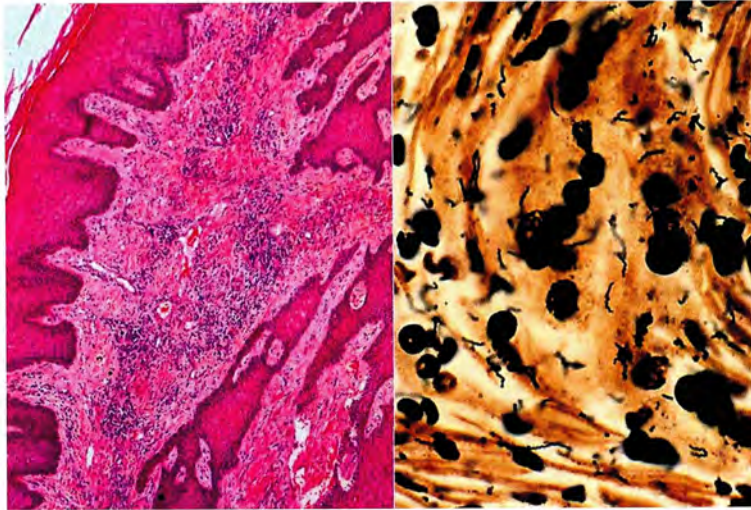


Figure II-BB-2. Histology of frambesia. Pseudoepitheliomatous hyperplasia of the epidermis and infiltration of lymphocytes and plasma cells are observed (left: H&E). Warthin-Starry's silver shows spiral pathogens in the surface exudates (right). The courtesy by Late Prof. Robin A. Cooke, Mayne Medical School, University of Queensland and Pathology Queensland, Brisbane, Australia.



Figure II-BB-3. Pinta in Mexico. Advanced leg skin lesions of pinta show marked scar fibrosis with an admixture of pigmentation and depigmentation. The courtesy by Prof. Emeritus Yasutake Yanagihara, Shizuoka Prefectural University, Shizuoka, Japan.

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CC) Borreliosis

1) Relapsing Fever

Borreliosis, categorized in human spirochaetosis, is mediated by arthropod vectors. There are two types of borreliosis affecting the human: Lyme disease and relapsing fever. Relapsing fever, a recurring and protracted febrile illness, is further divided into two forms. Louse-borne relapsing fever is caused by *Borrelia recurrentis*, while tick-borne relapsing fever is caused by at least 15 different *Borrelia* species such as *B. hermsii*, *B. turicatae*, *B. parkeri* and *B. duttonii*.

Louse-borne type and tick-borne type show significant epidemiological differences. The former transmitted by *Pediculus humanus* causes epidemic relapsing fever in tropical and subtropical areas, particularly in Africa, while the soft tick (*Ornithodoros*)-transmitted one is categorized in endemic relapsing fever with worldwide distribution. Humans are the sole host of *B. recurrentis*, while mammals (cattle, pigs, prairie dogs, squirrels and chipmunks) and reptiles (lizards, snakes and gopher tortoises) may serve as a reservoir for tick-borne *Borrelia* species. The mortality rate is higher in the former.

Borrelia species can vary the expression of outer surface antigens during the disease course. Alteration of the proteins, encoded in plasmid DNA, prevents elimination of the spirochetes by the immune system, leading to recurrent febrile episodes. It is known that interleukin-10 plays a protective role in down-regulating inflammation and spirochete load.

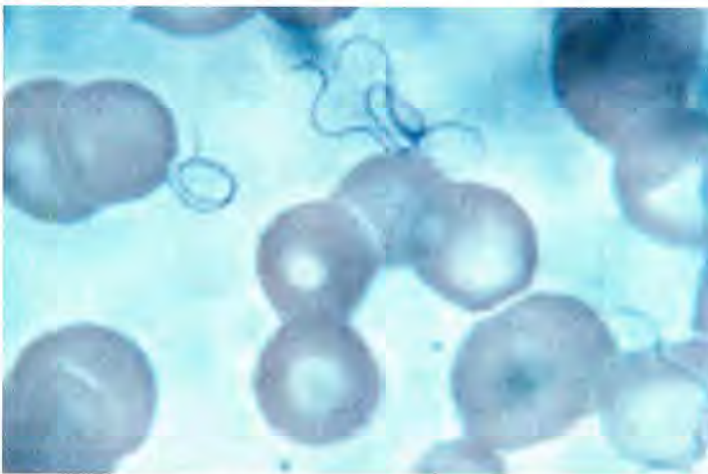


Figure II-CC-1. Peripheral blood smear in relapsing fever. Large-sized spirochetes appear in the peripheral blood at the initial stage of illness. *Borrelia* is easily stained with May-Giemsa when compared with other spirochete species. The courtesy by Prof. Emeritus Tomio Yamaguchi, Hirosaki University School of Medicine, Hirosaki, Japan.

After exposure to an infected louse or soft tick, spirochetes enter the dermis and gain access to the bloodstream where they infect the endothelium. The latent period is 4-14 days. There is an acute onset with high fever, arthralgia, myalgia, headache and dry cough. After the primary illness lasting for 3 days, a fever-free period for about 7 days follows. Usually, there are 3-4 relapses. The organisms disseminate to the liver, spleen, bone marrow, lung, kidney and central nervous system to eventually provoke disseminated intravascular coagulopathy. In the initial stage, spiral pathogens appear in the peripheral blood (Figure II-CC-1). Meningitis, hepatitis and myocarditis may be complicated.

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2) Lyme borreliosis

Lyme borreliosis is a multi-organ infection caused by *Borrelia* spp., particularly *Borrelia burgdorferi*, which is transmitted by the hard tick *Ixodes scapularis* or other *Ixodes* species. Lyme disease was named after the community in Connecticut, USA, where the disease was originally recorded. Common skin manifestations of Lyme borreliosis include erythema chronicum migrans, acrodermatitis chronica atrophicans and low-grade B-cell lymphoma. The latter two conditions are usually caused by *B. garinii* and *B. afzelii*, respectively, which are more common in Europe than in America. Late extracutaneous manifestations, very rare in Japanese cases, include carditis, neuroborreliosis and arthritis.

The pathognomonic skin lesion is centrifugally spreading erythema at the site of tick bite. The annular lesion measures 5-20 cm in diameter and is seen within 3 months of the tick bite (Figure II-CC-2-1). The diagnosis is fundamentally based on clinical findings and confirmed serologically. Diagnostic problems arise from patients with non-specific symptoms but with positive IgG serology. In 80% of the patients, the disease can be cured by antibiotic therapy.

Histological features of erythema chronicum migrans are nonspecific. There is superficial and deep perivascular and interstitial infiltration of small lymphocytes (Figure II-CC-2-2). Plasma cells and eosinophils may be intermingled. The pathogenic spiral microbes are very sparse, so that it may be difficult to identify the pathogens within the biopsied tissue. PCR has been applied for the molecular detection of *B. burgdorferi* in formalin-fixed, paraffin-embedded skin biopsy specimens.

In acrodermatitis chronica atrophicans, inflammatory edema is seen on the extensor surfaces of the extremities and areas around the joint. Skin atrophy gradually progresses with hypopigmentation and loss of the elastic fibers and appendages. Juxta-articular fibrous nodules may be seen. Cutaneous low-grade B-cell lymphoma (previously reported as pseudolymphoma) may be complicated.

Rash similar to Lyme disease has been observed in the human following tick bites by *Amblyomma* spp. The disorder is seen in the southeastern or central parts of USA. The rash may be associated with fever, fatigue, headache, muscle and joint pains. The condition has been called Southern tick-associated rash illness (STARI). Borreliac infection is not proven. Local allergic reaction to tick components is suspected. The concept "TARI" (tick-associated rash illness) has recently been presented in Japan. Refer to Chapter VII-C-1.



Figure II-CC-2-1. Centrifugal eruption in Lyme borreliosis. A centrifugally spreading erythematous lesion (migrating erythema) is seen around the site of tick bite (left-sided abdomen) (left). At the 7th week, the erythema reached the neck and thigh (right). At the 8th week, the erythema disappeared spontaneously. The courtesy of Dr. Shun-ichi Baba, Baba Dermatology Clinic, Tokyo, Japan.

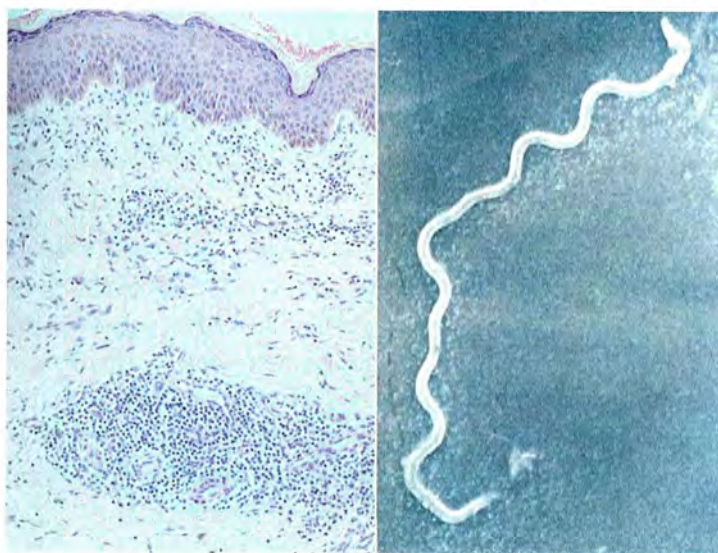


Figure II-CC-2-2. Histology of Lyme borreliosis and electron micrograph of *Borrelia*. The histologic features are nonspecific. Perivascular infiltration of small lymphocytes and some plasma cells is seen in the entire thickness of the dermis (left, H&E). Warthin-Starry's silver was negative. Negatively stained electron micrograph of *Borrelia burgdorferi* shows the morphology of loosely coiled spirochete (right). The courtesy of Dr. Shun-ichi Baba, Tokyo, Japan.

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DD) Bartonellosis

1) Cat scratch disease

Cat scratch disease is caused by infection of *Bartonella henselae* (old term: *Rochalimaea henselae*). Bartonellosis of this type manifests as a painless swelling of the axillary or cervical lymph node. The skin lesion may be seen at the site of cat scratch or cat bite. The latent period is 5 days to 2 weeks. Cat scratch disease is often seen in young male keeping a kitten (infant cat) and the peak incidence is in the autumn through winter. The rickettsia-like bacteria are kept in cat flea.

Figures II-DD-1-1 and II-DD-1-2 illustrate a cat bite-induced ulcerative skin lesion on the toe in a pediatric patient, who is complicated with a splenic lesion. A bullous skin lesion is associated with microvascular proliferation in the upper dermis. Endothelial growth causes thrombotic obstruction of the dermal arterioles. With a monoclonal antibody, *Bartonella* antigen was demonstrated in some endothelial cells within the lesion. The histological similarity to bacillary angiomatosis (see below) can be pointed out.

“Suppurative granuloma” characterizes the lymph nodal lesion in bartonellosis. Epithelioid granulomas surround the central abscess cavity. *B. henselae* is histologically visible in the suppurative zone by Warthin-Starry’s silver, though technically meticulous. The suppurative granulomas are also seen in such infectious lesions as tularemia, yersiniosis, melioidosis, *Alkaligenes xylosoxidans* infection, lymphogranuloma venereum, listeriosis, chromomycosis, sporotrichosis and *Mycobacterium abscessus* infection.

Systemic dissemination may also occur, often presenting multiple suppurative granulomas in the liver and spleen. The histologic features are very similar to those of melioidosis. The splenic lesion of bartonellosis in the pediatric case presented above is illustrated in Figure II-DD-1-3. The *Bartonella* antigen was focally identified in the cytoplasm of the epithelioid cells.

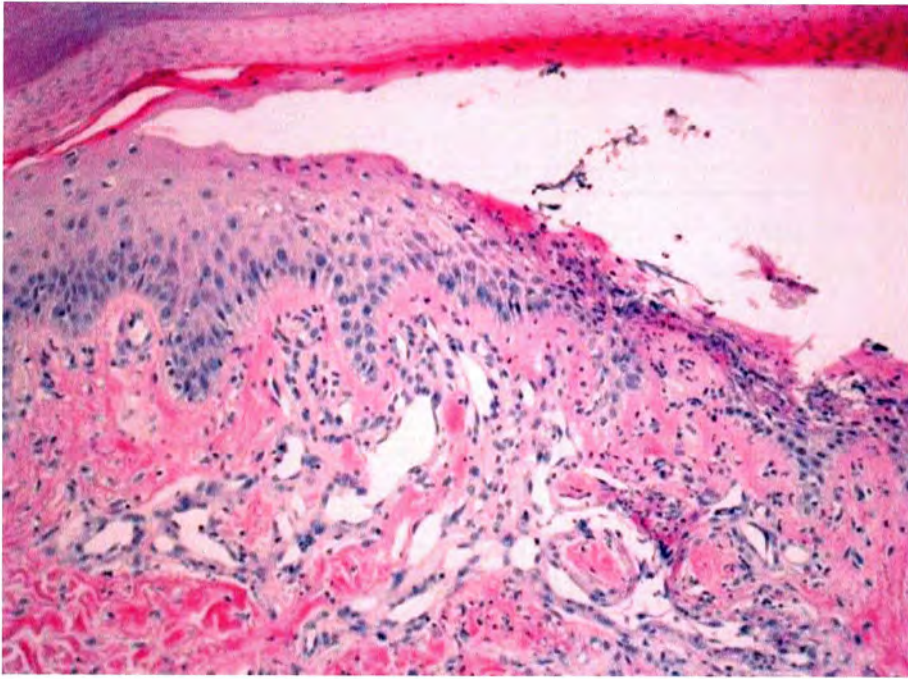


Figure II-DD-1-1. Low power view of skin biopsy in bartonellosis. A cat bite-induced bullous and ulcerative skin lesion on the toe shows microvascular proliferation in the upper dermis. The similarity to bacillary angiomatosis can be pointed out. The courtesy by Dr. Masafumi Ito, a pathologist at Japanese Red Cross Nagoya Daiichi Hospital, Nagoya, Japan.

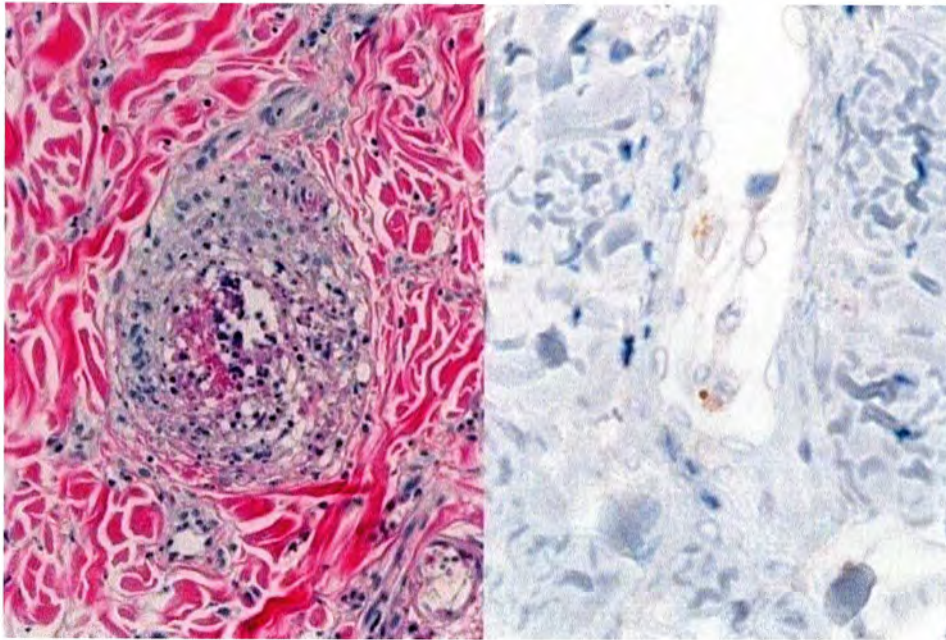


Figure II-DD-1-2. Higher power view of skin biopsy in bartonellosis. Endothelial growth causes thrombotic obstruction of a dermal arteriole (left: H&E). With a monoclonal antibody, *Bartonella* antigen was demonstrated in some endothelial cells (right: immunostaining).

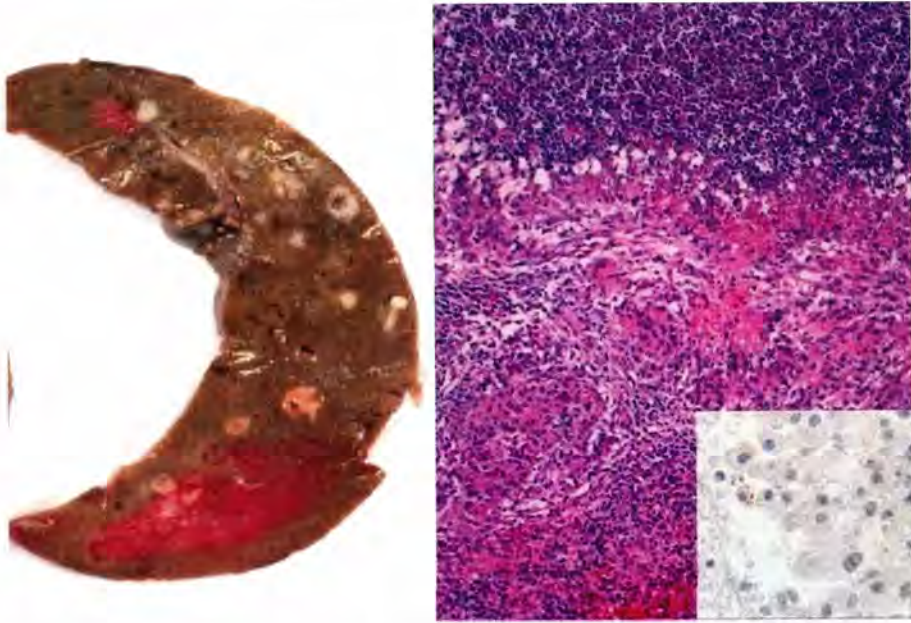


Figure II-DD-1-3. Splenic bartonellosis in the same pediatric case. Multiple small abscesses are distributed in the spleen (left). Formation of suppurative granuloma is characteristic of bartonellosis (right: H&E). Dot-like immunoreactivity is recognized in the cytoplasm of some epithelioid cells (inset: immunostaining for *Bartonella* antigen). The courtesy by Dr. Masafumi Ito, Nagoya, Japan.

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2) Bacillary Angiomatosis

Bacillary angiomatosis is known to be caused by a tiny rickettsia-like organism *Bartonella* species, with equal frequency by *B. henselae* and *B. quintana*. The Gram-negative bacilli are difficult to be cultured. Exposure to flea-infested cats leads to infection of *B. henselae*, and exposure to the human body louse results in *B. quintana* infection.

Peliosis hepatis (localized angiectasia of the liver, a form of extracutaneous presentation of bacillary angiomatosis) and lymph node involvement are seen in *B. henselae* infection. *B. quintana* can also cause urban trench fever and endocarditis in immunocompetent persons.

In 1987, Cockerell, et al. described 6 AIDS patients showing unusual cutaneous papules and nodules distinct from Kaposi sarcoma. They called it epithelioid angiomatosis, because the deeper dermis has microscopic changes similar to epithelioid hemangioma. The term bacillary angiomatosis was proposed by LeBoit, et al. in 1989. The patient may accompany signs and symptoms of chills, headache, fever, malaise and anorexia with or without weight loss. Bacillary angiomatosis often responds to therapy with oral erythromycin. The extracutaneous lesions include the oral and anal mucosal lesions and widespread visceral lesions in the liver, spleen, lymph nodes, bone marrow, gastrointestinal tract, peritoneum, etc.

This vascular-proliferative or pseudoneoplastic form of *Bartonella* infection is prone to occur in AIDS patients. In recent years, the incidence of bacillary angiomatosis has declined considerably as a result of effective antiretroviral therapy. Patients with a renal transplant are also susceptible to this disorder. Clinically, the skin lesions of bacillary angiomatosis vary from a single lesion to thousands, and appear as bright-red round papules, subcutaneous nodules, or as cellulitic plaques. When biopsied, the lesion tends to blanch out, bleed and cause pain.

Histologically, it resembles granuloma pyogenicum, Kaposi's sarcoma and angioma with secondary inflammation. Fibrinoid deposits and minute eosinophilic granular interstitial masses, corresponding to *Bartonella* aggregates, are seen adjacent to the vascular component. The organisms can be visualized with Warthin-Starry's silver.

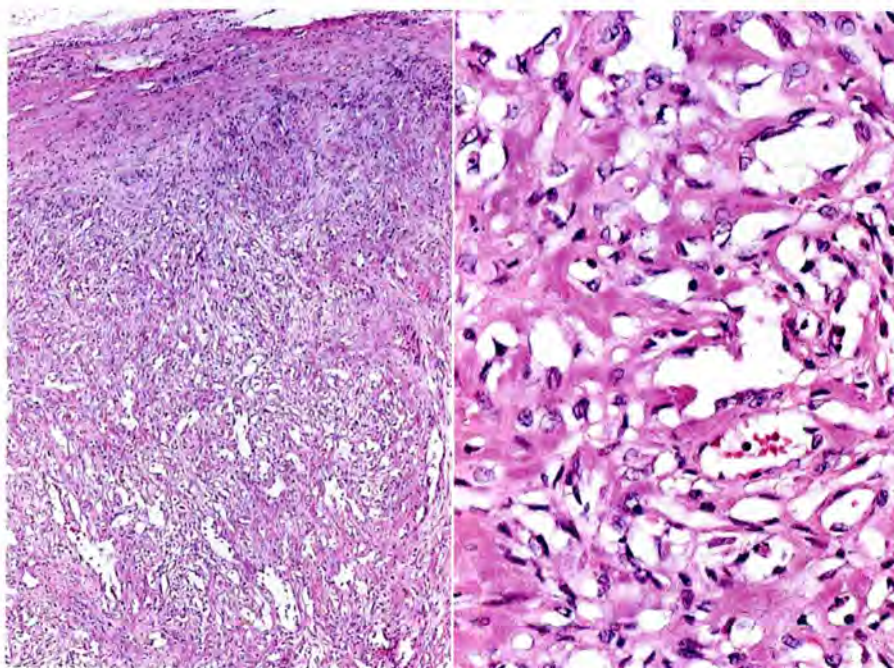


Figure II-DD-2. Bacillary angiomatosis in AIDS. Vascular-proliferative reaction in the dermis is evident in bartonellosis in AIDS. Fibrinoid deposits and minute eosinophilic granular interstitial masses, corresponding to *Bartonella* aggregates, are seen adjacent to the vascular component (H&E). The courtesy by Director Toshiaki Manabe, Shiga Medical Center Research Institute, Moriyama, Japan.

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EE) Rickettsial Infections

Rickettsial infections are caused by obligate intracellular, Gram-negative bacteria in the genera *Rickettsia*, *Orientia*, *Ehrlichia*, *Neorickettsia*, and *Anaplasma*. *Coxiella*, *Bartonella*, *Francisella* and *Brucella* belong to bacteria phylogenetically akin to the rickettsia family. *Rickettsia* in a narrow sense is further classified into the typhus group and the spotted fever group. *Orientia* makes up the classic scrub typhus group.

Most rickettsial pathogens are transmitted by ectoparasites such as fleas, lice, mites and ticks during feeding or by scratching infectious feces into the skin. In endemic areas, transmission occurs during outdoor activities in the spring and summer when ticks and fleas are most active. The incubation period is 5 to 14 days for most rickettsial diseases.

Rickettsial infection commonly associates macular or maculopapular skin rash. The presence of eschar (tache noire), a crusted ulcer at the site of arthropod bite, is quite characteristic. *Rickettsia* proliferates in the vascular endothelial cells, provoking a release of cytokines which damage endothelial integrity. This leads to a cascade of events, terminating in occlusive endangiitis.

Q fever (Q representing “query”) is a zoonotic systemic infection of *Coxiella burnetii* common in cattle, sheep, goats, dogs and cats, and infection of *C. burnetii* is notorious as a cause of abortion in these animals. In the human, Q fever is characterized by 1) atypical pneumonia with flu-like symptoms, and 2) systemic infections, including granulomatous

hepatitis and endocarditis. The incubation period is 9-40 days. Q fever has been thought to be differentiated clinically from spotted fever group rickettsiosis or typhus group rickettsiosis because of the lack of cutaneous eruptions. More recent clinical descriptions have emphasized that skin lesions are encountered in 5-21% of Q fever patients. The Q fever rash is nonspecific, and corresponds to pink macular lesions or purpuric red papules of the trunk.

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Note 29. Epidemic typhus, Dr. Prowazek and Dr. Ricketts

Epidemic typhus often causes epidemics following wars and natural disasters, so that the infection has also been called camp fever or jail fever. The causative organism is *Rickettsia prowazekii* transmitted by the human body louse, *Pediculus humanus corporis*. *R. prowazekii* grows in the louse's gut and is excreted in the feces. Transmission occurs when one scratches the itchy louse bite and rubs the feces into the wound. Symptoms include high fever, cough, rash, severe headache, muscle pain, shock and delirium. Without treatment, the mortality rate is very high.

Dr. Stanislaus JM von Prowazek, a Czech zoologist and parasitologist, described chlamydial inclusion bodies in trachoma and also identified microbes of epidemic typhus. Dr. Howard T. Ricketts, an American pathologist, discovered that the tick was a vector of Rocky Mountain spotted fever. The pathogen causing Rocky Mountain spotted fever, *Rickettsia rickettsii*, was named after him. Dr. Ricketts also identified the microbe in the blood of epidemic typhus.

In 1915, Dr. Prowazek was killed by *R. prowazekii* in Germany at the age of 41. Dr. Ricketts died of epidemic typhus in Mexico in 1910. He was just 39 years old.

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Note 30. *Sennetsu* and *Neorickettsia sennetsu*

Sennetsu, a rickettsial infection endemic in the Kyushu Island, Japan and also in Malaysia, means “lymph node fever” in Japanese. Clinical features resemble those of infectious mononucleosis (an acute infection of Epstein-Barr virus). The causative microbe is now classified in *Neorickettsia sennetsu*, and the disease is termed as sennetsu ehelichiosis. Unlike most other closely related diseases, *N. sennetsu* is transmitted by ingestion of a metacercaria stage of Trematode (flake) infesting in raw fish (gray mullet fish). The ecology and transmission of *Neorickettsia* spp. are very unique among bacteria, in that this agent parasitizes both trematodes and mammals. *N. sennetsu* resides within cytoplasmic vacuoles, primarily in monocytes in the blood and in macrophages of lymphoid or other tissues, eventually causing systemic diseases.

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1) Tsutsugamushi Disease and Scrub Typhus

There are two rickettsioses endemic in Japan: Tsutsugamushi disease caused by *Orientia tsutsugamushi* and Japanese spotted fever caused by *Rickettsia japonica*.

“Tsutsuga or tsutsumi” is an old Japanese word (noun) meaning the disease, and tsutsugamushi (chigger) represents the worm (mushi) causing a serious disease termed Tsutsugamushi disease (the author's name “Tsutsumi” using a different Chinese character means a river bank, not the disease!). The Japanese phrase “tsutsuganaku” (to do without tsutsuga) has been widely used for hundreds of years, indicating that this life-threatening infectious disease has been seen commonly in the northern part of Japan facing to the Sea of Japan (we call this river-associated endemic disease as the classic Tsutsugamushi disease). The trias includes high fever, skin rash and eschar. The mortality rate was 35-50% without antibiotics of the tetracyclin group. Tsutsugamushi disease also occurs in the southeastern Asia, Micronesia, Siberia, China and Korea, and has been called as “scrub typhus or rural typhus” and as new-type Tsutsugamushi disease in Japan.

The causative agent is *Orientia tsutsugamushi*, a subgroup of *Rickettsia* family, and the transmission is mediated through a bite by a 0.3-0.8 mm larval stage mite Tsutsugamushi (*Trombiculid* mite or red mite). Most patients have a history of visiting or playing in grassy fields or mountains. The young and adult worms are non-parasitic, while the larva sucks the tissue fluid from varying vertebrates. The microbe is transferred from the female adult mite to the larva via ovum (transovarial transmission). The latent period is 7-10 days. No human-to-human transmission occurs.

In Japan, this disorder is regarded as a re-emerging infection, since the number of reported patients keeps to be high (600-900 per year) since 1978. The patients are now widely

distributed throughout Japan, except for Hokkaido and Okinawa. The illness seen in the northern part of Japan (classic type) shows two seasonal peaks in April to June and October to November. The endemic season in the other warmer areas (new-type Tsutsugamushi disease) is from October to January. The new-type disease is predominant at present. Many strains of *O. tsutsugamushi* are known serologically: Kato, Gilliam, Karp, Irie/Kawasaki, Hirano/Kuroki, Shimokoshi, Ikeda, Boryong, etc (Figure II-EE-1-1). The Kato strain represents the microbe for the classic Tsutsugamushi disease, and the remaining ones for the new type. In Japan, JP-1 and JP-2 subtypes of Karp strain are now used for the serological analysis. Infection of Gilliam strain is rare in Japan. It is transmitted by the mite *Leptotrombidium akamushi* in the classic type, and by *L. scutellare* or *L. pallidum* in the new type.

At the site of larval mite bite, the microbes invade the skin to form eschar (Figure II-EE-1-2). They proliferate in the cytoplasm of endothelial cells and macrophages. Systemic hematogenous spreading follows to provoke multifocal micro(per)angiitis. The main symptoms (trias) include high remitting fever (39-40°C), spotted exanthemas (roseola) 5 mm in size mainly seen on the body trunk, and the eschar, an ulcerative and crusted induration measuring 5-10 mm (Figure II-EE-1-3). Lymphadenopathy, arthralgia and myalgia are frequently encountered.

In severely ill patients, such lethal complications as encephalitis, myocarditis or interstitial pneumonitis is seen in association with disseminated intravascular coagulation. The symptoms are allegedly provoked by superantigens in the microbes. Representative laboratory data include lymphopenia with atypical lymphocytes and thrombocytopenia.

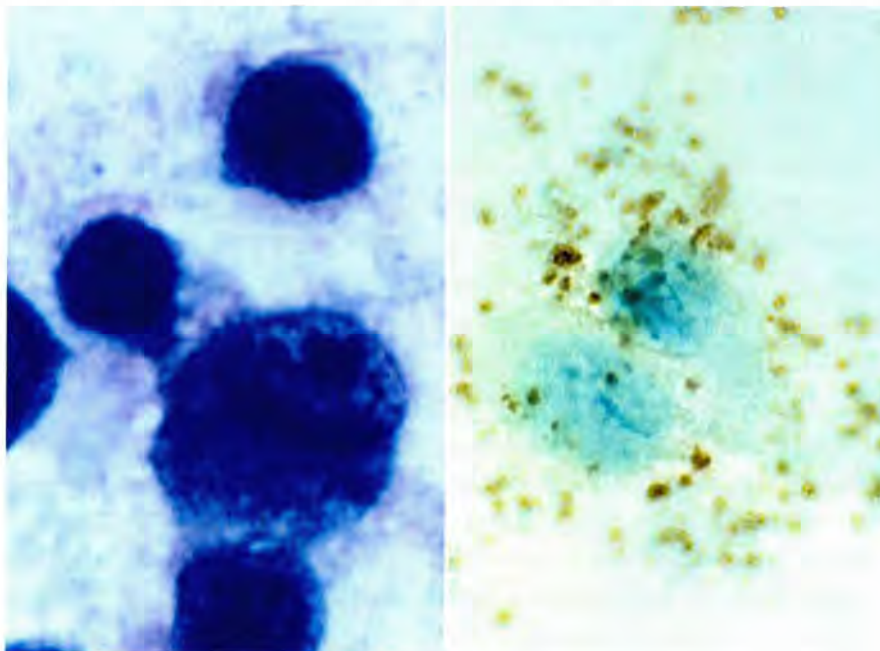


Figure II-EE-1-1. Cultured *Orientia tsutsugamushi*. L929 cells infected with the Gilliam strain of *O. tsutsugamushi* contain rickettsia particles in the cytoplasm (left: Giemsa). Immunostaining using antiserum against the Gilliam strain clearly demonstrates the pathogens (right).

The diagnosis is confirmed by a) the detection of strain-specific serum antibodies, b) blood inoculation into mouse abdominal cavity, and c) the demonstration of specific DNA sequence in the blood by PCR. Taiwanese investigators (Tseng et al.) have identified the microbes immunohistochemically. The histologic features are nonspecific. Mononuclear infiltration around small vessels is seen in a variety of organs and tissues. Biopsy specimens of the eschar (Figure II-EE-1-4) reveal deep dermal ulceration with accumulation of macrophages and neutrophils. Perivascular lymphocytic infiltration is associated.

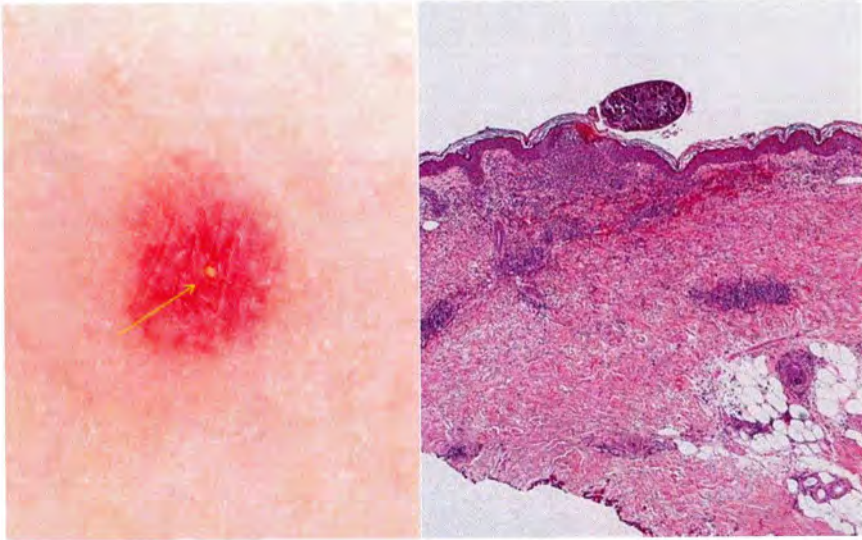


Figure II-EE-1-2. Experimental bite by a Tsutsugamushi larva (investigator's own challenge). At the site of a tiny larval bite (arrow) on the forearm, a non-itchy red-colored eruption occurs (left). Biopsy reveals that the larva provokes a small epidermal defect and perivascular lymphocytic infiltration in the dermis (right: H&E). The courtesy by Dr. Masaru Natsuaki, Department of Dermatology, Hyogo Medical University, Nishinomiya, Japan.



Figure II-EE-1-3. Eschar and eruptions in Tsutsugamushi disease. The trias of this mite-mediated infection includes high fever, roseolar eruptions (left and right top) and eschar at the site of mite bite (right bottom).

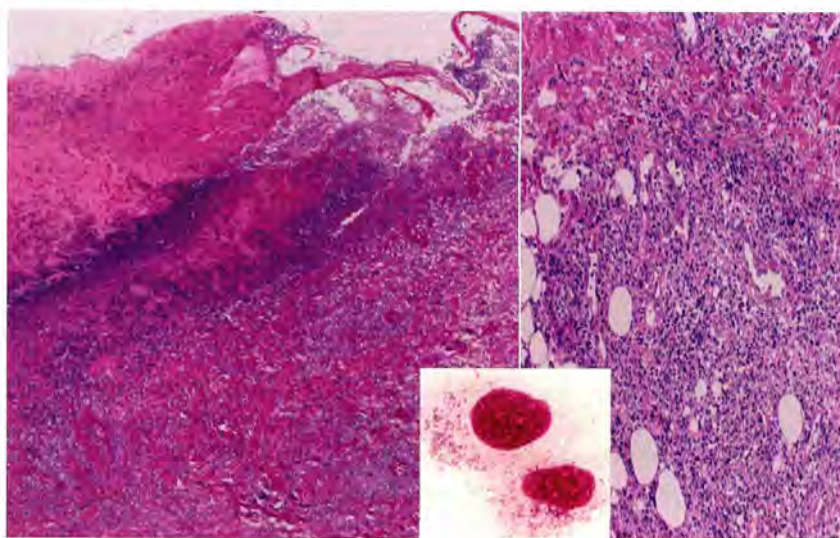


Figure II-EE-1-4. Histology of biopsied eschar. At the site of *Tsutsugamushi* bite (eschar), necrotic ulceration is formed (left: H&E). The mononuclear infiltration extends to the subcutaneous tissue (right: H&E). The inset illustrates *Orientia tsutsugamushi* growing in mouse intraperitoneal macrophages (Giemsa).

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2) Japanese Spotted Fever

Spotted fever group rickettsial infections are endemic in many spots of the world: Mediterranean spotted fever in southern Europe and Africa, Indian tick typhus in India, Astrakhan fever in southeastern Europe and central Africa, Israeli tick typhus in Mediterranean countries, Thai tick typhus in Asia and Australia, Far Eastern spotted fever in Russia, northern China and eastern Asia, Queensland tick typhus and Australian spotted fever in eastern Australia, tickborne lymphadenopathy in European countries, north Asian tick typhus in China and Russia, Rocky Mountain spotted fever and *Rickettsia parkeri* in the Americas, and African tick-bite fever in Africa and the Caribbean islands. Contact with tick-infested dogs may increase the risk of disease in areas endemic for certain rickettsiae.

Japanese spotted fever (JSF) is a newly found emerging infectious disease caused by *Rickettsia japonica*. This previously unknown infection was first reported in 1984 by Dr. Fumihiko Mahara, a science-eyed local physician in Tokushima prefecture, Shikoku Island, Japan. He noticed that the serum antibody was negative for *O. tsutsugamushi*, and the Weil-Felix reaction using *Proteus vulgaris* antigens showed a pattern of the spotted fever group: positive for OX2 and OX19 and negative for OXK. This disease is actually categorizable in the spotted fever group, such as Rocky Mountain spotted fever caused by *R. rickettsii* (Figure II-EE-2-1). The vector tick was found to be a larva or nymph (young form) of *Haemaphysalis flava* (Figure II-EE-2-2). The incidence is 160-180 cases per year, and the disease distribution is reportedly in the warmer areas along the Pacific Ocean.

The main symptoms (trias) of JSF are fundamentally similar to those of Tsutsugamushi disease, including high fever, skin rash and eschar (site of larval/young tick bite) (Figure II-EE-2-3). Hemorrhagic (purpuric) rash is occasionally observed. Characteristically, no tick is visible at the site of eschar, since the microbe is not transmissible through the adult tick. The spotted fever occurs in April through October. The latent period is 2-8 days. The site of tick bite tends to be smaller in size and thus may be overlooked. The eruptions are commonly observed on the extremities. In contrast to Tsutsugamushi disease, lymphadenopathy is infrequent. In addition to tetracycline/Minomycin/doxycycline, new quinolones are effective to the spotted fever, while the latter is ineffective to Tsutsugamushi disease. Fatal JSF cases have recently been seen in southwest Japan.

Serological screening of IgM and IgG antibodies against *R. japonica* and detection of *R. japonica* DNA in blood by PCR have comprised the main diagnostic tests for JSF. Serology requires a minimal 2-fold increase of antibody titers between the initial and second blood samples. It takes a 2-week period; therefore this cannot be used in emergency situation. Indeed, fatal JSF cases have occurred within 24 hours of hospitalization.

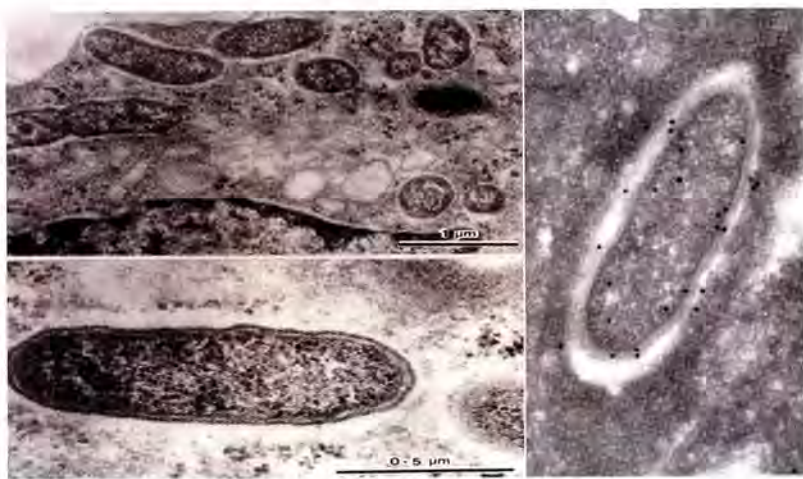


Figure II-EE-2-1. Electron micrographs of *Rickettsia japonica*. This Gram-negative rods exclusively proliferate within the cell (left top and bottom). Post-embedding immunoelectron microscopy using a monoclonal antibody demonstrates positive colloidal gold signals on the cell wall of the bacteria (right). The courtesy by Dr. Yasuhiro Yano, Department of Parasitology, University of Fukui Faculty of Medical Sciences, Eiheiji, Japan and by Dr. Hirotoishi Utsunomiya, Central Research Laboratory, Wakayama Medical University, Wakayama, Japan (for immuno-EM).

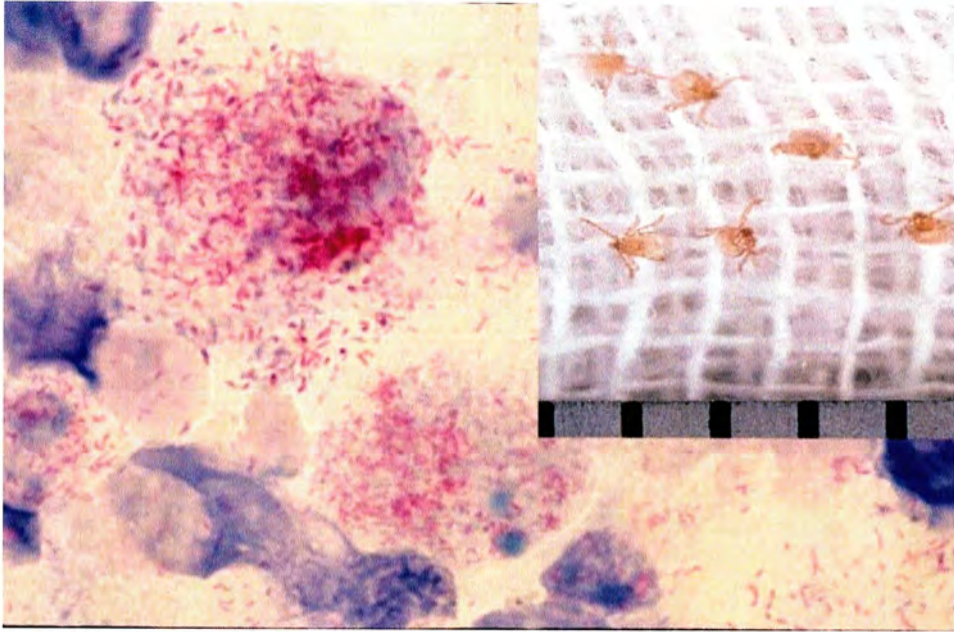


Figure II-EE-2-2. *Rickettsia japonica*-infected macrophages and the larval vector, *Haemaphysalis flava*. The infected rickettsiae are visualized in the cytoplasm (Giménez stain). Inset illustrates a larval stage of the tick vector, 1 mm in size. The transmission occurs during the summer season. The courtesy by Dr. Hiromi Fujita at the Acari Institute, Mahara Clinic, Anan, Tokushima, Japan.

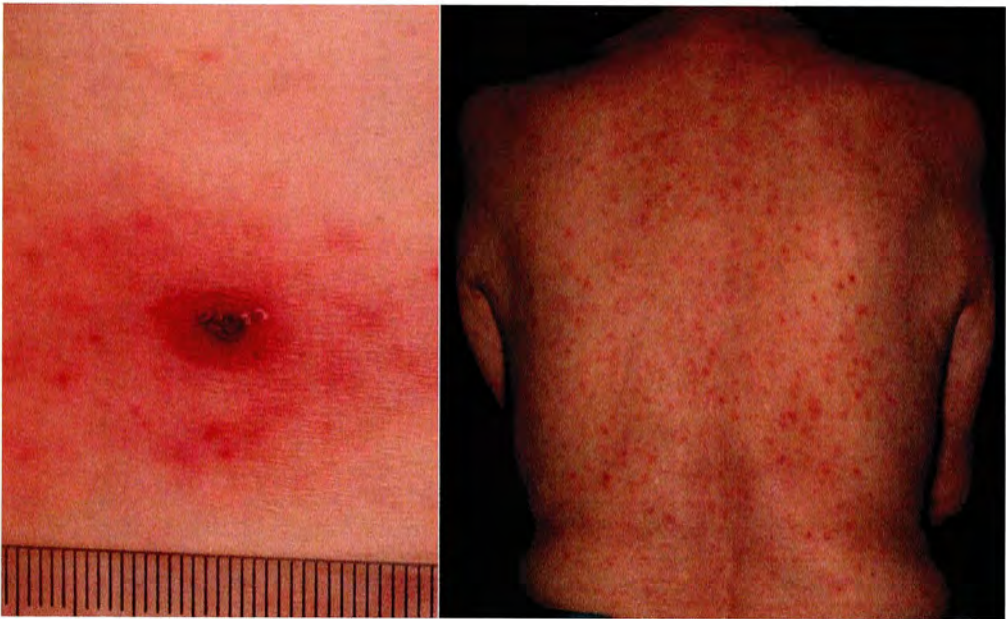


Figure II-EE-2-3. Japanese spotted fever. The eschar in JSF is smaller than in Tsutsugamushi disease (left). The skin rash is often hemorrhagic and dispersed on both the trunk and extremities (right). Usually, the endemic spots in Japan are reciprocal between JSF and Tsutsugamushi disease. The courtesy by Dr. Fumihiko Mahara, Mahara Clinic, Anan, Tokushima, Japan.

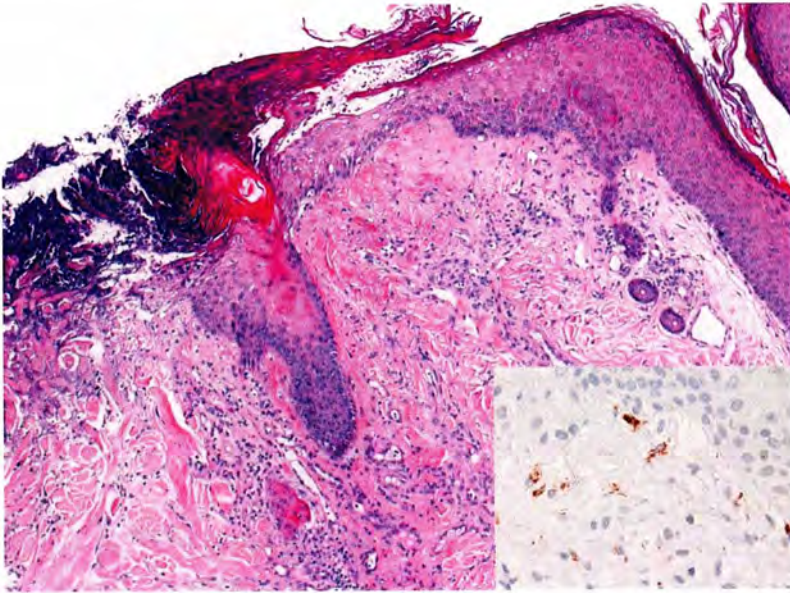


Figure II-EE-2-4. Biopsied eschar in JSF. Crusted ulceration with accumulation of macrophages, capillary growth and moderate perivascular lymphocytic infiltration are noted. The rickettsial antigen common in the spotted fever group shows dot-like immunolocalization in the cytoplasm of macrophages and vascular endothelial cells (inset).

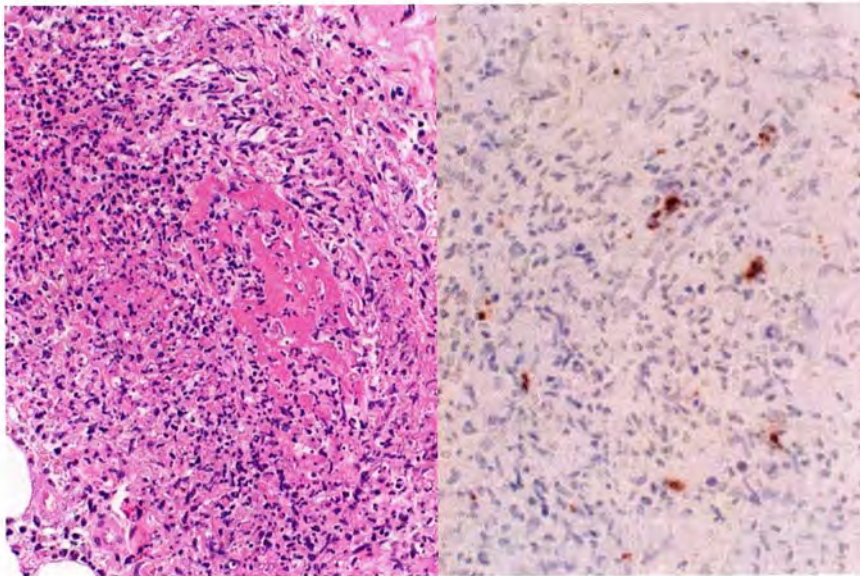


Figure II-EE-2-5. Biopsied hemorrhagic rash in JSF. Features of necrotizing vasculitis with fibrinoid necrosis and neutrophilic reaction are observed (left). Immunostaining for the rickettsial antigen of the spotted fever group demonstrates distinct dot-like positivity in the endothelial cells (right).

We have established diagnostic immunohistochemistry using two mouse monoclonal antibodies (S3 and X1) in heat-retrieved formalin-fixed, paraffin-embedded sections biopsied from eschar and eruptions (Tamakuma et al). The antibodies have been shown to be cross-reactive to other rickettsia of the spotted fever group. In the biopsied eschar, dotted positive

signals are seen in the cytoplasm of endothelial cells and macrophages (Figure II-EE-2-4). In the hemorrhagic skin rash, features of necrotizing vasculitis may be seen (Figure II-EE-2-5).

When immunostaining is combined with real-time PCR technique targeted at 17k genus common antigen gene to yield short length products less than 100 base pairs, good diagnostic accuracy is available. The diagnosis can be made earlier by IHC and/or RT-PCR than serology. We found that in some cases of JSF, serum antibody titers were not raised, when the early diagnosis prompted early treatment.

Recently, other types of spotted fever caused by *R. tamurae* or *R. heilongjiangensis* have been reported in Japan. Japan is a country of woods and forests full of ticks, it is plausible that emerging tickborne rickettsiosis may be newly recognized.

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Note 31. Does measles acutely kill a healthy adult?

A Japanese male aged 60's became acutely ill with high fever and systemic exanthemas, and soon died of disseminated intravascular coagulopathy. Eight days before disease manifestation, he went to a small uninhabited island facing to Tokushima, Shikoku Island, Japan, as a team member for topographical investigation. A local general practitioner made the diagnosis of measles as the cause of death.

His daughter was a medical student in Osaka, and 2 years earlier she took a special lecture by Dr. Fumihiko Mahara on the emerging rickettsial disease, Japanese spotted fever, first reported by himself in Tokushima. The clever medical student doubted the diagnosis given to her father, and she made a direct phone call to Dr. Mahara, practicing as a local medical doctor in Anan city in Tokushima. This disease founder quickly responded to her request to visit the wake (a Japanese ceremony to keep an all-night watch over the dead body) held in his house. Dr. Mahara identified the eschar on the body, and got a small aliquot of heart blood from the dead body in a coffin. Originally he was an experienced cardiovascular surgeon! The final PCR diagnosis was Japanese spotted fever. The epidemiological survey of the island confirmed the presence of *Rickettsia japonica* in tick vectors.

It should be noted that Rocky Mountain spotted fever in the United States has been called as “black measles”. The author sincerely respects Dr. Mahara and his action for the academic curiosity and daily warm mild to patients and their family.

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Note 32. Infectious disorders entitled with the word “Japanese”

There are a total of 3 infectious diseases entitled with the word “Japanese”. Japanese schistosomiasis, complicating liver cirrhosis and caused by a trematode, *Schistosoma japonicum*, has been totally eradicated in Japan in 1976, and no new cases are reported for years. At present, Japanese encephalitis, mosquito-mediated arbovirus infection, is very rarely experienced in west Japan. However, both diseases are endemic and often lethal in tropical and subtropical east/southeast Asian countries. In addition, the new rickettsial disease named Japanese spotted fever, so far endemic in Japan, is now reported from Korea and Thailand.

To our regret, all these serious disorders may give erroneous impression or image to Asian people, patients and their family as if the illness were imported from Japan during the World War II, when the Japanese army troops occupied these areas.

My deep regret and my protest with smile to Dr. Mahara are that he did give the novel rickettsial disease he found the name of “Japanese” spotted fever, instead of “Mahara” spotted fever.

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FF) Other Bacterial Infections with Skin Rash

Typhoid fever and paratyphoid fever are systemic and life-threatening illness still common in the developing world, and are caused by systemic infection of *Salmonella typhi* and *S. paratyphi*, respectively. Most cases in the developed countries suffer from the illness while traveling internationally. Infection is achieved when the individuals eat food or drinking water contaminated with the pathogen. The bacteria live only in the human. The patients carry the bacteria in the bloodstream and intestinal tract, and some become carriers who continue to carry the bacteria in the bile and stool. The symptoms include a sustained high fever (39 to 40°C), weakness, epistaxis, headache and abdominal pain. Relative bradycardia, leukopenia and hepatosplenomegaly are characteristic. In addition, the patients have skin rash of flat, rose-colored spots. The rose spots represent bacterial emboli in the skin, and appear on the lower chest and abdomen in one third of the patients. Typhoid and paratyphoid fevers are microscopically characterized by the formation of typhoid nodules (Figure II-FF-1), which represent aggregates of macrophages phagocytizing the pathogens.

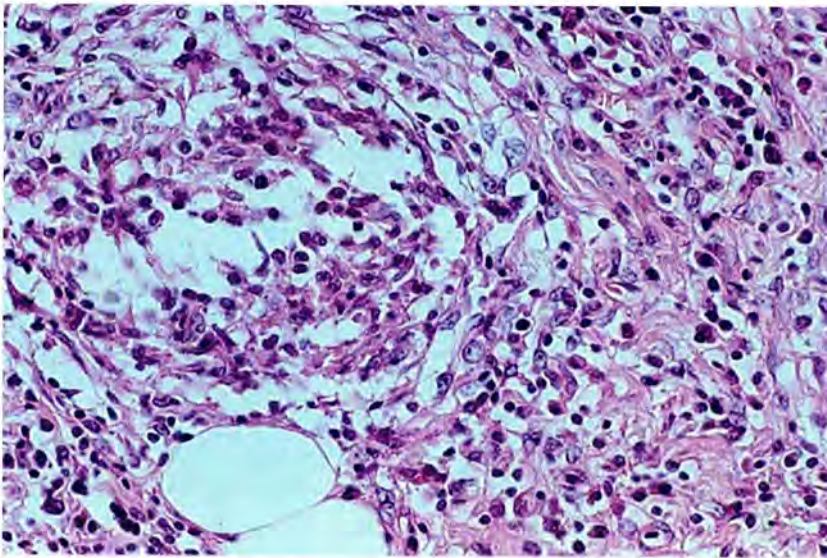


Figure II-FF-1. Typhoid nodule. Microgranulomatous reaction (typhoid nodules) is seen in the ileal wall with multifocal ulceration (H&E). The lesion represents embolic spread of the *Salmonella* pathogen.

Listeriosis is a rare systemic infection of *Listeria monocytogenes* named after the English pioneer of sterile surgery Joseph Lister (1827-1912), a Gram-positive coccobacillus, growing even in a refrigerator. This intracellular pathogens replicates in macrophages in the human body, and causes high fever, diarrhea and muscle pain. Neonates, the aged, immunosuppressed patients and pregnant women are susceptible to the microbe (Figure II-FF-2). Cutaneous lesions may be papular, pustular or purpuric. Histologically, the epidermis reveals mild spongiosis with lymphocytic exocytosis. Subcorneal pustules may be seen. The dermis is infiltrated by macrophages, phagocytizing Gram-positive pathogens.

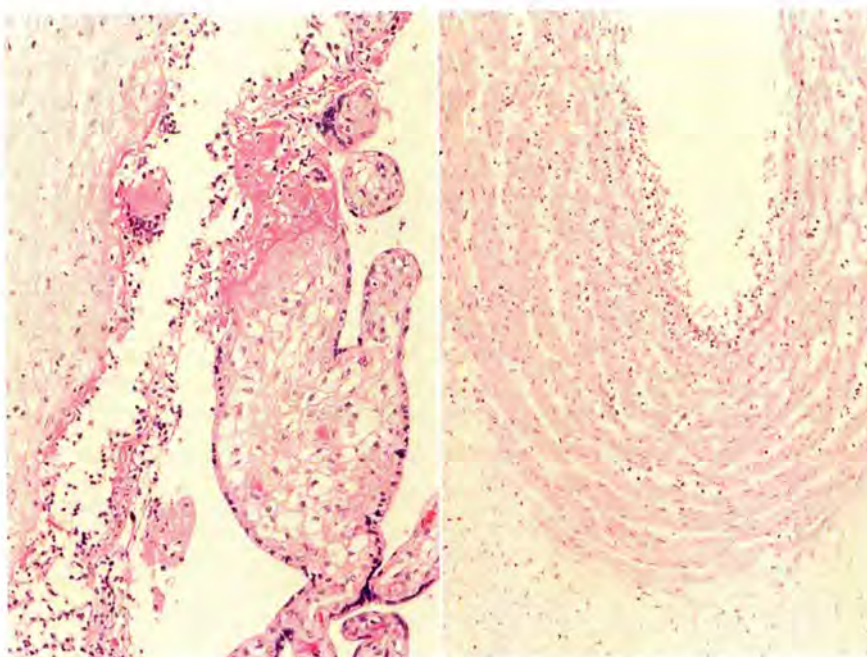


Figure II-FF-2. *Listeria*-induced villitis. Abscess formation on the villi of the placenta at the 23rd week of gestation (left: H&E) and endophlebitis of the umbilical cord (right: H&E) resulted in transplacental neonatal meningitis. Microbial culture from the amniotic fluid and blood of the mother and neonate was positive.

Brucellosis is a systemic infection caused by *Brucella melitensis* and less frequently by *B. abortus*, *B. suis* or *B. canis*. The Gram-negative aerobic short bacilli grow intracellularly and are transmissible through the intact skin. They primarily cause diseases in animals, such as sheep, pigs, cattle, goats and dogs, and this zoonotic pathogen is responsible for enormous economic loss due to abortion of the livestock. Humans can become infected by 1) eating or drinking contaminated food, particularly unpasteurized milk and cheese, 2) direct contact with an infected animal, and 3) inhaling the microorganism as occupation-related infection among laboratory workers or those who work with animals or animal carcasses. The incubation period is 1-8 weeks. Flu-like nonspecific symptoms occur. Serious complications of chronic brucellosis include endocarditis, hepatitis, splenitis, meningoencephalitis, orchitis and arthritis, forming abscesses and granulomas. Skin complications generally affect about 5% of patients with systemic brucellosis. Widespread red-purple papulonodular eruptions or maculopapular rash are observed. Histologically, nonspecific perivascular lymphocytic reactions are seen in the upper dermis. Abortive granulomatous change may be seen. Occasionally, erythema nodosum-like nodules develop.

Legionnaires' disease is an acute infection caused by *Legionella pneumophila*, primarily involving the lung. The other species of *Legionella* may cause a less severe lung infection known as Pontiac fever. Extrapulmonary manifestations include gastrointestinal symptoms, liver dysfunction, renal failure or central nervous system complications. Skin manifestations are uncommon but may accompany erythematous, maculopapular or petechial skin lesions. Toxic or immunological pathogenesis is postulated.

Whipple's disease rarely accompanies skin lesions related to a malnutrition state or immune reactions to infection of *Tropheryma whipplei*. Pellagra-like hyperpigmentation on the sun-exposed area is an example in the former, while eczema, nodular prurigo and erythema nodosum belong to the latter. Reportedly, *T. whipplei* DNA can be proven from skin biopsy specimens, even in cases without skin manifestation, suggesting that the skin is a reservoir for *T. whipplei*.

Mycoplasma pneumonia, a kind of "atypical pneumonia", is a pulmonary infection of *Mycoplasma pneumoniae*. Unlike other bacteria, it lacks the cell wall. Outbreaks are common in groups of young adults, particularly in military facilities and college dormitories. Droplet transmission occurs when persons have a close contact with the sick person. As a result of direct invasion by the bacteria or the immune response to the infection, *M. pneumoniae* may result in complications in other organs, such as encephalitis, hepatitis, myocarditis and hemolytic anemia. Skin rash affects up to one-third of patients with Mycoplasma pneumonia. Most frequently, the rash is non-specific exanthem, showing short-lasting red patches on the trunk and limbs, and spontaneously disappears without treatment. Vesicles and blisters, petechiae, urticarial rash and bullous erythema multiforme (Stevens-Johnson syndrome) may also be encountered.

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Fungal Infections

General features of fungal infection (mycosis) are summarized below.

- (i) Most mycosis belongs to non-serious superficial infection caused by dermatophytes and yeasts.
- (ii) The diagnosis of deep mycosis is difficult, and the effective protective procedures and treatments have apparent limitations. This category is of life-threatening importance in immunocompromised patients.
- (iii) There is almost no contagious mycosis.
- (iv) Fungi exist in two forms: unicellular “yeasts” and branching filamentous “molds”. Some fungi are dimorphic: they change from one form to another depending upon the environment. There are more than 50,000 fungal species in the environment, but only 20-25 species cause human infection.
- (v) Historically, mycosis was described earlier than bacterial infection. Bassi reported mycosis of silkworms as early as in 1836, Schoenlein described favus, skin infection of *Trichophyton schoenleinii*, in 1839, and Lagenbeck recorded thrust, oral mucosal infection of *Candida albicans*, in 1839.
- (vi) It may take several weeks until features of fungal colonies become evident on the culture plates. Accordingly, direct examination of scraped specimens in 10% potassium hydroxide and histopathological examination of biopsy specimens are needed for rapid diagnosis.
- (vii) Periodic acid Schiff (PAS) and Grocott’s methenamine silver are reliable methods of detecting fungi in histological and cytological specimens. When necessary, immunostaining and polymerase chain reaction (PCR) are used for fungal identification.

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A) Dermatophytosis

Dermatophytosis (tinea, ringworm) represents infection of dermatophytes. The dermatophytes are a group of related hypha-forming fungi (molds) showing the ability to invade the keratinized tissues of human and animals to colonize. Clinical manifestations vary considerably, based upon the species of fungus, the site of infection, the immunologic state of the patients, and the prior use of topical steroids. Common style of clinical presentation is an itchy superficial infection. Occasionally, hair may be lost and nails may be deformed. Deep dermal mycosis is also infrequently observed. Diabetes mellitus, ichthyosis, atopic dermatitis, collagen disease and Cushing's syndrome may predispose to chronic and recurrent dermatophytosis.

Three genera of dermatophytes include *Epidermophyton*, *Microsporum* and *Trichophyton*. There are three ecological groups according to their natural habitats, as follows.

- a) Anthropophilic dermatophytes preferentially affect humans. *T. rubrum* is the most common anthropophilic isolate. *T. violaceum*, *T. mentagrophytes*, *E. floccosum*, and *M. gypseum* are also isolated. The anthropophilic fungi cause a mild disease, but resist to therapy to be a chronic persistent disease.
- b) Zoophilic dermatophytes primarily affect animals. *M. canis*, *M. audouinii*, *T. verrucosum* and *T. tonsurans* represent zoophilic dermatophytes, and tend to evoke more intense inflammatory response than anthropophilic fungi.
- c) Geographic dermatophytes live in the soil as saprophytes, not infective to the human.

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1) Tinea

Tinea pedis (athlete's foot) is the most common dermatophytosis in adolescents and adults. *Trichophyton rubrum* and *T. mentagrophytes* are the most common isolates. In tinea barbae (beard), tinea manis (hand), tinea corporis (body) and tinea cruris (crotch rot) caused by *T. rubrum*, small itchy vesicles are clustered on the red-colored papule. Sharp scaling border is observed, hence the nickname of ringworm (Figure III-A-1-1). Regarding tinea capitis, involving the haired skin of scalp, see the next heading. Note that *T. rubrum* is not a scalp pathogen.

Histologically, intraepidermal spongiotic vesiculation with neutrophilic reaction is associated with elongation of the rete ridges and chronic dermal inflammation. Mycelia of anthropophilic dermatophytes stay in the cornified (hyperkeratotic) layer and do not invade the hair follicle, while *T. rubrum* may show ectothrix-type follicular involvement. Representative features are illustrated in Figure III-A-1-2.



Figure III-A-1-1. Gross appearance of tinea. Tinea pedis (athlete's foot, left) gives dry, hyperkeratotic macules on the 5th toe (left). An itchy elevated and indurated lesion with sharp scaling borders is seen on the back of the hand (tinea manis: center). Clustered hyperkeratotic vesicles are seen in tinea corporis (right). The courtesy by Dr. Soichi Tanaka, Tanaka Dermatology Clinic, Ohtsu, Japan.

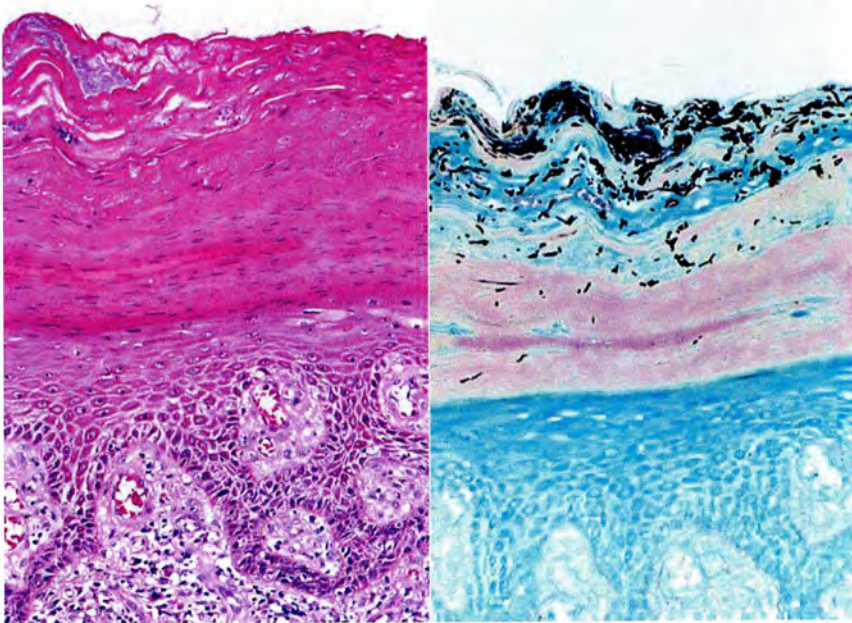


Figure III-A-1-2. Histology of tinea. Biopsy from an interphalangeal skin (tinea manis) shows intracorneal proliferation of mycelia within the hyperparakeratotic layer (left: H&E, right: Grocott).

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2) Tinea Capitis and Trichophytosis

There are three types of tinea capitis, namely, microsporiasis, trichophytosis and favus (Note 33). These are based on the causative pathogens and the nature of symptoms. Typical gross appearance of microsporiasis and trichophytosis is presented in Figure III-A-2-1.

In microsporiasis (gray-patch ringworm), small red, dry and scaly papules are formed around the hair shaft (exothrix infection), and eventually the hairs break off 1-3 mm. The round-shaped lesion is demarcated. The causative fungi are *Microsporum canis* and *M. audouinii*. The disease is mainly seen in children. Typically, sick cats and kittens carry these fungi, and the infection may be spread through person to person contact, or by sharing contaminated brushes and combs. The lesion caused by *M. canis* shows a bright yellowish green fluorescence by Wood lamp test, while *Trichophyton* infection provokes no fluorescence.

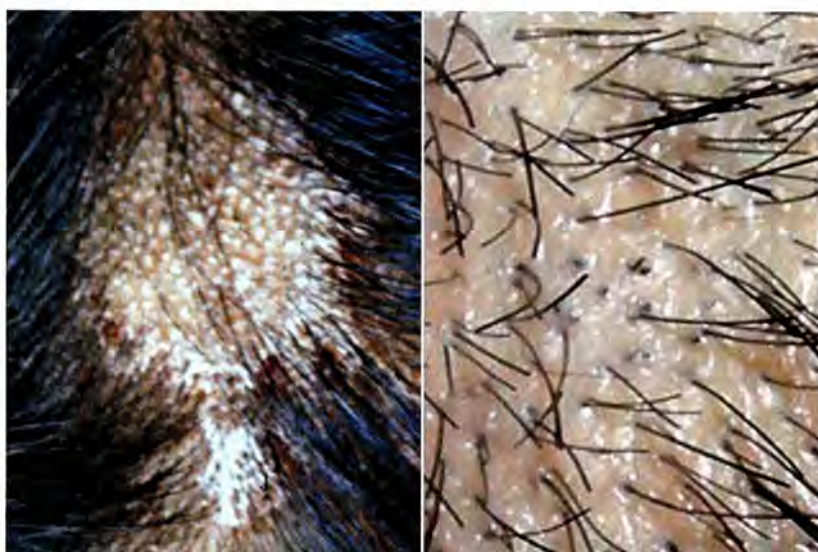


Figure III-A-2-1. Two forms of tinea capitis: exothrix-type microsporiasis versus endothrix-type trichophytosis. Microsporiasis caused by *M. canis* shows a demarcated pauci-hair patch with dry, gray and scaly papules around the hair shaft (left). In trichophytosis caused by *T. tonsurans*, an ill-demarcated, non-scaly, pauci-hair patch accompanies black dots, corresponding to the diseased hair follicles (right). The courtesy by Dr. Soichi Tanaka, Tanaka Dermatology Clinic, Ohtsu, Japan.

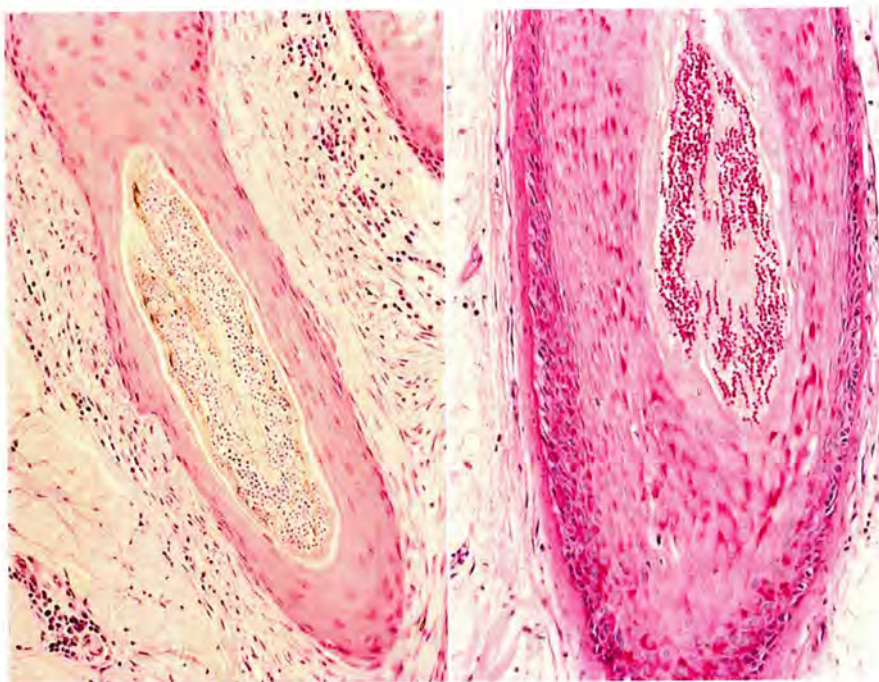


Figure III-A-2-2. Histology of trichophytosis. Endothrix infection of the mycelia is evident. Mild inflammatory reaction is noted around the hair follicle (left: H&E, right: PAS).

Trichophytosis (black-dot ringworm) is caused by *Trichophyton tonsurans* and *T. violaceum*. The fungi cause dry, non-scaly, non-inflammatory patches. The lesion, often angular in shape, is ill-demarcated. After the hairs break off at the opening of the hair follicle, black dots remain. This type of dermatophytosis, accompanying endothrix type hair invasion of the fungus, is characterized by intractable alopecia on the scalp, frequently seen in Japanese Judo and wrestling players, particularly in those who are ranked at a high level. Contact transmission may occur from foreign players in the international tournament. The fungi invade the hair, entering the hair cortex just above the hair bulb (Figure III-A-2-2). The endothrix infection is evident by PAS and Grocott stains.

Majocchi's granuloma represents a nodular and plaque-like dermatophytic lesion on the lower leg, histologically showing a granulomatous perifolliculitis. Females predominate over males. The subcutaneous lesion in the scalp is called as trichophytic granuloma. The causative dermatophytes include *T. rubrum*, *T. mentagrophytes*, *M. canis*, *T. violaceum* and *T. tonsurans*. The condition is often seen in the immunocompromised patients.

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Note 33. Favus and piedra

Favus is a chronic infection of the scalp in childhood, characterized by foul-smelled yellow crusts (scutula) overlying an erythematous base. A honeycomb-like mass of round dry and cup-shaped crusts is formed (favus means honeycomb in Latin). Favus is still seen in some developing countries. The infection of *Trichophyton schoenleinii* persists for life. Wood lamp examination may demonstrate a dull green fluorescence. It is of note that favus is the oldest infectious disease, in which the etiologic agent was proven by Schoenlein in 1839.

Piedra, which means "stone" in Spanish, is an asymptomatic superficial fungal infection of the hair shaft. Small bumps or nodules are attached to the hair. Piedra ultimately may lead to hair breaking. Two types of piedra are known. Black piedra is an infection of *Piedraia hortae*. White piedra is caused by *Trichosporon asahii* or *T. ovoides*. The pathogens are seen in soil. Black piedra is most common in tropical regions such as Brazil and Southeast Asia, while white piedra is distributed in temperate and semitropical areas in South America, Asia, Europe and the southern United States. Black piedra usually affects scalp hair, and white piedra commonly affects pubic hair, axillary hair, beards, mustaches, eyebrows and eyelashes. *Trichosporon* spp. commonly colonizes the perigenital skin.

Trichomycosis, a bacterial infection of axillary or pubic hair, is caused by *Corynebacterium tenuis*. Refer to Chapter II-F-3.

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3) Kerion Celci

Kerion celci, deeply situated trichophytosis on the scalp, showing suppurative and destructive folliculitis. Severe folliculitis and perifolliculitis accompany pain and fever, and eventually result in boggy violaceous inflammatory area with suppuration and alopecia. Erythema nodosum may be associated as a remote skin reaction. Histologically, perifollicular abscess formation is evident. The pathogens are hardly identified within the lesion. Endothrix and/or zoophilic fungi, such as *Trichophyton verrucosum*, *T. tonsurans* or *Microsporum canis*, are the causative agents of kerion. *M. gypseum* may also cause kerion celci. Representative features are shown in Figure III-A-3.

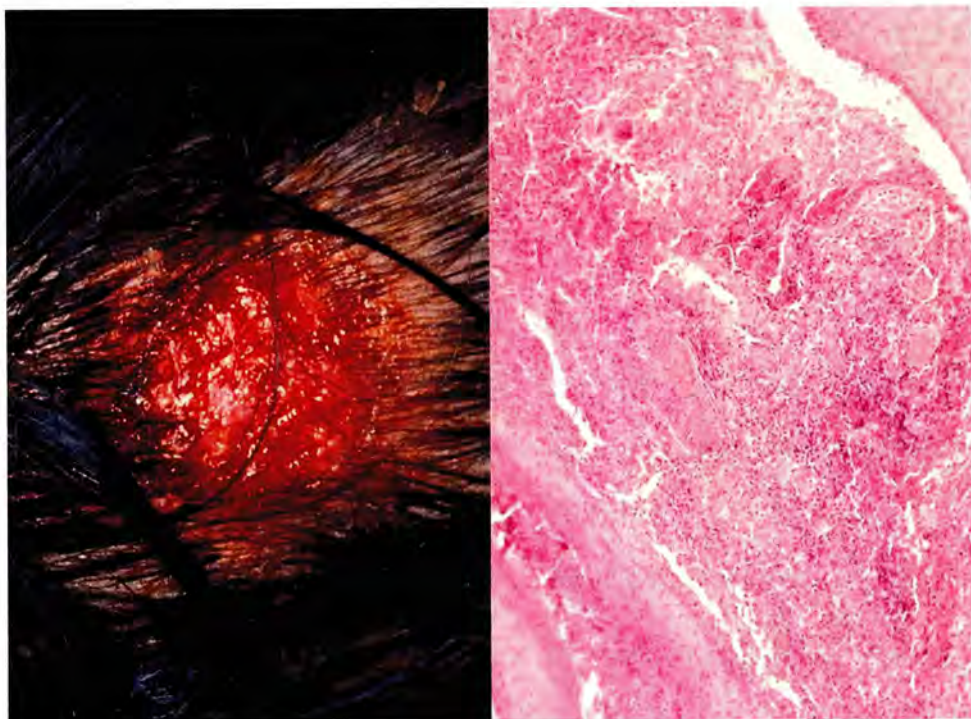


Figure III-A-3. Kerion celci. Active and severe folliculitis has provoked localized suppuration and alopecia. The mycelia are scarcely identified within the inflammatory lesion (left: gross finding, right: H&E).

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4) Onychomycosis

Onychomycosis is a general term for fungal infection of the nail, and makes a serious health burden in older persons and diabetics. Onychomycosis constitutes about a half of all nail abnormalities.

Dermatophytosis caused by *T. rubrum* or *T. mentagrophytes* often targets toe nails (Figure III-A-4-1). Deformity and turbidity of the involved nails are noted. Prolonged use of steroid ointment may lead to onychomycosis. The disease is commonly seen in the temperate western countries. The dermatophytic elements occur mostly in the deeper portions of the nail plate and in the hyperkeratotic nail bed. Nail surfaces are scarcely affected. This is the reason why systemic anti-fungal treatment is needed for onychomycosis. Occasionally, a thick hyperkeratotic nodule containing numerous clumped hyphae (dermatophytoma) is formed beneath the nail.



Figure III-A-4-1. Onychomycosis. Deformity and turbidity are observed in the infected toe nails. No itchy sensation is associated in the nail lesion. The courtesy by Dr. Soichi Tanaka, Tanaka Dermatology Clinic, Ohtsu, Japan.

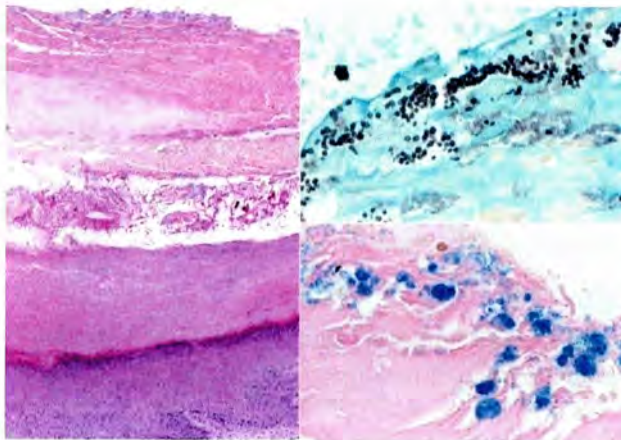


Figure III-A-4-2. Histology of candidal onychomycosis. Infection of *Candida* (predominantly yeast form) is seen in the superficial layer of the destroyed nail (left: H&E). Grocott stain clearly identifies yeast-form fungi (right top). Biofilm formation with acidic matrix is evident in the *Candida* colonies (right bottom, colloidal iron stain).

Candidiasis caused by *Candida albicans* mainly involves the fingers, and usually occurs in persons who frequently immerse their hands in water (Figure III-A-4-2). Biofilm formation is observed. Infection requires the prior damage of the nail by infection or trauma. The disease is common in the tropics and subtropics with a hot and humid climate.

Onychomycosis may also be caused by *Fusarium* species, most commonly *F. oxysporum* and *F. solani*.

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B) Malassezia Infection

1) Normal Flora and Secondary Colonization

Malassezia spp., including *M. globosa*, *M. restricta*, *M. furfur* and *M. sympodialis*, etc., is a dimorphic and lipophilic fungus, and is a normal inhabitant at the infundibulum of the hair follicle of the upper trunk, upper arm and face (in the seborrheic skin). *Malassezia* is often found in the dilated hair follicle. Budding yeasts (blastoconidia) and short hyphae (pseudomycelia) are observed in the cornified layer, giving a so-called "spaghetti and meat balls" appearance. The mycelial form is not so clearly seen in the state of normal flora (Figure III-B-1-1). It is very difficult to culture *Malassezia* fungi on the plate.

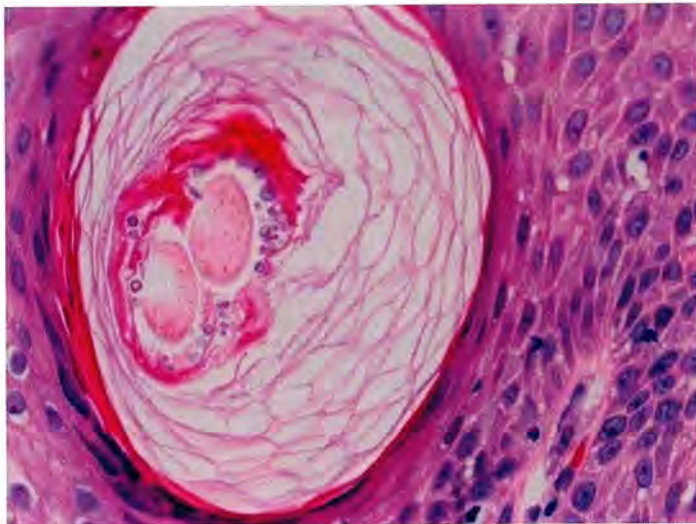


Figure III-B-1-1. *Malassezia* as normal flora in the hair follicle. In the hair follicle on the seborrheic skin (face), yeast-type fungi with basophilia are clustered around the hair shaft (H&E).

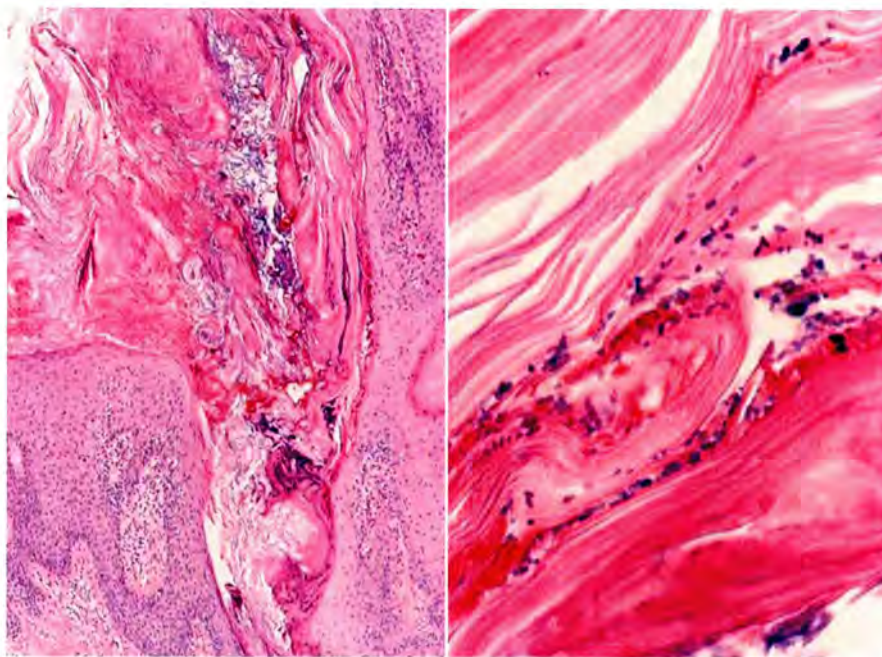


Figure III-B-1-2. Secondary colonization of *Malassezia* in the hyperkeratotic layer of seborrheic keratosis on the facial skin. Infection of *Malassezia*, forming both the yeast form and short mycelia, is observed in the hyperkeratotic layer (H&E).

Malassezia colonization is an exacerbation factor of seborrheic dermatitis, which manifests chronic scaling erythema on the sebum-rich areas such as the scalp, face and trunk. In the hyperkeratotic lesions on the seborrheic skin, secondary co-infection of *Malassezia* and *Staphylococci* is frequently encountered (Figure III-B-1-2).

The severity ranges from mild dandruff to exfoliative erythroderma. *Malassezia* is often identified in the lesion of seborrheic dermatitis, and anti-fungal therapy relieves the symptoms. Relationship between *malassezia* colonization and atopic dermatitis has also been proposed. Allegedly, specific IgE antibodies to *Malassezia* are elevated in the serum of atopic dermatitis patients.

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2) Tinea Versicolor

Tinea versicolor (pityriasis versicolor) is a superficial form of cutaneous mycosis (Figure III-B-2). The fungus, predominantly *Malassezia globosa*, needs the sebum to grow, so that the seborrheic skin is the site of inhabitation.

Usually, no itchy sensation is noted. The affected skin shows a golden yellow fluorescence with a Wood's lamp test. Microscopically, both yeast and mycelial forms are observed in the lesion of tinea versicolor, to give "spaghetti and meat balls" appearance. The affected skin varies in color from red-brown to white. The white skin becomes pigmented, while the black skin becomes depigmented. Hence the name "versicolor" (versi = version or change).



Figure III-B-2. Tinea versicolor. Wide areas of the chest wall through axilla (left) and the flexor part of the arm (right) show color change (increase of pigmentation). The patient does not complain of any specific symptoms.

References

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Note 34. Wood lamp test

Wood's lamp test is a diagnostic test for superficial fungal infections, frequently used in dermatology clinics. The ultraviolet light (wave length at 365 nm) is shined over the part of the skin in a dark room. It is also called "black light test". Normal skin will not shine or fluoresce. The expected results in fungal, bacterial and pigmented skin lesions are listed below.

golden yellow color : tinea versicolor
bright yellowish green color: *Microsporum canis* infection
pale green color: *Trichophyton schoenleinii* infection
coral red color: *Corynebacterium* infection
aqua green or blue color: *Pseudomonas aeruginosa* infection
bluish white color: leprosy
pink or pinkish orange color: porphyria cutanea tarda
pale white color: hypopigmentation
purplish brown color: hyperpigmentation
bright white color: albinism

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<http://www.skinsight.com/cosmetics/woodsLampExamination.htm>

3) Malassezial Folliculitis (pityrosporum folliculitis)

Malassezial folliculitis, or pityrosporum folliculitis, caused by *Malassezia restricta* or less frequently *M. globosa* presents as erythematous papules and pustules, 2-4 mm in size, with a predilection for the seborrheic skin, such as the upper back, shoulder, chest and upper arm. The pruritic skin lesions are common in females over the age of 30.

Underlying conditions that predispose the patient to malassezial folliculitis include diabetes mellitus, immunodeficiency and pregnancy. Interestingly enough, patients with spontaneous pneumothorax are often accompanied by malassezial folliculitis on the chest wall or back of the involved side. The affected individuals show high serum titer antibodies against *Malassezia*.

Histologically, suppurative granuloma (granulomatous folliculitis with central abscess formation) is seen around the ruptured hair follicle. The dilated follicle contains neutrophils and basophilic granular debris, and spherical to oval yeast-like organisms can be seen by PAS and Grocott stains. Pseudohyphae are hardly noted. The 1:500 diluted patient own serum is reactive to small yeasts present within the abscess cavity, revealed by the indirect immunoperoxidase method.

Typical examples are shown in Figures III-B-3-1 and III-B-3-2.

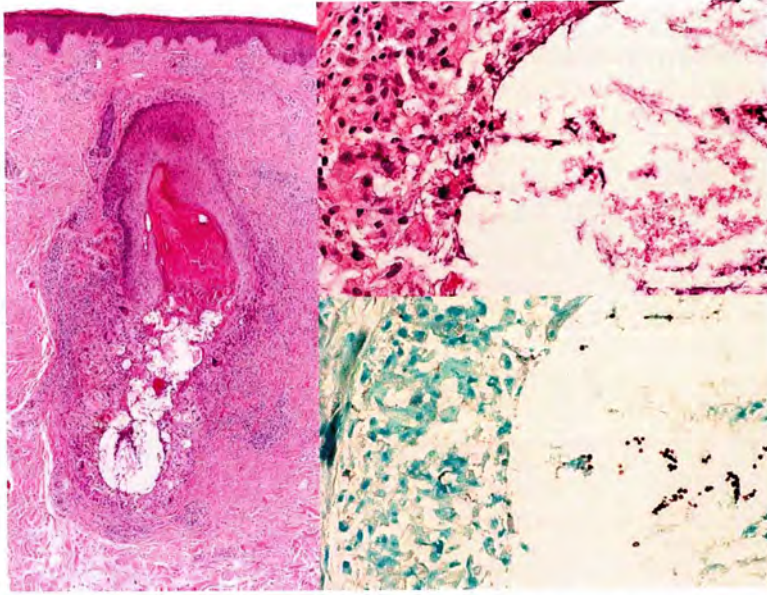


Figure III-B-3-1. Malassezian folliculitis (I). Granulomatous folliculitis is formed on the face (left: H&E). Higher powered views demonstrate infection of yeast-form fungi in the dilated follicular lumen surrounded by epithelioid granulomas (right top: H&E, right bottom: Grocott).

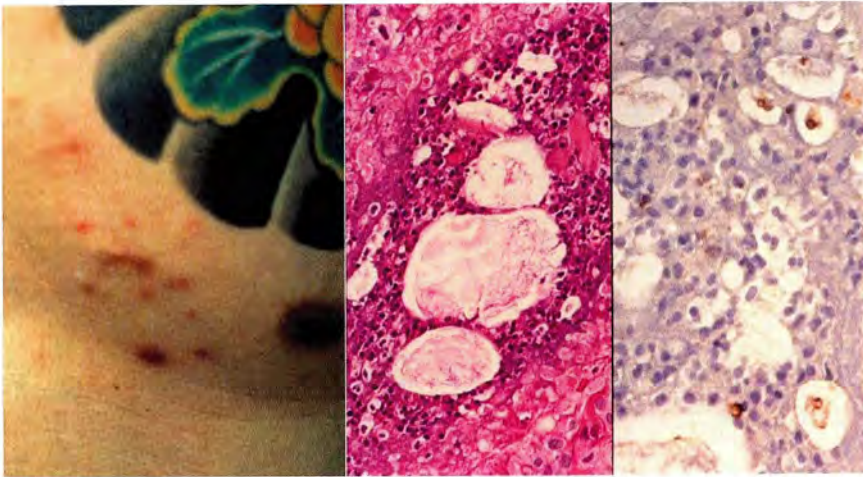


Figure III-B-3-2. Malassezian folliculitis (II). Tattooed chest wall skin shows multiple red-colored papules (left). Infection of yeast-form fungi is visualized within granulomatous folliculitis (center: H&E). The indirect immunoperoxidase staining using 1:500 diluted patient's own serum detects the microbes (right).

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C) Sporotrichosis

Sporothrix schenckii is a fungus that can be found worldwide in decaying vegetative matter. Sporotrichosis is a chronic infection of both humans and animals, characterized by nodular lesions in cutaneous and subcutaneous tissues with or without lymphatic involvement.

Ant sting, as well as animal bites by squirrel or cats, may cause the fungal infection. In immunosuppressed patients, systemic spread may occur with bone, muscle, brain and lung involvement. The diagnosis is made based on the clinical presentation, skin biopsy histology and microbial culture of the etiologic agent. Safe effective therapy for cutaneous sporotrichosis includes oral potassium iodide administration and local heat therapy. Itraconazole, one of the newer triazole antimycotic agents, appears effective against *Sporothrix schenckii* and may provide a means of reducing both the length of therapy and the relapse rate.

Grossly, sporotrichosis is experienced in two forms. A solitary papule is often formed on the face of children. The lymphangitic form is seen mainly in the upper limb of adults (Figure III-C-1). Occasionally, chronic, persistent and ulcerative mycotic infection provoked pseudoepitheliomatous hyperplasia (active downward proliferation) of the overlying epidermis, so that differential diagnosis of squamous cell carcinoma is needed. Careful observation in the markedly inflamed dermis, particularly at the site of foreign body giant cell reaction, is requested.



Figure III-C-1. Gross appearance of sporotrichosis. A solitary papule on the eye lid of a child (left) and multiple chained papules (lymphangitic form) on the chest wall of an adult (right) are presented.

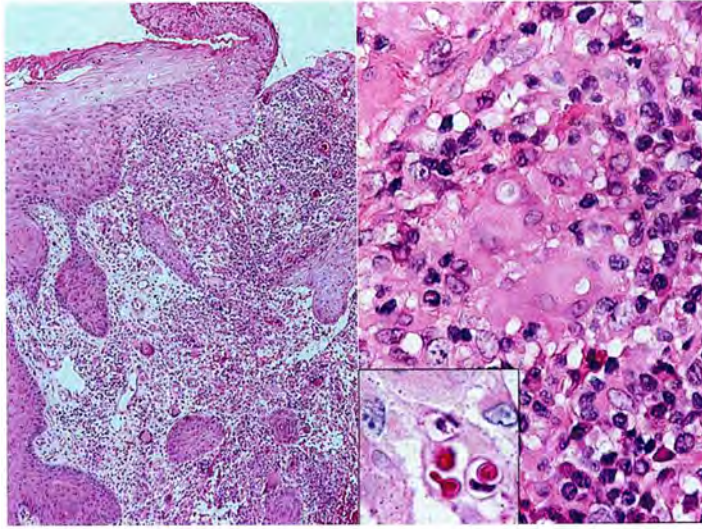


Figure III-C-2. Sporotrichosis mimicking squamous cell carcinoma. Biopsy specimen of this chest wall lesion was erroneously misdiagnosed as squamous cell carcinoma (left: H&E). Rebiopsy demonstrated translucent yeast-form fungi in multinucleated giant cells (right: H&E high-power), confirming the diagnosis of sporotrichosis with pseudoepitheliomatous hyperplasia. Inset: PAS-stained fungi.

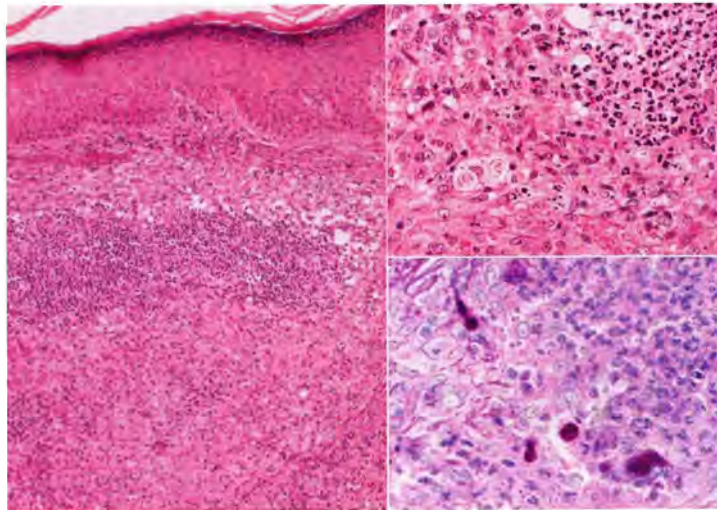


Figure III-C-3. Typical histology of sporotrichosis. Intradermal formation of suppurative granuloma is evident (left: H&E). Non-pigmented yeast-form fungi with nucleated appearance are observed in the cytoplasm of epithelioid cells (right top: H&E, right bottom: PAS).

A few transparent (non-pigmented) small, yeast-type fungi are phagocytized by multinucleated giant cells. The pathogens are PAS- and Grocott-positive, and show a budding-type division pattern (Figure III-C-2). Typical cellular reaction against the pathogen is suppurative granuloma. The blastospores phagocytized by macrophages may show nucleated appearance (Figure III-C-3). Indirect immunoperoxidase staining using a 1:500 diluted patient's own serum identifies yeast-type microbes in paraffin section. Usually, the serum is widely cross-reactive with many kinds of fungi, as was illustrated in Chapter I-B (Figure I-B-1).

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D) Chromomycosis

Infectious lesions caused by black fungi (dematiaceous fungi), producing a visible amount of melanin, are categorized into the following three groups.

- (i) Chromomycosis or chromoblastomycosis is characterized by cutaneous suppurative granulomas containing “sclerotic cells” or “muriform cells” without hypha formation. *Fonsecaea pedrosoi* and *Exophiala dermatitidis* are the causative agents.
- (ii) Pheohyphomycosis (pheo = brown, hypho = mycelium) is the term for infection of mycelium-forming black fungi. It is divided into the dermal/subcutaneous type and systemic type clinically. The causative agents are often *E. dermatitidis*, *E. jeanselmei* and rarely *F. pedrosoi*.
- (iii) Madura foot (mycetoma) forming black grains.

Chromomycosis is most often encountered. It is caused by traumatic or incidental inoculation of the fungus into the exposed skin. Grossly, painless, infiltrative and verrucous lesions are formed on the extremities.



Figure III-D-1. Chromomycosis. An infiltrative and verrucous lesion is formed on the back of the hand (left). Pigmented (melanotic) sclerotic cells are identified in the suppurative granuloma lesion. No mycelia are noted (right: H&E).

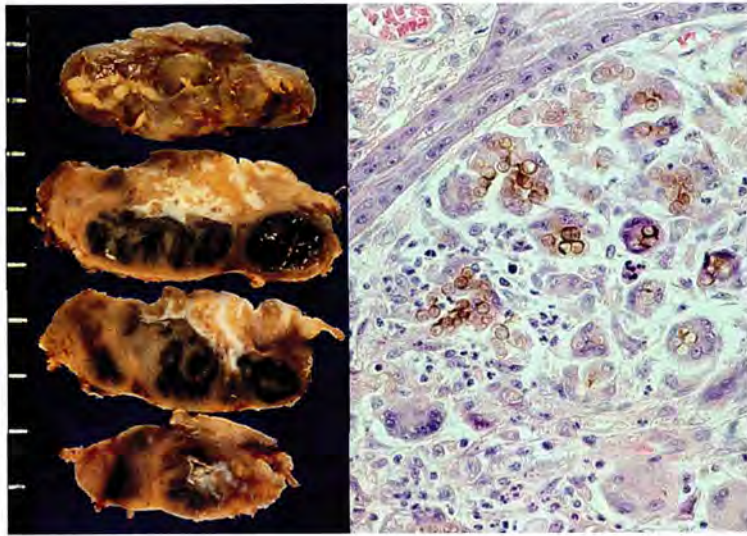


Figure III-D-2. Chromomycosis in a renal failure patient on hemodialysis. Accelerated growth of the pigmented fungal cells provokes a black colored nodular skin lesion near the arterio-venous shunt (left). Histologically, numerous sclerotic cells are observed within the cytoplasm of multinucleated giant cells (right: H&E). The courtesy by Dr. Osamu Miyaishi, a pathologist at National Center for Geriatrics and Gerontology, Obu, Japan.



Figure III-D-3. Electron micrograph of a sclerotic cell in the cytoplasm of a multinucleated giant cell. The cell wall of the fungal cell is electron-dense due to melanin production.

Histologically, suppurative granuloma (granuloma surrounding central abscess cavity) is formed against *Fonsecaea pedrosoi* or *Exophiala dermatitidis* (*Phialophora dermatitidis*). Brown-colored rounded sclerotic cells (muriform cells) are phagocytized by multinucleated giant cells in the inflamed dermis. Chains of pigmented sclerotic cells are noted occasionally. Mycelia are never formed within the tissue. Figure III-D-1 illustrates a representative case.

Proliferation of the pigmented fungi is accelerated by the immunosuppression due to chronic renal failure and hemodialysis (Figure III-D-2). Multiple large black nodules are grossly observed on the cut surfaces of the opportunistic skin lesion formed near the arterio-venous shunt for hemodialysis. Microscopically, numerous sclerotic cells are seen in and

outside phagocytic macrophages. Electron micrograph of the sclerotic cells phagocytized by a multinucleated giant cell is shown in Figure III-D-3.

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E) Pheohyphomycosis

Typical example of pheohyphomycosis (infection of hypha-forming pigmented fungi) in the dermis is illustrated in Figure III-E-1.

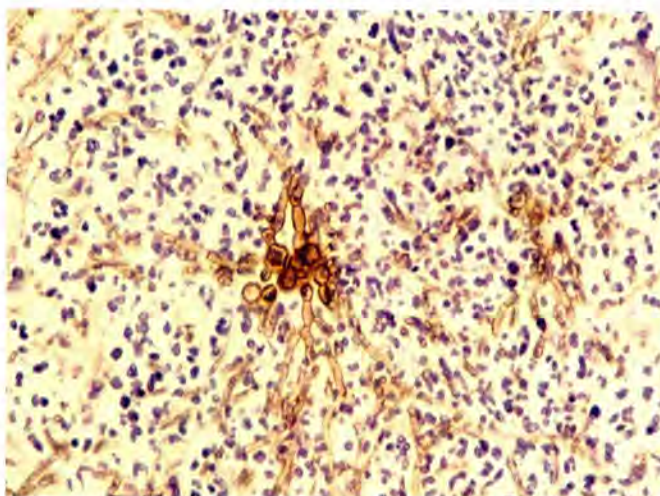


Figure III-E-1. Pheohyphomycosis in an immunocompromised case of acute leukemia. Hypha-forming pigmented fungi are observed in an intradermal abscess lesion (H&E).

Growth of hyphae-forming pigmented fungi is seen in the abscess cavity. Infection of hypha-forming *Exophiala jeanselmei* is promoted by an immunosuppressed state. This lesion is seen in a case of acute leukemia. Secondary brain abscess may be encountered.

Pheohyphomycosis may occur in para-articular location. Subcutaneous formation of an encapsulated cyst (synovitis/bursitis) is a common presentation of this mycosis. The lesion is histologically composed of admixture of abscess and epithelioid granuloma. Pigmented

hyphae positively signaled with PAS and Grocott stains are phagocytized by macrophages (Figure III-E-2). The lesion seen in the immunocompetent patient is usually caused by traumatic implantation of a wood splinter.

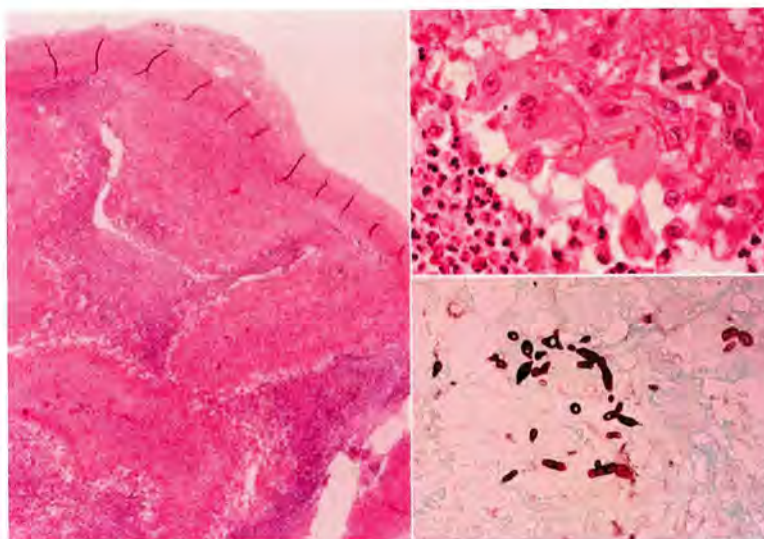


Figure III-E-2. Pheohyphomycosis presenting encapsulated synovitis of the finger. In the encapsulated cystic lesion, suppurative granuloma is formed, and the pigmented mycelia are seen in the cytoplasm of macrophages (epithelioid cells) (left and right top: H&E, right bottom: Grocott).

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F) Mycetoma (Madura Foot)

Mycetoma, “eumycetoma” or madura foot, a special form of pheohyphomycosis, is characterized by long-lasting, fistule-forming inflammatory nodules on the leg and foot. Black grains, consisting of pigmented fungal mycelia and matrix, are excreted from the fistula. The prevalence is high in tropical areas, including sub-Saharan Africa, tropical Asia, and Central and South America. The lesion is slowly progressive, occasionally resulting in bone ulceration. Causative black fungi often belong to *Madurella grisea*, *M. mycetomatis* and *Exophiala jeanselmei*. Treatments include 5-fluorocytosine administration, injection of Amphotericin B, local heat therapy and surgical resection.

A surgical case of mycetoma on the ankle is presented (Figures III-F-1 and III-F-2). This Japanese male patient had a history of dog bite at this location 25 years earlier. Cut surfaces of the surgically removed nodule show black granules (grains) in the center of yellow-colored xanthogranulomas, and extensive fibrosis white in color is seen between the granulomas.



Figure III-F-1. Mycetoma on the ankle. A fistule-forming nodular lesion is observed on the ankle (left). Cut surfaces of the surgically removed nodule show black grains in the center of yellow-colored xanthogranulomas in the subcutaneous tissue (right). The granulomas are surrounded by white-colored dense fibrosis.

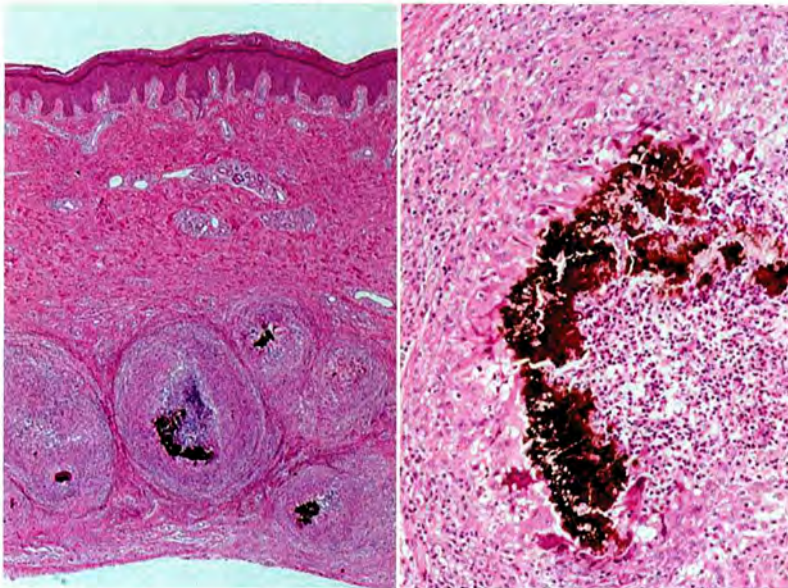


Figure III-F-2. Histology of mycetoma on the ankle. The demarcated xanthogranulomas in the subcutaneous tissue accompany central abscess formation and contain brown-colored grains (left: H&E). The grain, consisting of pigmented hyphae and eosinophilic hyaline matrix (Splendore-Hoeppli material), is surrounded by granulomatous reactions (right: H&E).

Microscopically, demarcated granulomas showing central abscess formation and containing brown-colored (melanotic) grains are formed in the subcutaneous tissue. The grain consists of pigmented hyphae and eosinophilic hyaline matrix (Splendore-Hoeppli material).

The pigmented hyphae forming a clump secrete pigmented grain matrix. Foreign body giant cells surround the grain.

Mycetoma is rarely encountered in other locations, including the finger and conjunctiva. A finger lesion in a Cambodian case is illustrated in Figure III-F-3. Figure III-F-4 presents a Japanese case of conjunctival mycetoma accompanying several black grains.

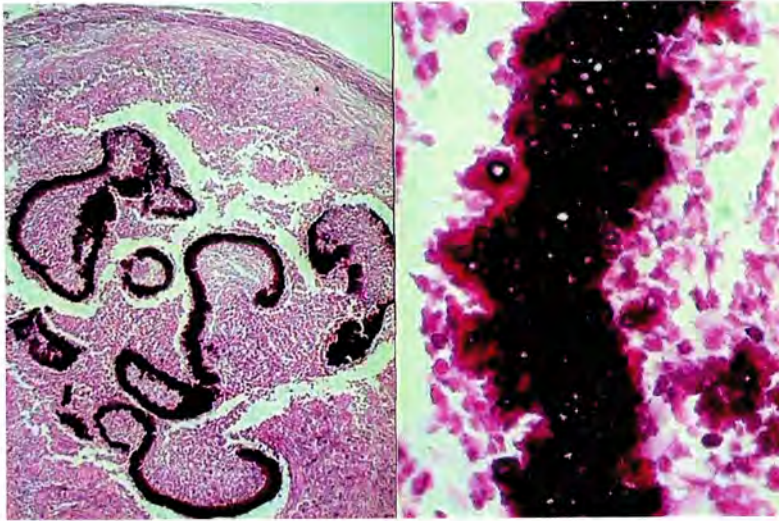


Figure III-F-3. Mycetoma on the finger of a Cambodian male. An encapsulated subcutaneous lesion contains ringed or crescentic melanotic grains. Suppurative granuloma is observed (PAS, left: low power, right: high power). The courtesy by Dr. Chhut Vathana, a pathologist at Sihanouk Hospital Center of HOPE, Phnom Penh, Cambodia.

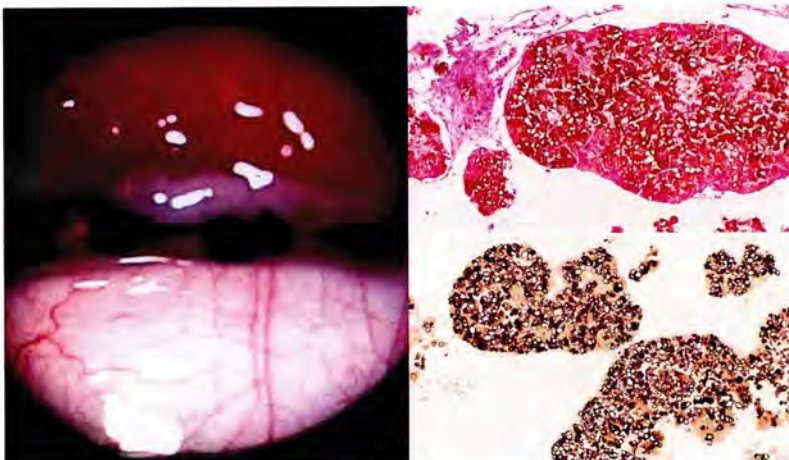


Figure III-F-4. Mycetoma on the conjunctiva. Multiple black grains are formed in the conjunctival mucosa (left). The grains consist of clustered pigmented hyphae and Splendore-Hoeppli material (right top, H&E). The fungi are clearly reactive with Grocott stain (right bottom). The courtesy by Dr. Masaki Moriyama, a pathologist at Nippon Medical University, Tokyo, Japan.

The color of the grains is white or yellowish white when they derive from *Phaeoacremonium kraidenii*, *Pseudallescheria (Petriellidium) boydii*, *Actinomedura madurae*, etc. Red-colored grains are observed, when *Actinomedura pelletieri* is infected.

Actinomyces israelii, *Nocardia asteroides* and *Staphylococcus aureus* may cause actinomycetoma, nocardial mycetoma and botryomycosis, respectively, producing white grains. *Streptomyces somaliensis* may produce yellow or red discharge.

The term “mycetoma” is occasionally and confusingly used as a synonym of the “fungus ball” in aspergillosis. *Aspergillus* fungus balls are commonly formed in the lung cavity and paranasal sinus.

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G) Alternariosis

Alternaria alternata is a saprophytic pigmented plant pathogen, and is usually non-pathogenic to the human. *Alternaria* is nominated as one of the major fungal allergens, including *Aspergillus*, *Penicillium*, *Trichosporon* and *Cladosporium*. Currently, about 70 fungal allergens have been approved by the International Allergen Nomenclature Committee, and are capable of causing IgE-mediated hypersensitivity in the human. The pigmented spores or hyphae of *Alternaria* species, floating in the air, occasionally appear as cytology contaminants (Figure III-G-1).

Alternariosis, a special form of pigmented mycosis, may rarely be encountered in both healthy and immunocompromised individuals, including solid-organ transplant recipients. Combined surgical and medical treatments seem to be useful in the management of infection. The infiltrative lesion on the knee shown in Figure III-G-2 is observed in an otherwise healthy adult female, and interesting enough, similar skin lesions of alternariosis have repeatedly been recorded among three sisters. Histologically, intradermal abscess is formed, and the surrounding dermis reveals chronic fibrosing inflammation. Granulomatous reaction surrounds the abscess lesion. Indirect immunoperoxidase staining using 1:500 diluted patient's serum discloses positively labeled round-shaped pathogens in the abscess cavity. It should be noted that the staining specificity is low, while the sensitivity is high enough.

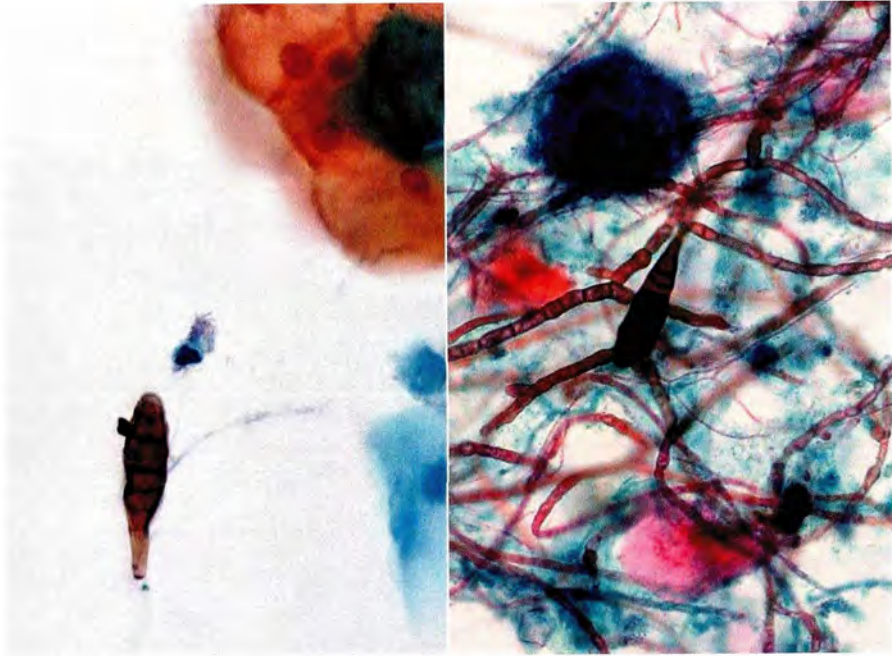


Figure III-G-1. Pigmented spores and hyphae of *Alternaria*, contaminants in the cytology specimen. It should be noted that *Alternaria* of spore form (left) or mycelial form (right) happen to be contaminated in the cytology preparation (Papanicolaou).

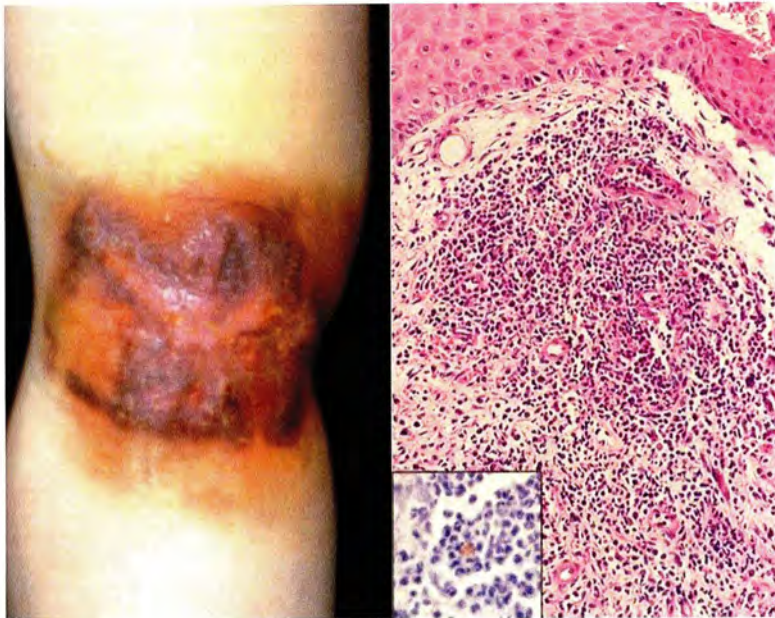


Figure III-G-2. Alternariosis in an immunocompetent adult female, accompanying an infiltrative and sclerotic lesion on the knee. The lesion persisted for 13 years. Of special note is that the skin lesions are seen in three sisters (left). Histologically, intradermal small suppurative granulomas are surrounded by inflammatory fibrosis (right). Immunostaining using 1:500 diluted patient's own serum identifies a few spores within the lesion (inset). The courtesy by Prof. Chikako Nishigori, Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan.

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H) Tinea Nigra

Tinea nigra is a rare superficial infection of pigmented fungus, *Hortaea werneckii* (*Exophiala werneckii*). In this superficial pheohyphomycosis, asymptomatic dark brown or black patches are observed on the palm and infrequently on the sole. Neither itch nor inflammatory reaction is associated. The fungi are widely distributed in the soil and water in tropical and subtropical countries. Infection is mediated through minor trauma in the endemic area. In Japan, tinea nigra is mainly distributed in the southern regions, including Kyushu Island and Okinawa. The latent period is 2-7 weeks.

Direct microscopy of the scraped material in 20% potassium hydroxide is useful for the diagnosis of mycotic infection. Biopsy specimen reveals that the cornified layer is slightly more compact than usual, and brown hyphae are distributed in the superficial layer. There is no inflammatory reaction in the dermis. A representative case is shown in Figures III-H-1 and III-H-2.



Figure III-H-1. Tinea nigra. An asymptomatic black macule is seen on the palm of a young female living in Kyushu Island, Japan (left). Scratch smear treated with 20% KOH shows numbers of fungal bodies (right top). Slide culture study morphologically identifies *Hortaea werneckii* (right bottom). The courtesy by Dr. Mohito Matsushita at Department of Dermatology of Kagoshima University Graduate School of Medicine, Kagoshima, Japan.

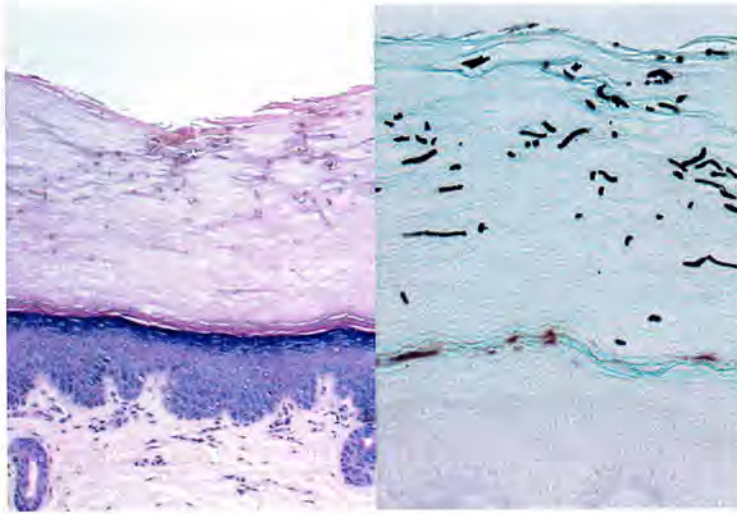


Figure III-H-2. Biopsy histology of tinea nigra. In the thick corneal layer of the palm, pigmented mycelia are randomly distributed. No inflammatory reaction is noted in the dermis (left: H&E, right: Grocott). The courtesy by Dr. Mohito Matsushita, Kagoshima.

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I) Candidiasis

Cutaneous candidiasis (moniliasis) is very common and can involve almost any area of the skin on the body. It most often occurs in warm, moist and creased areas such as the armpits and groin. Most lesions of candidiasis are caused by *Candida albicans*. Diaper dermatitis in newborns and the elderly is a representative of cutaneous candidiasis. The skin folds (intertrigo), the corner of the mouth (angular cheilitis), the edge of the nails (paronychia) and finger nails (onychomycosis: see Figure III-A-4-2) are the prerequisite sites of candidiasis. As a sexually transmitted infection, *Candida* causes vaginitis, balanitis and perianal dermatitis. Gross features of candidal cheilitis and candidal balanitis are demonstrated in Figure III-I-1. Thrust (candidal stomatitis) is commonly seen in infants, being not necessarily considered abnormal.

The candidal infection is prone to occur in patients with a weakened immune system, diabetes mellitus, pregnancy, obesity and AIDS. Antibiotics therapy may provoke the microbial substitution, resulting in mucosal candidiasis of the oral cavity, pharynx, esophagus, gastrointestinal tract and vagina. *Candida* species are not part of the normal flora

of the skin, while they are seen throughout the gastrointestinal tract (from the mouth to anus) as the normal commensal flora. The frequency of intravascular catheter-mediated infection (*Candida* septicemia) is high in case fed by hyperalimentation. High sugar content of the solution accelerates infections. Candidal endophthalmitis may follow.



Figure III-I-1. Gross appearance of candidiasis. The lip of a child shows multiple milky spots (left). The penile lesion caused by sexually transmitted infection is characterized by diffuse and dry reddish change on both the glans penis and penile shaft (right).

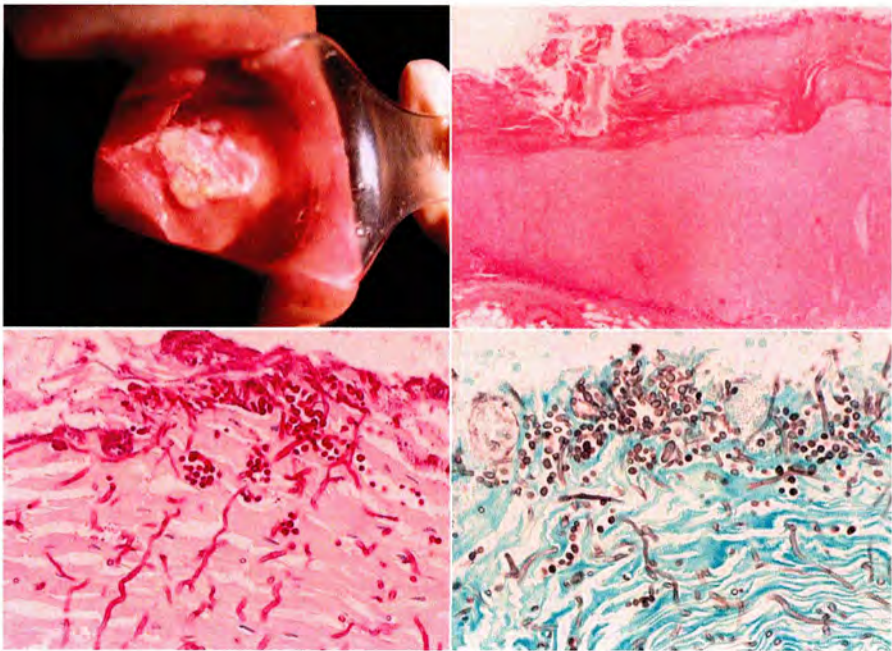


Figure III-I-2. Candidiasis of the oral cavity. A demarcated white and granulated plaque is noted on the buccal mucosa (left top). Fungal infection is observed on the surface of the hyperparakeratotic squamous mucosa (right top). The fungi are composed of both the yeast form (blastospores) and pseudohypha form (left bottom: PAS, right bottom: Grocott). The courtesy by Prof. Keiichi Tsukinoki, Kanagawa Dental College, Yokosuka, Japan.

Candida microscopically appears as two microscopic forms (dimorphic fungi). Yeast-type (blastospores) versus pseudohypha form (with focal cytoplasmic vacuolation). These two forms are clearly seen in the cytology specimens and histopathological sections. As one typical example, candidiasis of the oral cavity is presented in Figures III-I-2. When *Candida* is observed solely in the yeast form, it is highly likely that the fungi are non-pathogenic. When active inflammation is provoked by *Candida* infection, both yeast-form and pseudohypha-form are observed under a microscope.

Recently, the frequency of infection by non-albicans *Candida* species is increasing, and among them, *Candida glabrata* is the most important pathogen. *C. glabrata* is characterized by the innate drug resistance and unique non-dimorphic appearance without forming pseudohyphae (solely forming the yeast-form) (Figure III-I-3).

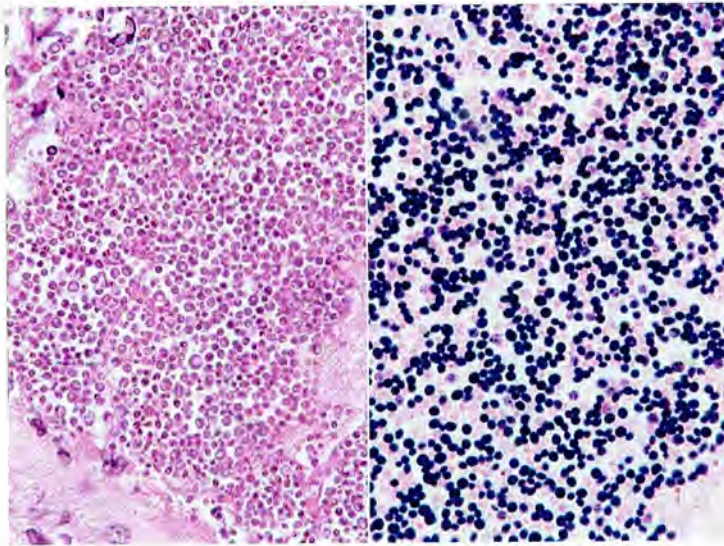


Figure III-I-3. *Candida glabrata* endocarditis. Monomorphous growth of yeast-form fungi is observed on the mitral valve in a case of disseminated *Candida glabrata* infection (left: H&E). Many but not all of the yeast-form fungi are Gram-positive (right).

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Note 35. *Candida (Torulopsis) glabrata*

Candida glabrata, also known as *Torulopsis glabrata*, is a relatively non-pathogenic commensal fungal organism of human mucosal tissues. However, with the frequent use of immunosuppressive agents, mucosal and systemic infections caused by *C. glabrata* have increased significantly, especially in the human immunodeficiency virus-infected population. A major obstacle in *C. glabrata* infection is their innate resistance to the azole antimycotic therapy, which is effective in treating infections caused by the other *Candida* species.

C. glabrata contrasts with other *Candida* species by its non-dimorphic blastoconidial morphology and haploid genome. Myceliform fungi are not seen. *C. glabrata* currently ranks second or third as the causative agent of nosocomial superficial infection (oral, esophageal, vaginal and urinary) or systemic candidal infection.

Candida vaginitis is most commonly caused by *C. albicans* (>85%). *C. glabrata* may cause symptomatic vaginitis, not rarely accompanying bacterial vaginitis. Vaginitis due to *C. albicans* responds well to available therapy, while *C. glabrata* vaginitis is associated with a high treatment failure rate. Treatment of *C. glabrata* vaginitis with vaginal boric acid suppository results in clinical improvement or mycologic eradication in about 80% of cases.

Meanwhile, *C. glabrata* is often seen as normal flora on the vaginal mucosa, so that the presence in the cervical cytological smear preparations does not mean a pathological condition (Figure III-I-4). In such an occasion, normal vaginal flora, *Lactobacillus* or Döderlein bacillus, is well retained.

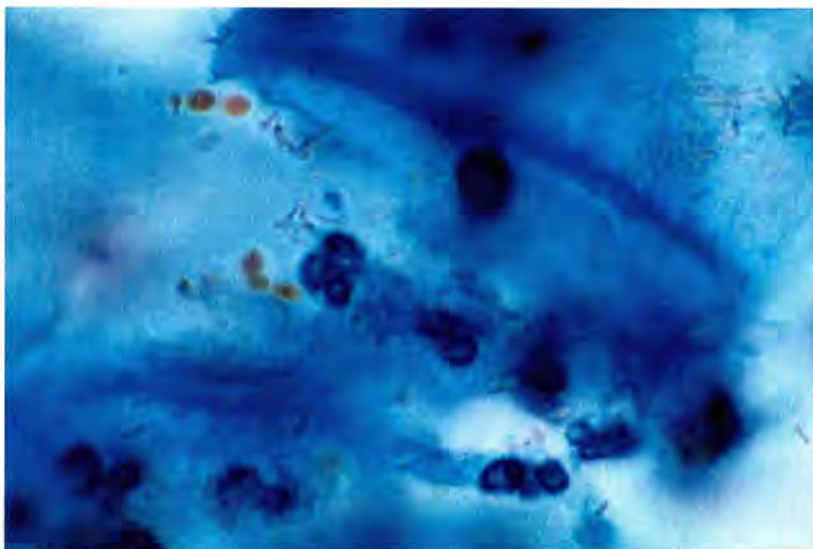


Figure III-I-4. *Candida glabrata* as normal flora of vaginal mucosa. Yellowish orange-colored paired yeasts are observed in a Papanicolaou-stained cervical smear preparation. In the background, long rods (Döderlein bacilli or *Lactobacillus*) are retained, thus indicating a non-pathogenic status.

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J) Trichosporonosis

Trichosporon spp. is a yeast-type fungus microscopically similar to *Candida* spp., forming both blastospores and pseudohyphae. It widely distributes in the soil, water, vegetables, birds and mammals. *Trichosporon* spp. is notorious as allergens to provoke summer-type hypersensitivity pneumonitis, mainly seen in the western part of Japan. In the human, *Trichosporon* spp. such as *Trichosporon cutaneum* (*T. beigelii*), *T. asteroides*, *T. ovoides*, *T. inkin*, *T. mucoides* and *T. asahii*, is a member of normal flora of the mouth and skin, particularly around the genitals of the aged, and can be the causative agent of superficial and deep infections. *T. asahii* superficially infects the pubic hair to form white piedra (refer to Note 33), and systemic and lethal infection of *T. cutaneum* happens in immunocompromised patients (Figure III-J-1). Secondary skin manifestations may be seen in cases of disseminated trichosporonosis. In Figure III-J-2, cutaneous trichosporonosis on the surface of the necrotic scrotum is shown. The patient suffered from Fournier's gangrene (see Chapter II-C-2).

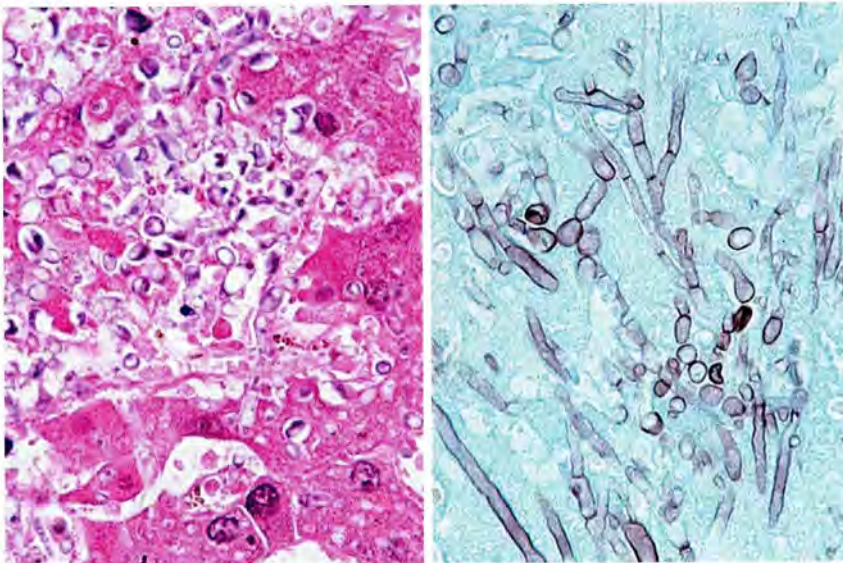


Figure III-J-1. Opportunistic systemic trichosporonosis. Secondary systemic spread of *Trichosporon cutaneum* occurred in a leukemia case post bone marrow transplantation. Both yeast-form and mycelia are observed in the liver (left: H&E, right: Grocott).

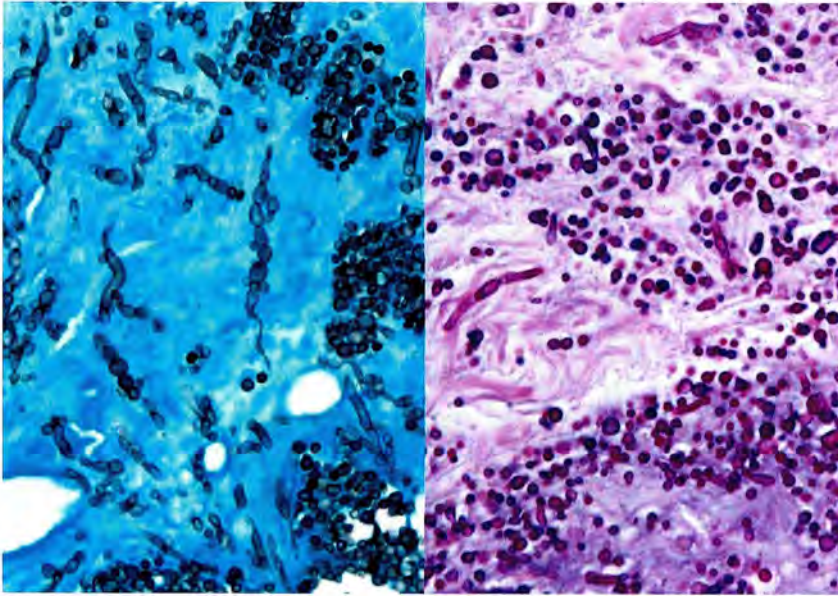


Figure III-J-2. Cutaneous trichosporonosis on the surface of the necrotic scrotum suffering from Fournier's gangrene. Microscopic features of trichosporonosis resemble those of candidiasis. Triangular tapering appearance at the end of the mycelia is characteristic of *Trichosporon* spp. (left: Grocott, right: PAS). The courtesy by Dr. Takaaki Chikugo, Department of Pathology, Kinki University Faculty of Medicine, Sayama, Japan.

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K) Aspergillosis

Aspergillosis is common in the lung and paranasal cavity, often forming a "fungus ball" or aspergilloma. The most common species is *Aspergillus fumigatus*. *A. flavus*, *A. niger*, *A.*

terreus and *A. nidulans* may cause infection. Aspergillosis of the skin occurs predominantly in the external meatus, except for rare secondary cutaneous involvement of systemic aspergillosis. Traumatic inoculation may cause primary cutaneous aspergillosis, at the site associated with intravenous access catheter, occlusive dressings, burns or surgery. Traumatic corneal infection is also encountered.

Otomycosis manifests as intractable otitis externa, and histologically, numbers of branching hyphae and a few conidial heads are intermingled with neutrophilic and fibrinous exudation. When the round-shaped conidiophores (conidial heads) produce pigmented (melanotic) spores, we can make a diagnosis of aspergillosis caused by *Aspergillus niger* (Figure III-K). *Candida albicans* and *Penicillium* spp. also cause surface otomycosis in the human. In poorly controlled diabetic patients, *Aspergillus* may provoke lethal malignant otitis externa (see also Chapter II-D-2).

Aspergillus species, widely distributed in the environment and often colonizing the sealing and wall of the hospital room, are a representative environmental pathogen. For immunosuppressed patients suffering from hematological disorders, *Aspergillus* becomes an important opportunistic pathogen, so that the airborne exogenous infection of the microbes should be cut off by the filtration of the room air. Flower gift is prohibited, because fungi are easily attached to the leaves and flowers. Inhalation of outside dusty air and the air supplied from hospital ward construction should also be avoided as much as possible.

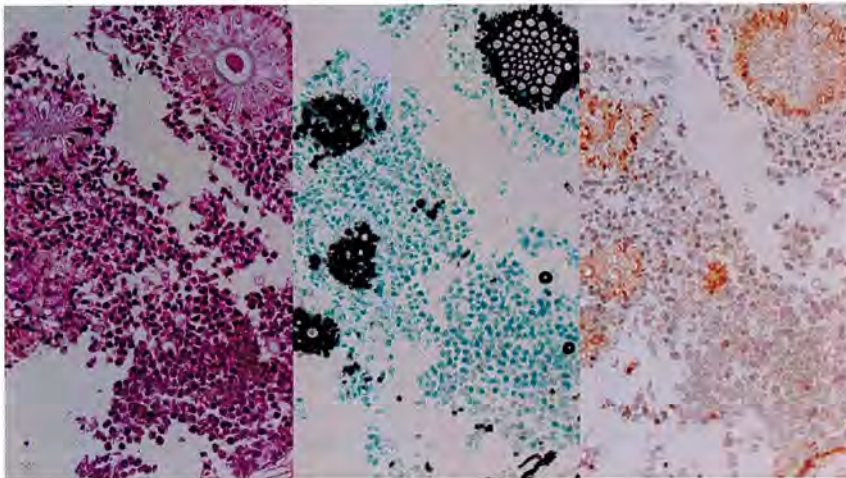


Figure III-K. Aspergillosis on the external meatus (otomycosis). Spore-producing conidiophores are observed. Neutrophilic cellular reaction is evident (left: H&E, center: Grocott stain, right: immunostaining using anti-*Aspergillus* monoclonal antibody). Regarding the histochemical detection of *Aspergillus*, see also Figure I-C.

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L) Mucormycosis

Mucormycosis (zygomycosis) is an infection by the class *Zygomycetes*, mainly *Mucor ramosissimus*, *Rhizomucor pusillus* and *Rizopus oryzae*. Some 16 species of *Zygomycetes* infect the human. *Zygomycetes* are commonly observed molds growing in a moist environment. Most fungi have chitin as structural polysaccharide, while *Zygomycetes* synthesize chitosan, a deacetylated homopolymer of chitin. Hence, serum β -D-glucan, a laboratory marker of fungal infection, is negative in mucormycosis.

The main sites of localized mucormycosis are the lung and paranasal cavity. Formation of conidiophores is rarely encountered in case of paranasal cavity infection (Figure III-L-1). Typical gross features of systemic mucormycosis include hemorrhagic infarction of the involved tissue and organ. Microscopically, weakly basophilic wide hyphae, showing lack of septum formation and wide angle of lamification, are seen among the mycotic thrombus. Stamp smear preparations reveal typical microscopic morphology of mucormycosis (Figure III-L-2). *Zygomycetes* are microscopically featured by angioinvasiveness and weak reactivity with Grocott staining (Figure III-L-3). However, some species reveal strong basophilia with strong Grocott reactivity, as shown in Figure III-L-4.

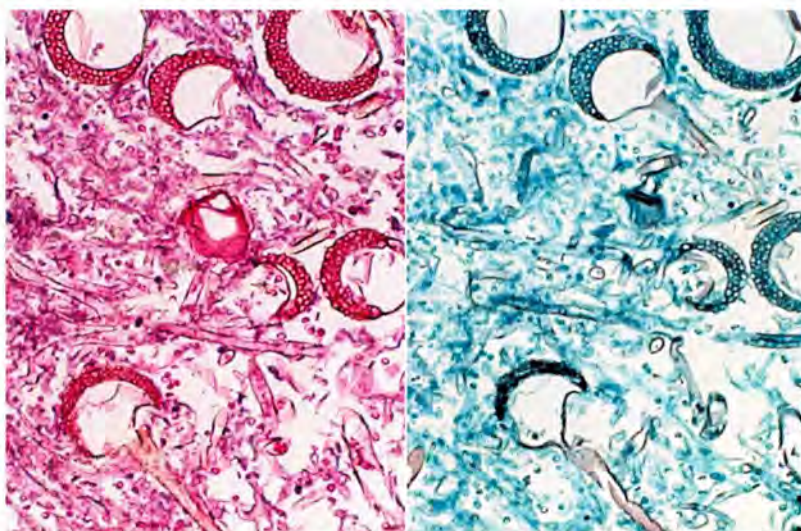


Figure III-L-1. Paranasal mucormycosis. Exceptionally, conidiophores are formed in the paranasal cavity. Aerated growth condition within the cavity is essential for conidiophore formation (left: H&E, right: Grocott).

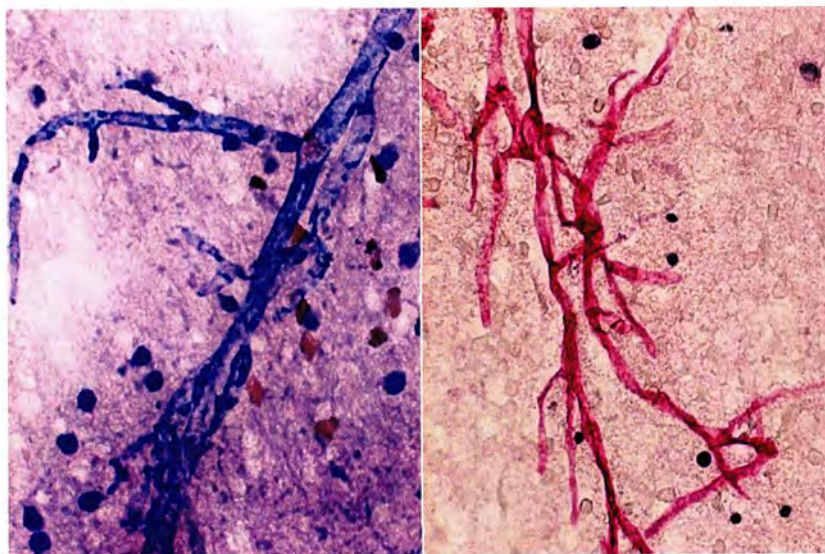


Figure III-L-2. Stamp preparation of cerebral mucormycosis in pediatric acute leukemia case. Non-septating hyphae show variable thickness. Wide angle of lamification is distinct from *Aspergillus* hyphae (left: Giemsa, right: PAS stain). The courtesy by Dr. Suzuko Moritani, a pathologist at Nagoya Medical Center, Nagoya, Japan.

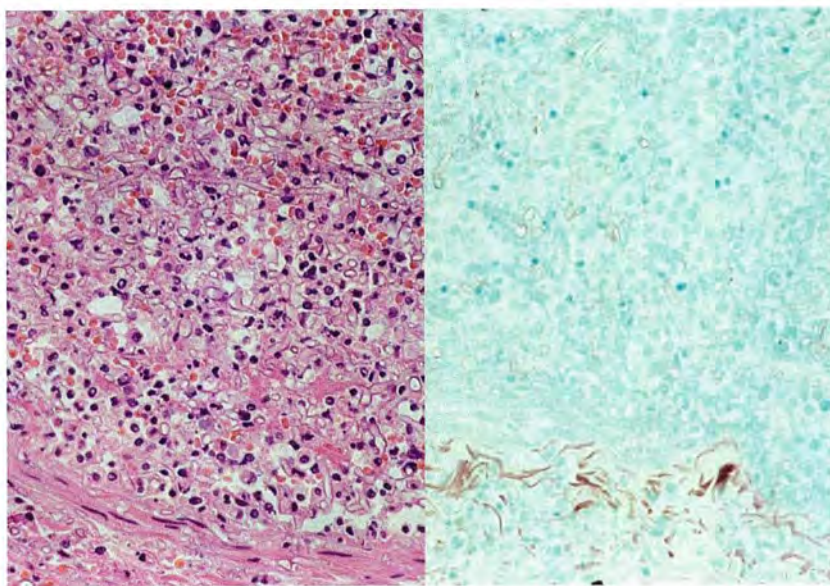


Figure III-L-3. Angioinvasive mucormycosis (I). *Zygomycetes* frequently show angioinvasion, resulting in hemorrhagic infarction of the organ. Weak staining reactivity with H&E and Grocott stain is characteristic of this opportunistic fungus (left: H&E, right: Grocott stain).

Cutaneous mucormycosis is infrequently seen as a skin manifestation of systemic mucormycosis. Localized mucormycosis may be provoked by animal bites. The rare lethal variant is rhinocerebral mucormycosis, seen as a complication of poorly controlled diabetes mellitus. Angioinvasive infection of *Zygomycetes* progressed from the paranasal cavity to the overlying facial skin and a lower part of the frontal lobe of the brain (Figure III-L-5).

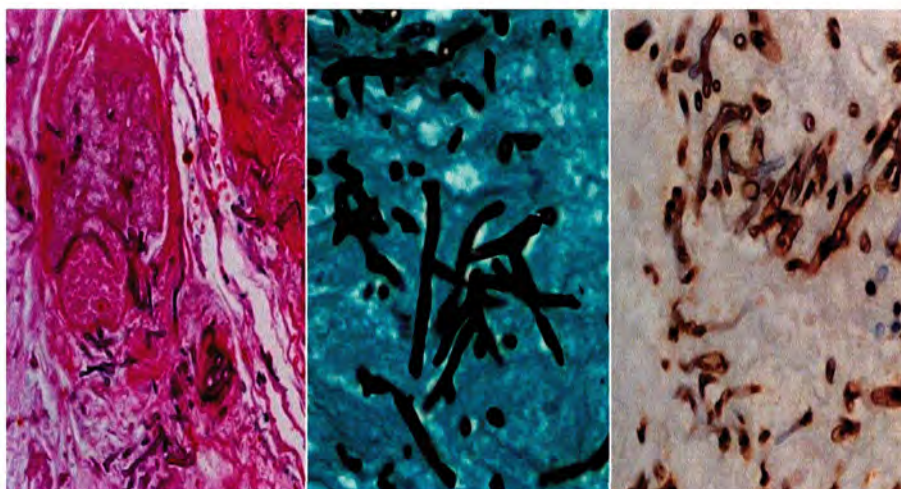


Figure III-L-4. Angioinvasive mucormycosis (II). Another case of angioinvasive mucormycosis exhibits deep basophilia (left: H&E) and strong reactivity with Grocott stain (center). The hyphae are immunoreactive with anti-*Rhizomucor* monoclonal antibody, which is cross-reactive with *Zygomycetes* but not with *Aspergillus* or *Candida* (right).

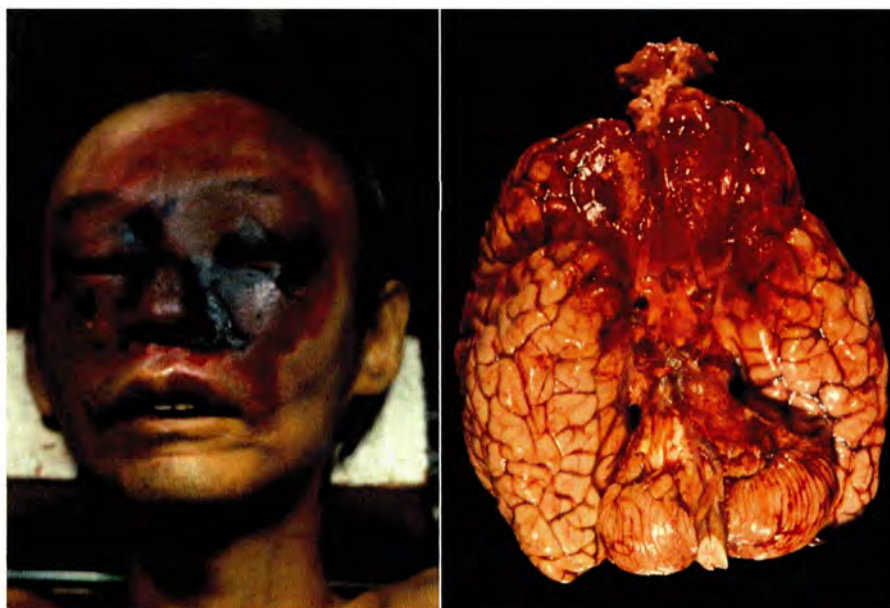


Figure III-L-5. Lethal mucormycosis of rhinocerebral type in a poorly controlled diabetic patient. Angioinvasive mycosis resulted in hemorrhagic necrosis of the face (left) and anteroinferior part of the brain (right). Infection had been extended from the paranasal cavity.

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M) Fusariosis

Fusarium species is ubiquitously found in the soil, air and on plants. This saprophytic fungus is notorious as a plant pathogen. In humans, in addition to mycotoxicosis due to ingestion of fungus-colonized food, *Fusarium* species causes localized, focally invasive and disseminated mycosis in the immunocompromised individuals, particularly with systemic burn, hematologic malignancy and persistent neutropenia. The most common fusariosis in the human is corneal infection (keratitis), caused by eye trauma (particularly, plant thorn stick) or contact lens use. The fungal keratitis is seen in immunocompetent individuals. *Fusarium*-induced onychomycosis has also been reported.

The pathogens of fungal keratitis include *Fusarium*, *Aspergillus* and *Candida*. A rare human case of fungal keratitis caused by *Cryosporium parvum*, a pathogen in lower animals and birds, has been reported. *C. parvum* characteristically forms round-shaped, thick-walled “adiaspores” as large as 250 µm in diameter. *Paecilomyces lilacinus* may also rarely cause fungal keratitis.

Invasive fusariosis includes endophthalmitis, arthritis, osteomyelitis, cystitis and brain abscess. Common species involved are *Fusarium solani*, *F. oxysporum* and *F. moniliforme*. The diagnosis of fusariosis is made on histopathology, mycology or blood culture. Portals of the entry of disseminated infection are the respiratory and gastrointestinal tracts and most commonly the skin. Typical skin lesions are multiple, painful red or violaceous nodules, the center of which becomes ulcerated and covered by black eschar, mainly on the trunk and extremities. The most frequent presentation of invasive fusariosis is a combination of the cutaneous lesion and positive blood culture, with or without lung or nasal sinus involvement. The prognosis is poor.

Histology of the skin lesion shows acute necrotizing inflammation. Vascular invasion with mycotic thrombosis frequently occurs. As is so in aspergillosis, both hyaline hyphae filaments (phialides) and unicellular form spores (phialoconidia) are observed (hyalohyphomycosis). In *Aspergillus*, hyphae show regular 45-degree branching and uniform diameter. In *Fusarium*, hyphae branching from 45 to 90 degrees are noted, and the hyphae diameter is smaller and irregular (Figure III-M-1).

A Cambodian teenager case of ophthalmic fusariosis is shown in Figure III-M-2. The severe infection was seen not only in the cornea but also in the surrounding tissues such as the lens, palpebra and orbit, so that the eye ball was removed surgically. Microscopically, active growth of hypha-forming *Fusarium* is observed in the necrotic tissue. On the surface of the

lesion, formation of several-celled (chained or beaded), fusiform- to sickle-shaped macroconidia is characteristically recognized.

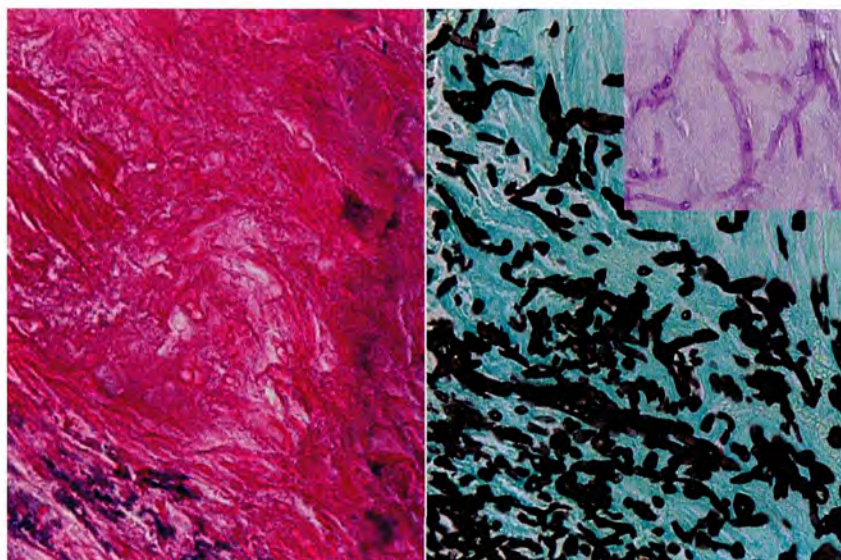


Figure III-M-1. Fusariosis of the nasal cavity. The necrotic nasal mucosa is infiltrated by poorly basophilic hyphae (left: H&E). Grocott stain identifies short-chained hyphae with variable angles of branching (right). Inset: PAS reactivity of the fungal hyphae.

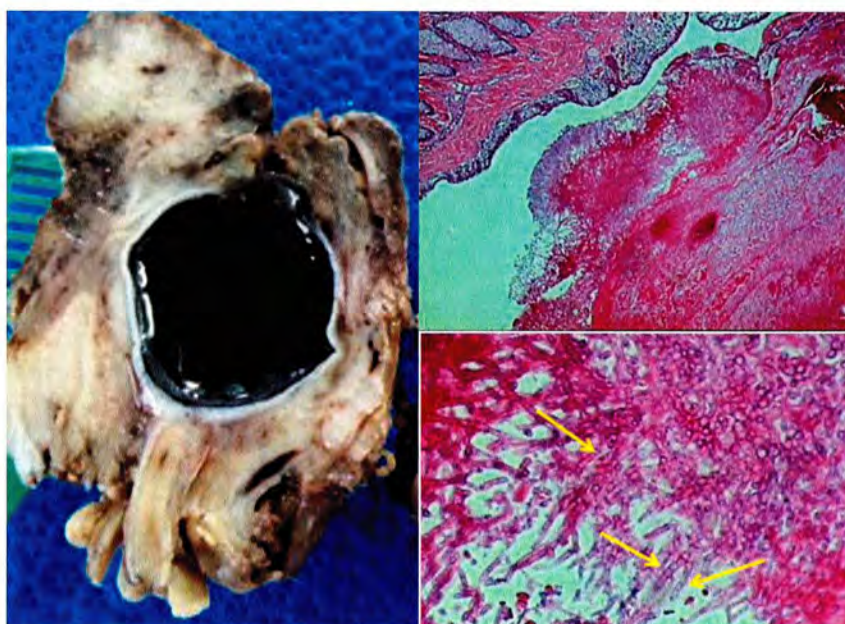


Figure III-M-2. Fusariosis of the eye ball of a Cambodian young. The eye lid, conjunctiva and cornea are heavily involved by necrotic infective process (left). Infection of hypha-forming fungi is evident in the necrotic conjunctival mucosa (right top: H&E). High-powered view shows dense infection of chained or beaded (several-celled) hyphae characteristic of *Fusarium* (arrows, right bottom: H&E). The courtesy by Dr. Chhut Vathana, a pathologist at Sihanouk Hospital Center of HOPE, Phnom Penh, Cambodia.

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N) Cryptococcosis

Cryptococcus neoformans, solely yeast-forming fungi, is often contained in the feces of birds, particularly pigeons. The main target organs of cryptococcosis are the lung and meninges. Cryptococcal granuloma is recognized as a coin lesion in the lung, and may histologically resemble a tuberculous lesion. Yeast-type pathogens are phagocytized by macrophages, to form foreign body granulomas. Capsule formation by the round-shaped yeasts is characteristic of this fungus, but the capsules are poorly formed within the cytoplasm of macrophages. The yeasts are translucent and unstained by H&E preparations. Vulnerability is increased when the suppression of cellular immunity occurs. One typical example is seen in AIDS.

Cutaneous cryptococcosis manifests papules, acne-like eruptions, subcutaneous nodules or ulcers, mainly in the head and neck region. It rarely occurs in immunocompetent individuals, and provokes marked granulomatous reaction in the dermis. A few small unstained (transparent) yeast-form fungi are phagocytized by the multinucleated giant cells (Figure III-N-1). In the immunosuppressed patient, the granulomatous reaction is abortive, and the small microbes appear to be floating in the mucoid or gelatinous matrix, representing extracellular growth (Figure III-N-2). The size of the yeasts is much larger and capsule formation is much more apparent than those seen in the cytoplasm of macrophages in cryptococcal granuloma.

The microbes, containing thick capsules, are stained with PAS and Grocott, and they are alcianophilic and colloidal iron-positive. Mucicarmine stain is classical method to see the mucoid material in the fungi. Fontana-Masson argentaffin silver reaction is also positive on the cell wall, indicating production of melanin-type argentaffin pigment, though invisible microscopically.

Infection of highly virulent *Cryptococcus gattii* has recently been documented, primarily in the tropical and subtropical areas, including Papua New Guinea and northern Australia. It should be noted that *C. gattii* principally affects immunocompetent people and healthy animals such as dogs, koalas and dolphins. The lungs and meninges are the main target of

infection. Skin infection by *C. gattii* has also been reported. Endemics were recorded in Vancouver Island in Canada and Washington and Oregon States in the USA.

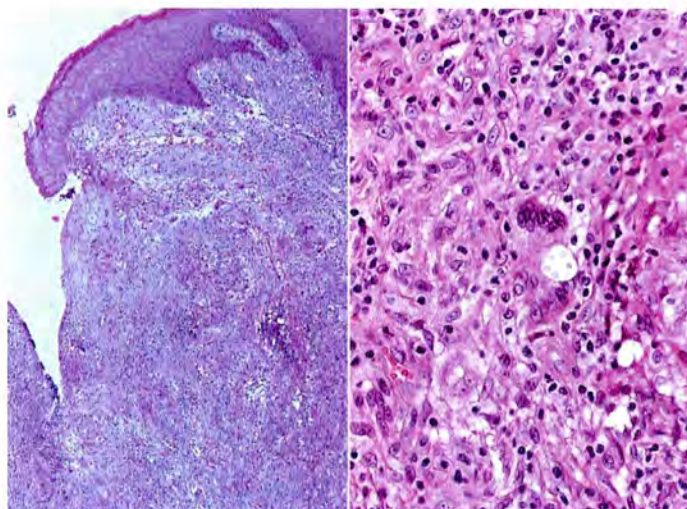


Figure III-N-1. Cryptococcal granuloma. An ulcerated skin lesion shows marked granulomatous reaction in the dermis. A few small unstained (transparent) yeast-form fungi are phagocytized by the multinucleated giant cells (left: low power, right: high-power, H&E).

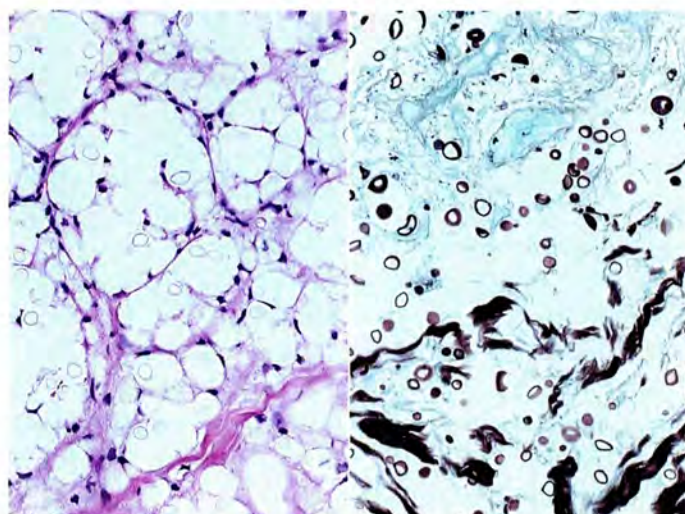


Figure III-N-2. Cutaneous cryptococcosis in an immunocompromised patient. In this pediatric case of acute lymphoblastic leukemia after chemotherapy, granulomatous reaction is absent, and the small microbes forming thick capsules are floating in the mucoid or gelatinous matrix (left: H&E, right: Grocott).

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Note 36. Two types of immunosuppressed state: neutropenia vs. lymphopenia

Two types of cellular defense mechanisms against microorganisms should be distinguished. Neutrophils principally protect extracellularly growing pathogens such as pyogenic bacteria (*Staphylococci*, *Streptococci*, *Enterobacteriae*, *Pseudomonas*, etc) and mycelium-forming fungi such as *Aspergillus*, *Candida* (candidal deep mycosis) and *Zygomycetes*. Infection of these microbes provokes phlegmone and abscess (neutrophilic clustering), so that the neutropenic state (bone marrow suppression) accelerates infection by these microbes. In diabetes mellitus, prolonged hyperglycemia provokes neutrophil dysfunction. Therefore, patients with poorly controlled diabetes mellitus are susceptible to the extracellularly growing pathogens.

In contrast, intracellularly growing pathogens are fought by functions of lymphocytes, particularly killer T-cells. Macrophages are also activated by cytokines secreted from the lymphocytes. The intracellular microbes include all the viruses, certain bacteria such as *Mycobacteria*, *Salmonella typhi*, *Legionella pneumophila*, *Chlamydia* and *Rickettsia*, yeast form fungi such as *Cryptococcus*, *Histoplasma* and *Coccidioides*, and some protozoa, including *Toxoplasma* and *Leishmania*. It is of note that mucosal candidiasis, featured by infection of yeast (blastospore) form, is mainly protected by lymphocytes, not by neutrophils. The cellular response pattern is lymphocytic infiltration and granulomatous reaction (when macrophages are activated). Neutrophils are useless in these states. In *Legionella* infection, lung macrophages are activated without association of lymphocytes.

After prolonged steroid administration and in AIDS, the patients with lymphopenia are susceptible to these intracellular pathogens, but not susceptible to the pyogenic pathogens, because neutrophils are usually kept normal in function. Mucosal candidiasis can be the initial sign of AIDS. In reactively swollen lymph nodes at a stage of AIDS-related complex, the p25 gag protein antigen of human immunodeficiency virus (HIV) is immunohistochemically observed along the follicular dendritic cells in the enlarged germinal center, and thus can be a diagnostic clue for HIV infection (Figure III-N-3).

In pathogens against which vaccination is effective, such as exanthematous viruses (measles, rubella, varicella and mumps), hepatitis B and yellow fever viruses, capsule-forming bacteria and exotoxin-producing bacteria, neutralizing antibodies in the serum are an important blocker of infection. The common feature of viruses of this group is the presence of viremia stage before infecting the target organs.

After bone marrow transplantation, the patient in an early stage is susceptible to pyogenic pathogens because of neutropenia, and in the following stage, the patient has an increased risk for intracellular pathogens due to T-cell dysfunction. In the late stage, the risk for capsule-forming bacteria remains high, because of the improper production of neutralizing antibodies.

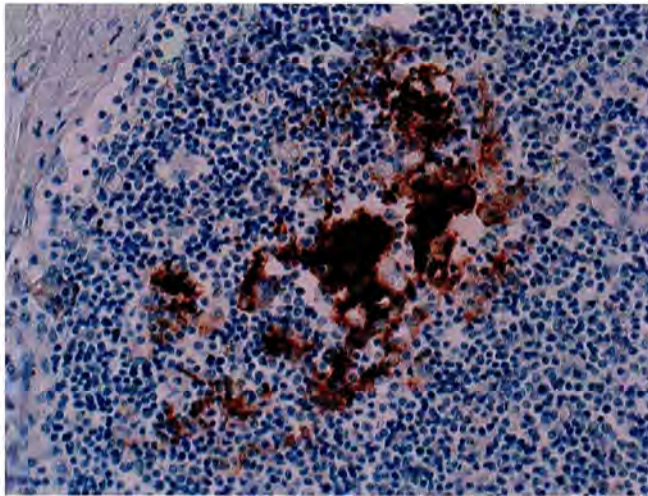


Figure III-N-3. p25 gag protein of HIV expressed in the lymph node. In patients with AIDS-related complex or AIDS, p25 gag protein antigen is immunohistochemically demonstrated along the follicular dendritic cells in the germinal center of enlarged lymph node.

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O) Histoplasmosis

Histoplasmosis is a systemic mycosis of the human and animals, including the dogs, cats, cattle, horses, pigs, rodents and bats. The classic type, a small-celled type, is caused by *Histoplasma capsulatum*. Lung infection is initiated by inhaling airborne infectious spores of *H. capsulatum* originating from the soil. The distribution of the microbe is widespread, including in both the North and South American continents, particularly in the regions along

the Mississippi River. The disease is also endemic in South Asia and South and West Africa. The pathogen grows in soil and material contaminated with bird or bat droppings. In childhood and an immunodeficient state, an acute (often fatal) disseminated disease is encountered. Histoplasmin skin reaction turns to be positive.

Histologically, both the clinical and microscopic features are very similar to tuberculosis. Formation of histoplasma (the encapsulated caseous focus) is characteristic. Small-sized (2-6 μm) transparent yeasts are observed in the necrotic area and in the cytoplasm of Langhans giant cells in the granulomatous lesion. PAS and Grocott stains are positive. The pathogens for which differential diagnosis is needed include *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Penicillium marneffei*, *Leishmania* spp., *Trypanosoma* spp. and *Toxoplasma gondii*.

In Africa, histoplasmosis of large-celled type caused by *H. capsulatum* var. *duboisii* is encountered. The size of the yeasts is much larger than the classic type. Skin and bone lesions may be associated.

Cutaneous histoplasmosis caused by *Histoplasma capsulatum* is infrequently seen as skin manifestation of systemic histoplasmosis in AIDS patients. Multinodular skin lesions are formed. When the patient is immunocompetent, the histology is indistinguishable from tuberculosis or non-tuberculous mycobacteriosis. With Grocott stain, small-sized, translucent rounded yeasts are seen in the necrotic tissue in the center of granulomas (Figure III-O).

In Japan, this type of mycosis is fundamentally categorized as the imported mycosis, mainly from the United States and southeastern Asia. However, a few domestic cases of histoplasmosis have been reported recently. Imported dogs or domestic bats may be carriers of this zoonotic mycosis, though no fungal DNA was detected in bats in Japanese caves.

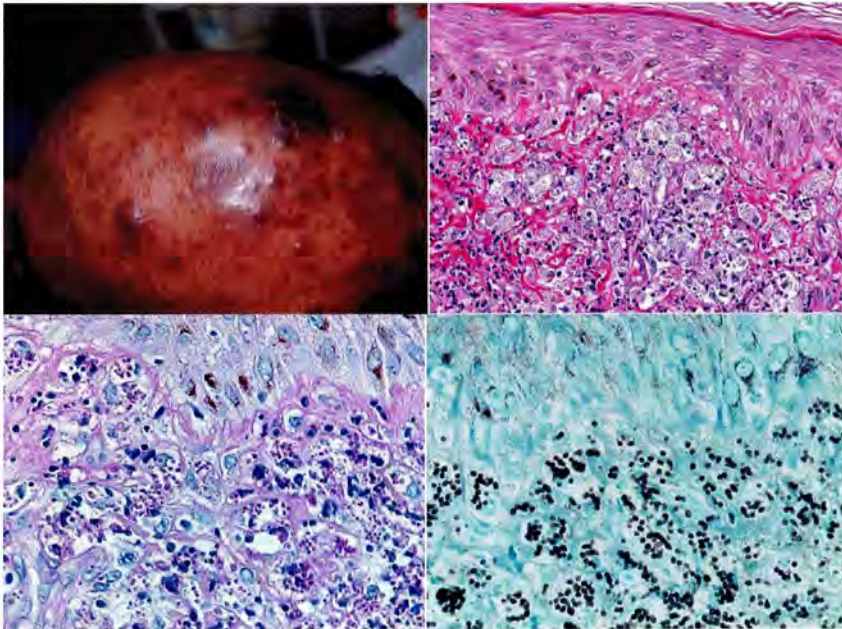


Figure III-O. Cutaneous histoplasmosis in an AIDS patient. Multiple nodular hemorrhagic lesions are observed on the scalp (left top). Histologically, small round, yeast-form fungi proliferate in the cytoplasm of macrophages in the dermis (right top: H&E, left bottom: PAS, right bottom: Grocott). The courtesy by Dr. Suzuko Moritani, a pathologist at Nagoya Medical Center, Nagoya, Japan.

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P) Paracoccidioidomycosis

Paracoccidioidomycosis, also called as South American blastomycosis, is endemic in the South American continent. The causative microbe is *Paracoccidioides brasiliensis*. The pathogen involves the lung, skin and mucosal surfaces, including the oral, nasal, anal or conjunctival mucosa. The skin lesion usually shows ulceration and pseudoepitheliomatous hyperplasia of the epidermis, somewhat resembling squamous cell carcinoma. In the dermis, abscess is formed among the granulomatous reaction (to be categorized as suppurative granuloma), and large-sized, yeast-form fungi, 5-60 μm in size, are phagocytized by multinucleated giant cells.

P. brasiliensis showing a double-contoured appearance is morphologically resembles *Blastomyces dermatitidis*. Fungal cells of *P. brasiliensis* are mononucleated and reproduce by single or multiple budding. The multiple budding forms of *P. brasiliensis* show the following two features. 1) The buds (daughter cells) are smaller than the parent cell to show “ship’s steering wheel-like appearance” (Figure III-P-1). 2) The buds are larger than the parent cells and are of unequal sizes and shapes (Figure III-P-2).

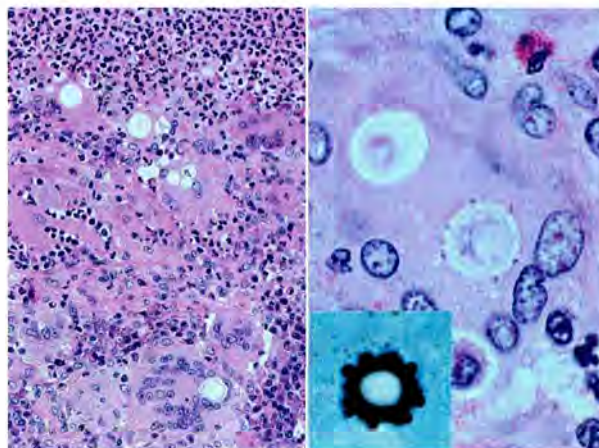


Figure III-P-1. Cutaneous paracoccidioidomycosis (I). In the dermis, suppurative granuloma is formed, and large-sized, yeast-form fungi are phagocytized by multinucleated giant cells. The budding daughter cells are smaller than the parent cell to show “ship’s steering wheel-like appearance” (left: low power, right: high power, H&E, inset: Grocott stain). The courtesy by Kenji Koda, a pathologist at Fujieda Municipal General Hospital, Fujieda, Japan.

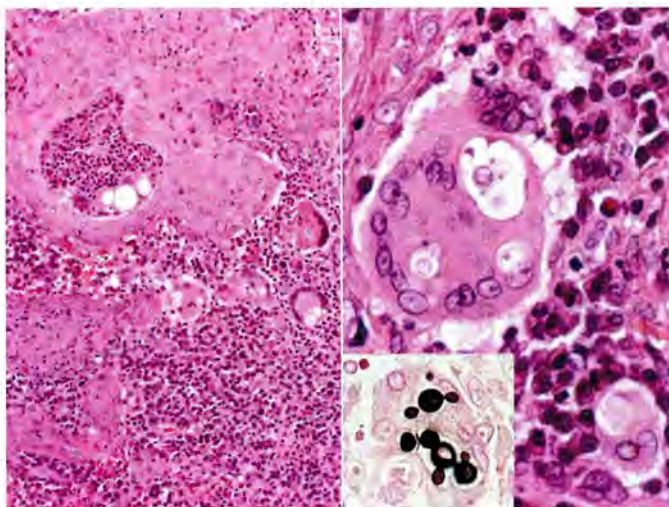


Figure III-P-2. Cutaneous paracoccidioidomycosis (II). Another case exhibits formation of dermal suppurative granuloma with multinucleated giant cells phagocytizing transparent yeast-type fungi. Pseudoepitheliomatous hyperplasia is noted in the epidermis (H&E, left and right). Grocott stain shows that the buds are larger than the parent cells and are of unequal sizes and shapes (inset). The courtesy by Soichiro Nose, a pathologist at Okayama Saiseikai General Hospital, Okayama, Japan.

Careful microscopic observation discloses the multiple budding forms, characteristic of *P. brasiliensis*. Grocott stain is especially effective in clearly illustrating multiple budding yeasts.

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Q) North American Blastomycosis

North American blastomycosis is a chronic granulomatous and suppurative disease of the human and animals, caused by *Blastomyces dermatitidis*. The disease is endemic in the North America, particularly around Chicago and along the Mississippi valley, and also in Africa, India and Israel. Inhalation of spores initiates lung infection. Formation of tuberculosis-type encapsulated caseous foci is exceptional. Instead, disseminated and bilateral lung lesions with mixed granulomatous and purulent inflammatory reaction are formed. Systemic dissemination may occur, and the prognosis is poor.

Another clinical form is cutaneous blastomycosis. It usually develops hematogenously from a primary lung lesion, but does not become systemic. Primary cutaneous blastomycosis due to traumatic inoculation of the pathogen is rare. Dog bite or specimen handling by pathologists may be biohazardous. A crusted verrucous nodule with central scarring or ulcerated plaque is formed.

Histological features are common with sporotrichosis and chromomycosis. Microabscess-forming lesions with or without suppurative granuloma are seen in the dermis and subcutis. The fungal cells appear as round or oval yeasts with thick and refractile cell walls. They generally range from 7 to 15 μm in size. In H&E preparations, the protoplasm readily stains basophilic or amphophilic and is separated from the rigid, unstained cell wall by a clear space. The yeasts proliferate by simple budding. They may be small in size, ranging from 2 to 4 μm , and the differential diagnosis from *H. capsulatum* and *C. neoformans* is needed. Figure III-Q represents typical microscopic features.

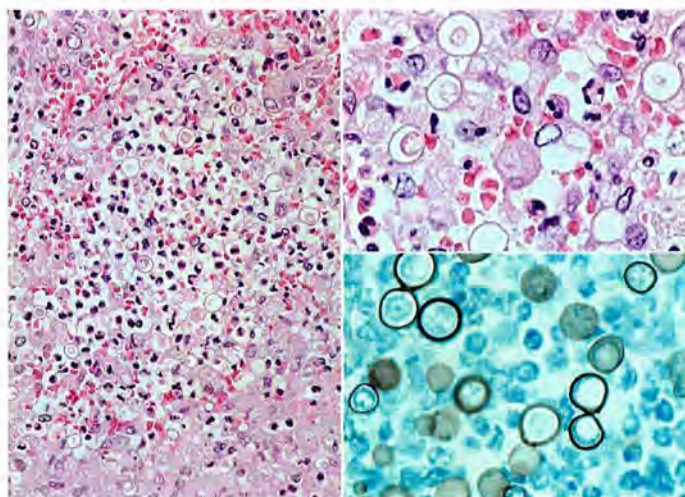


Figure III-Q. North American blastomycosis. Round to oval yeasts with thick and refractile cell walls, 7-15 μm in size, are seen in microabscess. The basophilic or amphophilic protoplasm is separated from the rigid, unstained cell wall by a clear space (left and right top: H&E, right bottom: Grocott). The courtesy by Director Toshiaki Manabe, Shiga Medical Center Research Institute, Moriyama, Japan.

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R) Coccidioidomycosis

Coccidioidomycosis is caused by *Coccidioides immitis*, endemic in Arizona and New Mexico states in the USA and also in Central and South America. The pathogen is not endemic in Japan. Airborne inhalation of the arthroconidia of the *C. immitis* in the soil may lead to pulmonary infection. An encapsulated caseous focus similar to tuberculosis and histoplasmosis is called as coccidioidoma. The pathogens are presented as a large (60-100 μm -sized) sporangium (spherule) containing small (2-5 μm -sized) endospores. PAS and Grocott' silver stain the endospores but not the capsule of the sporangium (Figure III-R).

In immunocompromised patients, dissemination of the infection results in skin lesions, as a verrucous plaque on the face, subcutaneous abscess, or pustular lesions. Primary cutaneous coccidioidomycosis may occur at the site of minor trauma due to inoculation of the microorganism.

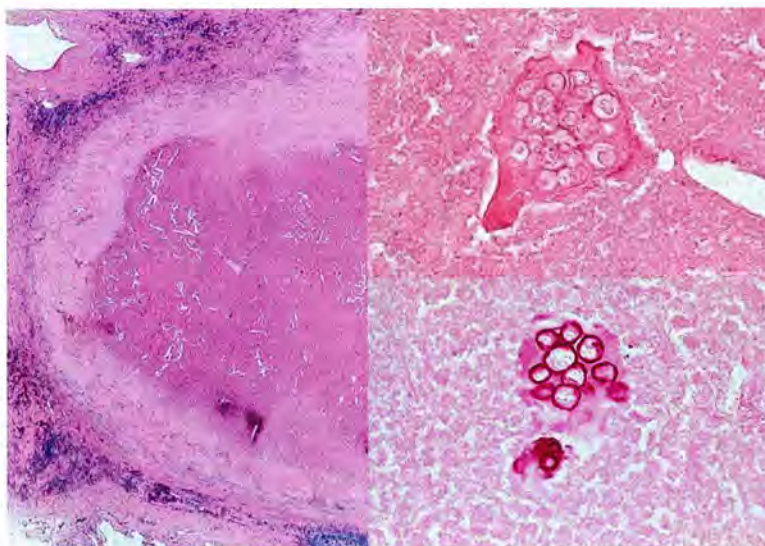


Figure III-R. Pulmonary coccidioidomycosis. Within a focally calcified encapsulated caseous focus formed in the lung, a large sporangium contains small endospores (left and right top: H&E). PAS stains the endospores but not the capsule of the sporangium (right bottom). The courtesy by Director Toshiaki Manabe, Shiga Medical Center Research Institute, Moriyama, Japan.

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S) Lobomycosis

Lobomycosis, also called as lacaziosis, is a chronic fungal disease, showing slowly growing and refractile keloid-like nodules on the exposed skin. Other nicknames of this disorder include Jorge Lobo's disease and keloidal blastomycosis. Lymph nodal involvement may be seen. This long-lasting cutaneous mycosis is caused by *Lacazia loboi* (old term: *Loboa loboi*) and endemic in low altitude areas of Central and South America. Dolphins are also affected with this fungus, and the infected dolphins have also been found in Japan. Phylogenetically, *Lacazia loboi* is closely related to *Paracoccidioides brasiliensis*, but is not culturable on the plate.

Histologically, the keloidal nodule in the dermis is mainly composed of mononuclear macrophages and multinucleated giant cells of Langhans or foreign body types, and numerous unstained fungal cells, 6-12 μm in size, are seen in their cytoplasm. The microscopic features are called as "sieve-like appearance". Some nucleated (cored) fungal cells show basophilia. Short chains of fungal cells may be formed by budding. Lymphocytic and plasmacytic reactions are poor. The organisms are stained with PAS and Grocott stains. Fontana-Masson's silver gives argentaffinity, indicating the melanin production by the fungal cells. Figure III-S illustrates typical microscopic features.

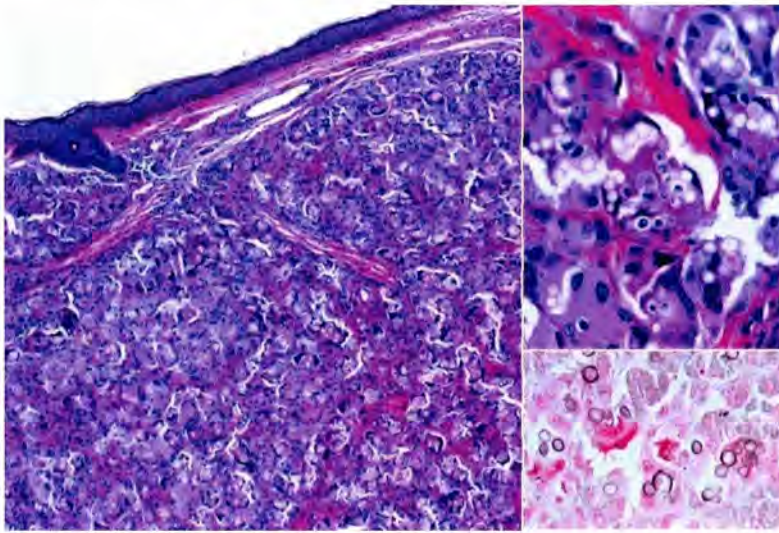


Figure III-S. Lobomycosis. The lobulated dermal nodule is packed with mononuclear and multinucleated macrophages phagocytizing unstained yeast-form fungi. Most fungal cells appear clear and empty, giving "sieve-like appearance". Some are cored to show basophilia. Lymphoplasmacytic reactions are poor (left and right top: H&E). The organisms are stained with Fontana-Masson's silver (right bottom). The courtesy by Dr. Masatomo Kimura, Department of Pathology, Kinki University Faculty of Medicine, Sayama, Japan.

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T) Rhinosporidiosis

Rhinosporidiosis, forming an inflammatory polypoid nodule in the nasal mucosa, is caused by *Rhinosporidium seeberi*. The disease is endemic in India, Nepal and Bangladesh but rare in other areas. Involvement of the facial skin and oropharyngeal and conjunctival mucosa, as a result of autoinoculation, has infrequently been reported. *R. seeberi* fails to grow in any of the artificial media used, but can be maintained through its life cycle in tissue culture.

Microscopic features of the infected tissue reveal a granulomatous reaction with pseudocystic abscesses and fibrosis around the causative organisms, but the inflammatory reaction is mild in degree. Sporangia of varying sizes are formed just beneath the ciliated or squamous metaplastic epithelium. Mature sporangia contain numerous spores, while smaller immature sporangia contain amorphous basophilic cytoplasm. The mature sporangium is Grocott-positive, while the immature one is unreactive. The walls of the sporangia are trilaminated, whereas those of the immature one are bilaminated. A representative features are shown in Figure III-T.

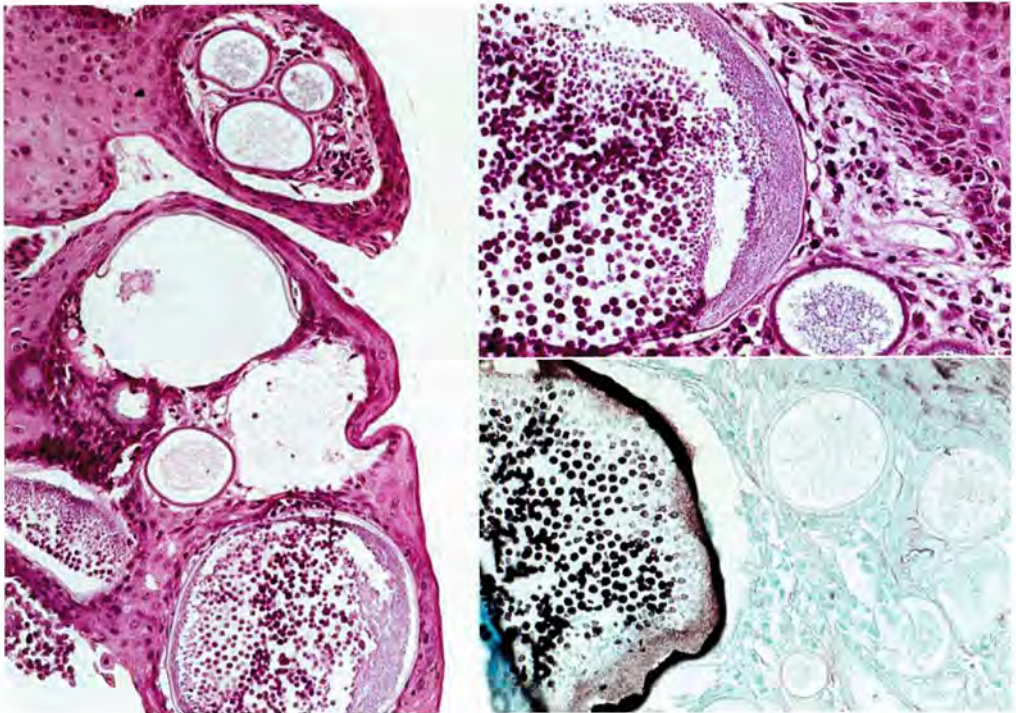


Figure III-T. Rhinosporidiosis. Sporangia of varying sizes are formed just beneath the squamous metaplastic mucosa. Mature sporangia contain numerous spores, while smaller immature ones contain amorphous basophilic cytoplasm. Inflammatory reaction is minimal (left and right top, H&E). Grocott stain gives positivity in the mature sporangium but not in the immature one (right bottom).

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U) Penicilliosis Marneffeï

This opportunistic systemic fungal infection caused by *Penicillium marneffeï* is endemic in Southeast Asia, including Thailand, the Guangxi region of China, Hong Kong, Vietnam and Indonesia. *P. marneffeï* is identified in the soil in the endemic areas, and inhalation of the conidia may lead to lung lesions, subsequently resulting in systemic dissemination to the liver, spleen and lymph nodes. Cutaneous and subcutaneous lesions are encountered in two thirds of cases. Involvement in the oral mucosa and joint tissue may also be seen. Penicilliosis marneffeï is one of the leading causes of death in AIDS patients in northern Thailand.

Penicillium (blue mold) is ubiquitously distributed in the environment and usually non-pathogenic, except for surface infection on the external meatus. *P. marneffeï* is the only species known to be dimorphic among the genus *Penicillium*, and exceptionally causes deep mycosis in immunocompromised individuals. Infection is frequently seen in a rainy season. On the culture plates, *P. marneffeï* grows as a mycelial (hypha-forming) fungus. Within human tissues, globose, ovoid and elongated yeast-like cells measuring 2–3 x 2–6 µm are seen extracellularly or within macrophages. Oil immersion observation discloses nucleated basophilic granules in the cytoplasm of macrophages. Extracellular sausage-shaped cells up to 8–13 µm long may rarely be seen. Characteristically, the multiplying cells form a single central septum (schizogony), visualized with Grocott stain, being the highly pathognomonic microscopic feature (Figure III-U). Granulomatous reactions which resemble tuberculosis take place in immunocompetent patients, but in AIDS patients, anergic and necrotizing lesions rich in phagocytizing macrophages are formed.

No specific and effective treatment is known. The mortality rate of penicilliosis marneffeï is 100% in AIDS patients.

Differential Diagnosis

Clustered small round pathogens phagocytized by macrophages are seen histologically in the following infectious diseases. These include a) protozoan infections such as leishmaniasis

and toxoplasmosis, b) deep mycosis such as penicilliosis marneffeii, histoplasmosis, North American blastomycosis, and cryptococcosis, and c) bacterial infections such as rhinoscleroma and granuloma inguinale.

Protozoan parasites such as *Leishmania* and *Trypanosoma* can be distinguished by the negative reaction with PAS and Grocott stains. It should be noted that cysts of *Toxoplasma gondii* may be positive with PAS and Grocott stains, although cutaneous toxoplasmosis is exceptional. Opportunistic systemic infections by yeast-like fungi listed above can be encountered in AIDS patients. Septum formation (schizogony) is quite characteristic and pathognomonic of penicilliosis marneffeii, though few in number. Careful observation of Grocott stained sections is thus requested. Budding-type growth is noted in the other fungi, including *Histoplasma capsulatum*. In histologic sections, *H. capsulatum* appears to be clear and less basophilic than *P. marneffeii*. Histoplasmosis is endemic in the USA, particularly along the Mississippi River, but may also be seen in Thailand. In Japan, almost all the human cases have imported from endemic countries, whereas domestic canine histoplasmosis has been reported.

Formation of alcianophilic capsules is a feature of cryptococcosis, and large-sized yeasts are embedded in the mucinous material in cases where granulomatous reactions are absent. The cellular size of *Blastomycosis dermatitidis* and *Cryptococcus neoformans* is larger than the former two fungi.

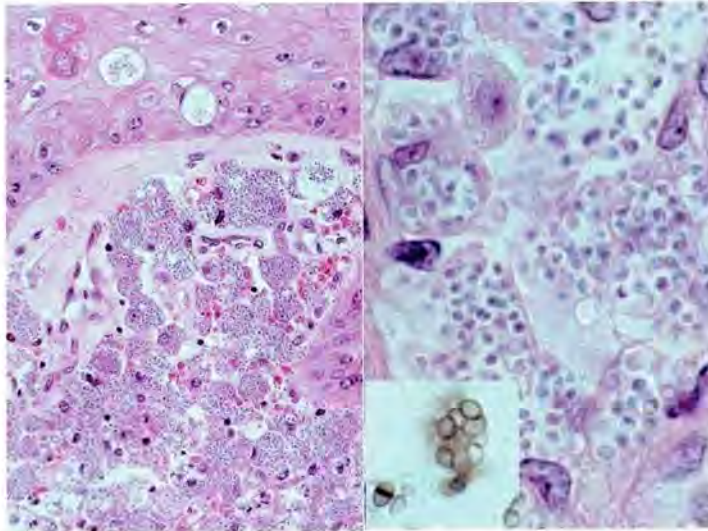


Figure III-U. Penicilliosis marneffeii infection of the skin in AIDS. Globose or ovoid yeast-like cells (nucleated basophilic granules) are seen in the cytoplasm of macrophages (left and right: H&E). Grocott stain occasionally discloses a single central septum (schizogony), the highly pathognomonic microscopic feature (inset). The courtesy by Dr. Nirush Lertprasertsuke, Department of Pathology, Chiang Mai University School of Medicine, Chiang Mai, Thailand.

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V) Protothecosis

Protothecosis is not a mycotic disease, but is tentatively described in this Chapter. Protothecosis is a rare infection by ubiquitous achlorophyllous algae, resulting from traumatic inoculation to the skin. *Prototheca* is widely distributed in the tree, soil and the gut of the fish, so that the traumatic insertion of a wood splinter may cause the infection.

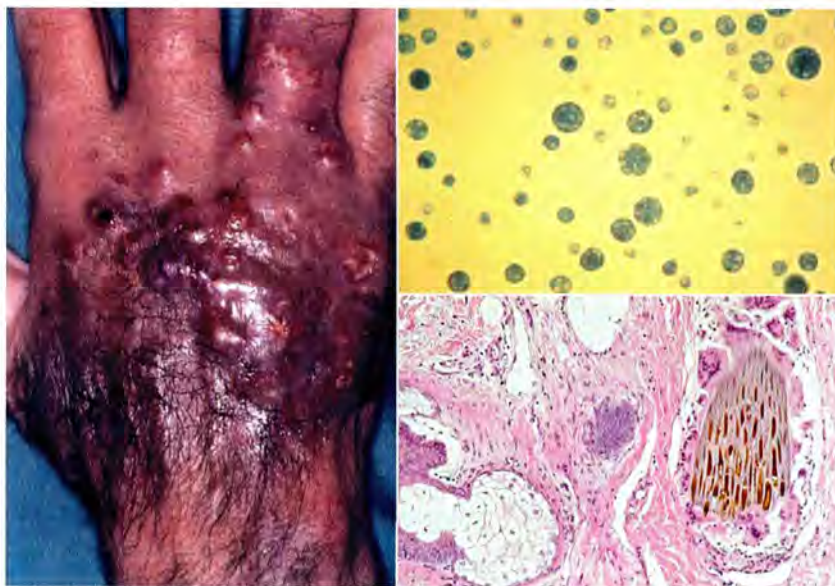


Figure III-V-1. Protothecosis. On the back of the hand, pustules and fistulas are formed in the infiltrative plaque, and multiple metastatic nodules are associated at the periphery (left). Sporangia of *Prototheca wickerhamii* cultured on Sabouraud's medium showing morule-like internal septation are shown in Papanicolaou stain (right top). No chlorophylls are formed. The traumatic insertion of a wood splinter is identified in the biopsy specimen (right bottom, H&E). The courtesy by Dr. Shinji Okano, Okano Dermatology Clinic, Hiroshima, Japan.

Four pathogenic species are known, including *Prototheca wickerhamii*, *P. salmonis*, *P. stangnora*, *P. zopfii*. Main causative agent is *P. wickerhamii*. Protothecal sporangia do not belong to fungi, but are culturable on Sabouraud's medium, stained with PAS and Grocott, and treatable with Amphotericin B. In the cow and dog, protothecal mastitis and ophthalmitis are known. The human infection is often encountered as an opportunistic infection: Patients with a mild degree of immunosuppression such as diabetes mellitus, renal failure and steroid administration are susceptible to this microorganism.

Human protothecosis has been classified into three clinical forms: a) cutaneous and/or subcutaneous infection, particularly on the extremities, b) synovitis of olecranon bursa or other fibrous tissue, and c) systemic infection.

On the exposed skin, particularly the hands and arms, pustules and fistulas are formed in an infiltrative plaque, and multiple metastatic nodules are secondarily associated.

Histologically, epithelioid granulomas with central necrosis are seen in the dermis through subcutaneous tissue. The sporangium contains morule-like internal septation or several thick-walled autospores. Protothecal sporangia are much smaller than those of *Coccidioides immitis*. PAS and Grocott stains demonstrate endospores (thick-walled spherical bodies).

Representative features are illustrated in Figures III-V-1 and III-V-2.

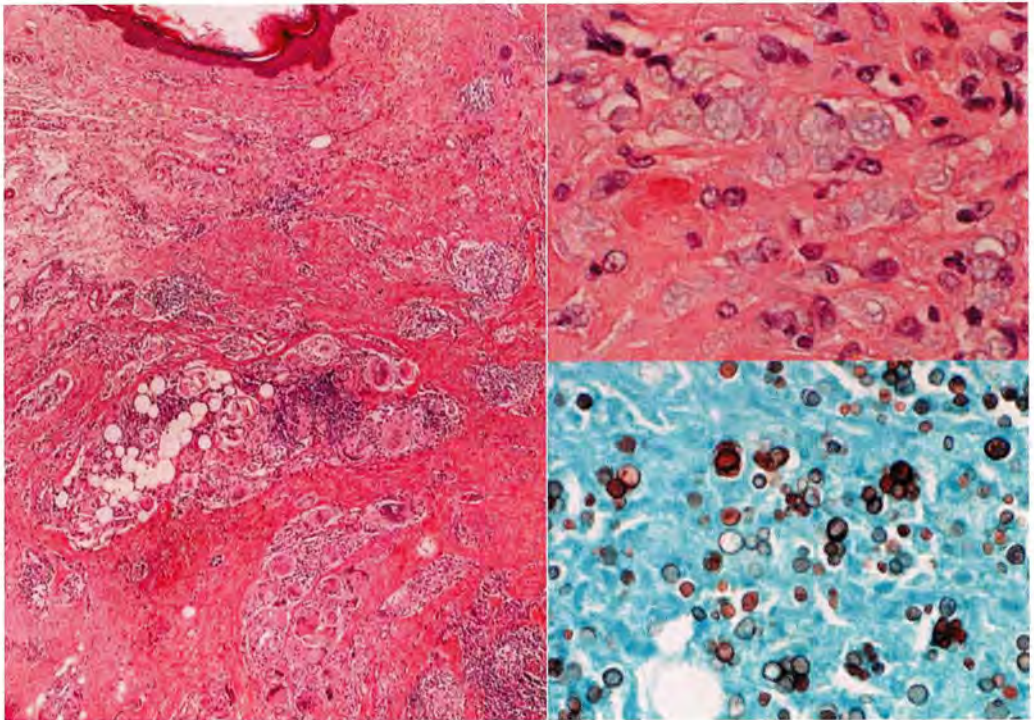


Figure III-V-2. Histology of protothecosis on the hand. Epithelioid granulomas are formed in the fibrotic dermis (left: H&E). The sporangia containing morule-like internal septation are observed within the granuloma (right top: H&E). Grocott stain demonstrates endospores, thick-walled spherical bodies (right bottom). The courtesy by Dr. Shuji Yonehara, a pathologist at Onomichi General Hospital, Onomichi, Japan.

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Note 37. *Microcystis aeruginosa*

Microcystis aeruginosa, also known as *Anacystis cyanea*, is a photosynthetic (oxygen-producing) bacterium called cyanobacterium. The chlorophyll gives it a green color, so that it has long been erroneously thought to be a blue-green alga. Cells are usually spherical, pale blue-green and average 4–6 µm in diameter. It grows well in water that is nutrient rich, warm and stagnant, and often forms thick blooms. Blooms of cyanobacteria can cause water quality and management issues. The colonies are moist and sticky, free-floating and usually 600–900 µm long. The colonies can look net-like with holes, and have irregular outlines.

Microcystis aeruginosa produces toxins, such as microcystins, nodularins and cylindrospermopsins, which are harmful to the human and animals. Acute exposure to high concentrations of microcystins due to inadvertent ingestion or inhalation can lead to epidemic gastroenteritis, liver failure and even death. Young children, splashing vigorously in the water, are susceptible to the internal infections. In 1996, Caruaru syndrome occurred at a hemodialysis unit in Caruaru, Brazil, accompanying an outbreak of acute liver failure. Body contact during water recreation may lead to minor skin irritations or allergic reactions of the skin, including eye irritation, rhinorrhea and blistering of the lips. Polysaccharides derived from the cell wall of the bacterium cause these external irritations.

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Viral Infections

Viruses may reach the skin by direct inoculation as in warts and orf or by spread from other locations as in herpes zoster. Acute viral exanthemas result from generalized infection via droplet or airborne (measles and varicella) transmission. The virus is commonly infected in the epidermis or endothelial cells in the dermis. Viral skin infections are mainly caused by three DNA virus families of poxviruses, herpesviruses and papovaviruses and one RNA virus family of picornaviruses. The three major DNA virus families show histologically or cytologically diagnostic inclusion bodies. The other viruses produce histologically nonspecific changes, such as perivascular lymphocytic infiltration and mild epidermal spongiosis. For the diagnosis of viral infections, serology and polymerase chain reaction (PCR) are of clinical importance. Immunostaining and *in situ* hybridization technique can be used for diagnostic pathology, when needed.

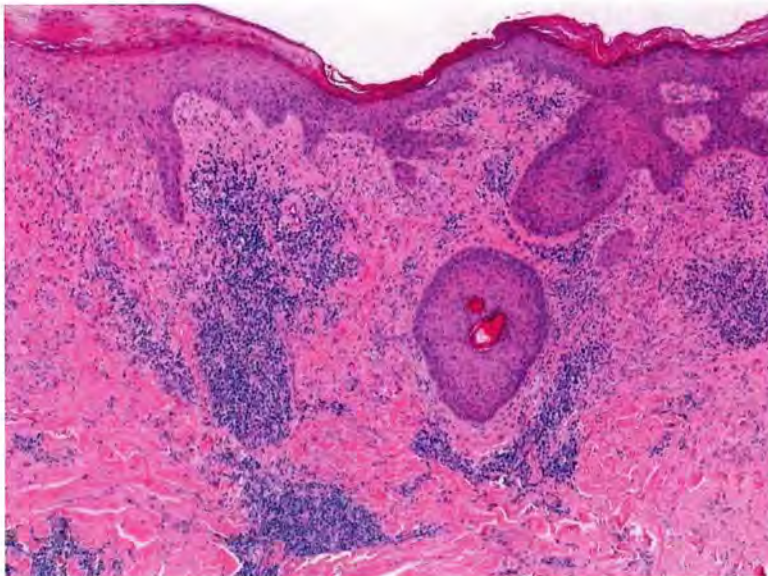


Figure IV-0. Post-viral dermatitis. An adult case accompanied persistent skin eruptions after herpes zoster. Biopsy reveals lymphocytic vasculitis and lichenoid interphase dermatitis with reactive acanthosis (H&E). Herpes zoster antigen is negative in the lesion.

Of special note is that DNA viruses and retroviruses may cause malignant neoplasms. Examples include human papillomavirus, Epstein-Barr virus, human herpes virus 8, Merkel cell polyomavirus, and human T-cell leukemia virus.

Erythema multiforme, erythema nodosum or lichenoid lymphocytic vasculitis may follow the preceding viral infections, as a result of an immunological reaction to the virus. This is called as "post-viral dermatosis". One example is demonstrated in Figure IV-0, where lymphocytic vasculitis and lichenoid interphase dermatitis are observed in an adult case of herpes zoster.

A) Human Papillomavirus Infection

Human papillomavirus (HPV) is a member of papovaviruses, encompassing papillomavirus, polyomavirus and vacuolating agent. HPV, a group of wart viruses, represents a small (8,000 base pair-sized) DNA virus containing double-stranded circular genome, and principally infects squamous epithelial cells and induce papillomatous growth. More than 150 types of HPV have been described. The classification is based on the traditional criteria that the sequence of the L1 gene should be at least 10% dissimilar from the closest known HPV. When the difference is between 2% and 10%, it is defined as a subtype. HPV is grouped into skin-type and mucosal-type. The mucosal-type HPV is further divided into the high-risk (carcinogenic) type and low-risk types. The high-risk HPVs include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 69, 73 and 82. Types other than 16 and 18 may be categorized in the intermediate-risk HPVs.

It is well known that almost all uterine cervical carcinomas are caused by high-risk HPV of mucosal-type. Vulvar, vaginal, anal, penile and oropharyngeal squamous cell carcinomas may be caused by high-risk HPV infection. Squamous cell carcinoma of the lung may also be related to the HPV etiology. Most (85-90%) of the HPV infections in young females are temporary, and when the infection persists, precancerous dysplastic lesions occur in the uterine cervix in 10-15 years. Papillomavirus vaccines against high-risk HPV types 16 and 18 are now available for the effective prevention of cervical cancer.

It should be realized that the *in situ* hybridization (ISH) detection of HPV is dependent upon the probe specificity. We used the following biotinylated cocktail probes for HPV 6/11 for benign papillomatous lesions, HPV 16/18 and HPV 31/33/35 for precancerous and cancerous lesions commercially available from Life Technologies (Gaithersburg, MD, USA). Biotinylated cocktail probes for HPV of mucosal type (reactive with HPV 6, 11, 16, 18, 31, 33 and 51) from Enzo Life Sciences (Farmingdale, NY, USA) were also used for screening. All these probes do not hybridize with HPV of skin (wart) type.

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1) Verruca Vulgaris

Verruca vulgaris, a common wart caused by HPV types 2, 4 and 7, makes a papillomatous skin lesion with normal skin color, particularly during the childhood. Contact transmission or autoinoculation occurs, so that the extremities, particularly hands and fingers, are the prerequisite site of infection. Histologically, the polypoid skin lesions show active papillomatosis and hyperparakeratosis. Hypergranulosis with smudgy nuclear inclusions and perinuclear koilocytic changes is evident. Vacuoles dispersed in the cornified layer are suggestive of HPV infection. Immunostaining for papillomavirus capsid antigens using Dako's antiserum against bovine papillomavirus demonstrates positive nuclear signals in the granular and parakeratotic layers. Electron micrographs show small-sized (40 nm) viral particles (virions) in the nuclear matrix. Representative microscopic features are illustrated in Figure IV-A-1-1. Immunostaining for papillomavirus capsid antigens is not almighty. Old verrucous lesions tend to be devoid of viral particle formation in the nuclei. Some verrucae in an active phase are negative with the immunostaining, but the nuclei of the keratinocytes are filled with virions ultrastructurally (Figure IV-A-1-2).

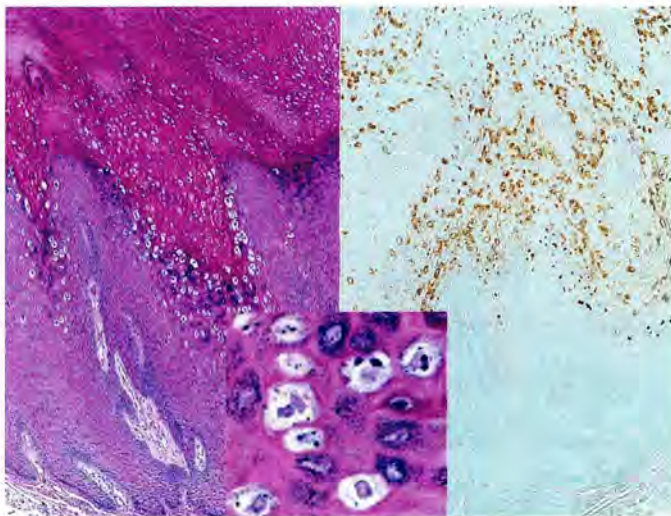


Figure IV-A-1-1. Verruca vulgaris. Marked papillomatosis and hyperparakeratosis with koilocytosis in the granular layer are characteristic (left: H&E). Papillomavirus capsid antigens are densely localized in the nuclei of the granular and hyperparakeratotic layers (right: immunostaining). Inset illustrates the koilocytic cells in the granular layer. Nuclear viral inclusions of smudgy type are noted (H&E).

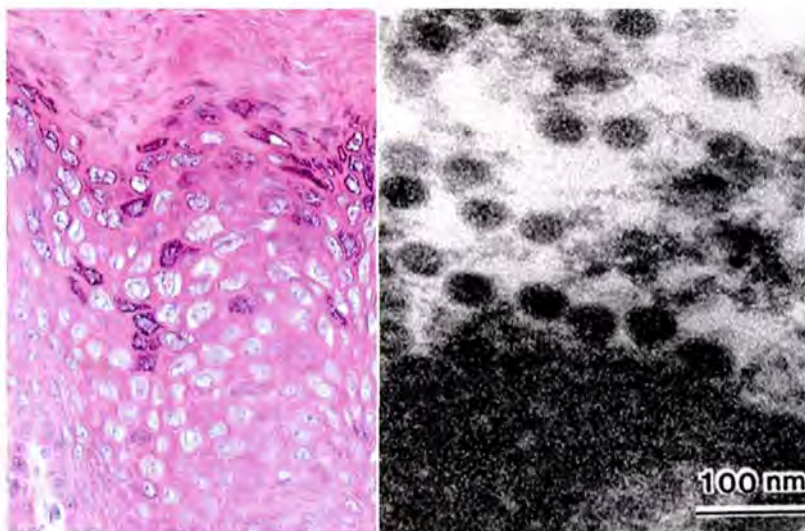


Figure IV-A-1-2. Viral antigen-negative verruca vulgaris. Immunostaining for papillomavirus capsid antigens is negative in this wart with nuclear inclusions (left: H&E), but electron microscopy identifies numbers of small viral particles sized around 40 nm in the nuclei (right).

Rarely, multiple skin involvement with oral or genital mucosal verruca vulgaris may be encountered, particularly in immunocompromised patients.

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Note 38. Immunostaining and *in situ* hybridization of HPV in verrucous and precancerous squamous lesions at the ultrastructural level

Human papillomavirus (HPV) antigens or genomes were ultrastructurally localized by either the immunostaining or the non-isotopic *in situ* hybridization technique in formalin-fixed, paraffin-embedded archival specimens of verruca vulgaris of the skin, condyloma acuminatum of the penis and severe dysplasia of the uterine cervix.

A commercially available rabbit antiserum against capsid antigens of bovine papillomavirus (Dako) localized the viral antigens. Biotinylated DNA probe cocktails (7 kb long) were employed for the visualization of HPV-DNA, types 6 and 11 (HPV 6/11) and types 16 and 18 (HPV 16/18) (Life Technologies, Carlsbad, CA, USA). The pre-embedding beam capsule method was utilized for the ultrastructural localization of the viral antigens and genomes.

In verruca vulgaris, HPV antigen-positive 50-60 nm particles of mature viral size were observed in the nuclei of the granular cells and parakeratotic cells with perinuclear haloes (Figure IV-A-1-3, inset), whereas HPV 6/11 and HPV 16/18 DNA were negative. In condyloma acuminatum, the nuclei were positive for the HPV antigen and HPV 6/11 DNA, but were negative for HPV 16/18 DNA. More cells were labeled for the viral DNA than for the viral antigens (refer to Figure IV-A-6-3). The ultrastructural observation indicated the presence of the naked (plasmid) viral DNA as fine particles sized 15-20 nm (Figure IV-A-1-3).

In the dysplastic cervical mucosa, dot-like positivity of HPV 16/18 DNA was recognized (refer to Figure IV-A-11-1). The HPV antigens and HPV 6/11 DNA were negative. HPV 16/18 DNA was localized in part of nuclear chromatin (Figure IV-A-1-4). This pattern of localization may suggest the integration of viral DNA into host cell DNA.

Compare the intranuclear localization of the virus in each lesion.

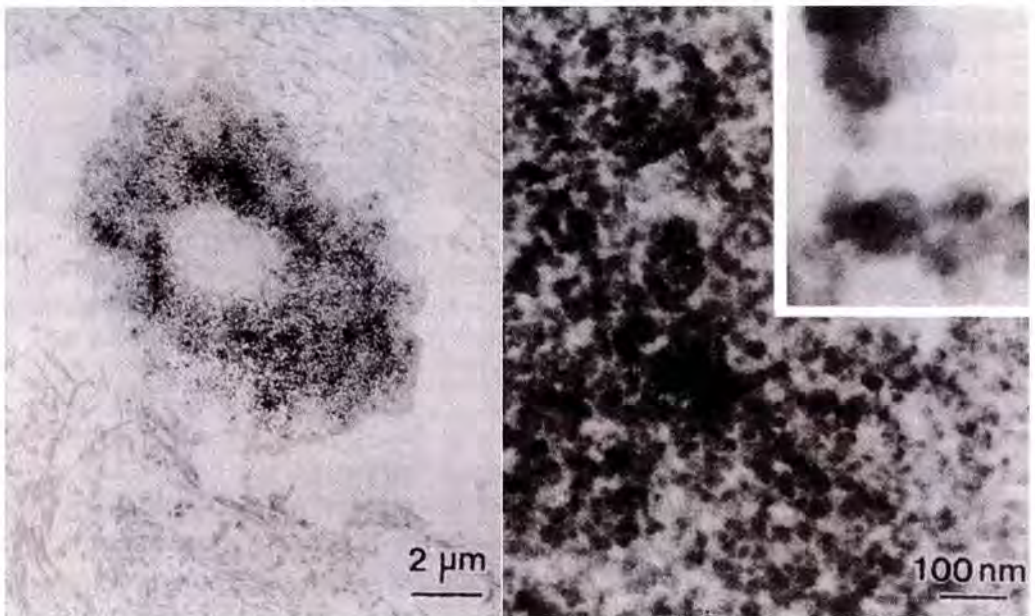


Figure IV-A-1-3. HPV 6/11 DNA localization in condyloma acuminatum. The viral genome is distributed as 15-20 nm-sized fine particles in the nuclei, probably representing naked (plasmid) viral DNA (ISH at the ultrastructural level). Inset indicates immunoelectron microscopic localization of papillomavirus capsid antigens in verruca vulgaris. Compare the immunoreactive intranuclear viral particles around 50-60 nm in size with viral DNA of the plasmid-type (15-20 nm in size) in condyloma.

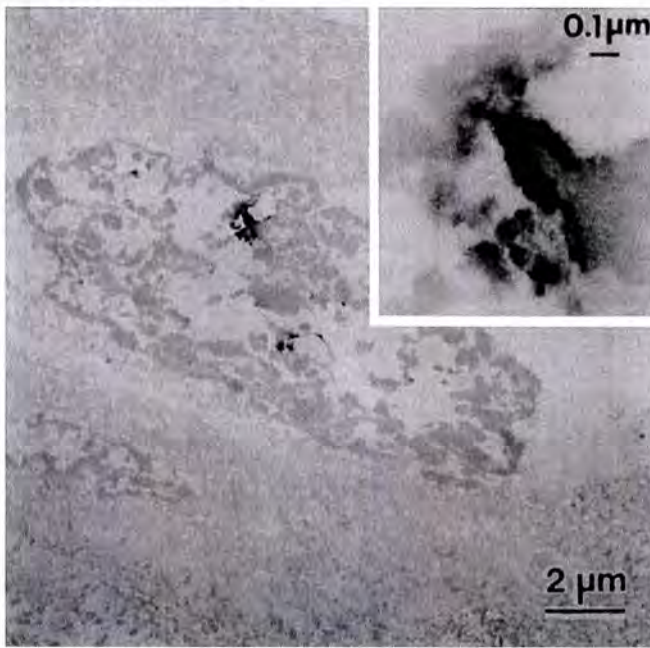


Figure IV-A-1-4. HPV 16/18 DNA localization in cervical severe dysplasia. The carcinogenic viral genome is seen on the nuclear chromatin. This pattern of localization suggests the integration of viral DNA into host cell DNA (ISH at the ultrastructural level).

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2) Verruca Plana

Verruca plana (plane wart) is flesh-colored or brownish, flat-topped papules, a few millimeter in size, occurring most frequently on the back of the hands and on the face of the young (Figure IV-A-2-1). The warts are caused by infection of HPV types 3 and 10. They may disappear suddenly after a few weeks or months, and the involution is usually preceded by erythematous changes in the warts. Extensive skin manifestation may be seen in immunocompromised patients.

Histologically, hyperkeratosis and acanthosis with koilocytosis are seen, and the stratum corneum shows “basket-weave appearance”. Immunohistochemically, the positivity of papillomavirus capsid antigens is noted in the koilocytic nuclei and vacuolated cornified layer component. Figure IV-A-2-2 represents microscopic features of a facial lesion in the young.



Figure IV-A-2-1. Gross appearance of verruca plana. Flesh-colored or brownish, flat-topped papules, a few millimeter in size, are clustered on the chin (left) and neck (right) of the young.

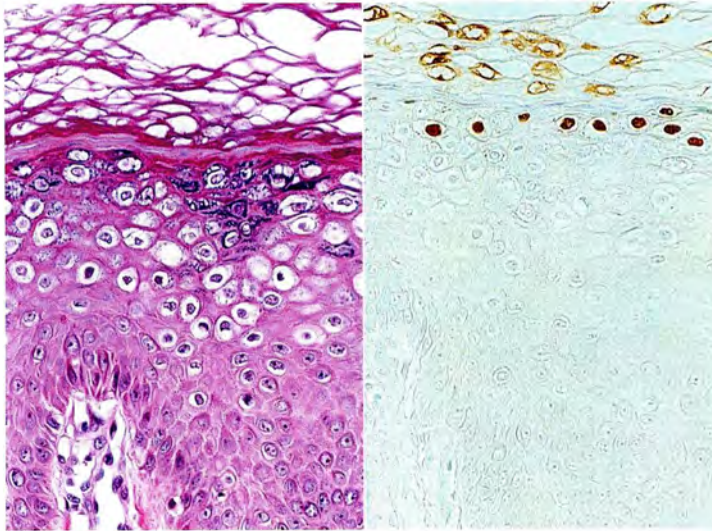


Figure IV-A-2-2. Histology of verruca plana. "Basket-weave appearance" in the hyperkeratotic layer and koilocytic change in the keratinocyte layer are characteristic (left: H&E). Immunohistochemically, papillomavirus capsid antigens are positive in some koilocytic nuclei and vacuolated cornified layer component (right).

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3) Epidermodysplasia Verruciformis

Epidermodysplasia verruciformis (also called Lewandowsky-Lutz dysplasia) is a rare autosomal recessive hereditary skin disorder with abnormal susceptibility to HPV infection and a high risk of squamous cell carcinoma of the skin. Inactivating mutations in transmembrane channel-like protein-6 (TMC6) or TMC8, playing a role in regulating the distribution of zinc in the cell nucleus, are the genetic cause of this disorder. Zinc is a necessary cofactor for many viral proteins, and the activity of TMC6/TMC8 complex restricts the access of viral proteins to cellular zinc stores, eventually limiting the viral growth.

The onset is in infancy or childhood (1-20 years). More than 20 HPV types are associated with this disease. Two clinical forms are known. In one form, infection of HPV 3 or HPV 10 provokes multiple plane warts. The difference from verruca plana is wider distribution and the persistence of the lesion. Another form is related to infection of HPV 5, 8, 9, 14, 20, 38, 47 or others. In addition to verruca plana, lesions resembling pityriasis versicolor and seborrheic keratosis are associated. The keratinocytes in the upper part of the acanthotic epidermis are swollen to be pale with perinuclear haloes in appearance. In the second form, Bowen's disease, invasive squamous cell carcinoma or basal cell carcinoma may develop on the sun-exposed area in one fourth of the patients.

Histologically, HPV 3 or 10-induced lesions are indistinguishable from verruca plana. In the second form caused by a variety of HPV types, including HPV 5 and 8, microscopic features resemble verruca plana or condyloma acuminatum accompanying a unique epidermal change with swollen large cells and perinuclear haloe in the upper part of the acanthotic epidermis. Basal cell carcinoma is associated in the lesion presented as Figure IV-A-3-1.

In AIDS patients, epidermodysplasia verruciformis-like disseminated eruptions are caused by HPV infection (Figure IV-A-3-2).

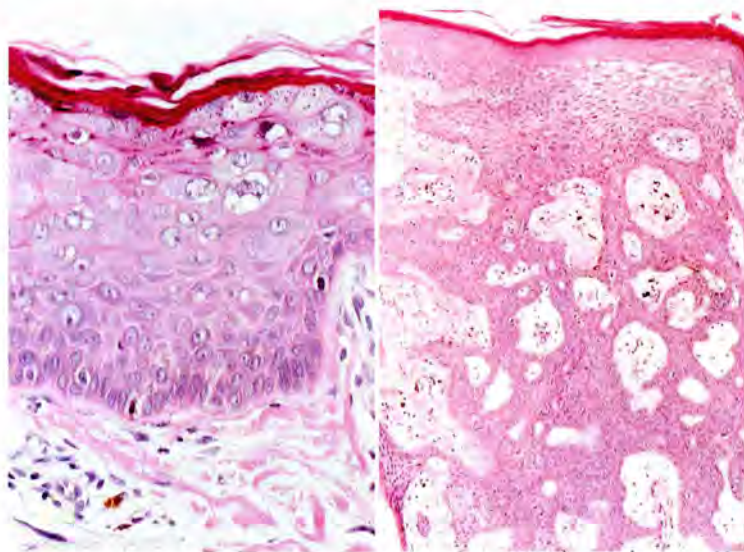


Figure IV-A-3-1. Epidermodysplasia verruciformis associated with basal cell carcinoma. The microscopic features resemble verruca plana but with swollen large and pale atypical cells showing perinuclear haloe in the upper part of the acanthotic epidermis (left: H&E). Basal cell carcinoma is associated in this case (right: H&E).

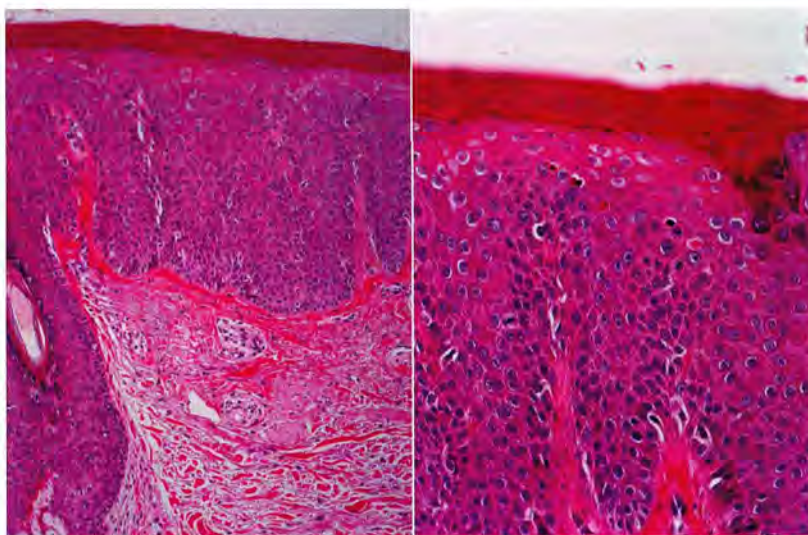


Figure IV-A-3-2. Disseminated epidermodysplasia verruciformis-like eruptions in an AIDS patient. Downward acanthosis with parakeratosis is observed microscopically (left). Pale cells with perinuclear haloes and dyskeratotic cells are distributed in the upper part of the spinous layer (right: H&E). The courtesy by Dr. Noboru Onoda, a pathologist at Yamato Municipal Hospital, Yamato, Japan.

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4) Cystic Wart

Warts found on the palm or sole are often painful, and may occur beneath pressure points. Several variants of palmoplantar warts are known. HPV 60-associated plantar cystic wart resembles epidermal cyst (so-called atheroma). Other types of HPV such as HPV 57, 63, 65, 66 and 156 may also be involved. HPV 63 infection is associated with a whitish keratotic wart, and HPV 65 provokes a pigmented verrucous variant. In AIDS patients, a large verrucous plantar wart is formed by HPV 66 infection. Myrmecia is caused by HPV 1 infection (see below).

The warts on the palm and sole tend to form painful intradermal cystic inclusions because of pressuring. It should not be confused with traumatic epidermal cyst. Histologically, hyperkeratotic squams in the cystic lesion contain round, vacuolated structures, characteristic and pathognomonic of HPV 60-related plantar cystic wart. The vacuoles are immunoreactive

for the papillomavirus capsid antigens (Figures IV-A-4-1). In addition, the plantar cyst wall contains a few eosinophilic cytoplasmic inclusions within the lining keratinocytes. The overlying epidermis also reveals formation of the eosinophilic cytoplasmic inclusions (Figure IV-A-4-2). Note the difference from staining in myrmecia. The viral antigens are expressed in some nuclei with eosinophilic inclusions, while the cytoplasmic inclusions are not immunoreactive. Electron microscopically, the eosinophilic inclusion is composed of interlacing bundles of cytokeratin filaments (Figure IV-A-4-3). The compressed nucleus shows clustering of virions (viral inclusion bodies). The intranuclear viral inclusion is solely composed of 40 nm-sized viral particles. Ubiquitin immunostaining is negative.

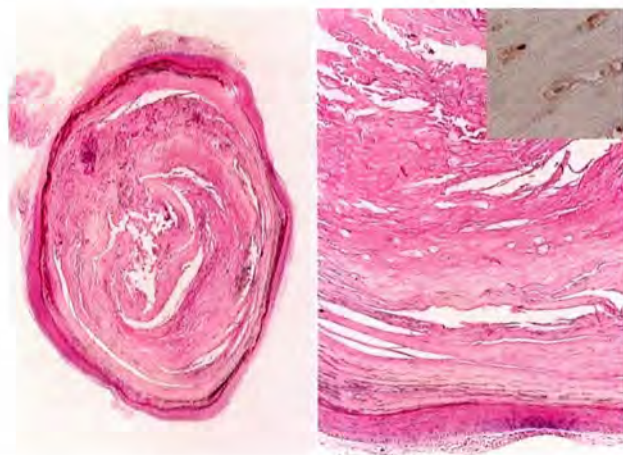


Figure IV-A-4-1. Plantar cystic wart (I). A cystic inclusion resembling epidermal cyst is formed in the sole skin (left: H&E). Round, vacuolated structures are distributed in the hyperkeratotic layer (right: H&E), and are immunoreactive for the viral capsid antigens (inset).

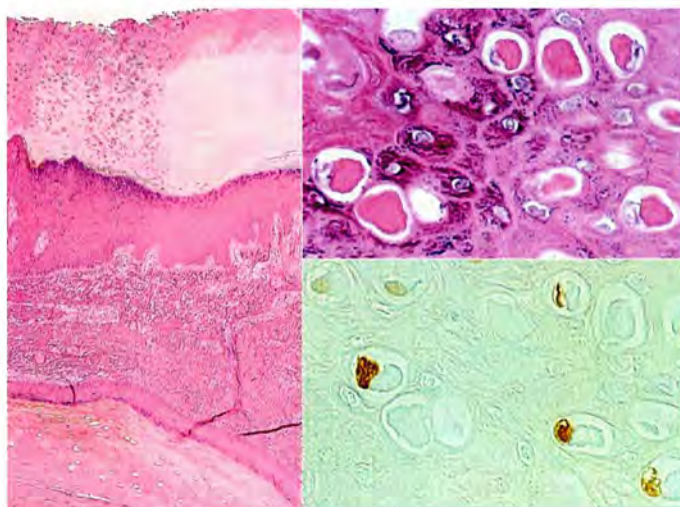


Figure IV-A-4-2. Plantar cystic wart (II). Round and eosinophilic cytoplasmic inclusions are observed in the keratinocytes lining the plantar cyst wall. Vacuolated structures are noted in the hyperkeratotic layer (left: H&E). The overlying epidermis with hyperparakeratosis also reveals regional formation of the eosinophilic cytoplasmic inclusions (right top: H&E). Papillomavirus capsid antigens are positive in the nuclei but negative in the cytoplasmic inclusions (right bottom).

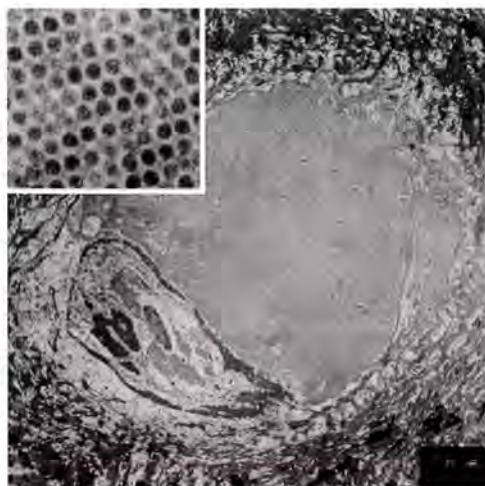


Figure IV-A-4-3. Electron micrographs of plantar cystic wart. The eosinophilic cytoplasmic inclusion in the keratinocyte is composed of interlacing bundles of cytokeratin filaments, and the nucleus contains clustering of small-sized virions. Inset demonstrates a close-up view of the 40 nm-sized virions in the nucleus.

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5) Myrmecia

Myrmecia (deep palmoplantar wart) is a special form of palmoplantar wart, caused by HPV 1. The name is derived from its supposed resemblance to the anthill. The lesion is usually seen in children and young adults. Dome-shaped undermining growth of the thickened keratinocytes is observed. Large basophilic cytoplasmic inclusions (granular inclusions) and ground-glassed nuclei of smudge type are diagnostic of myrmecia. Electron-dense, keratohyaline-like myrmecia inclusions are associated with HPV1 E4 gene products. Vacuolated structures are seen in the hyperkeratotic layer. Immunostaining for papillomavirus

capsid antigens reveals strong positivity in the nuclei of the inclusion body-carrying cells. The cytoplasmic inclusions remain unstained. Figures IV-A-5-1 and IV-A-5-2 illustrate representative lesions on the sole.

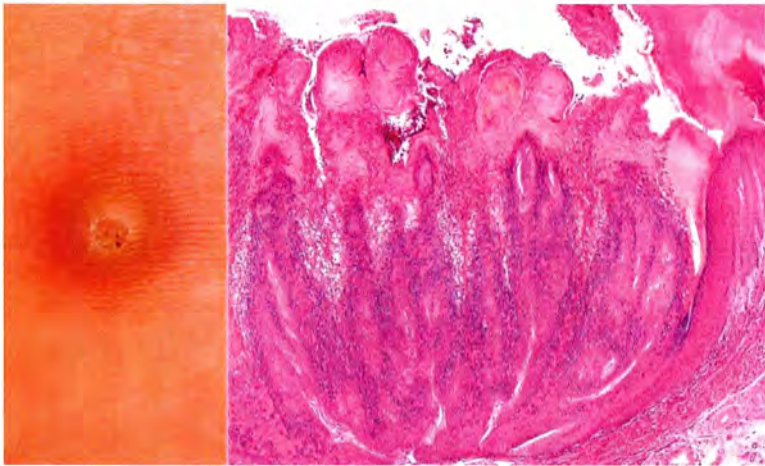


Figure IV-A-5-1. Myrmecia (I). Gross appearance reveals invaginated hard nodule formation (left). A localized invaginated nodule is observed in the sole. Dome-shaped undermining growth of the thickened keratinocytes is observed. Hyperkeratosis and koilocytosis are characteristic (right).

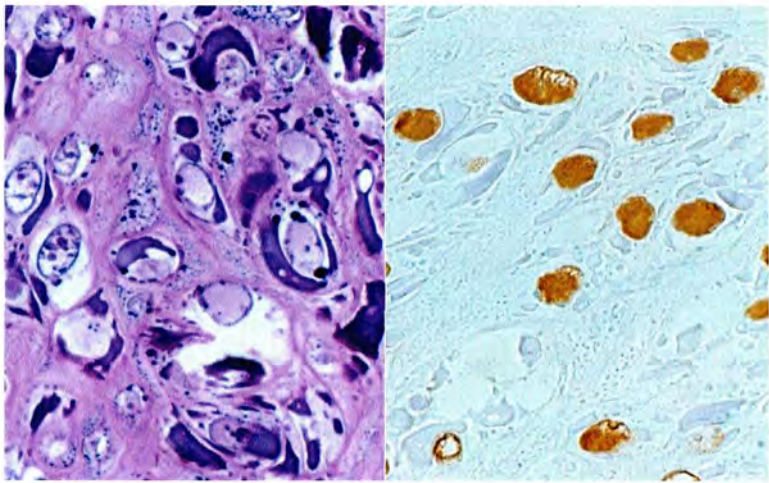


Figure IV-A-5-2. Myrmecia (II). The papillary growing keratinocytes with koilocytic change reveal characteristic large basophilic (keratohyaline-like) cytoplasmic inclusions. The nuclei possess smudgy inclusion bodies (left: H&E). Papillomavirus capsid antigens are expressed in the ground-glassed nuclei, but the cytoplasmic inclusions remain unstained (right: immunostaining).

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6) Condyloma Acuminatum

Condyloma acuminatum represents one of the most common sexually transmitted infections, mostly caused by infection of HPV 6 and 11. Other HPV types have also infrequently been implicated, including HPV 2, 16, 18, 31, 33, 35, 39, 41-45, 51, 56 and 59. Clinical manifestation includes multiple papillomatous lesions in the external genitalia (penis and vulva). Both the mucosa and skin part of the genitalia are involved by multiple polypoid (verrucous/warty) lesions (Figure IV-A-6-1). Uterine cervical and vaginal lesions may also be seen.

Histologically, the polypoid lesion consists of active papillomatosis of non-keratinizing squamous mucosa or the epidermis. Koilocytosis with perinuclear haloe and mild nuclear enlargement, characteristic in the superficial squamous layer, is a useful indicator in cytology diagnosis with Papanicolaou stain. Typical cytological features of koilocytic atypia are demonstrated in Figure IV-A-6-2. With non-isotopic *in situ* hybridization technique, numbers of nuclei are positively stained with cocktail probes to HPV type 6/11, and papillomavirus capsid antigen-positive nuclei are usually much less than the HPV 6/11 genome-positive nuclei (Figure IV-A-6-3, see also Figure IV-A-1-3 in Note 38).

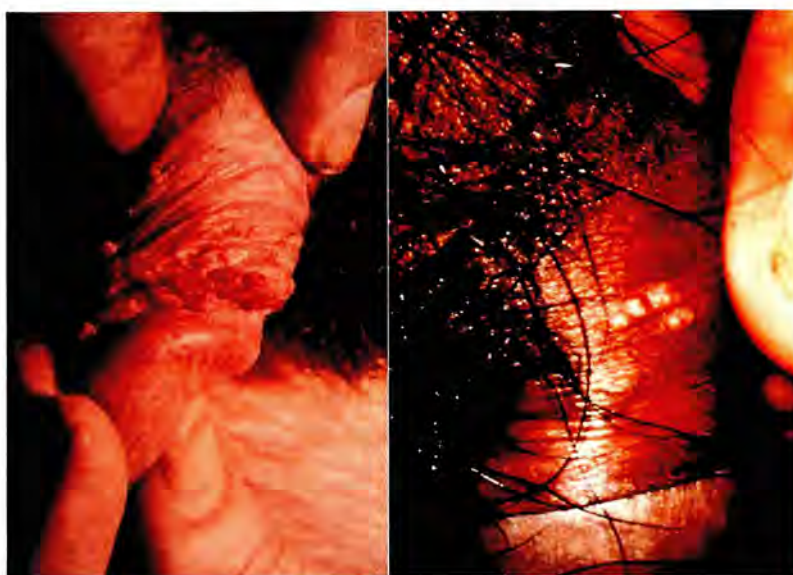


Figure IV-A-6-1. Condyloma acuminatum. Sexually transmitted verrucous lesions are seen on the penile mucosa (corona glandis) (left) and penile shaft skin (right).

It is known that early lesions are strongly immunoreactive to the viral capsid antigens because of active formation of virions in the nuclei. With time, naked (plasmid) DNA without the immunoreactivity remains in the nuclei. HPV 16/18 genomes are undetectable.

Anal and oral sex induces extragenital condylomatous lesions in the perianal skin, lip, throat and conjunctiva (see Figure IV-A-9). A Japanese male aged 60's complained of multiple verrucous lesions on the neck skin. By biopsy, the lesions were proven to be condyloma rather than verruca vulgaris, because of the positivity of *in situ* hybridization signals for HPV genomes of mucosal type (Figure IV-A-6-4).

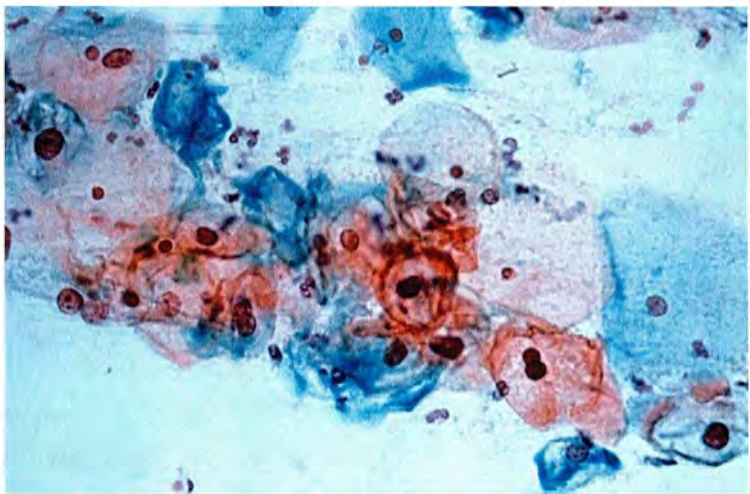


Figure IV-A-6-2. Koilocytosis in the cervical smear preparation. Koilocytic atypia (perinuclear halo plus mild nuclear enlargement) is characteristic cytological features of the condylomatous lesion or mild dysplasia (Papanicolaou).

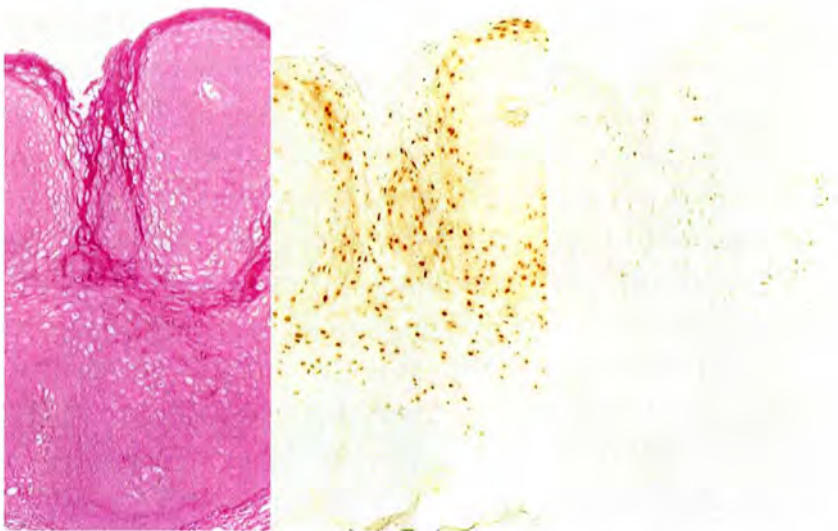


Figure IV-A-6-3. Histology of condyloma acuminatum on the glans penis. Active papillomatosis with koilocytic change are pathognomonic of condyloma acuminatum (left: H&E). A good number of nuclei are labeled for HPV genomes of types 6 and 11 (center: *in situ* hybridization), while the papillomavirus capsid antigens are positive in fewer nuclei (right: immunostaining).

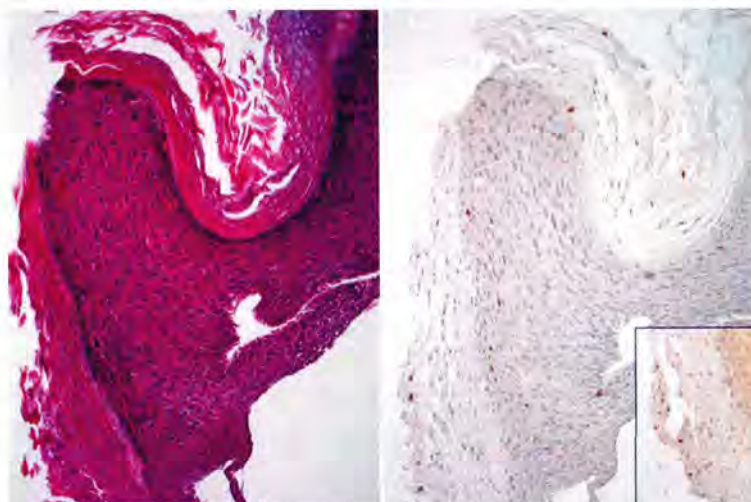


Figure IV-A-6-4. Multiple clustered condyloma acuminatum on the neck skin. Verrucous growth of the parakeratotic epidermal keratinocytes is observed in the biopsy specimen (left: H&E). The unusual localization of the condylomatous lesion in this male patient aged 60's was confirmed by the positivity for HPV genomes of mucosal type (right: *in situ* hybridization). Inset indicates focal nuclear reactivity of papillomavirus capsid antigens (immunostaining).

The cocktail probes used for the mucosal-type HPV screening can detect a total of 7 HPV types of mucosal type (including HPV 6, 11, 16, 18, 31, 33 and 51), but skin type HPVs are unreactive. The guy might have a unique sexual behavior.

In contrast, rare genital mucosal involvement of verruca vulgaris can be confirmed by positivity of the viral capsid antigens but with negativity for HPV genomes of mucosal type.

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7) Giant Condyloma Acuminatum and Condylomatous Carcinoma

Giant condyloma acuminatum of Buschke and Loewenstein is a slow-growing, locally destructive verrucous plaque typically seen in the glans penis. It also occurs on any anogenital mucosal surface, including the vulva, vagina, anus and scrotum. HPV 6 and 11 and occasionally types 16, 18 and 54 have been identified in the lesion, but the virus is scarcely demonstrable histochemically. Nuclear atypia is minimal, but is locally invasive (Figure IV-A-7-1). The polypoid lesion is now regarded as a variant of verrucous carcinoma, but disputation is still there. Invasive squamous cell carcinoma may follow.

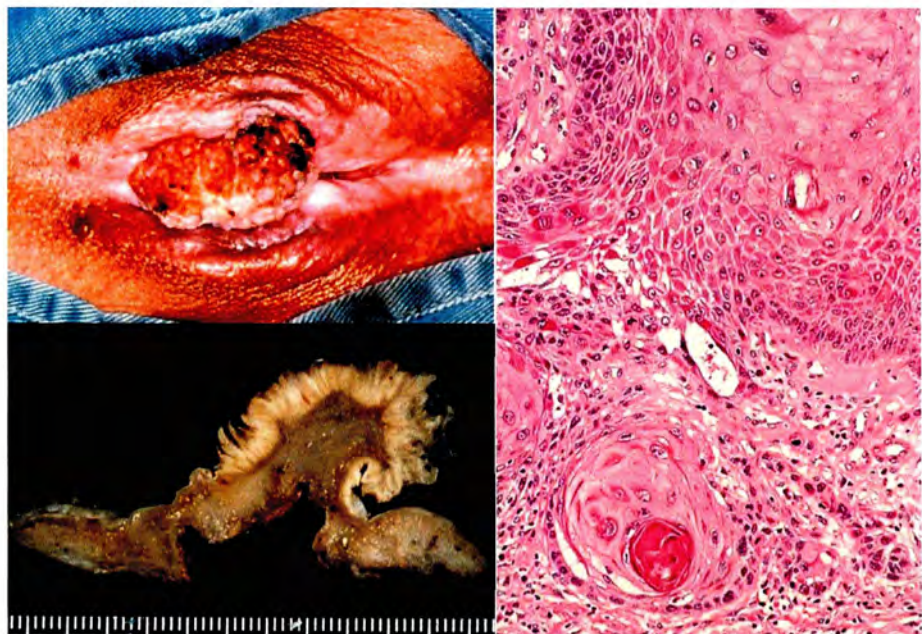


Figure IV-A-7-1. Giant condyloma (verrucous carcinoma) of the vulva. A large verrucous lesion is seen on the vulva of an aged Japanese female (left top). The cut surface of the surgical specimen after formalin fixation reveals active papillary proliferation (left bottom). Microinvasion is microscopically confirmed, while nuclear atypia is minimal (right: H&E). HPV is undetectable histochemically.

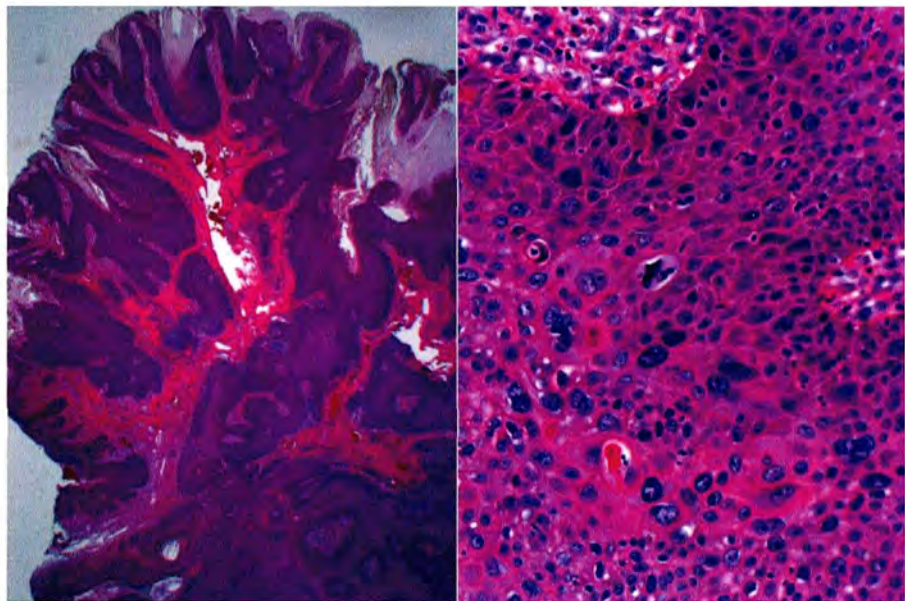


Figure IV-A-7-2. Condylomatous carcinoma of the vulva (I). A large verrucous lesion is seen on the vulva of a female aged 30's. Active papillary downward growth of epidermal keratinocytes is evident (left: H&E). High-powered view demonstrates marked nuclear atypia (pleomorphism) and atypical mitosis (right: H&E).

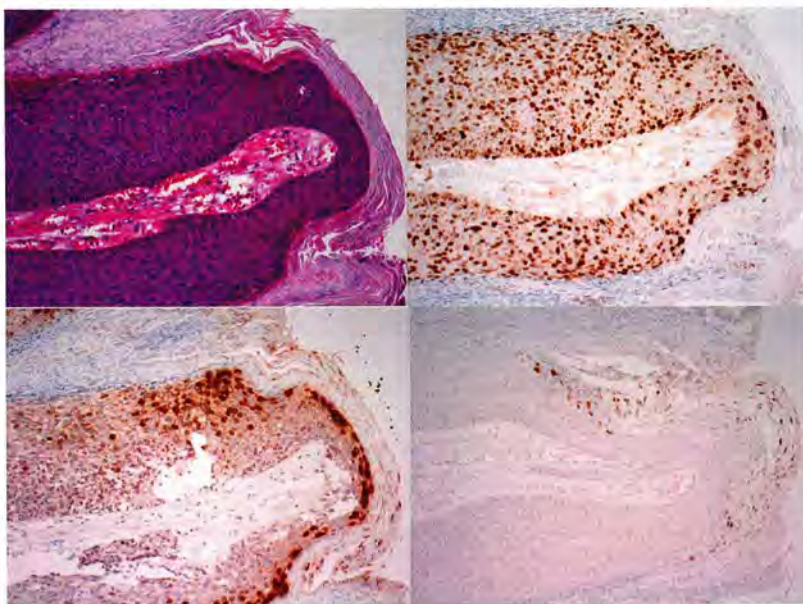


Figure IV-A-7-3. Condylomatous carcinoma of the vulva (II). The papillary and hyperparakeratotic cancer cells (left top: H&E) are actively proliferative with a high Ki-67 labeling index, evenly distributed in the entire layer (right top). Both the HPV genomes of mucosal type (left bottom) and the papillomavirus capsid antigens (right bottom) are visualized histochemically. More cancer cells are positive for the genomes than for the antigens.

Condylomatous carcinoma (warty carcinoma) grossly resembles verrucous carcinoma with its large and exophytic appearance. Both male and female genitalia are involved. Intraepithelial precancerous lesions are frequently associated. Histologically, the cancer cells display nuclear pleomorphism and evident cytologic atypia. In the superficial layer, koilocytic cells are observed. High-risk HPV, particularly HPV 16 is identified. Figures IV-A-7-2 and IV-A-7-3 show typical microscopic features of condylomatous carcinoma of the vulva, with distinct positivity for intranuclear localization of both papillomavirus capsid antigens and HPV genomes of mucosal type.

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8) Heck's Disease

Heck's disease, also known as focal epithelial hyperplasia, is characterized by multiple soft white or pink papules or confluent sessile plaques on the mucosa of the lip and cheek. Histologically, the squamous mucosa is acanthotic with clubbing of the rete ridges. The keratinocytes in the upper part of the mucosa show characteristic cytoplasmic pallor with pyknotic nuclei (Figure IV-A-8). Mitosis-like changes within otherwise mature and well-differentiated epithelium are observed. Binucleated cells are scattered, but without intranuclear inclusion bodies.

High incidence among Inuits (Eskimos) and American Indians is noted. Reportedly, the etiological agent is HPV 13 or 32. The viral genome is detectable with *in situ* hybridization or polymerase chain reaction.

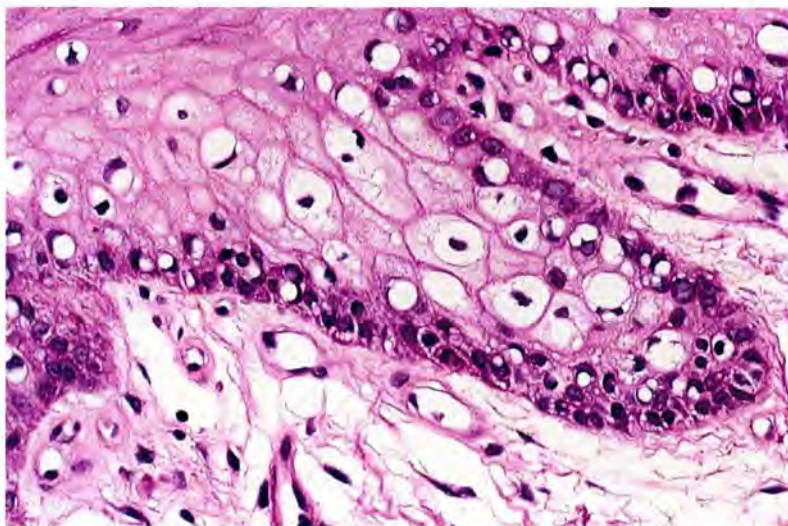


Figure IV-A-8. Heck's disease. The squamous mucosa of the lip is mildly acanthotic with elongation of the rete ridges. The suprabasal keratinocytes show characteristic cytoplasmic pallor with pyknotic nuclei. Binucleated cells are focally seen. No nuclear inclusions are discerned (H&E). The courtesy by Dr. Yoshito Ueyama, a pathologist at Inagi Municipal Hospital, Inagi, Japan.

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9) Conjunctival Papilloma

Conjunctival papilloma is caused by inoculation of HPV 6 and 11 via finger contact. The lesion presents a pedunculated or sessile polyp, with a glistening surface with geometrically arranged red dots. The young aged is relatively susceptible to this sexually infective lesion. Proliferated conjunctival mucosa reveals minimal nuclear atypia. HPV genomes of mucosal type are more easily detectable in the nuclei than the viral capsid antigens (Figure IV-A-9).

Papillomatous lesions may be seen in the eyelids and in the lacrimal duct (located at the medial canthus).

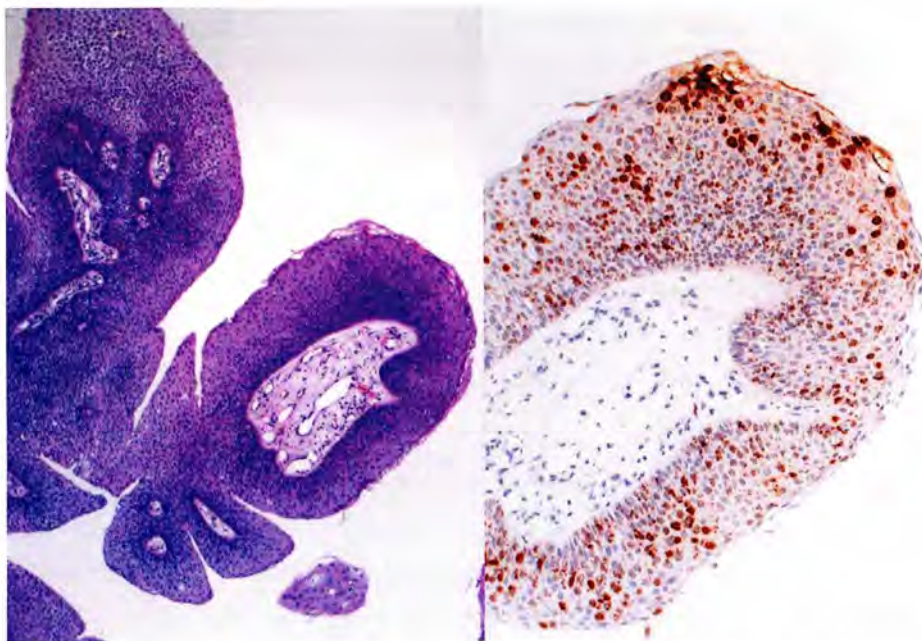


Figure IV-A-9. Conjunctival papilloma. Active papillary growth is discerned in the conjunctival mucosa (left: H&E). *In situ* hybridization demonstrates high nuclear labeling for HPV genomes of mucosal type (right).

References

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10) Bowenoid Papulosis

Bowenoid papulosis is an uncommon genital dysplasia resulting from infection of oncogenic HPV. Bowenoid papulosis is now considered to be a sexually transmitted infection caused mainly by HPV 16. Malignant transformation into squamous cell carcinoma has been documented. Female sexual partners of men with this disease may be at an increased risk of cervical cancer.

Clinically, it resembles persistent warts, multiple small pigmented lesions with verrucous projections (papules), on the penis or vulva (Figure IV-A-10-1).



Figure IV-A-10-1. Bowenoid papulosis. Multiple small pigmented papules with verrucous projections are seen on the vulva. Clinically, the lesion resembles warts.

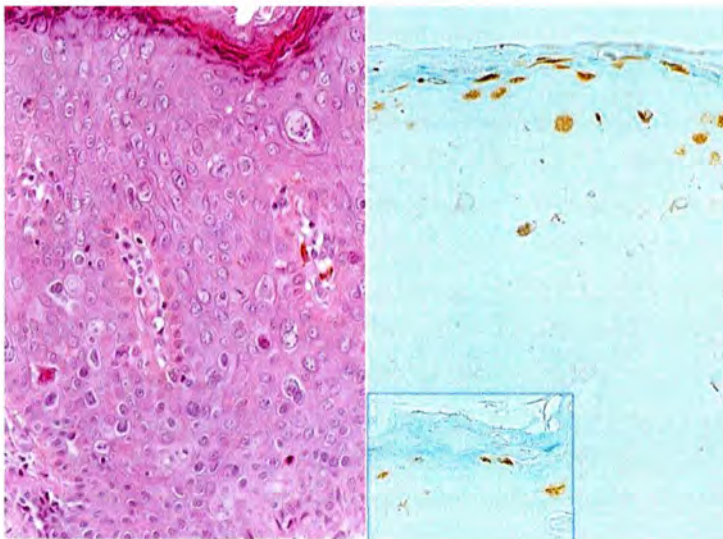


Figure IV-A-10-2. Histology of bowenoid papulosis. The lesion consists of atypical keratinocytes, and the microscopic resemblance to Bowen's disease is evident (left: H&E). In sharp contrast to Bowen's disease, carcinogenic HPV 16/18 genomes can be demonstrated within the lesion with the *in situ* hybridization technique (right). Inset demonstrates co-localization of HPV 31/33/35 genomes.

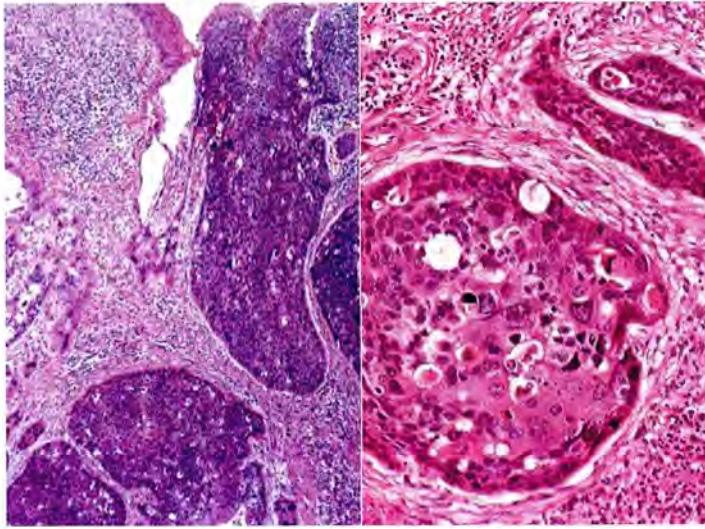


Figure IV-A-10-3. Secondary occurrence of squamous cell carcinoma in bowenoid papulosis. Five years later, invasive squamous cell carcinoma with groin nodal metastasis developed in this case (left: vulvar lesion, right: groin node, H&E). The viral genomes failed to be detected in the overt malignancy.

The acanthotic squamous mucosa histologically resembles Bowen's disease or squamous cell carcinoma *in situ*. Dyskeratotic cells are scattered. Multiplicity of the lesions, spontaneous regression and high-risk HPV genome identification in the lesion distinguish this condition from Bowen's disease or penile erythrodisplasia of Queyrat.

Carcinogenic HPV 16/18 or less frequently HPV 31/33/35 genomes are demonstrated in the superficial part of the lesion by *in situ* hybridization technique. Papillomavirus capsid antigens may also immunohistochemically be demonstrated in the lesion. Representative microscopic features are shown in Figure IV-A-10-2. In a long-term follow-up period, invasive squamous cell carcinoma with groin nodal metastasis developed in the same case (Figure IV-A-10-3).

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11) HPV and Squamous Cell Carcinoma of the Skin

HPVs of mucosal type are sexually transmitted to the genital area, while HPVs of skin type infect the skin via contact to form warts. High-risk HPVs of mucosal type are implicated in the development of cervical dysplasia (intraepithelial neoplasia). As described in Note 38,

dot-like positivity for carcinogenic HPVs is characteristic of cervical severe dysplasia (Figure IV-A-11-1). In the uterine cervix, papillary (polypoid) squamous cell carcinomas often show dot-like signals for carcinogenic HPV genomes in the nuclei (Figure IV-A-11-2). Both adenocarcinoma and neuroendocrine carcinoma of the uterine cervix are also frequently positive for the carcinogenic HPV genomes (Figure IV-A-11-3). It is of note that the expression of p16 INK4A immunoreactivity corresponds to high-risk HPV infection in the uterine cervical precancerous and cancerous lesions (Figure IV-A-11-4).

Anogenital pre-cancerous lesions, as well as vaginal, vulvar, penile and anal cancers, are also closely related to carcinogenic HPVs. As described above, bowenoid papulosis and warty (condylomatous) carcinoma are frequently associated with high-risk HPV infection, whereas verrucous carcinoma of the penis or vulva is commonly correlated with HPV 6 and 11 infection. Most of the anal and a half of penile squamous cell carcinomas are reportedly caused by high-risk HPV infection. In squamous cell carcinoma of the head and neck regions (particularly oropharyngeal carcinoma), p16 INK4A immunoreactivity can be a microscopic candidate of HPV infection and the non-keratinizing type is closely associated with HPV infection and better patient survival when compared to the keratinizing type. Some cases of squamous cell carcinoma of the lung have been linked to HPV 16/18 infection.

Squamous cell carcinoma of the skin is often curable when treated surgically. The most common cause is prolonged exposure to ultraviolet radiation. The possibility that HPV may play a role in the induction but not in the maintenance of cutaneous squamous cell carcinoma has been documented. Most precancerous and cancerous skin lesions express p16 INK4A immunoreactivity. Direct PCR detection of HPV genomes in the cancer lesion has been tried. However, conclusions for the viral etiology of non-anogenital skin cancer have not been obtained.

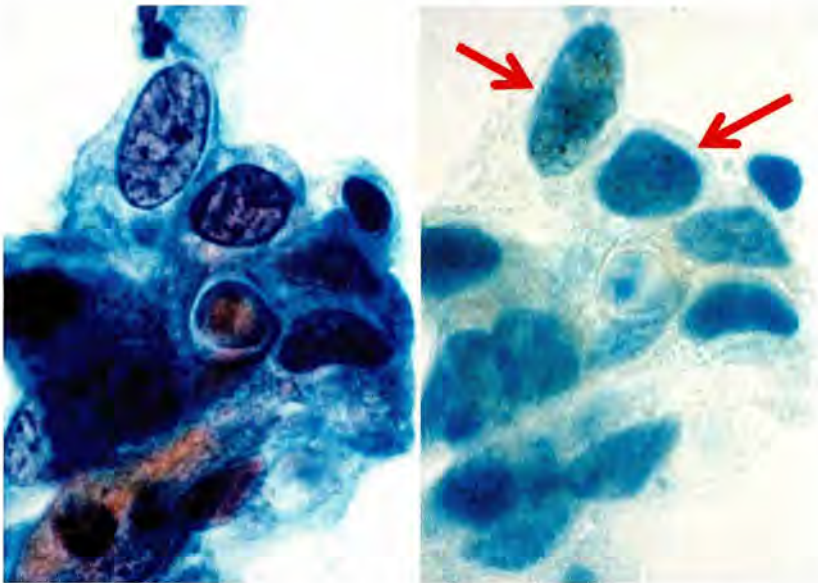


Figure IV-A-11-1. Intracellular dot-like localization of carcinogenic HPV genomes in severe dysplasia of the uterine cervix. The dyskeratotic cells of parabasal type in the cytology specimen (left: Papanicolaou) show dot-like reactivity of the carcinogenic HPV genomes in the nuclei (arrows) (right: *in situ* hybridization for HPV 16/18 using the re-staining method). Refer also to Figure IV-A-1-4.

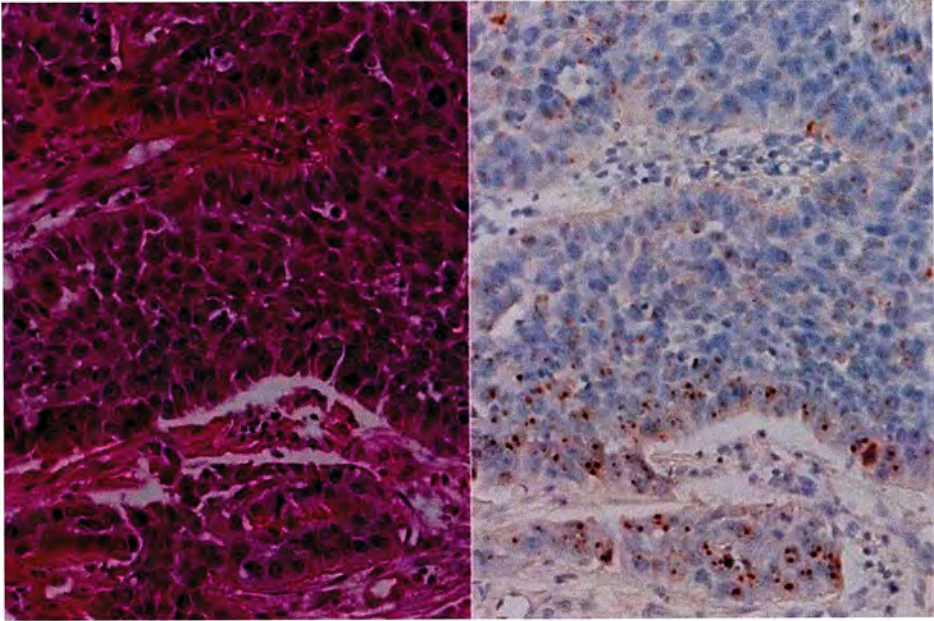


Figure IV-A-11-2. Polypoid squamous cell carcinoma of the uterine cervix. Dot-like reactivity of the HPV genomes of mucosal type is easily observed in the nuclei of cervical squamous cell carcinoma of polypoid type (left: H&E, right: *in situ* hybridization).

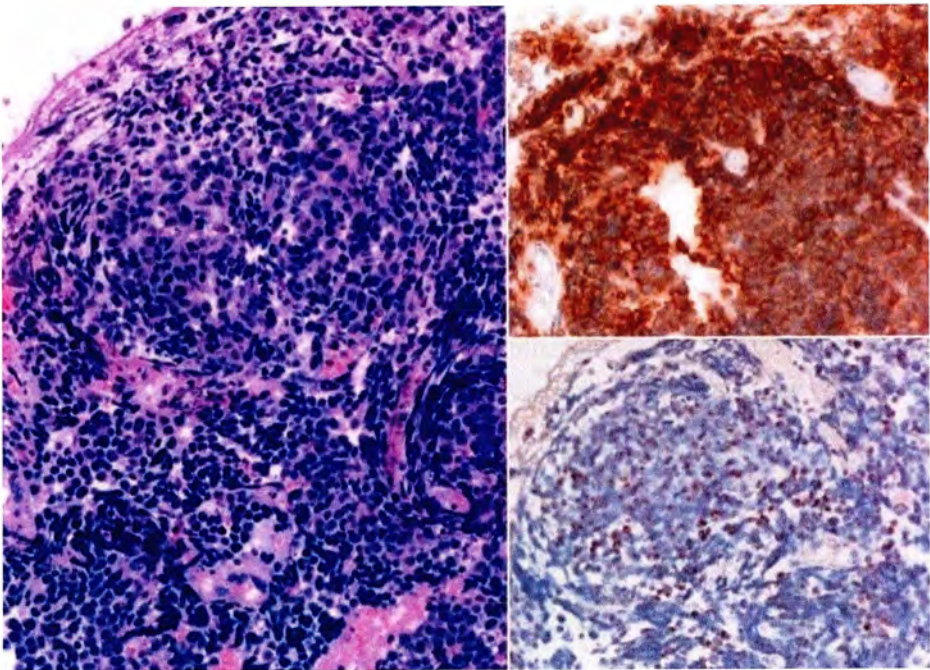


Figure IV-A-11-3. HPV genome identification in neuroendocrine carcinoma of the uterine cervix. Not only squamous cell carcinoma and adenocarcinoma of the uterine cervix but also neuroendocrine carcinoma show intranuclear dot-like positivity for the HPV genomes of mucosal type (left: H&E, right top: immunostaining for neuronal cell adhesion molecule, right bottom: *in situ* hybridization).

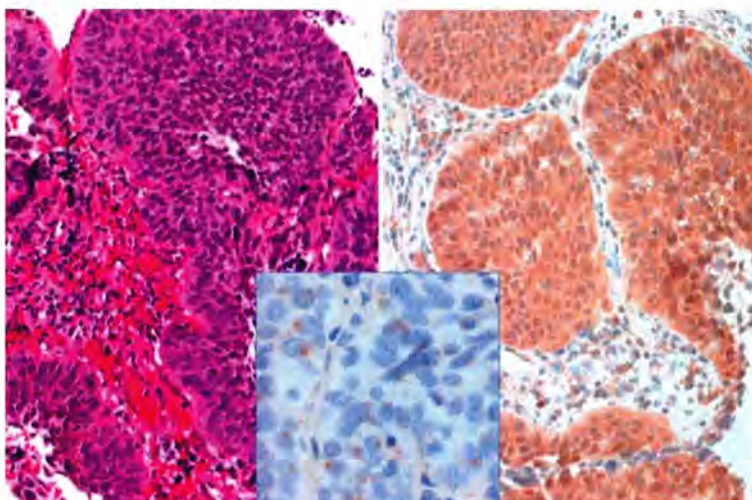


Figure IV-A-11-4. p16 INK4A immunoreactivity in invasive squamous cell carcinoma of the uterine cervix. The cytoplasmic expression of p16 INK4A immunoreactivity corresponds to high-risk HPV infection in the cervical lesions (left: H&E, right: immunostaining for p16 INK4A, inset: intranuclear dot-like reactivity with *in situ* hybridization for HPV genome of mucosal type).

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B) Molluscum Contagiosum

Molluscum contagiosum occurs as solitary or multiple dome-shaped waxy papules with a predilection for the head and neck, flexural areas or the genitalia of children and adolescents. Sexually transmitted infection may occur. The papules are 2–8 mm in size. Spontaneous regression is common. Extensive, disseminated lesions may be seen in AIDS patients. The disease is caused by molluscum virus, a large brick-shaped poxvirus. This double-stranded DNA virus of poxvirus group belongs to the largest virus, about 300 nm in the long axis.

Histologically, the lesion is characterized by inverted lobules of squamous epithelium with “molluscum bodies” maturing toward the surface. The keratinocytes possess large-sized cytoplasmic inclusion bodies. No nuclear inclusions are seen. The papules are often located at the infundibulum of the hair follicle. Sebaceous gland involvement indicates that the molluscum lesion occurred at the site of hair follicle.

The change of stainability of the “molluscum body” is characteristic. The keratinocytes at the Malpighian layer possess eosinophilic inclusion bodies, and at the level of the granular and cornified (parakeratotic) layers, they become homogeneous and basophilic and occupy the entire cell (Figure IV-B-1). Electron microscopically, the molluscum body consist of numerous oval-shaped viral particles, consisting of the outer envelope and internal dumbbell-shaped electron-dense nucleoid (Figure IV-B-2). The molluscum virus belongs to a large-sized, double-stranded DNA virus.

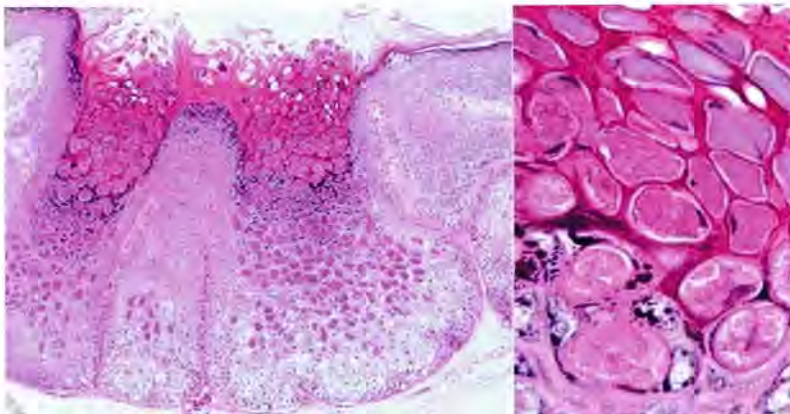


Figure IV-B-1. Molluscum contagiosum. Numerous large cytoplasmic inclusions “molluscum bodies” are seen in the keratinocytes of hair follicle origin. Clear-looking sebaceous gland cells are observed at the periphery of the downward growing lesion. The molluscum bodies show changes in stainability. Along with surface differentiation, eosinophilia turns to be basophilia in the parakeratotic cornified layer (H&E, left: low power, right: high power).

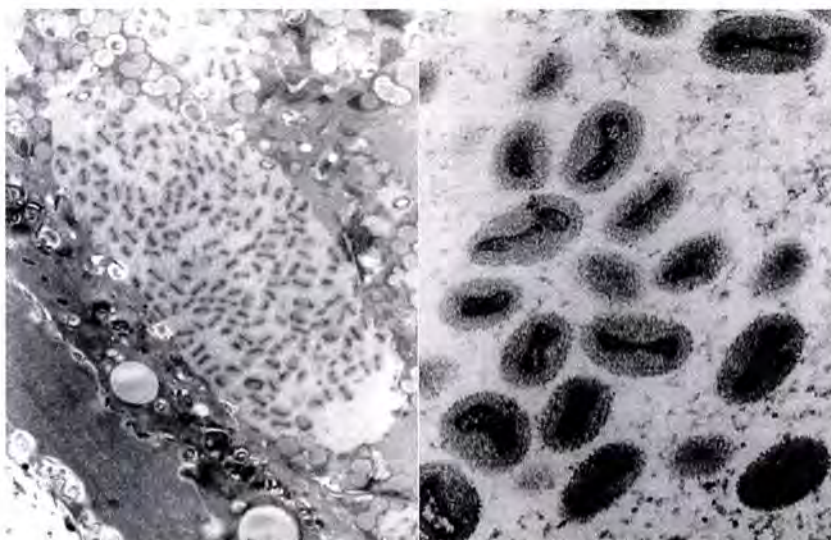


Figure IV-B-2. Electron micrographs of molluscum virus. The molluscum body in the cytoplasm of the involved keratinocyte consists of numerous oval-shaped particles, consisting of the outer envelope and internal dumbbell-shaped electron-dense nucleoid (left). The size of this double-stranded DNA virus is as comparable as the smallest bacterium (right).

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Note 39. Nuclear inclusions versus cytoplasmic inclusions

Generally speaking, DNA viruses form nuclear inclusion bodies. Examples include papovaviruses (human papillomavirus and polyomavirus such as JC virus and BK virus), herpes viruses (herpes simplex virus types 1 and 2, varicella zoster virus and cytomegalovirus), adenovirus, erythrovirus (parvovirus B19), and hepatitis B virus. Among herpes viruses, Epstein-Barr virus, human herpesvirus (HHV)-6, HHV-7 and HHV-8 do not form nuclear inclusion bodies. Cytoplasmic inclusions are also accompanied in infection of cytomegalovirus and hepatitis B virus (clustered HBs antigen). The simultaneous occurrence of nuclear and cytoplasmic inclusions are characteristic of enlarged CMV-infected cells. Poxviruses, including molluscum virus and orf virus, exceptionally accompany cytoplasmic inclusion bodies but no nuclear inclusions.

RNA viruses may form cytoplasmic inclusion bodies: Examples include Negri bodies seen in neurons in rabies, and eosinophilic inclusion bodies seen in Ebola hemorrhagic fever. However, no cytoplasmic inclusions are formed by most of the RNA viruses, hence RNA viral infection is not recognizable light microscopically. Measles virus is an exceptional RNA virus to accompany intranuclear inclusion bodies in multinucleated cells. The nucleoprotein (NP) antigen of influenza virus A is immunohistochemically located in the nuclei of type II pneumocytes in influenza pneumonia.

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C) Orf and Milker's Nodule

Orf, or ecthyma contagiosum, is primarily a disease of young sheep, goats and deer, involving the lip and perioral area. Orf is caused by a parapoxvirus and can be transmitted to the human by contact with the infected animals. Orf virus is a large-sized (180 x 300 nm), enveloped and double-stranded DNA virus. Grossly, a nodular lesion with central umbilication and erythematous halo is formed on the hand and forearm of the human. The size of the lesion is 1-3 cm, and multiple nodules may be seen. Regional nodal swelling is occasionally associated. In human orf, spontaneous regression occurs in 7 weeks.

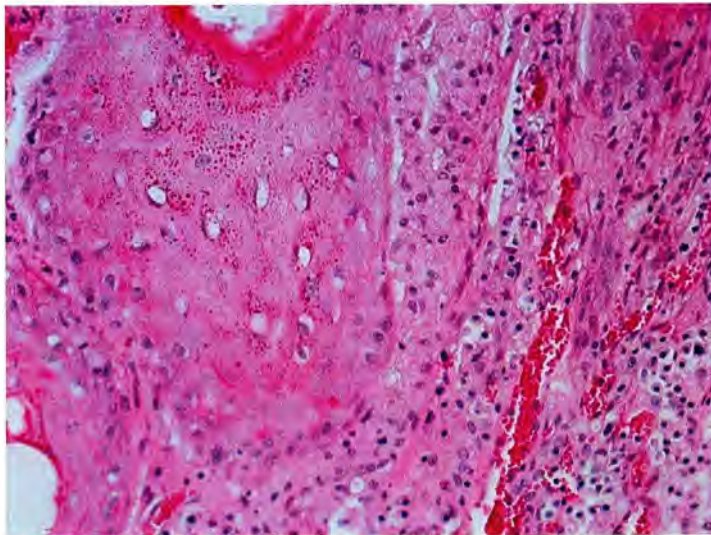


Figure IV-C. Orf. The perioral skin of deer shows downward acanthosis and vascularization in the papillary dermis. Small-sized eosinophilic viral inclusions are dispersed in the cytoplasm of the keratinocytes (H&E). No nuclear inclusions are noted. The courtesy by Dr. Makoto Haritani, Pathology Division of the National Agriculture and Food Research Organization, Tsukuba, Japan.

Histologically, intraepidermal vesicles or bullae are seen. In the upper part of moderately acanthotic epidermis, small-sized eosinophilic cytoplasmic inclusions are seen in the keratinocytes with pale vacuolated or ballooned cytoplasm (Figure IV-C). The papillary dermis between the elongated rete ridges is infiltrated by lymphoid cells and characteristically vascularized, resembling a vascular tumor. In a late stage, epidermal necrosis is observed.

Milker's nodule results from infection of parapoxvirus (paravaccinia virus), transmitted from the udders of infected cow. A solitary nodular lesion measuring 0.5-2 cm is seen on the hand, particularly the finger. Both the clinical and histologic features are similar to human orf. These two conditions are collectively called "farmyard pox".

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D) Herpes Simplex Virus Infection

Herpesviruses are ubiquitous and extremely well adapted pathogens, and there are 8 members of the human herpesvirus (HHV) family. Non-human herpesviruses are also known: for example, infection of koi herpesvirus shows high mortality of common carp and koi carp all over the world. The name comes from the Greek '*Herpein*' - 'to creep', describing a chronic, latent or recurrent nature of infections. Humoral neutralizing antibodies prevent reinfection, while the cellular immunity is responsible for the recovery from infection.

The double-stranded DNA genome and a protein core are surrounded by a complex icosahedral nucleocapsid, about 100 nm in diameter. Outside the capsid is the tegument, a protein-filled region seen amorphous in electron micrographs. On the outside of the particle is the lipid bilayered envelope containing rich glycoproteins. The whole viral size measures 180-200 nm. They belong to the following three families.

1. α -herpesviruses: herpes simplex virus (HSV) types I and II and varicella zoster virus (VZV), showing a relatively short reproductive cycle, variable host range, killing activity of the infected cells and latent infection in sensory ganglia.
2. β -herpesviruses: cytomegalovirus (CMV), HHV-6 and HHV-7, showing long reproductive cycles, a restricted host range and enlargement of the infected cell. Latency is maintained in leukocytes, endothelial cells, kidneys, secretory glands and others.

3. γ -herpesviruses: Epstein-Barr virus (EBV) and HHV-8, showing affinity for T or B lymphocytes, and latency is often seen in lymphoid tissue.

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1) Herpetic Ulceration

Herpes simplex virus (HSV) types I and II cause recurrent vesicular lesions at the mouth angle (herpes labialis) and in the external genitalia (vulva or less frequently, penis), respectively. Vesicles easily rupture to form painful ulceration. In general, angular cheilitis is caused by HSV, *Candida albicans* and *Staphylococcus aureus*.

Recently, half of the herpetic ulcer in the vulva is caused by acute infection of HSV type I. The genital lesion by HSV type I is transmitted through oral sex, and usually accompanies severer clinical manifestation than HSV type II. Recently, the author experienced a case of herpes simplex on the nipple, clinically presented as painful nipple erosions. Cytology specimen showed typical nuclear inclusion bodies in multinucleated keratinocytes.

Herpetic cheilitis is clinically distinguished, so that histopathological evaluation is an infrequent occasion. Vulvar herpes is grossly featured by multiple map-like ulcerations focally with bulla formation (Figure IV-D-1-1).



Figure IV-D-1-1. Genital herpetic ulcer. The gland penis (left) and the vulva (right) are involved by vesicular lesions. They are easily eroded, causing pain. HSV type I causes more severe clinical manifestations than HSV type II.

Histologically, intraepidermal vesicles reveal acantholysis, multinucleation and intranuclear viral inclusions (Figure IV-D-1-2). Cytology examination also demonstrates that squamous epithelia are often multinucleated and show intranuclear inclusion bodies. Ground-glassed (smudgy) nuclei are characteristic. Haloed intranuclear inclusion of Cowdry's type A is infrequently encountered. Immunostaining using anti-HSV antiserum demonstrates positive signals in both the nuclei and cytoplasm (Figure IV-D-1-3). Regarding the inflammatory reaction, vesicles are often infiltrated with neutrophils, and moderate dermal infiltration of neutrophils and lymphocytes is associated.

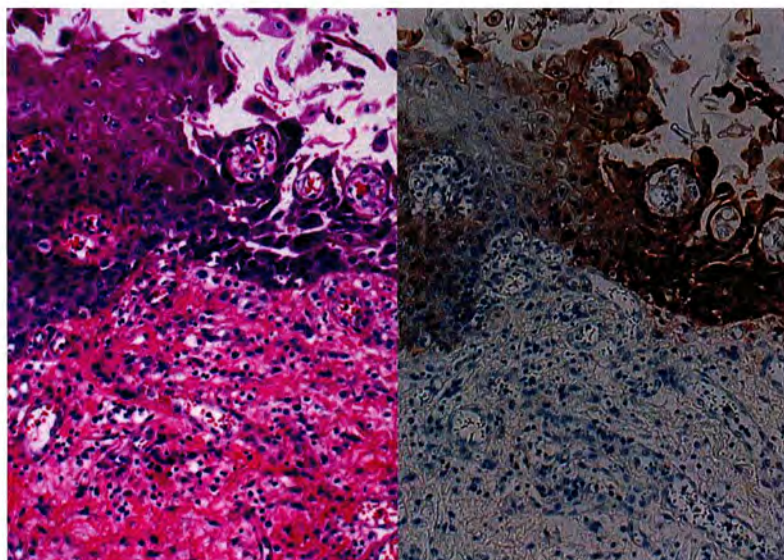


Figure IV-D-1-2. Histology of herpetic cheilitis. Acantholytic changes are seen in the epidermis with nuclear viral inclusions. Lymphocytic infiltration is evident in the dermis (left: H&E). HSV antigens are strongly positive in the nuclei and cytoplasm of the acantholytic zone (right: immunostaining).

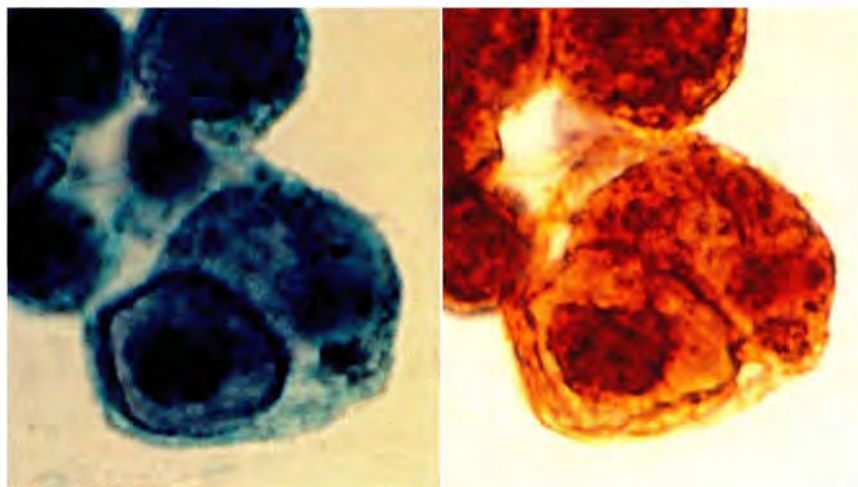


Figure IV-D-1-3. Cytological features of vulvar herpetic ulcer. Haloed inclusions of Cowdry's type A are observed in the Papanicolaou-stained cytologic smear (left). Re-staining for HSV antigens discloses both nuclear and cytoplasmic reactivity (right: immunostaining).

Among the causes of genital ulcer disease in women, HSV infection is most prevalent worldwide. Especially in some African countries, however, the prevalence of chancroid is still high. Granuloma inguinale and lymphogranuloma venereum should also be considered in tropical areas. Painless syphilitic ulcers should not be forgotten either.

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2) Herpetic Folliculitis

Focal and mild involvement of HSV in the pilosebaceous unit is common in recurrent herpetic skin lesions, but overt herpetic folliculitis is infrequently encountered. Herpetic folliculitis is seen in the perioral skin of male patients with recurrent herpetic cheilitis, and is characterized by pilosebaceous involvement of HSV type I.

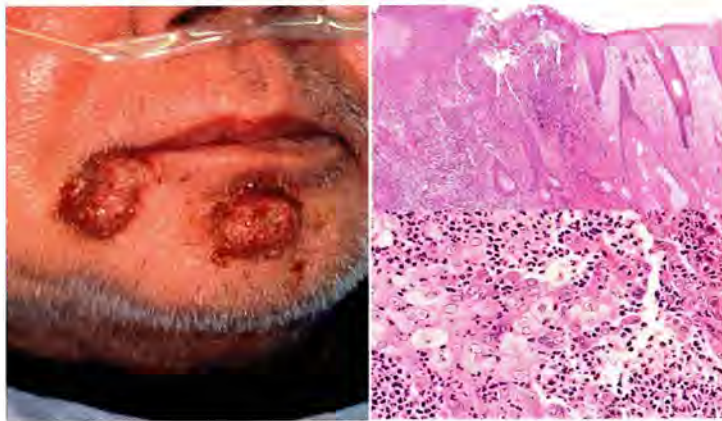


Figure IV-D-2. Herpetic folliculitis. Ulcerative nodular lesions are noted on the chin of an aged male (left). Histologically, follicle-centered inflammatory infiltration is heavily noted in the dermis (right top: H&E). Viral nuclear inclusions are seen in the hair follicle keratinocytes (right bottom: H&E).

Immunocompromised patients are vulnerable to this condition. Infrequently, eccrine glands and ducts are involved (herpetic syringitis). The dermal inflammatory infiltrate is heaviest in the deep dermis, a so-called “bottom-heavy” infiltrate. Severe destructive change of the hair follicle results in the ulceration and abscess formation. The destructive hair follicle epithelium contains smudgy or haloed intranuclear inclusions. Acantholytic change is evident. A representative case is presented in Figure IV-D-2.

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Note 40. Trichodysplasia spinulosa

Trichodysplasia spinulosa is another type of viral folliculitis seen in immunocompromised patients, caused by trichodysplasia spinulosa-associated polyomavirus (TSPyV). Erythematous and indurated papules on the face are associated with friable spinose processes eventually resulting in alopecia. TSPyV can be regarded as human viral skin flora. Histologically, the hair follicles are dilated with hyperparakeratotic plugging. The inner root sheath reveals enlarged irregular trichohyaline granules and apoptotic cells. When the hyperplastic areas show hair matrix differentiation with cellular disorganization and loss of nuclear polarity, it is called “pilomatrix dysplasia” or cyclosporine-induced follicular dystrophy. Inflammatory reaction is minimal.

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3) Kaposi's Varicelliform Eruption

Kaposi's varicelliform eruption, also called as eczema herpeticum, is a severe form of HSV type I infection. Commonly, the facial skin of infants suffering from atopic dermatitis is

involved with erythematous vesicles and pustules, resembling varicella eruptions (Figure IV-D-3). The prognosis is fair. The lesion may spread to the other skin areas.

When generalized vesiculopustular rash is developed, it resembles smallpox clinically. Histological features share with the common herpes simplex lesions. Originally thought to be a disorder of infants, Kaposi's varicelliform eruption is now known to occur in children at any age.



Figure IV-D-3. Kaposi's varicelliform eruption. The periaural skin of an infant suffering from atopic dermatitis show clusters of erythematous vesicles and pustules, resembling varicella eruptions. The courtesy by Prof. Tetsushi Yoshikawa, Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Japan.

Adult patients with atopic dermatitis are also susceptible to this illness. It has been proposed that the impairment in cutaneous immune responses and the defective mechanical barrier properties of the affected skin in atopic dermatitis are interrelated as the disease mechanism. Pemphigus vulgaris, Darier's disease, seborrheic dermatitis, psoriasis vulgaris and lupus erythematosus are nominated as the susceptible pre-existing dermatosis. HSV type II, Coxsackievirus A16 and vaccinia virus may infect those dermatoses to cause the similar conditions.

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4) Neonatal Herpetic Infection

Systemic HSV infection occurs in premature infants. The possibility of this serious infection should be considered if the neonate has obvious ulcerative lesions or if the mother has known HSV infection. Transvaginal HSV infection provokes multiple vesicular skin eruptions mainly in the head and neck region of the baby (Figure IV-D-4-1). Visceral involvement is highly likely. HSV type II is usually responsible for the neonatal infection, but one third is caused by HSV type I.



Figure IV-D-4-1. Neonatal herpetic infection. Transvaginal HSV infection provokes multiple vesicular skin eruptions mainly in the head and neck region of the baby. The patient died of massive hepatocellular necrosis due to systemic viral spread (see Figure IV-D-4-2).

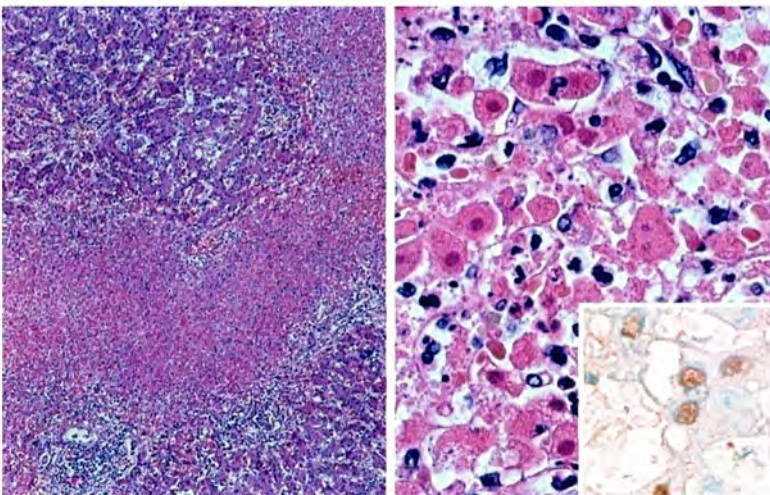


Figure IV-D-4-2. Massive liver necrosis in neonatal disseminated herpetic infection. Zonal coagulation necrosis of the hepatocytes is evident (left). Necrotic hepatocytes contain intranuclear inclusion bodies (right). Inset demonstrates HSV antigen immunoreactivity in the nuclei of the hepatocytes.

Neonatal HSV infection is categorized into three clinical forms: a) skin/eye/mouth infection, b) central nervous system infection, and c) disseminated disease with massive hepatocellular necrosis (Figure IV-D-4-2). Each disease category is responsible for roughly one third of neonatal infections. The mortality is highest in the disseminated disease, while the morbidity is highest for survivors of central nervous system infection. Unfortunately, antiviral therapy with acyclovir or vidaribine has to be started in an early stage of infection to be effective. Neonates who survive are usually severely disabled.

Systemic infection of HSV, accompanying massive liver necrosis, is also rarely seen in adults during pregnancy and in an immunosuppressed state.

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E) Varicella-Zoster Virus Infection

1) Varicella

Varicella-zoster virus (VZV), also called as human herpes virus type 3, electron microscopically consists of 4-layered 180 nm-sized virions, outermost envelop, tegument, capsid and the innermost core. Intranuclear virions lack the envelope which is produced in the cytoplasm. VZV often shows abnormal virions without the capsid component (aberrant form virions) (Figure IV-E-1-1). The initial infection of VZV causes varicella (chickenpox) in children, while reactivation of VZV that has latently been infected in ganglion cells (neurons) leads to herpes zoster (shingles) in adults. Varicella in pregnancy may cause an anomaly syndrome termed varicella embryopathy (hemilateral cutaneous scar, hypoplasia of extremities, adactylia, nervous system anomalies, and microphthalmia).

Varicella, one of the most common viral infections in the childhood, is caused by acute infection of VZV. Airborne infection occurs. Vaccination effectively protects VZV infection. Systemic skin involvement by small vesicles with red haloes is characteristic of varicella (Figure IV-E-1-2). Clinical diagnosis is not difficult. Tzanck test by scraping an ulcer base to look microscopically for multinucleated giant cells (Tzanck cells) is simple, quick and thus clinically useful (Figure IV-E-1-3). The scraped smear preparation is usually stained with quick Giemsa (Diff-Quik). Acantholytic multinucleated cells with nuclear inclusions are common between herpes simplex and varicella, so that an immunofluorescent study is added when necessary. In AIDS patients, chronic verrucous lesions may occur.

Biopsy is performed usually from adult cases (Figure IV-E-1-4). Histologically, the acantholytic squamous cells possess intranuclear inclusion bodies with smudgy (full) type (ground-glassed in appearance) or Cowdry's type A (cored and haloed in appearance). Multinucleation is consistently associated. Inflammatory reaction is scanty in the dermis, and

this makes a sharp contrast to herpes simplex. Immunostaining using monoclonal antibody to glycoprotein-1 (GP-1) antigen of VZV shows plasma membrane staining on the infected cells. Nuclear inclusions remain unstained. Indirect immunoperoxidase staining using a 1:500 diluted patient's serum from another adult case recovered from varicella also discloses the membrane reactivity. GP-1 antigen is known to be highly immunogenic.

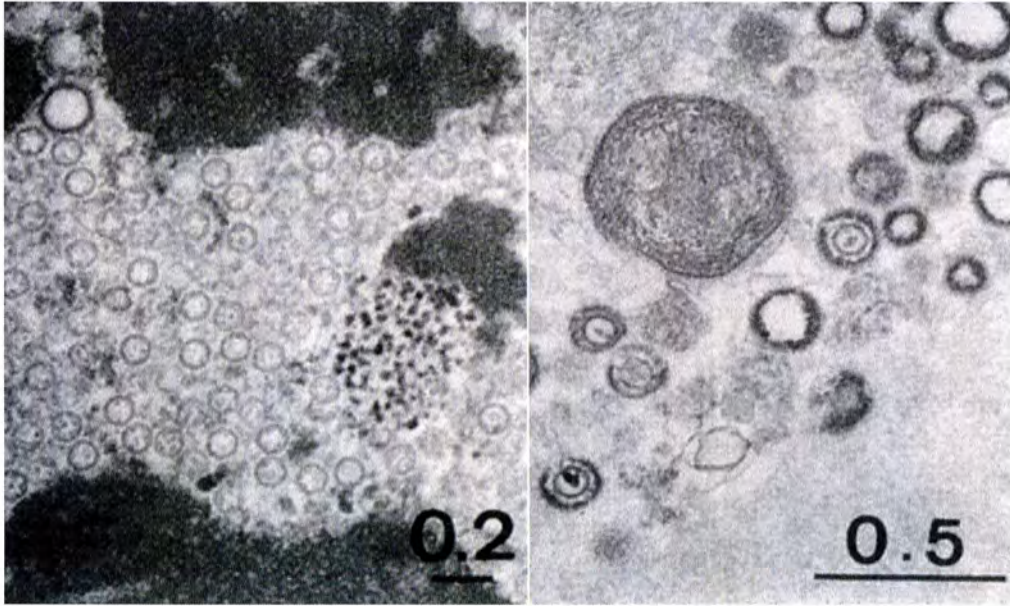


Figure IV-E-1-1. Electron micrographs of VZV virions. Intranuclear virions, 120-150 nm in size, contain the capsid antigen and the innermost viral DNA core. The envelope is formed in the cytoplasm. Abnormal empty virions without the capsid component are called as an aberrant form.



Figure IV-E-1-2. Gross appearance of varicella. Multiple small vesicles with red haloes are observed on the back of an infant. The courtesy by Prof. Tetsushi Yoshikawa, Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Japan.

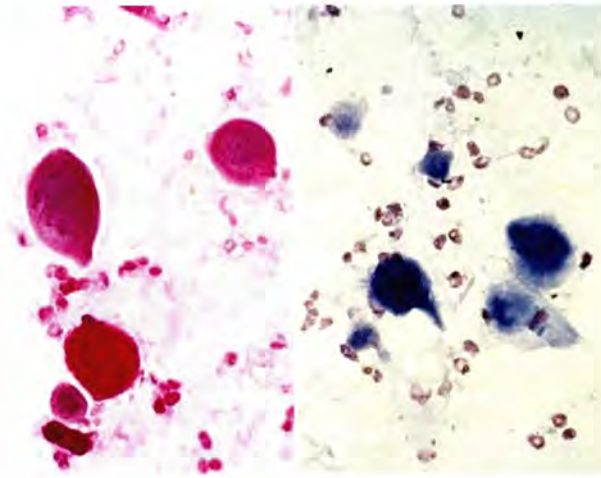


Figure IV-E-1-3. Tzanck test. By scraping an ulcer base of ruptured vesicles, inclusion body-positive multinucleated giant cells (Tzanck cells) are microscopically evaluated. The test is simple, quick and thus clinically useful for diagnosing infection by HSV and VZV (left: H&E, right: Diff-Quik).

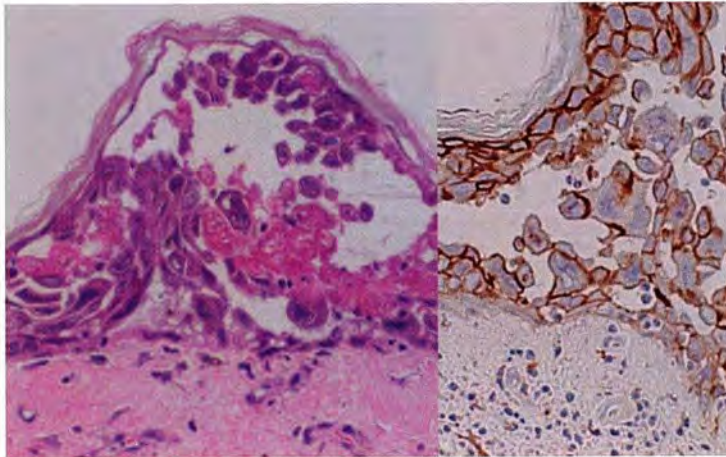


Figure IV-E-1-4. Biopsy histology of varicella in an adult patient. The vesicle consists of acantholytic squamous cells with intranuclear inclusions of either smudgy type or cored/haloed type. Inflammatory reaction is scanty in the dermis (left: H&E). Immunostaining using monoclonal antibody to glycoprotein-1 (GP-1) antigen of VZV shows plasma membrane staining on the infected cells (right).

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2) Hemorrhagic Varicella

Hemorrhagic varicella, also called as atypical varicella, is a lethal systemic infection of VZV, seen in an immunocompromised host. The vesicles are hemorrhagic and irregular-shaped. The vesicles in common varicella and herpes zoster never show hemorrhagic changes. It happens that non-vesiculated infective foci with viral nuclear inclusions are seen microscopically in the normal-looking epidermis adjacent to the hemorrhagic vesicle. Capillary endothelial cells in the dermis are also infected, reflecting the systemic dissemination of the virus. The mortality rate is very high.

Representative features seen in a pediatric case post bone marrow transplantation as the treatment for acute lymphoblastic leukemia are illustrated in Figure IV-E-2. Recurrence (reinfection or reactivation) of varicella occurred after chemotherapy and bone marrow transplantation. The vesicular skin lesions are characteristically hemorrhagic, and VZV pneumonia can be the cause of death. Pediatric hematologists often shout that varicella is the enemy of leukemia patients! Importantly, VZV shows airborne transmission. It is of note that the airborne transmission of VZV occur directly from the skin lesions of herpes zoster.

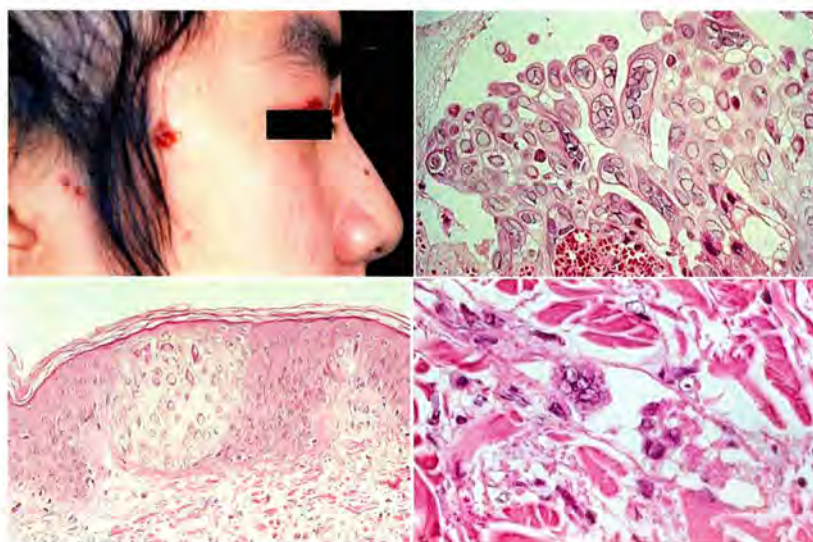


Figure IV-E-2. Relapsing hemorrhagic varicella after bone marrow transplantation. Hemorrhagic vesicles are observed on the facial skin of a leukemic boy (left top). Nuclear inclusions and multinucleated keratinocytes are characteristic of the acantholytic keratinocytes (right top). A small non-vesiculated infectious focus is seen in the biopsied epidermis (left bottom). Vascular endothelial cells in the dermis are swollen, and multinucleation is associated (right bottom).

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3) Herpes Zoster

Herpes zoster (shingles) represents reactivation of latent VZV in sensory ganglia, particularly at the lumbar and thoracic levels. The trigeminal nerve may also be involved. The virus travels through the sensory nerves to reach the skin (Figure IV-E-3). This acute disease is seen exclusively in adults. When children are infected from adults suffering from zoster, they develop varicella and not zoster. Recurrent attacks of zoster can occur, but cases suspected recurrent zoster are more likely to be recurrent herpes simplex. The illness is febrile and begins with pain along with the involved peripheral nerve. Red papules quickly transform into vesicles and pustules. Post-herpetic neuralgia is a serious and cumbersome complication.

Histological features showing intranuclear inclusion bodies are similar to those of varicella or herpes simplex. Inflammatory reactions in the dermis are much milder than herpes simplex.



Figure IV-E-3. Herpes zoster. Clusters of red vesicles or pustules are formed along the peripheral nerve (left). Sciatic nerve involvement is shown in the right panel. The courtesy by Prof. Tetsushi Yoshikawa, Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Japan.

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4) Disseminated Herpes Zoster

In immunosuppressed patients, systemic VZV infection may happen. Manifestation of bilateral or multifocal varicella zoster is a sign of systemic dissemination (opportunistic reactivation) of VZV. Gastric mucosal involvement can be seen endoscopically.

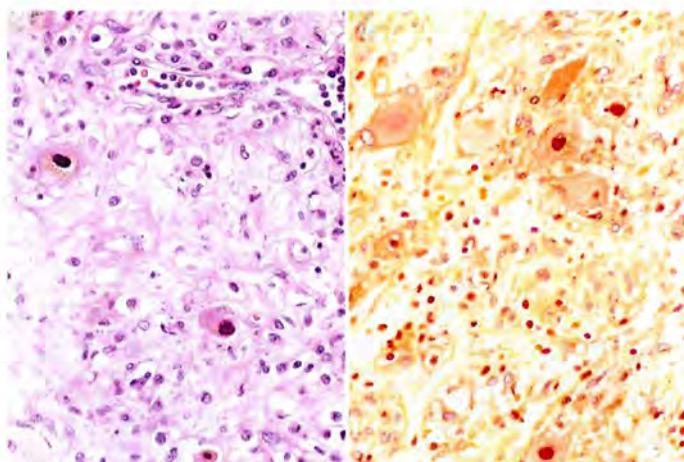


Figure IV-E-4-1. Disseminated herpes zoster involving internal organs (I). In a case of Hodgkin's lymphoma after chemotherapy, ganglion cells (neurons) contain distinct basophilic intranuclear inclusion bodies (left: H&E). Classical inclusion body staining demonstrates red-colored intranuclear inclusions (right: phloxine-tartrazine stain).

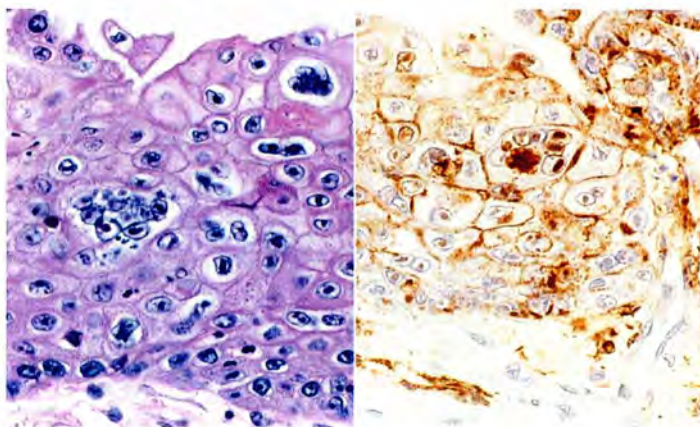


Figure IV-E-4-2. Disseminated herpes zoster involving internal organs (II). Esophageal involvement in a hemodialysis patient shows intranuclear inclusion bodies with multinucleation (left: H&E). Immunostaining for both glycoprotein-1 (GP-1) antigen and nuclear capsid protein-1 (NCP-1) of VZV shows positivity on the plasma membrane and in the nuclei (right).

Systemic infections of VZV may be identified at autopsy. Disseminated necrotic lesions are seen in a variety of internal organs. With phloxine-tartrazine stain for inclusion bodies (Figure IV-E-4-1), the intranuclear inclusions are stained red (phloxinophilic). This classic staining method can now be replaced by immunostaining employing specific antibodies. Immunoreactivities of both glycoprotein-1 (GP-1) antigen and nuclear capsid protein-1 (NCP-1) are shown in an esophageal lesion in Figure IV-E-4-2.

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F) Cytomegalovirus Infection

Cytomegalovirus (CMV), human herpes virus type 5, is an extremely common virus, and most individuals are asymptomatic carriers of this double-stranded DNA virus. Viral reactivation occurs in any organ or tissue. In particular, endothelial cells are the preferred site of infection (reactivation) (Figure IV-F). Epithelial cells, such as type II pneumocytes, gastrointestinal epithelia and salivary gland ductal cells, are also infected.

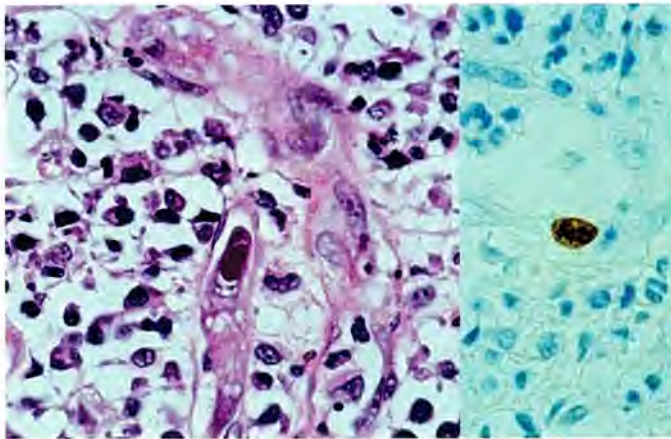


Figure IV-F. Cytomegalovirus infection. Granulation tissue seen in an endoscopic biopsy from the esophagus is demonstrated. An endothelial cell contains a large basophilic intranuclear inclusion, being pathognomonic histologically (left: H&E). Immunostaining for CMV antigen is confirmative (right).

When cytomegalic inclusions are seen in the endothelial cells in granulation tissue of biopsied samples of the gastrointestinal tract, one must suspect an immunosuppressed state of

the patient. Viewing from another angle, CMV-infected granulation tissue is somewhat atypical, either less inflammatory or prominent in endothelial swelling. As experienced pathologists feel something different from regular granulation tissue, it can be a hint of CMV infection. Often times, patients have received steroid or immunosuppressive therapy. Identification of cytomegalic inclusions in the biopsy specimen of the gastrointestinal mucosa may happen to lead to the diagnosis of AIDS.

Clinical manifestations of acute CMV infection in adults are fundamentally identical to infectious mononucleosis in Epstein-Barr virus infection. Those include morbilliform, urticarial or erythema multiforme-like eruptions, in addition to fever, splenomegaly and pharyngitis. In AIDS or post-transplantation patients, perianal ulceration or purpuric maculopapular eruption may be seen, and the biopsy confirms characteristic viral inclusions. Since such skin lesions are part of systemic and mixed infection, the prognosis is grave.

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Note 41. Exogenous infection versus endogenous infection

When we consider hospital-acquired infection and opportunistic infection, distinction whether or not the pathogen is exogenous in origin is quite important. Washing hands and wearing gloves, masks, cap and gown are effective to shut off the exogenous pathogens. If the pathogen in question is endogenous (that is, the normal flora or the microbes showing latent infection are the source of infection), it is meaningless to wear gloves and masks and to disinfect the environment.

That is the reason why the use of the “aseptic room” and the supply of “disinfected meal” for leukemia patients after extensive chemotherapy have been abandoned. Opportunistic pathogens for the immunosuppressed leukemia patients are mostly endogenous in origin. The filtration of the air is important to shut off the airborne exogenous infection of fungi, especially *Aspergillus*. Similarly, wearing a gown, cap and mask is unnecessary in the intensive care unit. By wearing a mask, what sort of infection is the patient protected from? Normal flora in the oral cavity which is shared by most individuals is not infectious even to the leukemia patient.

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G) Epstein-Barr Virus Infection

1) Infectious Mononucleosis and Epstein-Barr Virus-Associated Hemophagocytic Syndrome (EB-VAHS)

Infectious mononucleosis is an acute, self-limiting Epstein-Barr virus (EBV) infection to B lymphocytes. EBV is classified as human herpes virus type 4, categorizing in γ -herpesvirus. It often occurs in the youth or young adults, so that the nickname of the kissing disease is given. Clinical symptoms and signs include high fever, malaise, skin rash, arthralgia, myalgia, liver dysfunction (hepatitis), lymphadenopathy, tonsillar swelling with ulceration and increase of atypical lymphocytes in the peripheral blood. Gastric ulceration may also be associated (Figure IV-G-1-1). Similar illness is known to be provoked by acute infection of cytomegalovirus.

The incidence of infectious mononucleosis has been low among the Japanese, principally because of the early exposure to EBV in the infancy period. Recently, this disorder is increasing among the young Japanese due to westernization of the life style with a low frequency of the early exposure to EBV.

Another benign lesion related to EBV is Gianotti-Crosti syndrome (see Figure IV-P-3 in Chapter IV-P).

Virus-associated hemophagocytic syndrome (VAHS) is caused by abnormal activation of macrophages secondary to viral infection of herpes virus group, particularly EBV, to T lymphocytes. VAHS caused death of patients with 2009 influenza A pandemic. Hypercytokinemia results in a variety of clinical manifestations common with infectious mononucleosis. Marked hemophagocytosis leads to pancytopenia. VAHS is mainly seen in children and young adults, and the prognosis is not so good.

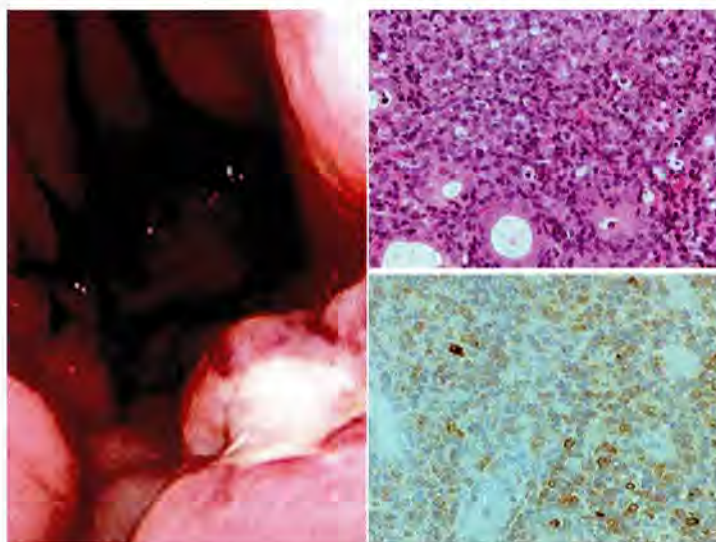


Figure IV-G-1-1. Gastric ulcer seen in infectious mononucleosis. A large ulcer is formed on the gastric mucosa (left). Biopsy reveals dense infiltration of atypical B lymphocytes with CD20 positivity (right top: H&E, right bottom: immunostaining). The courtesy by Dr. Yasuhiko Kitayama, a pathologist at Fujieda Municipal General Hospital, Fujieda, Japan.

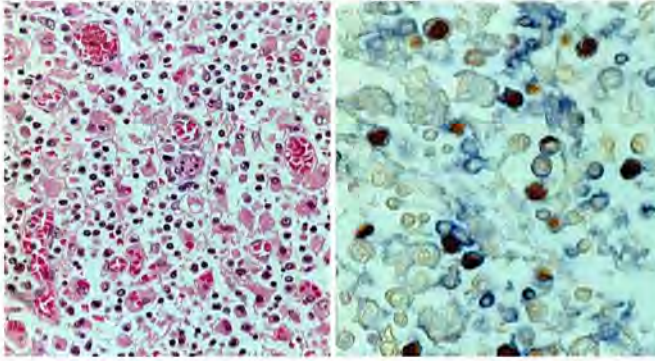


Figure IV-G-1-2. Lymph nodal involvement by EB-VAHS. In the swollen lymph node, hemophagocytosis is evident (left). EBV infection is seen in the nuclei of T lymphocytes (right: double staining for CD45RO, a T-cell surface marker, stained blue and EBER1 stained brown).

In case of EBV-induced VAHS (EB-VAHS), infection of EBV occurs in natural killer (NK)/T cells. Typically, increase of atypical lymphocytes (large granular lymphocytes = LGLs) is observed in the peripheral blood (granular lymphocytosis). LGLs often belong to CD56-positive natural killer (NK) cells. Histochemically, EBER-1 (EBV-related small nuclear RNA) is demonstrated in the nuclei of proliferating NK/T cells (Figure IV-G-1-2).

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Note 42. Regionality of EBV infection

The first contact with EBV occurs in an infancy period in most Asians and Africans as a form of latent infection. In contrast, infectious mononucleosis caused by the initial exposure to EBV is common in the adolescence of the western countries.

Infectious mononucleosis is thus common in the western countries, but infrequent in the developing countries. The regionality of EBV-related neoplasms is summarized, as follows. Hodgkin's lymphomas are common in the west, while it is infrequently seen in Japan. Burkitt's lymphomas are endemic in sub-Saharan Africa. Nasopharyngeal carcinoma (lymphoepithelioma) is prevailing in the southern part of China (Canton and Hong Kong). Pyothorax (tuberculous pleuritis)-associated B-cell lymphoma is mainly seen in Japan. Meanwhile, EBV-related lymphoepithelioma-like carcinoma (blue cell carcinoma) of the stomach is evenly distributed throughout the world, accompanying 7% of all the gastric cancers.

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2) Chronic Active EBV Infection

When the inflammatory process with hypercytokinemia caused by EBV infection persists more than half a year, the condition is termed as chronic active EBV infection. A lymphoproliferative disorder of NK/T-cell type often follows the state of chronic active EBV infection. The peripheral blood or effusion fluid contains atypical lymphocytes (granular lymphocytosis) (Figure IV-G-2). Hemophagocytosis and eosinophilia are associated. Variegated clinical manifestations somewhat resembling collagen disease are provoked by hypercytokinemia (the cytokine storm), and the prognosis is poor. Secondary CD56-positive NK-cell malignancy is not a rare occasion.

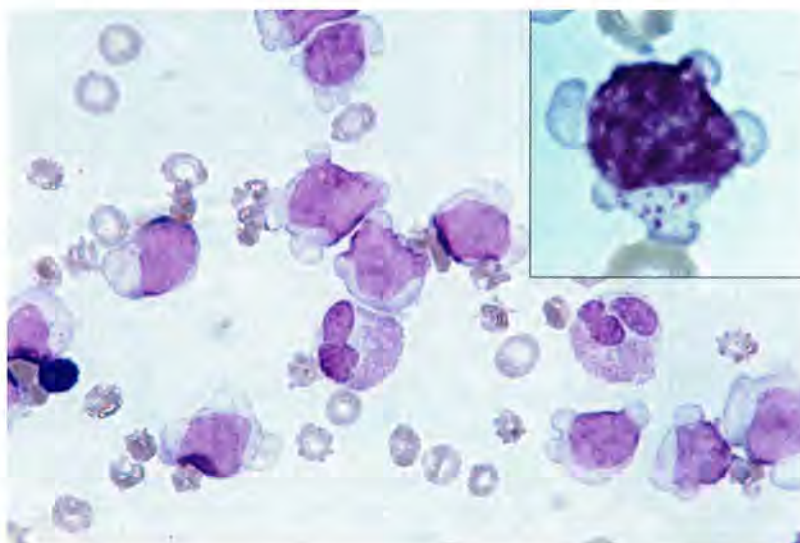


Figure IV-G-2-1. Large granular lymphocytes seen in ascitic fluid in a lethal case of chronic EBV infection. Granular lymphocytosis is associated with eosinophilia and hemorrhage (May-Giemsa). Inset demonstrates a close-up view of a granular lymphocyte.

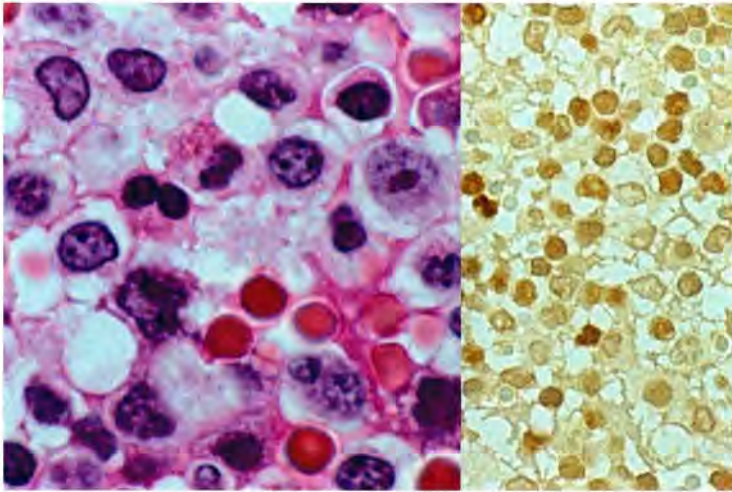


Figure IV-G-2-2. EBER1 positivity in large granular lymphocytes in ascitic fluid. Cell block preparation of the ascitic sediment illustrates atypical lymphocytes (left: H&E). EBER1 is positive in the nuclei of the CD56-positive granular lymphocytes (right: *in situ* hybridization).

It is known that EBV can monoclonally or oligoclonally transform normal peripheral blood B lymphocytes into *in vitro* culturable cell lines, “lymphoblastoid cell lines”. In an immunocompromised state such as AIDS and post-bone marrow transplantation, EBV activation may lead to malignant lymphoma of B-cell type. The brain is the prerequisite site of B-cell malignancy in AIDS patients.

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3) Hydroa Vacciniforme

Hydroa vacciniforme is a rare debilitating photodermatosis in children. Clinically, erythema and vesicles develop on the face within 1-2 days after sun exposure. After healing, varioliform scar may remain. Recently, this condition is regarded as EBV-associated bullous dermatosis. Latent EBV infection has been detected in the dermal infiltrate. Potential

propensity for developing EBV-associated lymphoma of the skin or subcutaneous tissue should be considered.

Histologically, intraepidermal vesiculation with peculiar reticulated features is characteristic (Figure IV-G-3). The vesicles are filled with serum, fibrin and inflammatory cells. Epidermal necrosis and ulceration may follow.

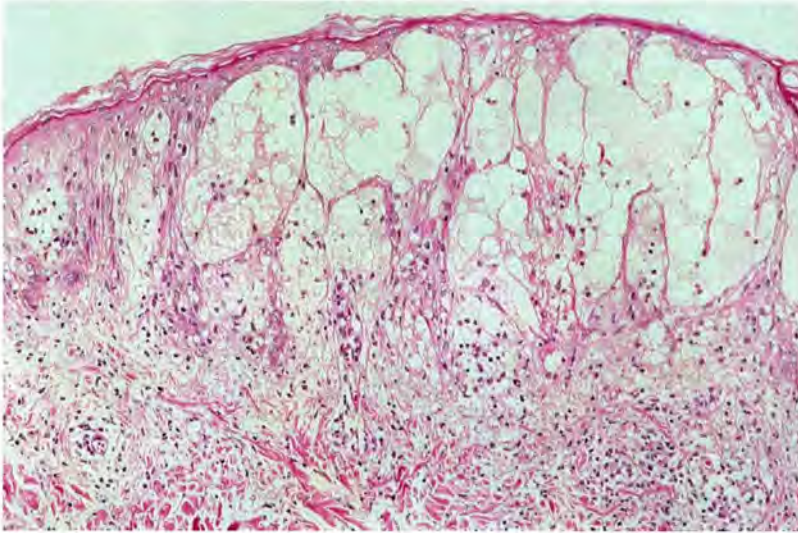


Figure IV-G-3. Hydroa vacciniforme. Intraepidermal vesiculation with peculiar reticulated features is observed. The vesicles are filled with serum, fibrin and inflammatory cells (H&E).

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4) Hairy Leukoplakia of Tongue

Representative oral mucosal lesions associated with AIDS include hairy leukoplakia, Kaposi's sarcoma and candidiasis. Hairy leukoplakia is a disease of the tongue mucosa occurring mostly in HIV-infected patients and due to the opportunistic proliferation of EBV within the squamous epithelium. The lesion may be seen in patients treating with hemodialysis.

Clinically, it shows as whitish patches with a shaggy (hairy) surface occurring on the lateral margins of the tongue. This may be prodromal to AIDS. Histologically, it shows

hyperparakeratotic acanthosis with koilocytoid cells (cells with an eosinophilic nuclear inclusion surrounded by a clear haloe and pale-stained cytoplasm) in the upper part of the squamous mucosa. Electron microscopy demonstrates abundant herpes-group virions within the nuclei. By *in situ* hybridization, EBER1, EBV-encoded small nuclear RNAs, can be demonstrated. This is an exceptional state in which EBV replicate intact viral particles in the infected cells to accompany nuclear inclusions. Typical features are shown in Figures IV-G-4-1 and IV-G-4-2.



Figure IV-G-4-1. Gross and ultrastructural features of hairy leukoplakia of the tongue in AIDS. Whitish patches with a shaggy (hairy) surface are observed on the lateral margin of the tongue (left). Electron micrograph demonstrates intranuclear formation of herpes viral particles, a quite exceptional phenomenon in EBV infection (right). The courtesy by Prof. Yukio Nakatani, Department of Diagnostic Pathology, Chiba University Graduate School of Medicine, Chiba, Japan.

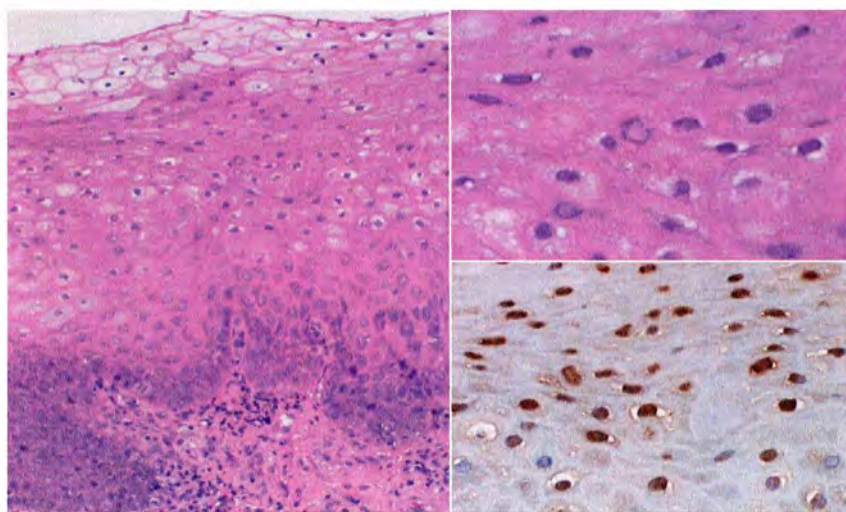


Figure IV-G-4-2. Histology of hairy leukoplakia of the tongue. In the acanthotic squamous mucosa, nuclear inclusion bodies are observed (left and right top: H&E). EBER1 is demonstrated in the nuclear inclusions (right bottom: *in situ* hybridization). The courtesy by Prof. Yukio Nakatani, Chiba, Japan.

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5) Mosquito Bite Hypersensitivity

Hypersensitivity to the mosquito bite is known to be one of the varieties of EBV-associated lymphoproliferative disorders. Some cases overlap with the entity of chronic active EBV infection and hydroa vacciniforme (a form of EBV-associated bullous dermatosis). It frequently predisposes to hemophagocytic syndrome or lymphoid malignancy with a clonal EBV replication. Pediatric patients manifest severe local skin reaction (deep skin ulcer), high fever, lymphadenopathy and liver dysfunction.

Histologically, lymphocytes, oligoclonally overexpressing EBV-encoded small nuclear RNAs (EBER1), and eosinophils infiltrate diffusely into the dermal lesion. The peripheral blood shows marked increase of the CD11b+, CD16+, CD56+ NK cells as atypical lymphocytes (large granular lymphocytes). The NK cells also aberrantly express CD25, a high affinity receptor for interleukin-2 (IL-2), and show an augmented *in vitro* proliferative response to IL-2. Mosquito bite hypersensitivity thus involves EBV-associated NK cell proliferation as a pathogenetic mechanism.

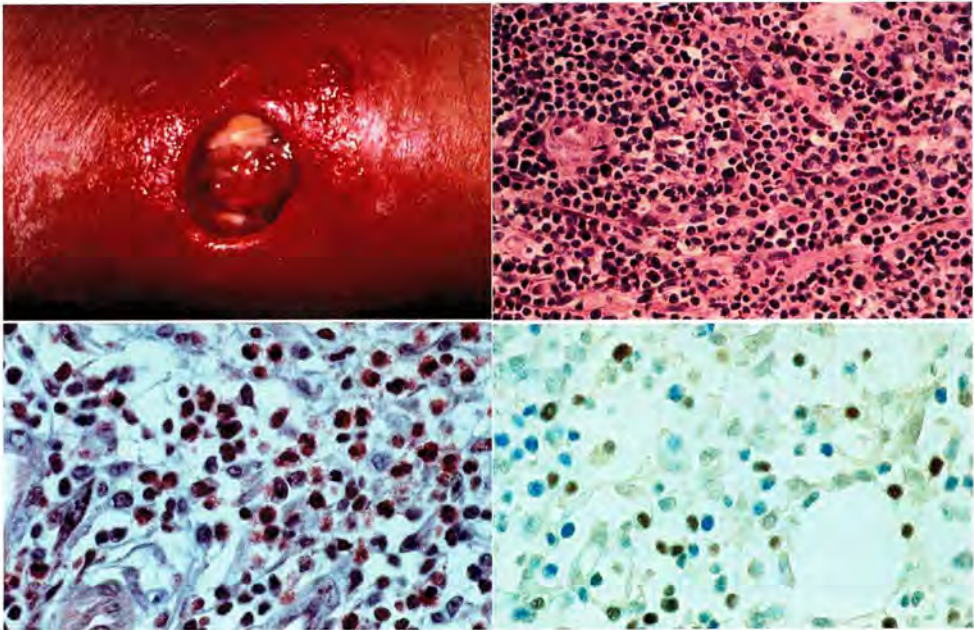


Figure IV-G-5. Mosquito bite hypersensitivity in a pediatric case. A deep and intractable ulcer is formed at the site of mosquito bite (left top). Biopsy reveals dense infiltration of small lymphocytes and eosinophils (right top: H&E, left bottom: Luna stain for eosinophils). EBER1 is expressed in some nuclei of lymphoid cells (right bottom).

Figure IV-G-5 illustrates a representative pediatric case. Deep ulceration is seen at the site of mosquito bite. Skin test to *Aedes* spp. was positive in this case. Elevation of serum IgE levels, positive antibody titers to EBV and 17% appearance of atypical (large granular) lymphocytes were also recorded. Biopsied skin tissue reveals dense infiltration of small lymphocytes. Mitoses are scattered in the lymphoid cells. Reactivity of EBER1 was observed in the nuclei of some of the small lymphocytes with the *in situ* hybridization technique. EBER1 positivity was detected in CD56+ NK cells. Luna stain disclosed marked infiltration of eosinophils, suggesting allergic nature of the ulcerative lesion. EBER1 positivity was also confirmed in the biopsied regional lymph node.

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6) EBV-Related Lymphoproliferative Disorders of the Skin

EBV has been implicated in the development of a wide range of B-cell lymphoproliferative disorders, including Burkitt's lymphoma, classic Hodgkin's lymphoma, and lymphomas arising in immunocompromised individuals (post-transplant and HIV-associated lymphoproliferative disorders). Among T-cell malignancies, a subset ($\gamma\delta$ -type) of peripheral T-cell lymphomas, angioimmunoblastic T-cell lymphoma, extranodal nasal type natural killer/T-cell lymphoma are EBV-positive (Figure IV-G-6-1).

Among cutaneous lymphomas, hydroa vacciniforme-like lymphoma and extranodal-type natural killer (NK)/T-cell lymphoma are prototypes of EBV-associated cutaneous T- or NK-cell lymphomas. Typical presentations include skin ulcerations and mass lesions confined to the extremities without systemic involvement. Secondary skin involvement of nasal NK/T lymphoma is also noted (Figure IV-G-6-2). Histologically, diffuse dermal and subcutaneous lymphomatous infiltration shows tumor necrosis and angioinvasion. The CD56 (neural cell adhesion molecule)-positive lymphoma cells of nasal NK cell type typically reveal features of "large granular lymphocytes" containing cytoplasmic killer granules, which are immunoreactive for TIA-1, perforin and granzyme B. EBV-related cutaneous NK/T-cell lymphomas in childhood and early adulthood are characterized by a protracted clinical course, but eventually with an aggressive phase.

It has been described that CD4+ cutaneous T-cell lymphoma of mycosis fungoides type, CD30+ anaplastic large cell lymphoma and lymphomatoid papulosis are poorly related to the

EBV etiology. Cutaneous B-cell malignancies may infrequently be associated with EBV infection.

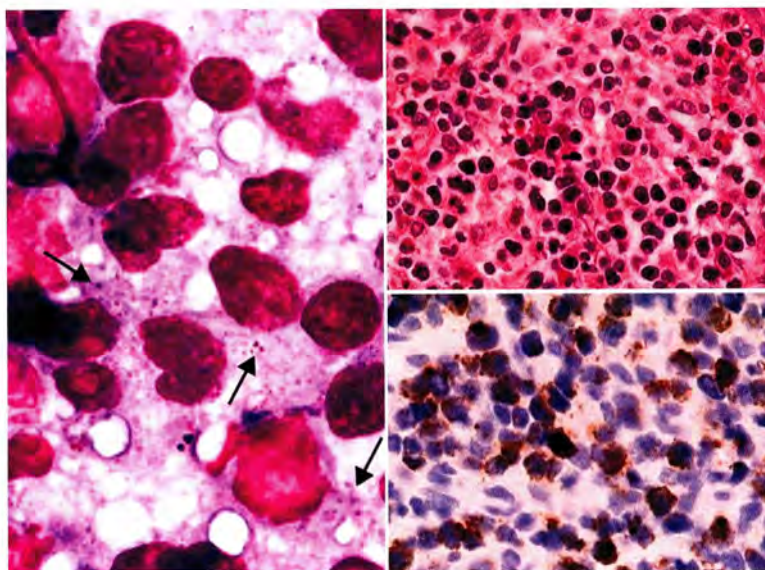


Figure IV-G-6-1. Nasal NK/T-cell lymphoma. The lymphoma cells are predominantly composed of atypical lymphoid cells containing fine granules (arrows), corresponding to large granular lymphocytes (left: May-Giemsa). Diffuse nasal mucosal infiltration of polymorphous medium-sized lymphoma cells is observed (right top: H&E). The lymphoma cells, positively labeled for EBER1, possess TIA-1-positive killer granules in the cytoplasm (right bottom). The courtesy by Prof. Yoshikazu Mizoguchi, Division of Diagnostic Pathology, Fujita Health University Ban-buntane Hospital, Nagoya, Japan.

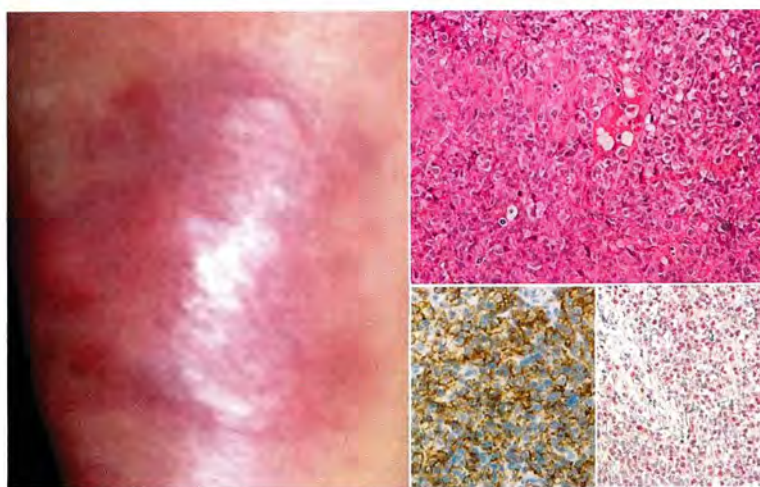


Figure IV-G-6-2. Secondary skin involvement by nasal NK/T-cell lymphoma in a Japanese aged female. One year after the diagnosis of nasal NK/T-cell lymphoma, ill-defined nodular skin infiltration of the lower leg was observed (left). Diffuse dermal infiltration of atypical lymphoid cells with clear and plump cytoplasm is noted microscopically (right top: H&E). The tumor cells express CD56 along the plasma membrane (right bottom, left half) and EBER1 in the nuclei (right bottom, right half). The courtesy by Prof. Yasuaki Harabuchi, Department of Head and Neck Surgery, Asahikawa Medical University, Asahikawa, Japan.

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H) Infection of Human Herpesvirus (HHV)-6 and HHV-7

Exanthem subitum (meaning sudden rash), also called as roseola infantum, pseudorubella or sixth disease, is caused by human herpesvirus (HHV) type 6 or 7. These two β -herpesviruses are collectively referred to Roseolovirus. There are two variants of HHV-6 (HHV-6A and HHV-6B), and exanthema subitum is caused by HHV-6B.

Exanthem subitum is an acute benign infection in infancy between six months and two years of age. Usually, it begins with a sudden high fever (39–40°C). In 3 days, fever subsides, and red rash appears on the trunk, spreading to the legs and neck. The rash is not itchy and lasts for 1 to 2 days. Nagayama macules (spots), also documented as uvulo-palatoglossal junctional ulcers, are characteristically observed on the soft palate (Figure IV-H-1). HHV-7 causes the disease less frequently than HHV-6B. HHV-7 infection may be associated with acute febrile respiratory disease. Neuroinvasion by HHV-7 has also been reported. Similar to other herpesviruses, asymptomatic infection of HHV-6 and HHV-7 is very common.

Reportedly, both HHV-6 and HHV-7 primarily infect CD4⁺ T cells. HHV-6A may foster the progression of human immunodeficiency virus (HIV) disease toward AIDS, since co-infection of HHV-6A and HIV-1 accelerates the depletion of CD4⁺ T cells. CD4 molecules on the cell surface function as the receptor of these viruses.

Reactivation of HHV-6A and HHV-7 occurs in one third to a half of patients after bone marrow transplantation, and may increase the severity of cytomegaloviral disease. In graft

versus host disease, HHV-6A/HHV-7 reactivation may also play a role in the exacerbation process (Figure IV-H-2). Infrequently, HHV-6/HHV-7 transmission may be provided from the transplanted donor allografts or organs. It should be noted that the salivary gland is the site of latent infection and the viruses are consistently shed in saliva.

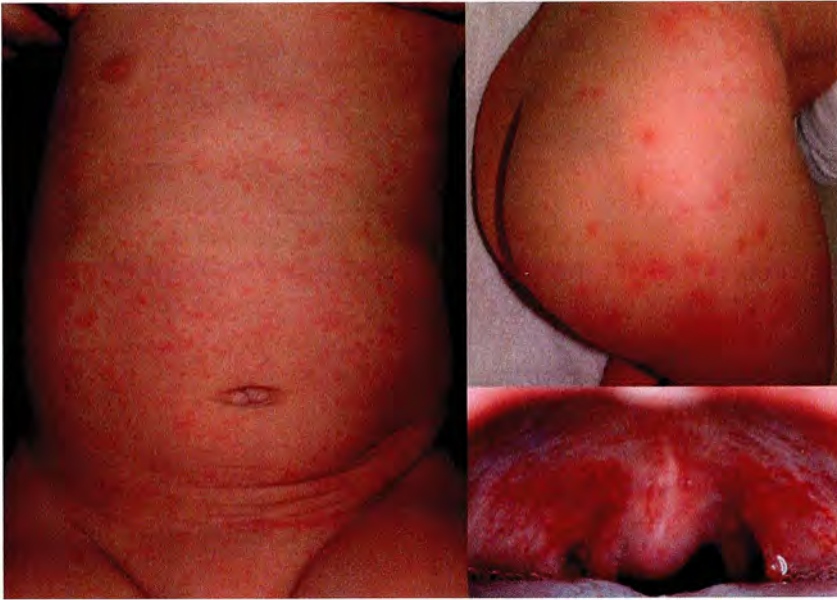


Figure IV-H-1. Exanthem subitum. Acute and benign red rash appears on the trunk and thigh (left and right top). Nagayama spots on the soft palate are characteristic (right bottom). The courtesy by Prof. Tetsushi Yoshikawa, Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Japan.

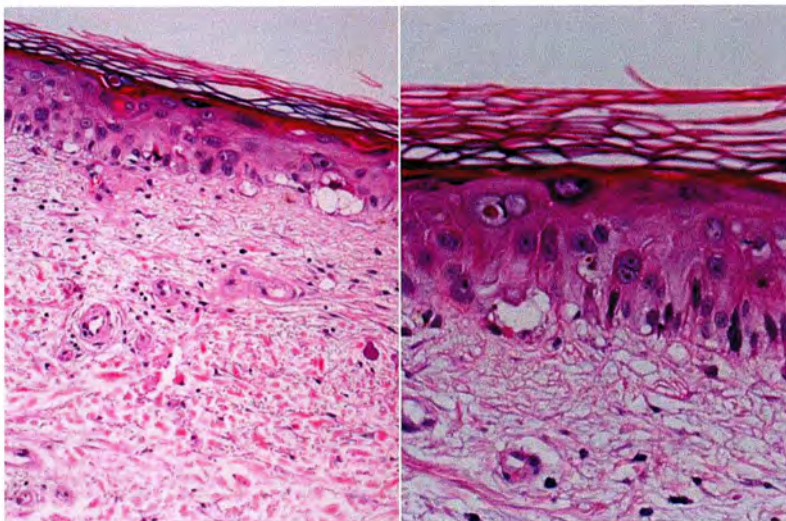


Figure IV-H-2. HHV6A-reactivated graft versus host disease after bone marrow transplantation. Biopsy reveals microscopic features of GVHD with basal liquefaction and apoptotic keratinocytes (H&E). HHV-6A reactivation plays a role in the exacerbation process of GVHD. The courtesy by Prof. Tetsushi Yoshikawa, Toyoake.

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I) Kaposi Sarcoma

Kaposi sarcoma is a mesenchymal malignancy involving blood and lymphatic vessels. Both the skin and extra-cutaneous (internal) organs are involved. The spindled vessel-forming tumor cells express endothelial markers.

Kaposi sarcoma is presented in four clinical and epidemiological forms: classic, African (endemic type), a variant associated with immunosuppressive therapy, and an HIV-associated (epidemic) type. All types of tumors are associated with infection of human herpesvirus 8 (HHV8) or Kaposi sarcoma-associated herpes virus (KSHV) in the original term. Kaposi sarcoma initiates in a context of immune dysregulation, characterized by CD8⁺ T cell activation and the production of Th1 type cytokines. The cytokines induce generalized activation of endothelial cells leading to adhesion and tissue extravasation of lymphocytes and monocytes, spindle cell formation and angiogenesis. The phenomena are triggered or enhanced by infection with HHV8 that, in turn, is reactivated by the same cytokines. In the era of highly active antiretroviral therapy (HAART), the incidence of AIDS-related Kaposi sarcoma has considerably declined, probably due to the enhanced immune reconstitution and anti-HHV8-specific immune responses. HHV-8 etiology is also indicated in the non-HIV cases. This oncogenic γ -herpesvirus also provokes a special form of B-cell lymphoma, primary effusion lymphoma involving the pleural and pericardial cavities, as well as multicentric Castleman's disease of the lymph nodes in AIDS.

Figure IV-I illustrates representative features of Kaposi sarcoma. Grossly, multifocal dark red/brown-colored skin lesions are formed. Histologically, one observes increase of dermal capillary vessels with swelling of endothelial cells, nuclear atypia, extravasation of red blood cells, hemosiderosis and hyaline globular inclusions in the cytoplasm. High-powered view

demonstrates swollen endothelial cells with prominent nucleoli and cytoplasmic hyaline globules with PAS reactivity.

Histological variations are noted: Some cases show proliferation of highly atypical polygonal cells focally with small lumen formation. Refer to the review by Grayson and Pantanowitz. Immunohistochemically, the tumor cells express both vascular endothelial cell markers (CD34, CD31 and factor VIII-related antigen) and a lymphatic endothelial marker D2-40. Immunohistochemistry using HHV-8 latent nuclear antigen-1 (LNA-1) has been shown to exhibit high sensitivity and specificity for diagnosing Kaposi sarcoma.

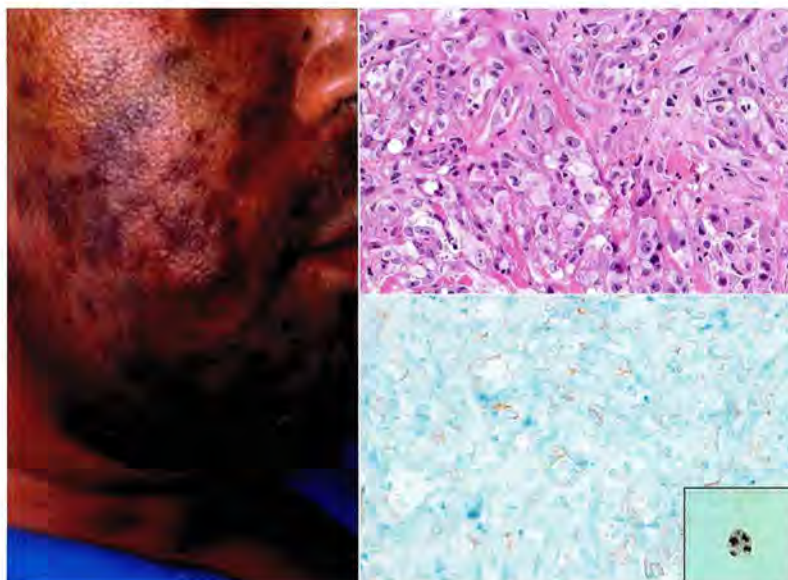


Figure IV-I. Kaposi sarcoma. Dark red/brown-colored infiltrative lesions are multifocally formed on the facial skin (left). Biopsy reveals intradermal proliferation of atypical endothelial cells (right top: H&E). The tumor cells are immunoreactive for factor VIII-related antigen (right bottom). Cultured cells express HHV-8 genome in the nuclei (inset: *in situ* hybridization). The gross feature was cordially supplied by Dr. Akifumi Imamura at Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan.

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J) Merkel Cell Carcinoma

Merkel cells, a normal epithelial component in the dermis, particularly rich on the face, possess neuroendocrine granules in the cytoplasm. Merkel cell carcinoma represents primary small (neuroendocrine) cell carcinoma of the skin, preferentially occurring on the sun-exposed skin of the aged. Males predominate over females. The tumor is microscopically very similar to small cell carcinoma of the lung, and homogeneously sanded nuclei and frequent mitoses are characteristic (Figure IV-J-1).

The epidermis is spared in most cases, so that differential diagnosis from metastatic small cell lung carcinoma is needed. Neuroendocrine markers and cytokeratin 20 are expressed in most tumor cells immunohistochemically, and neuroendocrine-type cored granules are observed ultrastructurally (Figure IV-J-2). Spreading to the regional lymph nodes and skin metastasis are common. The overall 5-year survival rate is 75%.

In 2008, Merkel cell polyomavirus (MCV) was identified in DNA preparations of Merkel cell carcinoma. In approximately 80% of cases, MCV infection has been observed. MCV is closely related to the African green monkey lymphotropic polyomavirus. Analysis of MCV isolated from Merkel cell carcinoma reveals tumor-specific mutations that truncate the MCV T-antigens. The virus is integrated into the host genome in a monoclonal fashion.

Viral detection is still at a research phase and is generally not available as a clinical test. Viral DNA has been detected by PCR or Southern blot analysis. Immunohistochemical approach using antibodies to the T-antigen has been applied to detect MCV-infected tumor cells specifically.

Human polyomaviruses, such as BK virus and JC virus, induce tumors in animal models, while their role in human cancer remains a matter of debate. MCV is the first polyomavirus etiologically associated with a human tumor. Refer to Note 43.

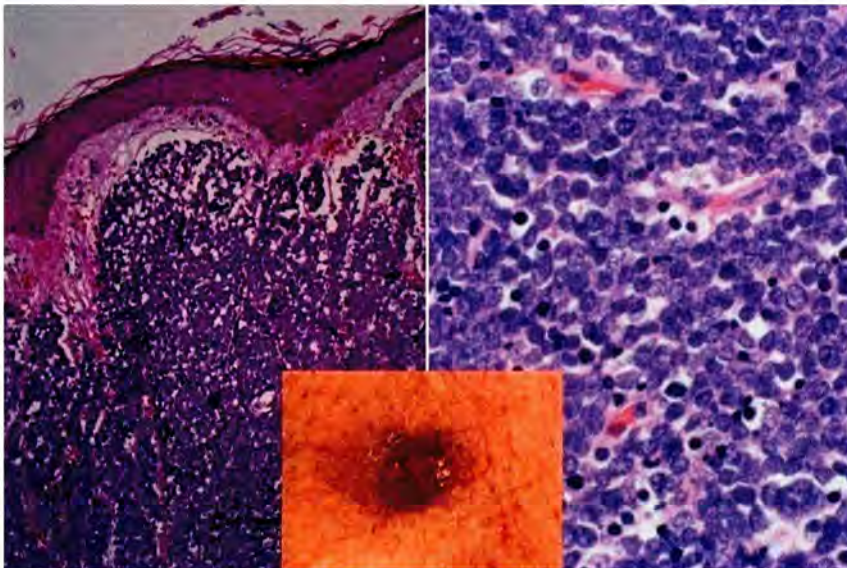


Figure IV-J-1. Merkel cell carcinoma (I). Dense and monomorphous growth of small-sized tumor cells resembling small cell carcinoma of the lung is seen in the dermis (H&E). The epidermis is spared. Inset illustrates gross appearance of Merkel cell carcinoma occurring on the facial skin.

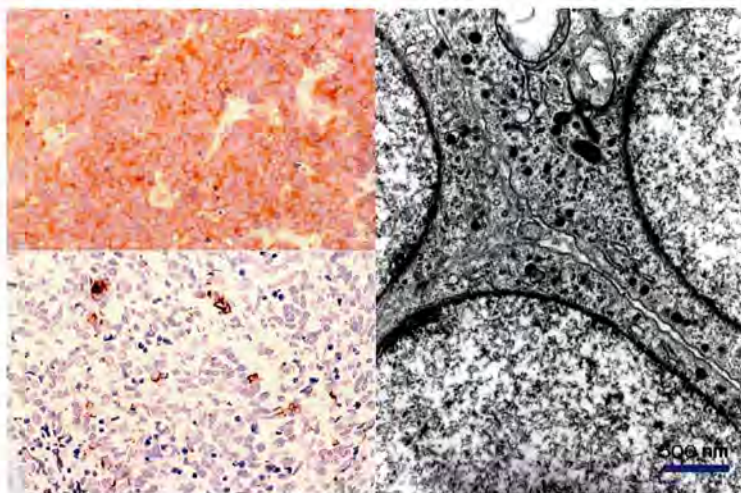


Figure IV-J-2. Merkel cell carcinoma (II). Immunohistochemically, the tumor cells diffusely express synaptophysin (left top), and some are positive for cytokeratin 20 (left bottom). Ultrastructurally, neuroendocrine-type cored granules of small size (mean: 120 nm in diameter) are observed in the cytoplasm of the tumor cells (right). Viral particles are undetectable.

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Note 43. Novel polyomaviruses and the skin

Polyomaviruses are small non-enveloped viruses with a double-stranded circular DNA genome, usually causing asymptomatic primary infections in childhood. In immunocompromised hosts, polyomavirus is reactivated to manifest clinical diseases, such as JC virus-related progressive multifocal leukoencephalopathy and BK virus-related cystitis/nephritis.

Merkel cell carcinoma-associated polyomavirus caused Merkel cell carcinoma, and trichodysplasia spinulosa-associated polyomavirus (human polyomavirus 8) causes trichodysplasia spinulosa (Note 40). Human polyomavirus 6, human polyomavirus 7 and human polyomavirus 9 are also members of the skin microbiome, and detected in skin diseases. Recently, Korup et al. have identified human polyomavirus 12 in the gastrointestinal tract tissue and in the stool. Further characterization of the biology and the possible impact on human health of the novel human polyomaviruses is needed.

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K) Adult T-Cell Leukemia/Lymphoma

Adult T-cell leukemia/lymphoma (ATLL) caused by the retrovirus, human T-cell leukemia virus-1 (HTLV-1), is endemic in the western part of Japan, particularly Kyushu Island, the Caribbean islands and in central Africa. Milk-mediated vertical transmission occurs from mother to infant. Latent infection (a healthy carrier state of HTLV-1) is common in endemic areas, and carriers are positive for anti-HTLV-1 antibody in the serum. A smoldering form of adult T-cell leukemia is also known.

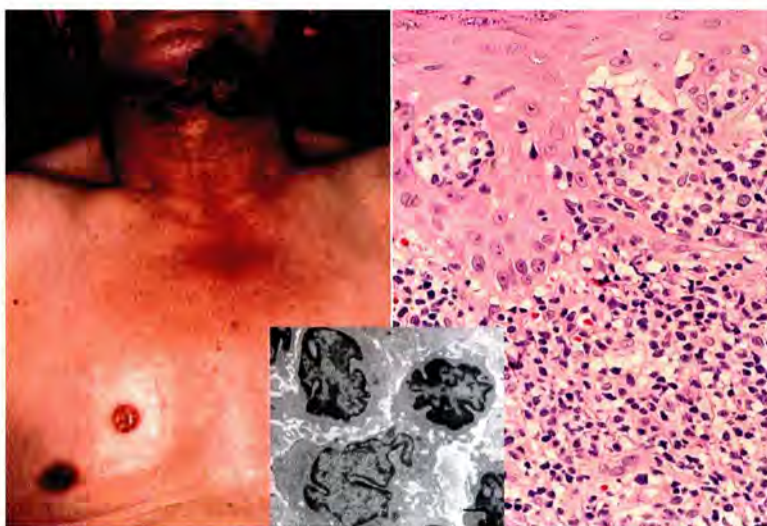


Figure IV-K-1. Adult T-cell leukemia/lymphoma. Multifocal ulcerated nodular skin lesions are formed on the neck and chest wall (left). Biopsy reveals epidermotropic infiltration of lymphoid cells with irregular-shaped nuclei (right: H&E). Inset demonstrates ultrastructural features of leukemia cells with convoluted (clover-shaped) nuclei.

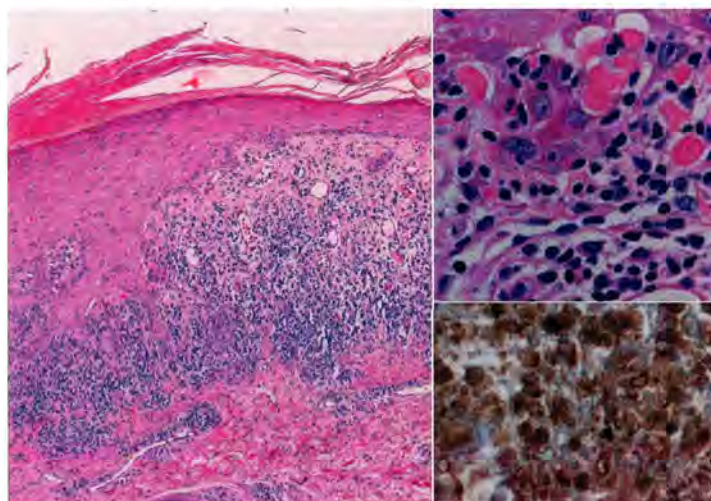


Figure IV-K-2. Lichen planus-like adult T-cell leukemia. Lichen planus-like infiltration of leukemia cells (basal liquefaction and band-like mononuclear accumulation in the upper dermis) is observed (left and right top: H&E). The tumor cells strongly express CD4 (right bottom: immunostaining). The courtesy by Dr. Masako Namisato, Auen Polyclinic, Tokorozawa, Japan.

ATLL is an aggressive CD4⁺ peripheral T-cell malignancy, often accompanying hypercalcemia. Circulating leukemia cells possess characteristic clover-shaped or convoluted nuclei. In contrast to other leukemias, bone marrow involvement is relatively mild in ATLL. The lymphoma-type shows highly aggressive clinical features. In the lymph node, pleomorphic morphology is diagnostic.

ATLL often involves the skin. A representative case accompanying nodule/tumor formation on the skin is presented in Figure IV-K-1. The atypical T-cells diffusely infiltrate in the dermis, in association with epidermotropism. The skin invasions accompany erythema, erythroderma or lichen planus-like lesions (Figure IV-K-2).

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L) Coxsackievirus Infection

Coxsackievirus is a member of the Genus Enteroviruses, encompassing Coxsackievirus, Echovirus and Poliovirus primarily infecting the enteric tract. Enteroviruses and Rhinoviruses (infecting nose and throat) belong to the Family Picornavirus, representing non-enveloped, positive-stranded small RNA virus (“pico” means small) with an icosahedral capsid.

Group A Coxsackieviruses cause acute vesicular eruptive diseases in childhood. These include hand-foot-and-mouth disease and herpangina, and both present small-sized vesicles. The etiological viruses (picornaviruses) are shared, including types A5, A6, A10 and A16 and enterovirus 71. Hemorrhagic conjunctivitis is also caused by Coxsackievirus A infection, mainly type A24 and enterovirus 70.

1) Hand-Foot-and-Mouth Disease

Hand-foot-and-mouth disease is mainly caused by acute infection of Coxsackievirus A16 and enterovirus 71. Other types have been described. When enterovirus 71 is a causative agent, encephalitic complication is problematic. This afebrile illness, seen in infants older than 6 months, is featured clinically by vesicle formation in the anterior part of the mouth, on the hands and feet and on the other parts of the body, including the diaper area (hip). The vesicles are small in size and sparse in distribution, and spontaneously disappear within 10 days. Histologically, spongiotic vesicles with prominent reticular changes are formed in the epidermis. Exocytosis of lymphocytes is also noted (Figure IV-L-1). Neither multinucleation nor viral inclusions are observed.

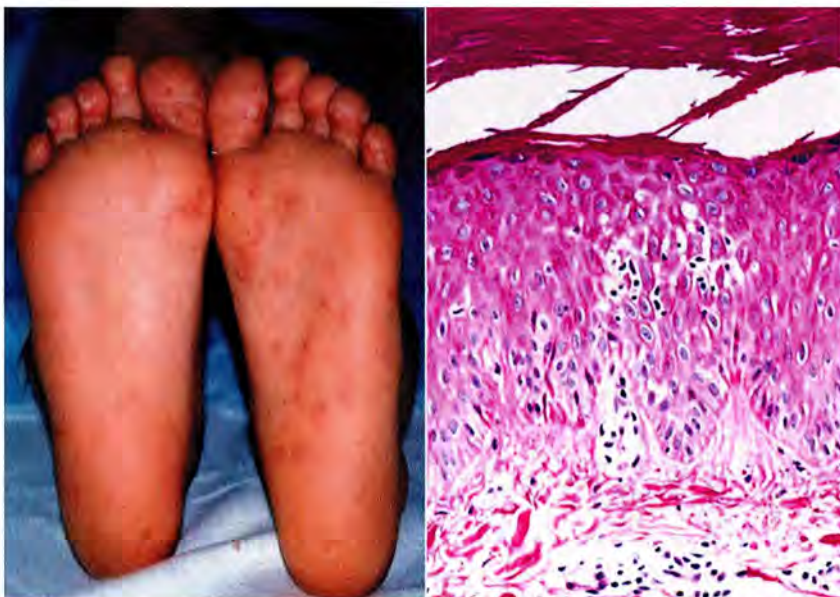


Figure IV-L-1. Hand-foot-and-mouth disease. Sparse distribution of vesicular eruptions is noted on the sole (left). Biopsy reveals spongiotic vesicle formation in the epidermis, in association with prominent reticular changes and lymphocytic exocytosis (right: H&E)

2) Herpangina

Herpangina (mouth blisters) is caused by acute benign infection of picornaviruses such as Coxsackievirus A5, enterovirus 71 or Coxsackievirus B. Types A6, A10 and A16 may also cause herpangina. It manifests sudden high fever and sore throat (painful mouth). Most cases of herpangina are seen in the early summer season, predominantly affecting children aged below 4 years. The incubation period is 4-14 days.

A small number of mouth lesions, formed on the soft palate or tonsillar pillars progress initially from red macules to vesicles and finally to 2-4 mm-sized ulcers (Figure IV-L-2). Anterior part of the mouth is not involved. The lesions heal in 7-10 days.



Figure IV-L-2. Herpangina. A small number of vesicles are formed on the soft palate of a 1-year old boy, finally resulting in 2-4 mm-sized painful ulcers. The courtesy by Prof. Tetsushi Yoshikawa, Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Japan.

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M) Erythema Infectiosum

Erythrovirus (previously called as parvovirus B19), a single-stranded small DNA virus, causes erythema infectiosum (fifth disease), a benign self-limiting exanthematous disorder in infancy. The disease is also referred to as slapped cheek syndrome, and in Japan, the infection is nicknamed as “apple sickness”. Bright red cheeks are defining symptoms. The rash extends over the bridge of the nose or around the mouth. Lacy red rash is also observed on the other parts of the body (Figure IV-M-1). The rash lasts for a few days and may itch. The disease is mild. Reticulocytes in the peripheral blood decrease during the clinical course.

When the pregnant women suffer from erythema infectiosum, erythrovirus vertically transmits via placenta to the fetus. The virus preferably proliferates in the nuclei of erythrocytes, resulting in severe anemia with circulatory failure, and thus abortion, stillbirth and hydrops fetalis. Hydrops fetalis is often seen when the infection occurs in the 19th to 29th weeks of gestation (Figure IV-M-2). In this period, hematopoiesis (erythropoiesis) is active in the liver. Eosinophilic nuclear inclusions are observed not only in the erythroblasts in the bone marrow and liver but also in the nucleated red cells distributed in the internal organs at this stage of development.

Family members of congenital hemolytic anemias, such as hereditary spherocytosis and sickle cell anemia, are susceptible to erythrovirus infection. Aplastic crisis may be seen after manifestation of erythema infectiosum. Eosinophilic nuclear inclusion bodies are characteristically seen in erythroblasts in bone marrow aspirates.



Figure IV-M-1. Erythema infectiosum. Apple-like bright red cheeks are defining symptoms. The rash extends over the bridge of the nose. Lacy red rash is also observed on the trunk and extremities. The courtesy by Prof. Tetsushi Yoshikawa, Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Japan.



Figure IV-M-2. Hydrops fetalis at the 25th week of gestation. The mother suffered from erythema infectiosum at the 20th week of gestation. Intrauterine fetal death provoked maceration in this hydropic baby (left). Pale color of the liver and systemic anemic change indicate erythropoietic failure (right). Inset illustrates eosinophilic nuclear inclusions in erythroblasts (nucleated red cells) within the capillary lumen of the lung (H&E).

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N) Measles

Measles (rubeola, morbilli) is an acute systemic infection of measles virus, a single-stranded RNA virus, and is closely related to rinderpest virus infective to cattle and canine distemper virus infective to dogs. Clinical manifestations of measles include fever with two

peaks, coughing, generalized skin rash, Koplik spots on the oral mucosa and involvement of a variety of internal organs. Measles is most common in children aged below 2 years, but adolescence and young adult cases are increasing in Japan, because of the lack of neutralizing antibodies in the serum. In developing countries, measles pneumonia often causes death of children. Measles encephalitis is another serious complication in the young. Otitis media may occur. Lymphoid tissue involvement (lymphoid hyperplasia) is common. Almost no latent infection is seen, so that viral infection equals disease manifestation. The latent period is 9-11 days. The infection is preventable by vaccination. The mode of airborne transmission necessitates a special care for the prevention of hospital-acquired infection.

Histologically, formation of multinucleated giant cells is characteristic. In the lymphoid tissue and Koplik spots, Warthin-Finkeldy giant cells are formed, where no nuclear inclusions are observed (Figure IV-N-1).

In contrast, measles pneumonia is featured by giant cell pneumonia, and multinucleated (cell-fused) pneumocytes reveal eosinophilic intranuclear inclusion bodies. Intranuclear inclusions are also seen in the lesion of measles encephalitis. Measles virus is an exceptional RNA virus accompanying intranuclear inclusion bodies (refer to Note 39).

In Figures IV-N-2, representative clinical and microscopic features of measles are presented. Biopsy histology of measles rash in an adult case is demonstrated in Figure IV-N-3. Inflammation is minimal, and a few intranuclear inclusions are noted in epidermal keratinocytes.

In the period of Edo era in Japan (17th -19th century), people deeply feared contracting measles rather than small pox, because it frequently killed healthy adults or caused visual loss. Small pox was regarded as the disease of children, and the adults had some resistance to this bullous and highly contagious disorder. Numbers of “anti-measles” Ikiyo-e printings for hoping the cure from this lethal infection are left in our hand.

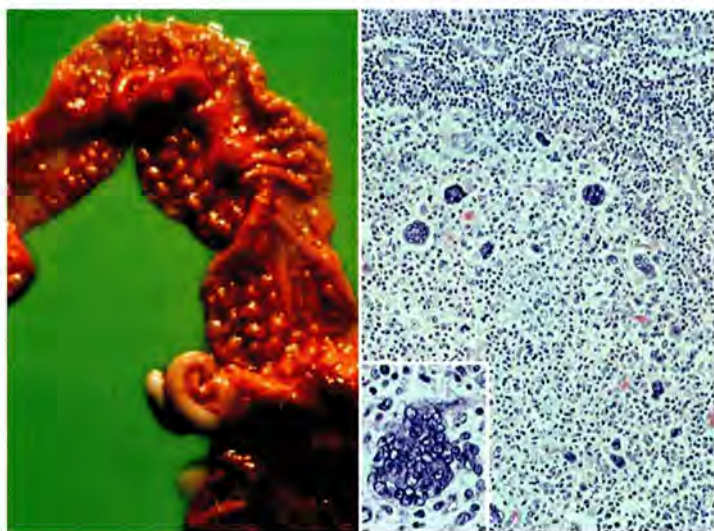


Figure IV-N-1. Lymphoid tissue involvement by measles virus. Ileal lymphoid apparatus are swollen (left). Microscopically, Warthin-Finkeldy-type multinucleated giant cells are clustered in the germinal center of the node. No nuclear inclusions are observed in this type of giant cells (right: H&E). Inset: high-powered view of the giant cell. The courtesy by Dr. Yoshihiro Nozawa, Department of Pathology, Fukushima Medical University, Fukushima, Japan.

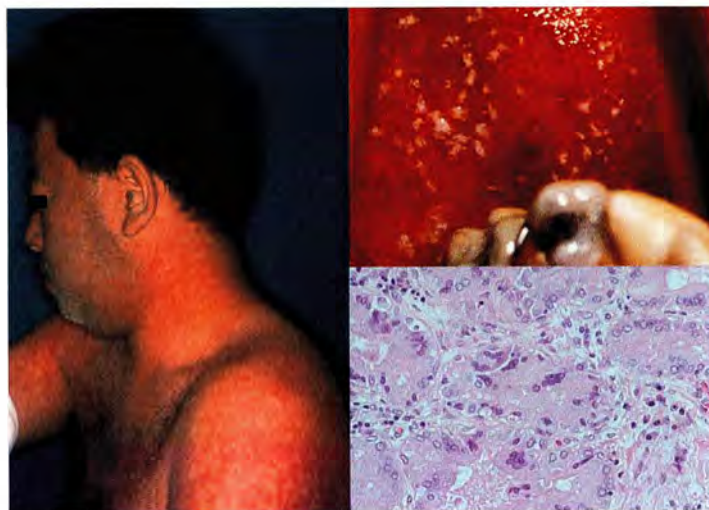


Figure IV-N-2. Measles in adult cases. Fine and commonly fused skin rash is formed on the whole body surface, starting from the face and neck to spread to legs (left). Multiple white spots (Koplik spots) on the parietal oral mucosa are pathognomonic of measles (right top). In another case of lethal measles pneumonia, multinucleated type II pneumocytes accompany intranuclear inclusion bodies (right bottom: H&E).

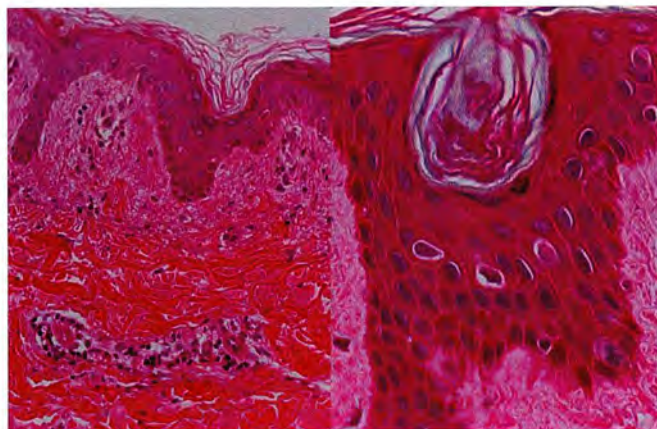


Figure IV-N-3. Biopsy histology of measles rash. Minimal perivascular infiltration of lymphocytes is seen in the edematous and mildly angiectatic upper dermis. Intranuclear inclusion-like appearance is noted in some keratinocytes (H&E, left: low-power, right: high-power).

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O) Rubella

Rubella is an acute febrile infection of rubella virus, a single-stranded RNA virus. It is also called as German measles (or “3-days measles” in Japan). Clinical trias includes fine non-fusing exanthema (Figure IV-O-1), fever and retroauricular lymphadenopathy. The mode of transmission is droplet transmission, and the latent period is 2-3 weeks. Vaccination is effective to prevent infection. Rubella is fundamentally a disease of children, but adults without neutralizing antibodies in the serum can be contracted. In adult cases, clinical manifestations tend to be severer than children.



Figure IV-O-1. Rubella. Generalized skin rash lasts for 3-5 days. The distribution is relatively sparse when compared with measles. The courtesy by Prof. Tetsushi Yoshikawa, Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Japan.

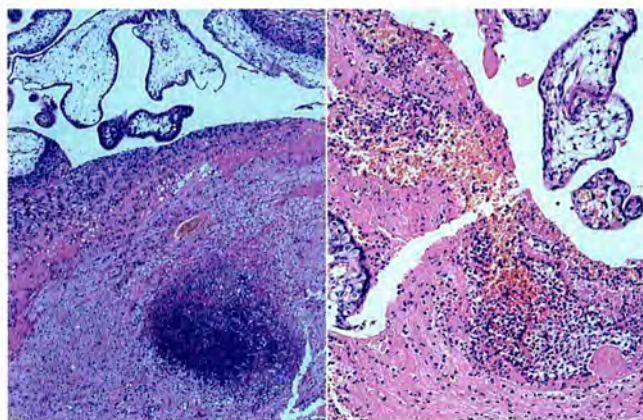


Figure IV-O-2. Rubella villitis. Curettage specimen for artificial abortion in early pregnancy in 1981, when rubella epidemic was recorded in Japan. Multifocal necrosis with fibrinous exudation and neutrophilic reaction is observed in and around the placental villi (H&E). No viral inclusions are seen. Features of villitis may indicate rubella viral transmission to the fetus.

Complications of rubella include arthritis and idiopathic thrombocytopenic purpura. The most problematic complication of rubella is congenital rubella syndrome. When women in the first trimester (up to the 16th week) of pregnancy suffer from rubella, intrauterine curettage specimens may show multifocal villitis (placentitis) (Figure IV-O-2), and the baby may manifest cardiac malformation, deafness and cataract. Cerebral palsy, glaucoma and microphthalmos may also be observed.

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P) Other Viral Infections Accompanying Acute Rash

The skin is the representative organ of signals for acute viral infections, including varicella, infectious mononucleosis, EB-VAHS, exanthem subitum, hand-foot-and-mouth disease, erythema infectiosum, measles and rubella. In 1980, the World Health Organization declared that small pox was completely eradicated from the world.

Other viruses such as mumps virus, Echovirus, poliovirus, adenovirus, Dengue virus may accompany exanthemas (Figure IV-P-1). Skin biopsy reveals nonspecific perivascular lymphocytic infiltration (Figure IV-P-2).



Figure IV-P-1. Skin rash associated with enterovirus (Echovirus) infection in infancy. Skin rash in children may suggest acute viral infection. The courtesy by Prof. Tetsushi Yoshikawa, Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Japan.

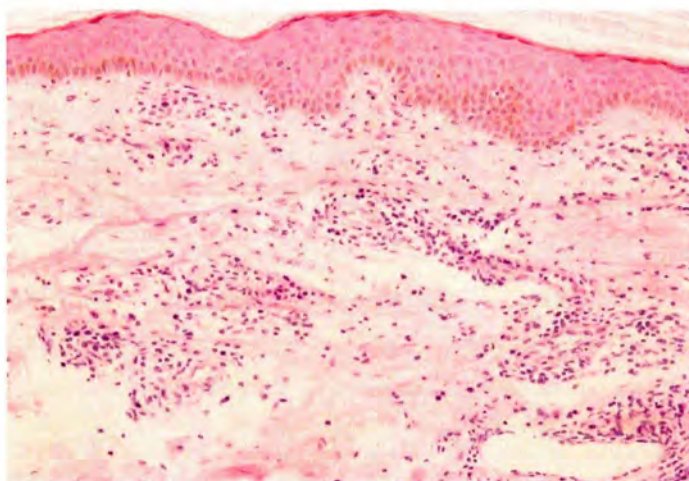


Figure IV-P-2. Histology of skin rash associated with Dengue fever. Perivascular lymphocytic infiltration is observed (H&E). The features are nonspecific.

Acute primary infection of human immunodeficiency virus (HIV) may lead to a transient, generalized, morbilliform eruption, commonly seen on the upper thorax and collar area, face, arms, scalp and thighs. The eruption appears as pink macules and papules measuring up to 1 cm. Acute HIV syndrome occurs 3-6 weeks after exposure in 50-70% of recently infected patients. Fever may be associated. Refer to Figure III-N-3.

Gianotti-Crosti syndrome is characterized by non-relapsing erythematous or papular rash on the face and limbs, lasting for 3 weeks. Lymphadenopathy may be associated. The disorder is mainly seen in children under the age 15 years, particularly below the age 4 years (Figure IV-P-3). Rarely, adult cases have been reported. Anicteric acute hepatitis may be associated.



Figure IV-P-3. Gianotti-Crosti syndrome. Non-relapsing erythematous or papular rash is seen on the face of an infant. Hepatitis B infection with mild hepatic dysfunction is proven. The courtesy by Prof. Tetsushi Yoshikawa, Toyoake.

The causative viruses include hepatitis A virus, CMV, EBV, adenovirus, rotavirus, parainfluenza virus, Coxsackievirus and live vaccines. Recurrent papular acrodermatitis may be seen in hepatitis B carriers. In Japan, hepatitis B virus is the most frequent cause of this syndrome, while EBV is said to be common worldwide.

Bacterial sources of similar skin reactions include scarlet fever, Far East scarlet-like fever (Izumi fever), Kawasaki disease, toxic shock syndrome, Tsutsugamushi disease and rickettsial spotted fevers manifest systemic rash, as already commented above.

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Note 44. Cutaneous manifestations in AIDS

A variety of skin lesions are associated with HIV disease and AIDS.

Cutaneous manifestations in AIDS result from human immunodeficiency virus (HIV) infection itself or from opportunistic disorders secondary to immunosuppression. Cutaneous disorders may be the initial signs of AIDS, and their recognition in an early stage allows initiation of appropriate antiretroviral therapy. A variety of infectious and noninfectious diseases and neoplasms cause skin manifestations during the course of HIV disease. In acute primary HIV infection, a transient, generalized, morbilliform eruption may develop on the trunk and the arms.

Kaposi sarcoma occurs prior to the onset of immunosuppression. When the immunosuppression starts, mucocutaneous disorders with atypical clinical features may occur, such as recurrent varicella zoster/verrucous herpes infection and hairy leukoplakia of the tongue. Treatment-resistant seborrheic dermatitis and generalized dry skin syndrome (xerosis) may be the initial cutaneous manifestations of HIV disease. The eruption may progress to erythroderma. Widespread warts (condyloma acuminatum, verruca vulgaris and verruca plana) are seen on the oral mucosa, face, neck, perianal region and vulva. Large plantar warts caused by HPV-66 and an epidermodysplasia verruciformis-like eruption may happen.

In the later stages, infections of HSV, CMV and molluscum contagiosum are characteristic. Trichodysplasia spinulosa, a polyomavirus infection of the hair follicle, may be accelerated. Mycobacterial (tuberculous and non-tuberculous) infections and persistent and recurrent mucocutaneous candidiasis are also common.

In childhood HIV infection, mucocutaneous candidiasis, such as recurrent and widespread diaper dermatitis and chronic paronychia, as well as impetigo and cellulitis caused by *Staphylococcus aureus*, are frequently encountered. Recurrent herpetic infection in the oral cavity, scabies and severe atopic dermatitis are also observed in pediatric cases.

Regarding superficial fungal infections, generalized dermatophytosis caused by *Trichophyton rubrum* and tinea capitis caused by *Microsporum canis* may also happen. Tinea versicolor becomes persistent and recurrent in HIV infection. Among deep mycosis, cryptococcosis, histoplasmosis, North American blastomycosis and coccidioidomycosis may cause papules, nodules and ulcers on the skin in AIDS patients. Penicilliosis marneffeii is a lethal systemic infection in Southeastern Asia.

Bacterial infections in AIDS include recurrent impetigo/folliculitis, disseminated furunculosis, and severe gingivitis/stomatitis. Reportedly, HIV-infected individuals have increased rates of cutaneous colonization by *Staphylococcus aureus*. Bacillary angiomatosis is caused by *Bartonella* infection. *Mycobacterium avium-intracellulare* complex (MAC) and, rarely, *M. kansasii* may cause cutaneous papules and crusted plaques. Disseminated cutaneous tuberculosis may rarely be seen in AIDS patients. Co-infection with syphilis and other sexually transmitted infections is observed in HIV-infected patients, particularly in those who are homosexual or bisexual. It is called "malignant syphilis" clinically.

Perianal or penile amebomas may be noted in homosexuals. Acanthamoebic infection may occur on the skin. Crusted (Norwegian) scabies, disseminated *Demodex folliculorum* folliculitis representing pruritic papular eruption on the face and the upper part of the trunk and disseminated cutaneous leishmaniasis may also happen.

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Protozoan Infections

The protozoa are unicellular organisms covering six categories: amebae, flagellates, coccidia, microsporidia, ciliates and sporozoa. Protozoan skin infections are mainly caused by the flagellates (*Leishmania* and *Trypanosoma*) and amebae (*Entamoeba* and *Acanthamoeba*). Cutaneous leishmaniasis is of particular importance.

A) Leishmaniasis

Leishmaniasis, one of the six infectious diseases in the program of the Tropical Disease Research and Training proposed by the World Health Organization, is caused by a flagellate protozoa, *Leishmania*, proliferating within mammalian cells (zoonosis). *Leishmania* was named after William B. Leishman, a British army pathologist, who microscopically identified oval bodies in splenic macrophages of fatal cases in 1901. Leishmaniasis is divided into three clinical types: visceral (kala-azar), mucocutaneous (espundia) and cutaneous (oriental sore). The disease is transmitted by sandfly *Phlebotomus papatasi*, a blood-sucking 2-3 mm-sized fly. Sandflies occupy damp, dark places like soil and tree bark. They usually bite at night. The latent period is 1-2 weeks.

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1) Visceral and Mucocutaneous Leishmaniasis

Visceral leishmaniasis or kala-azar is a systemic and often lethal disorder with a worldwide distribution, caused by *Leishmania donovani* complex. The parasite migrates to

the internal organs such as the liver, spleen and bone marrow (Figure V-A-1-1). Signs and symptoms include fever, weight loss, fatigue, diarrhea, edema, lymphadenopathy, hepatosplenomegaly, anemia/thrombocytopenia (hypersplenism) and mucosal ulcers. Immune complex-mediated glomerulonephritis may be associated. Co-infection of *Leishmania* and HIV accelerates clinical manifestations and modifies clinical course. The skin becomes pigmented (kala-azar means “black fever”). Liver biopsy is suitable for making histopathological diagnosis of visceral leishmaniasis (refer to Figure I-B-2 in Chapter I). Rectal biopsy is also utilized for the diagnosis. A new peroral drug miltefosine (Impavido) is expected to be effective to visceral leishmaniasis.

Visceral leishmaniasis is categorized into 4 types. 1) Indian type shows high mortality. The final host is the human. 2) East African type also reveals high mortality. The final host is the rodent. The pathogens causing Indian and African types are termed *L. donovani*. 3) Mediterranean and central Asian type caused by *L. infantum* affects children and shows a subacute to chronic clinical course. The final host is the dog. 4) American type caused by *L. chagasi* is seen in Amazonian Brazil. The final host is the rodent.

Mucocutaneous leishmaniasis or espundia, also called as Breda's disease, is an intractable and destructive mucocutaneous disease in the tropical area in South America. The infection is caused by *L. brasiliensis*. Especially, the nasal septum, mouth, palate, lip and pharynx are grossly destroyed and dysfigured (Figure V-A-1-2). It should be noted that the mucosal lesions are complicated by a metastasis from the skin lesions. The duration until metastatic mucosal lesions ranges from several weeks to years. In contrast, the original skin lesion disappears.

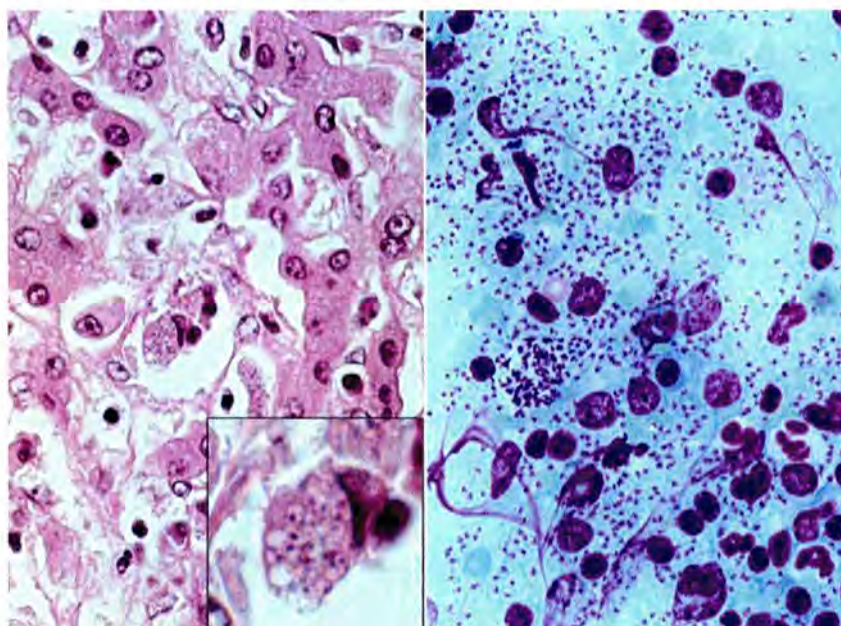


Figure V-A-1-1. Visceral leishmaniasis (kala-azar). Autopsied liver of visceral leishmaniasis in a Kenyan adult is illustrated. Activated Kupffer cells contain haloed small granules (protozoan bodies) in the cytoplasm (left and inset: H&E). Stamp smear of the spleen of a Chinese boy infected with *L. infantum* demonstrates numerous protozoan bodies phagocytized by macrophages (Giemsa). The courtesy by Prof. Jia Xin Shan, Chinese Medical University, Shenyang, China, for the Chinese case.



Figure V-A-1-2. Mucocutaneous leishmaniasis (espundia). Intractable and destructive mucocutaneous lesions are observed multifocally. The mucosal lesion is progressive. The disease caused by *L. brasiliensis* is endemic in the South America. The courtesy by Prof. Emeritus Yoshimasa Kaneda, Department of Parasitology, Tokai University School of Medicine, Isehara, Japan.

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2) Cutaneous Leishmaniasis

Cutaneous leishmaniasis is a benign, self-limiting skin infection of zoonotic leishmanian parasites. The lesion may heal spontaneously with atrophic scarring. Two types of cutaneous leishmaniasis are known (Figure V-A-2-1). The urban (Indian) type, mainly affecting the head and neck skin, is caused by *Leishmania tropica* and prevailing in India through the

Middle East. Solitary lesions occur, and dogs carry the protozoa. The rural (African) type, caused by *L. major*, is seen in the African continent, and multiple hemorrhagic and ulcerated skin lesions are seen on the extremities, and tend to expand to be fused. The natural reservoir of *L. major* is rodents. Microscopic distinction of these two protozoan parasites seen in macrophages is impossible.

Histologically, the upper to mid-dermis is densely filled with vacuolated macrophages. Numerous amastigotes (nucleated round particles) are phagocytized by vacuolated macrophages in the dermis (Figure V-A-2-2). In the deep dermis, foreign body granulomatous reaction is observed, with only a few microbes demonstrated. The morphology of the microbe resembles yeast-form fungi such as *Penicillium marneffei* and *Histoplasma capsulatum*, but PAS and Grocott stains are negative (refer to Chapter III-U). Indirect immunoperoxidase staining using a 1:1,000 diluted patient's serum identifies intracellular microbes in paraffin sections. The serum of a patient with *L. major* infection was not cross-reactive with *L. tropica*. Generally speaking, the specificity of the patient serum is high enough in case of protozoan or helminthic infection (Figure V-A-2-3). The number of protozoa in the lesion is dependent on the timing of biopsy. In the old regressing lesion, fewer pathogens are seen in granulomatous reactions.

Stamp cytology preparation shows round-shaped amastigotes phagocytized by macrophages. The nucleus and kinetoplast (paranucleus) can be recognized (Figure V-A-2-4). Electron micrograph of *Leishmania* in the cytoplasm of a macrophage reveals the 3 μm -sized amastigotes, containing a round nucleus with characteristic chromatin condensation, mitochondria, a blepharoplast, a kinetoplast without forming flagella, and a row of microtubules just beneath the plasma membrane (Figure V-A-2-5).

Under the acellular culture condition, the protozoan body transforms into the promastigote, a flagellated and elongated form seen in the mid-gut of the vector. Figure V-A-2-6 illustrates promastigotes of *L. tropica* transformed in the culture medium.



Figure V-A-2-1. Two types of cutaneous leishmaniasis. The urban (Indian) type caused by *Leishmania tropica* (left) affects the neck skin. The lesion is solitary. The rural (African) type caused by *L. major* accompanies multiple hemorrhagic and ulcerated skin lesions on the forearm. Both are imported infections seen in Japanese males who stayed in India and Mali, respectively.

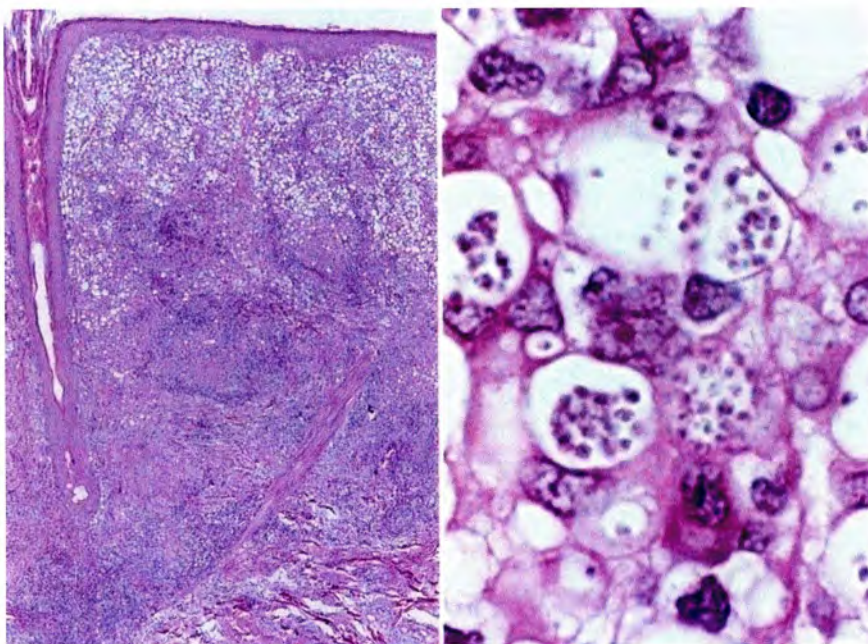


Figure V-A-2-2. Histology of cutaneous leishmaniasis. The upper to mid-dermis is densely filled with vacuolated macrophages. Numerous amastigotes (nucleated round particles) are phagocytized by vacuolated macrophages in the dermis. The nucleus and kinetoplast (paranucleus) are observed (H&E).

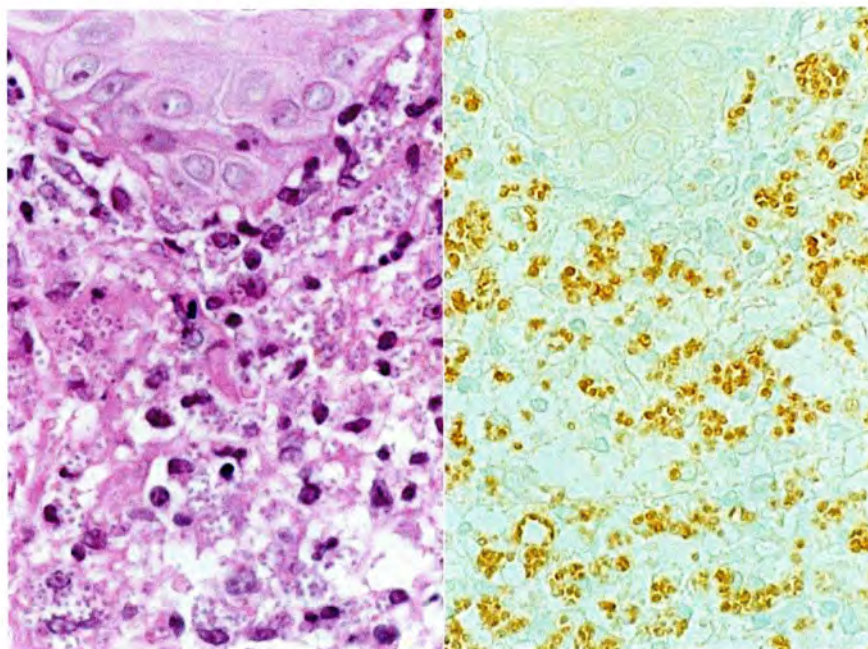


Figure V-A-2-3. Immunostaining using patient's own serum in cutaneous leishmaniasis. The 1:1,000 diluted patient's own serum clearly detects the protozoa phagocytized by macrophages (indirect immunoperoxidase). The serum of a patient with *L. major* infection was not cross-reactive with *L. tropica*.

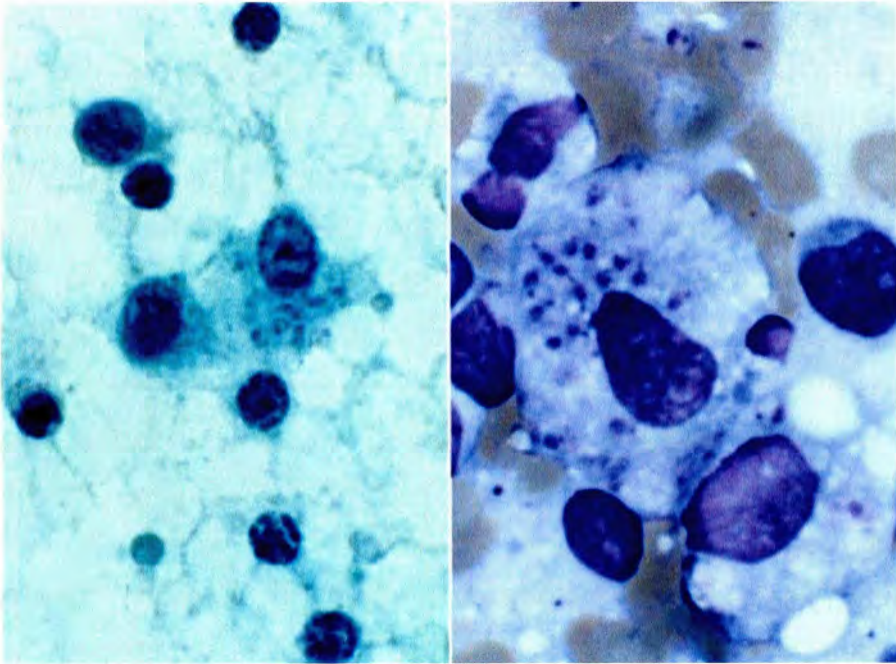


Figure V-A-2-4. Stamp cytology of cutaneous leishmaniasis. Protozoan bodies (amastigotes) are seen in the cytoplasm of macrophages. In Giemsa-stained preparation, the nucleus and kinetoplast are clearly recognized (left: Papanicolaou, right: May-Giemsa).



Figure V-A-2-5. Electron micrograph of *Leishmania tropica* in the cytoplasm of a macrophage. The 3 μm -sized amastigotes contain a round nucleus with characteristic central chromatin condensation, mitochondria, a blepharoplast, a kinetoplast without forming flagella, and a row of microtubules just beneath the plasma membrane.

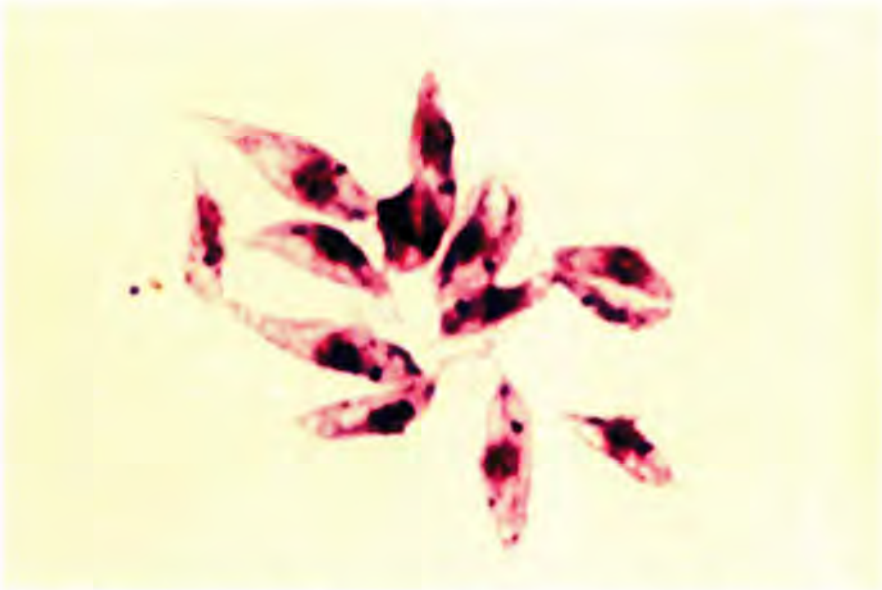


Figure V-A-2-6. Promastigote form of *L. tropica* in the culture medium. Under the acellular culture condition, the protozoan body transforms into the promastigote, a flagellated and elongated form seen in the mid-gut of the vector (May-Giemsa).

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B) Trypanosomiasis

Trypanosomiasis, caused by flagellated protozoa (belonging to *Mastigophora*), is transmitted by blood-sucking arthropods. The term *Trypanosoma* was derived from the Greek trypano- (borer) and soma (body), because of their corkscrew-like motion.

The peripheral blood smear preparation shows flagellated trypomastigotes, which possess a round nucleus in the center of elongated cell bodies. Kinetoplasts are located at the rearmost part of the body. Within the infected cells, *Trypanosoma* changes its morphology to an aflagellated form, amastigote, which is indistinguishable from *Leishmania*. Epimastigote and

promastigote are formed inside the insect, and not seen in the vertebrate cells. Trypanosomiasis is divided into two clinical forms: African trypanosomiasis (sleeping sickness) and American trypanosomiasis (Chagas disease). Both are severe, systemic and zoonotic disorders.

African sleeping sickness, mediated by tsetse fly (*Glossina*), is caused by two kinds of *Trypanosoma*: *T. gambiense* and *T. rhodesiense*. The latter is severer and more often fatal than the former. In an acute stage, fever, lymphadenopathy, hepatosplenomegaly, skin rash and edema are seen, and in a chronic stage, meningoencephalitis results in a coma state. The pathogens are observed in both the peripheral blood and cerebrospinal fluid (Figure V-B-1).

Infection of *T. cruzi*, mediated by a cone-nose bug or kissing bug, *Triatoma*, is called Chagas disease, and is distributed in the tropical areas of Central and South America. Acute disease shows parasitemia and accompanies lymphadenopathy, myocarditis and meningitis, but the clinical course is benign. Unilateral palpebral edema and facial swelling with regional lymphadenopathy associated with acute infection of *T. cruzi* is known as Romana's signs, and the pathogens are microscopically observed in the peripheral blood (Figure V-B-2).

Skin lesions at the site of inoculation are usually the earliest sign of Chagas disease, and may be recognized as chagoma, a boil-like, indurated, violet lesion with central edema. Chagoma persists for several weeks. Transient, non-itchy skin rash resembling measles is occasionally encountered. It is difficult to identify the infected protozoa within the tissue.

In a chronic stage, *T. cruzi* preferentially infects cardiomyocytes (Figure V-B-3). Chronic interstitial myocarditis with conduction disturbance, especially right bundle branch block and extrasystole, results in chronic heart failure (chronic chagastic cardiopathy). In Chagas disease, acute symptoms are often subtle and thus tend to be unrecognized by the patients. The asymptomatic period lasts for 10 to 30 years! This fact makes up the biggest barrier for the prevention of this incipient but lethal infectious disorder.

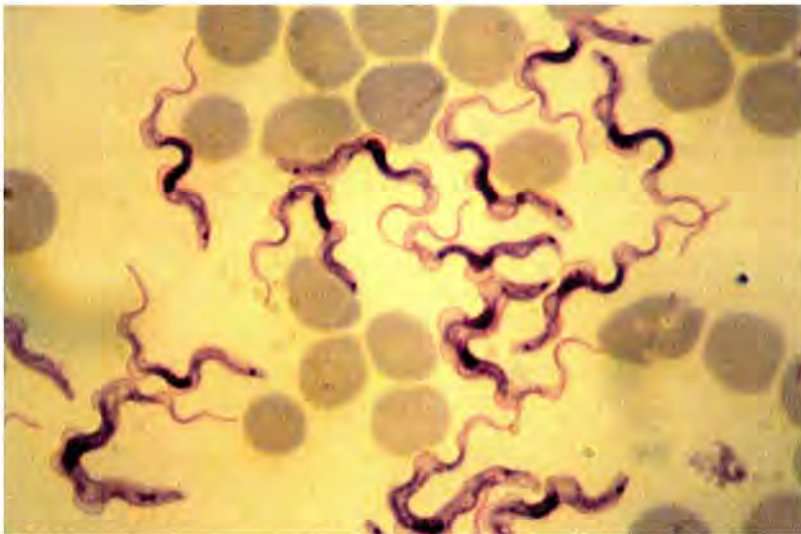


Figure V-B-1. *Trypanosoma gambiense* in mouse blood. African trypanosomiasis caused by *T. gambiense* or *T. rhodesiense* is known as "sleeping disease", resulting in lethal meningoencephalitis. The flagellated trypomastigotes of *T. gambiense* in the peripheral blood are larger than those of *T. cruzi*. Both the nucleus and kinetoplast (located at the rearmost part of the body) are recognized (May-Giemsa).



Figure V-B-2. Romana's signs and *Trypanosoma* in the peripheral blood. Unilateral palpebral edema and facial swelling with regional lymphadenopathy associated with acute infection of *T. cruzi* is known as Romana's signs (left). In the peripheral blood, flagellated protozoan bodies are observed (right: May-Giemsa). The courtesy by Prof. Emeritus Yoshimasa Kaneda, Department of Parasitology, Tokai University School of Medicine, Isehara, Japan.

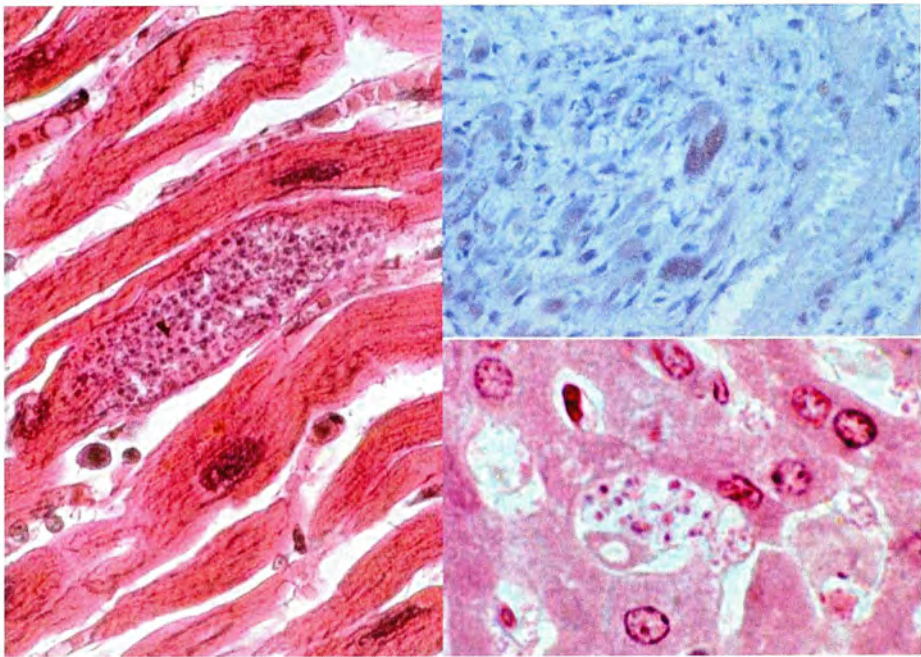


Figure V-B-3. Chagas disease. In chronic chagastic cardiopathy, interstitial myocarditis results in conduction disturbance and chronic heart failure. Cardiomyocytes infected with the aflagellated form of *T. cruzi* are dispersed (left). Monoclonal antibody identifies the infected protozoa in the cardiomyocytes (right top). Kupffer cells in the experimentally infected mouse liver contain the amastigotes with a nucleus and paranucleus, and the features are indistinguishable from kala azar (right bottom: H&E).

Schema of *Leishmania* and *Trypanosoma*

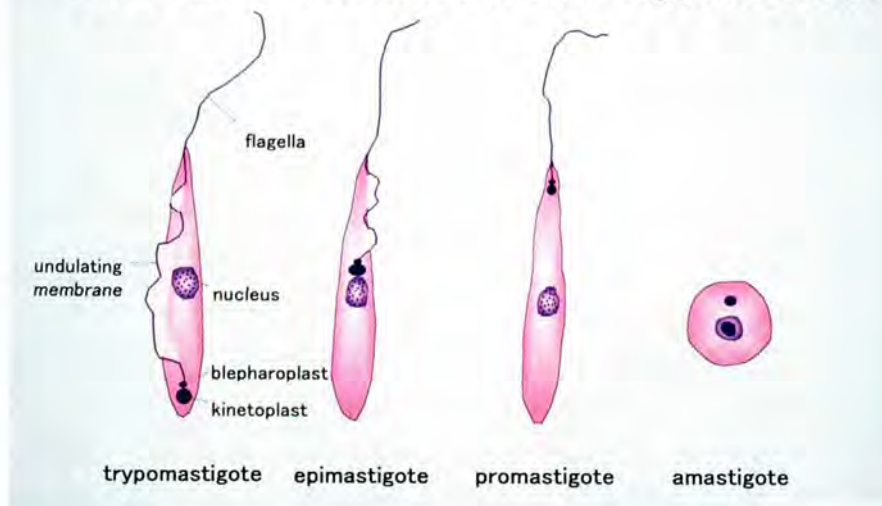


Figure V-B-4. Schematic presentation of morphological changes of *Mastigophora* (*Trypanosoma* and *Leishmania*). In the peripheral blood, *Trypanosoma* is seen as a flagellated trypomastigote, containing a round nucleus in the center of elongated cell body and a kinetoplast at the rearmost part of the body. Within the infected cell, *Trypanosoma* transforms into the aflagellated form, amastigote, indistinguishable from *Leishmania*. Epimastigote and promastigote are the forms in the vector, not seen in the vertebrate. In *Leishmania*, the promastigote is the form in the vector.

In South America (but not in Central America), *T. cruzi* infects the Auerbach's nerve plexus, leading to megacolon and esophageal ectasia. Any other part of the gastrointestinal tract, tracheobronchial tree, ureter and uterus may also be dilated (megavisceral phenomenon). In AIDS patients, massive opportunistic infection of the protozoa in the glial cells may be observed.

Morphological changes of *Trypanosoma* and *Leishmania* are schematically illustrated in Figure V-B-4.

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C) Entamebiasis

The preferred site of amebic dysentery, infection of *Entamoeba histolytica*, is the colorectal mucosa, particularly the cecum and then rectum. Resulting from secretion of lytic enzymes by the trophozoites, characteristic flask-shaped ulcers are formed in the large bowel. Early lesions show pin-point ulceration. Infective trophozoites microscopically resemble macrophages, but contain an eosinophilic nucleus and vacuolated cytoplasm with phagocytized red blood cells. Mucohemorrhagic stool, resembling strawberry jelly, is characteristic. Fever and tenesmus are milder than bacillary dysentery. Diarrheal stool is not very infective, because the pathogens are seen in the form of non-infective trophozoites. The trophozoite stage exists only in the host and in fresh loose feces, while cysts survive outside the host in water, in soil, and on food, especially under moist conditions. The cysts survive for a few months outside of the host.

Representative complications include colonic perforation and liver abscess. Perianal necrotic skin lesions (amebomas) may be formed. Penile skin lesions are increasingly seen in male homosexuals. It is known that a tumorous cecal ameboma resembles colon cancer (Figure V-C-1).

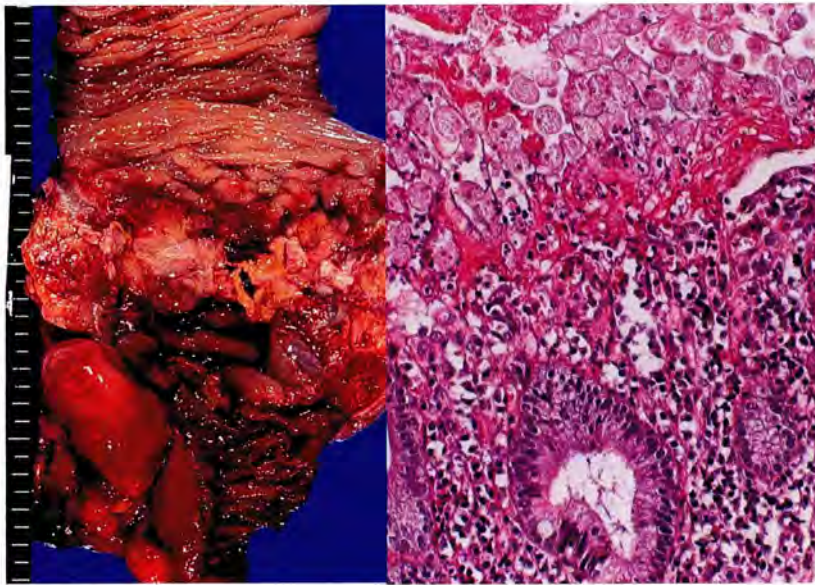


Figure V-C-1. Ameboma in the cecum. Colon cancer-like ulcerated tumor is caused by amebic infection. Right hemicolectomy was performed under the diagnosis of colon cancer (left). Microscopically, trophozoites are clustered on the eroded surface, and phagocytosis of red cells is occasionally observed (right: H&E). The courtesy by Dr. Takaaki Chikugo, Department of Pathology, Kinki University Faculty of Medicine, Sayama, Japan.

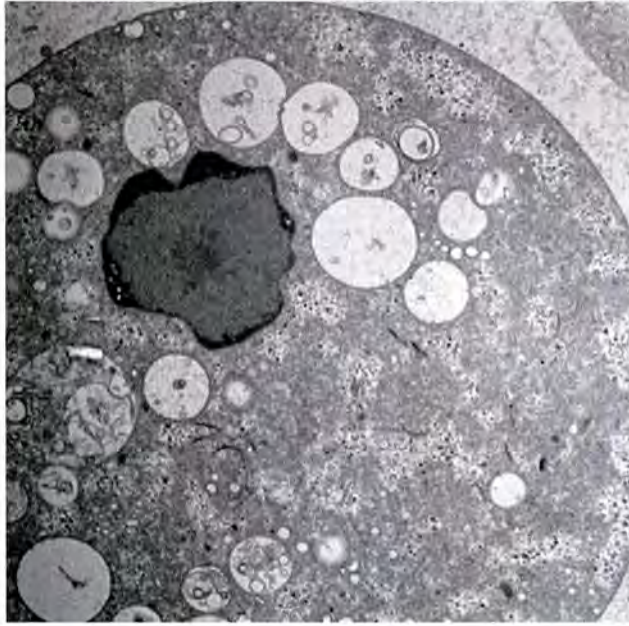


Figure V-C-2. Electron micrograph of *Entamoeba histolytica*. Cultured trophozoites showing cytoplasmic vacuolation and characteristic chromatin distribution in the nucleus. Anaerobic *E. histolytica* totally lacks mitochondria.

Amebic dysentery is endemic in tropical and subtropical countries, and thus it is nominated as one of the imported infectious diseases. However, in Japan, most of the patients of amebic dysentery are single males in the fifth decade, and careful interview discloses homosexuality in their sexual behavior. HIV antibody is often positive in their sera. The diagnosis is based upon biopsy histology and/or serologic study. The protozoan bowel infection seen in AIDS patients is called “gay bowel syndrome”, and encompasses amebic dysentery, giardiasis, cryptosporidiosis, isosporiasis and microsporidiosis.

E. histolytica lacks mitochondria, and is thus totally anaerobic (Figure V-C-2). PAS reactivity reflects rich glycogen content in the trophozoites. Encystation occurs in the bowel lumen. The cysts are resistant to heating. Zoonotic infection in the dog, cat, monkey and pig is seen.

In the human bowel lumen, non-pathogenic amebae are colonized, including *Entamoeba coli*. *E. dispar* has been distinguished as another non-pathogenic species of *Entamoeba* from *E. histolytica*. Traditionally, individuals with colonization of *E. dispar* have been called as asymptomatic carriers of a non-pathogenic strain of *E. histolytica*. Both are indistinguishable morphologically.

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D) Acanthoamebiasis

Three species of free-living ameba pathogenic to the human are known, *Naegleria*, *Acanthamoeba* and *Balamuthia* (*Leptomyxid*). *Acanthamoeba* and *Balamuthia* infect the immunocompromised hosts, and cause chronic granulomatous encephalitis via hematogenous spread of cysts, which are inhaled or skin-implanted. The incidence of amebic granulomatous encephalitis by the *Acanthamoeba* species is allegedly increasing in AIDS patients. Typically, chronic granulomatous reactions including multinucleated giant cells are formed in the centrifugal direction. Both trophozoites and cysts are observed within the lesion (Figure V-D-1).

Acanthamoeba also causes progressive keratitis in immunocompetent contact lens wearers. In *Acanthamoeba* keratitis, severely painful ring-shaped crowding and ulceration is formed. Cytological smears identify a cyst form of the microbes, and are useful in making the diagnosis (Figure V-D-2). Histologically, the cornea tissue is infiltrated mainly by acanthoamebic trophozoites (Figure V-D-3).

Pustular, chronic ulcerating or nodular skin lesions may be seen particularly in patients with AIDS. In AIDS patients, granulomatous reaction is suppressed, so that subcutaneous abscess is formed together with numbers of organisms in the form of trophozoites and cysts. The epidermis remains intact.

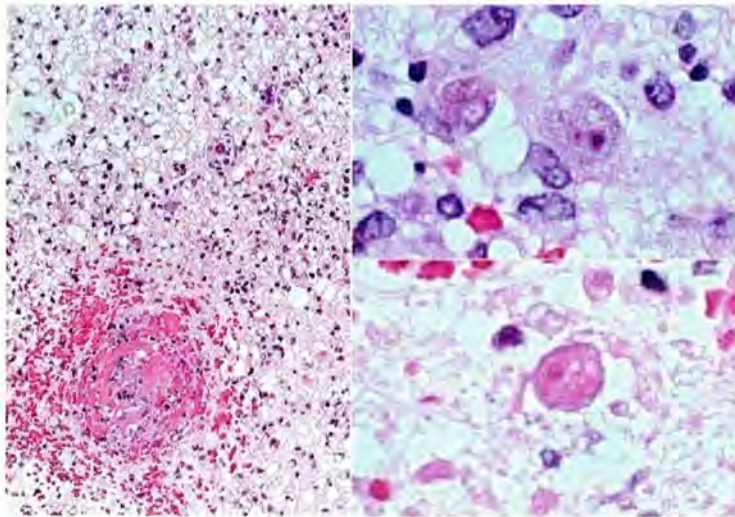


Figure V-D-1. Acanthoamebic encephalitis in a cirrhotic patient. Inflammatory infiltration is observed in the hemorrhage and edematous cerebral cortex (left). The perivascular macrophage-like cells with centrally situated karyosome correspond to the trophozoites of free-living *Acanthamoeba* (left top), and a smaller, round-shaped cell with eosinophilic cytoplasm, a round nucleus and wrinkled, double-layered cell wall belongs to the cyst form (right bottom). The courtesy by Dr. Rokuro Ariwa, a pathologist at Tokyo Metropolitan Ohtsuka Hospital, Tokyo, Japan.

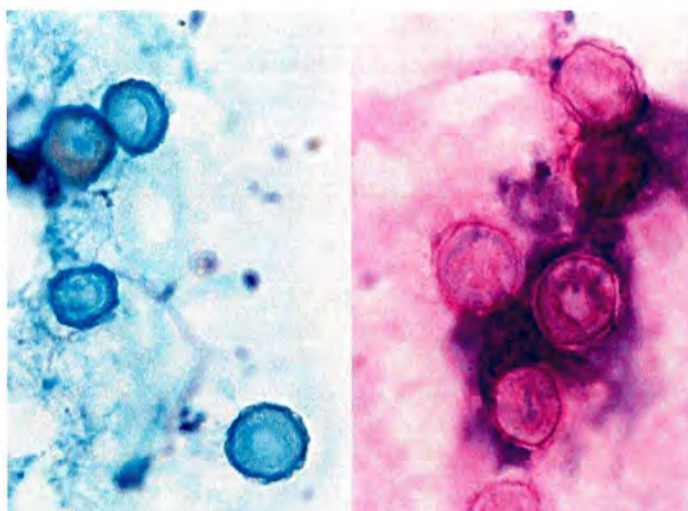


Figure V-D-2. Cytology of acanthamoebic keratitis. Brushing cytology from the diseased cornea shows numbers of acanthamoebic cysts. Wrinkled, double-layered cyst wall is pathognomonic (left: Papanicolaou, right: Giemsa). The courtesy by Dr. Yoshitaka Miyanaga, Nishi-Kasai Inoue Eye Clinic, Tokyo, Japan.

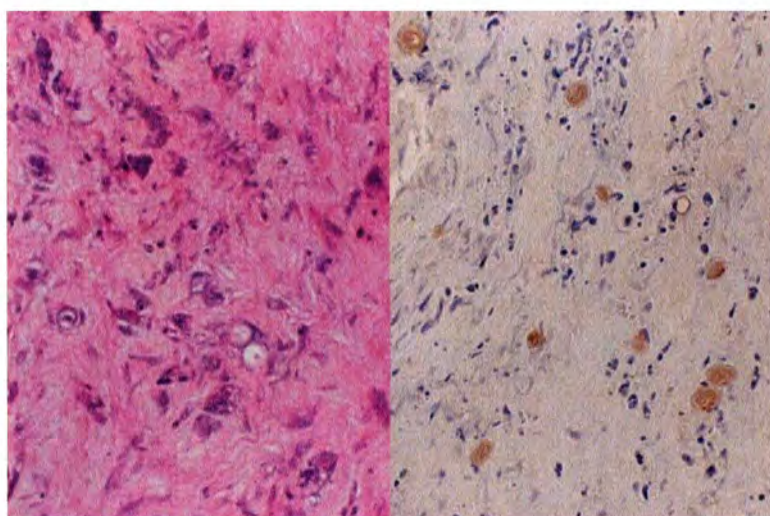


Figure V-D-3. Histology of acanthamoebic keratitis. The turbid cornea surgically removed for corneal transplantation shows infiltrating trophozoites of *Acanthamoeba* with a centrally located karyosome among the collagenous matrix (left: H&E). Antiserum raised in the mouse specifically detects the protozoan pathogen (right: immunostaining). The courtesy by Prof. Yoshitaka Miyanaga, Tokyo, Japan.

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Note 45. Protozoan hypothesis of Darier's disease

Darier's disease is an autosomal dominant hereditary disorder of the skin. Greasy, yellow to brown, crusted papular lesions develop in the seborrheic regions of the head, neck and trunk. The onset is in adolescence, and the disease runs a chronic course. There is preposition to bacterial, fungal and viral infections. Foul smell results from the secondary infection. Histologically, the papular lesion of Darier's disease shows suprabasal acantholysis with formation of lacunae. Irregular projections of the papillary dermis, covered with single-layered epidermal basal cells (so-called villi), extend into the lacunae. Two types of dyskeratotic cells are characteristic. Corps ronds with small pyknotic nuclei and eosinophilic cytoplasm represent solitary dyskeratotic cells in the upper Malpighian layer and the cornified layer (Figure V-D-4). The grains are small cells with elongated nuclei and scanty cytoplasm.

In 1889, Darier erroneously reported that the corps ronds represented protozoan (Coccidia) infection, and he regarded them as "*Psorospermia*". Actually, *Psorospermia* is a coccidian species, frequently infecting the liver of the rabbit.

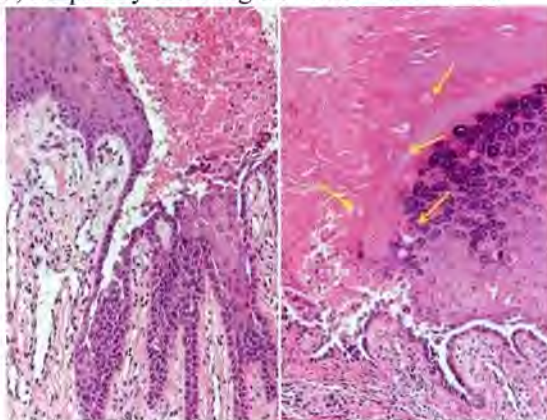


Figure V-D-4. Darier's disease. This hereditary bullous disease of the skin histologically shows suprabasal acantholysis and irregular villous projections of the papillary dermis. Dyskeratotic "corps ronds" are seen in the upper Malpighian and cornified layers (arrows), somewhat resembling protozoan bodies (H&E).

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Helminthic Infestations

Helminthic parasites encompass three major categories: nematode (threadworm), trematode (flake) and cestode (tape worm). Filariasis, onchocerciasis and duracunculiasis are examples of adult worm infestation, mediated by arthropods or cyclops. Others represent larval infestations, namely cutaneous larva migrans or creeping disease. It should be noted that the creeping disease is closely associated with the dietary habit or lifestyle of people.

A) Lymphatic Filariasis

Filariasis is a tropical parasitic disease caused by thread-like nematode, filariae. The disease is transmitted by mosquitos. Lymphatic filariasis is caused by *Wuchereria bancrofti*, and endemically by *Brugia malayi* and *Brugia timori*. These worms occupy the lymphatic system, particularly the lymph node, and elephantiasis develops at the chronic stage.

Elephantiasis, chronic thickening of the skin of the leg and scrotum, is a representative clinical feature of chronic filariasis. Histologically, marked lymphangiectasia is seen in the fibrotic dermal connective tissue. The epidermis is reactively acanthotic. Chronic lymph stasis and edema is caused by obstruction of lymphatic flow. The groin lymph nodes show eosinophilic abscess containing female adults of *W. bancrofti*. Numerous microfilariae are seen in the uterine cavity, and the gut component is also recognized. Microfilariae are seen in the peripheral blood, particularly during the night time. Typical appearance of bancroftian filariasis is demonstrated in Figure VI-A.

In the ancient time in the Edo era, lymphatic filariasis was common in Japan, and Hokusai Katsushika (1760-1849), one of the most famous Ukiyo-e painters, illustrated a comical print, on which a guy carries his own huge scrotum with a kago (palanquin) with his colleague. Mr. Takamori Saigo (1828-1877), a powerful politician born in Kagoshima located at the southernmost part of Kyushu Island, who significantly contributed to re-opening of modern Japan, suffered from this parasitosis. No patients are recorded after 1978 in Japan.

Brugiasis malayi, distributed in South and Southeast Asia, was once endemic in a small island in the Hachijo-jima islands located in the Pacific Ocean, Japan. In 1969, all the inhabitants of this island moved to the other islands, and the disease was thus eradicated. *B. timori* is endemic in some Indonesian islands.

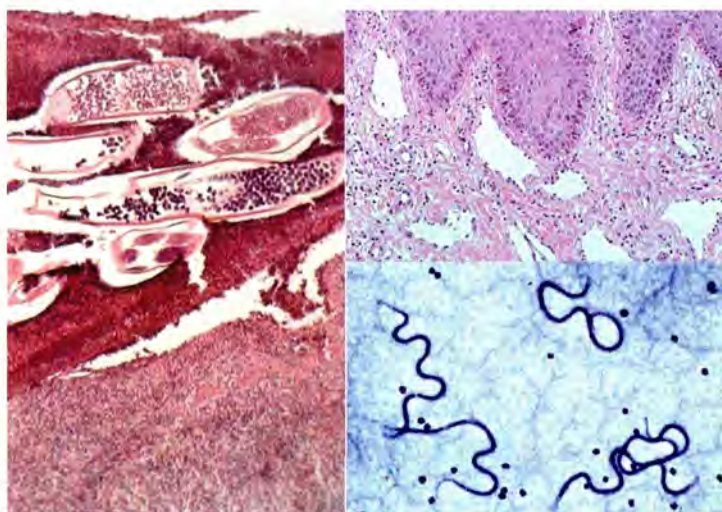


Figure VI-A. Lymphatic filariasis. Female adults of *W. bancrofti* are clustered in eosinophilic abscess in the groin nodes. Numerous microfilariae in the uterine cavity and the gut components are observed (left: H&E). The scrotal skin reveals microscopic features of chronic lymphedema, such as marked lymphangiectasia in the fibrotic dermis and acanthotic epidermis. Microfilariae are seen in the peripheral blood during the night time (right bottom: Giemsa).

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Note 46. The probability of establishing infection of filariasis

Wuchereria bancrofti is transmitted by varied species of mosquitos, and the microfilariae appear in the peripheral blood during the night time, accompanying fever and chilling. The probability of establishing infection of filariasis is extremely low. According to the Hairston and de Meillon's calculation, one should have 15,500 mosquito bites per year (or 42 times a day). The reasons are as follows. 1) low probability that the mosquito carries microfilariae, 2) a small number of microfilariae in the mosquito, 3) only one chance for the mosquito to transmit microfilaria to the human, 4) low probability (5%) of the percutaneous penetration of microfilaria during mosquito bite, 5) low probability of mating of adult male and female worms in the human body to succeed the next generation.

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B) Subcutaneous Filariasis

1) Loa Loa Filariasis

Loa loa filariasis (loiasis) is a skin and eye disease caused by the nematode, *Loa loa*. The disease, endemic in Central and West Africa, is transmitted through the bite of deer fly (*Chrysops* spp.). Microfilariae are seen in the peripheral blood, but microfilaremia is asymptomatic. The adult *Loa loa* (African eye worm) migrates in the subcutaneous fat tissue, frequently crossing into subconjunctival layer of the eye, where it can be observed grossly (Figure VI-B-1). *Loa loa* does not affect vision but may cause pain. Itchy skin lesions are called "Calabar swelling". The skin lesions represent localized angioedema and erythema usually on the extremities, characterized by a fugitive lump in the subcutaneous tissue due to adult filarial worm parasitism. The swollen areas migrate with the worm moving through the body. Painful nodule formation in the tendon sheath may last for 1-3 days, and accompany localized urticarial reactions.

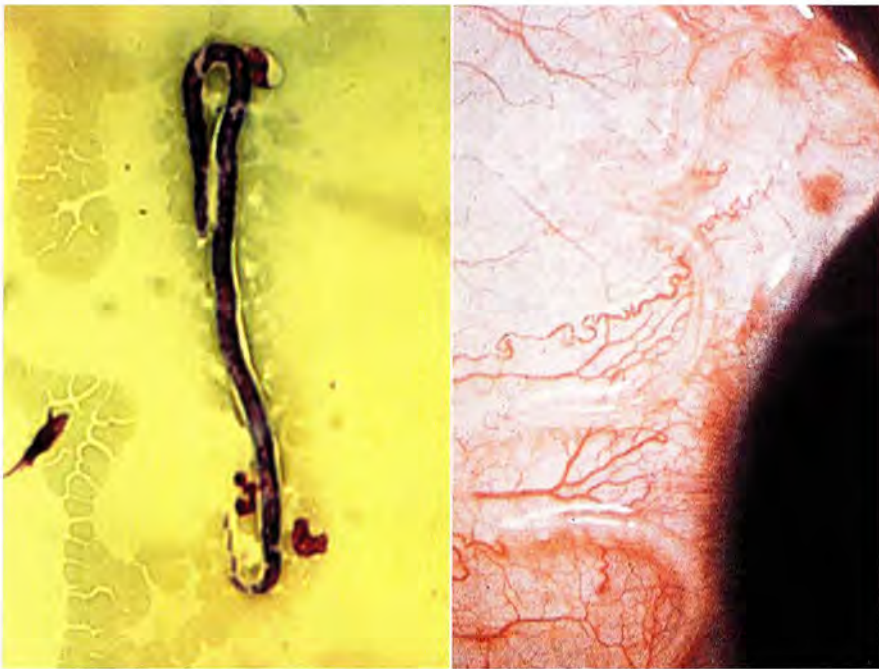


Figure VI-B-1. *Loa loa* filariasis. Asymptomatic microfilariasis is seen in the peripheral blood (left: Giemsa). The adult *Loa loa* (African eye worm) migrates in the subconjunctival layer of the eye (right). The courtesy by Dr. Satomi Yara, Department of Internal Medicine, University of Ryukyus Graduate School of Medicine, Okinawa, Japan.

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2) Onchocerciasis

The greatest burdens related to human onchocerciasis are the result of the eye and skin lesions caused by the microfilariae. The disease is transmitted by blackfly (*Simulium*), living along the river at the high altitude of 500-1,000 m. More than 17 million persons, mostly in Africa, are estimated to be infected with *Onchocerca volvulus*. Onchocerciasis has caused blindness in 270,000 and left another 500,000 with severe visual impairment. This disease is nicknamed as “river blindness”. Onchocerciasis can also cause disfiguring skin changes. Onchocercal skin lesions (onchocercal nodules, onchocercal dermatitis, hanging groin and scrotal elephantiasis) have been shown to be a major socio-economic burden, in terms of disability-adjusted life-years. The skin lesions commonly accompany severe itching. *O. volvulus* may cause localized lymphedema, but no typical elephantiasis is seen. Ivermectin effectively kills the microfilariae, and treatment with ivermectin relieves intense skin itching and halts the progression towards blindness.

Histologically, female adults of *Onchocerca volvulus* are clustered in the subcutaneous tissue (Figure VI-B-2-1). The uterus is filled with microfilariae. The cuticles are thick, while the muscle layer is thin.

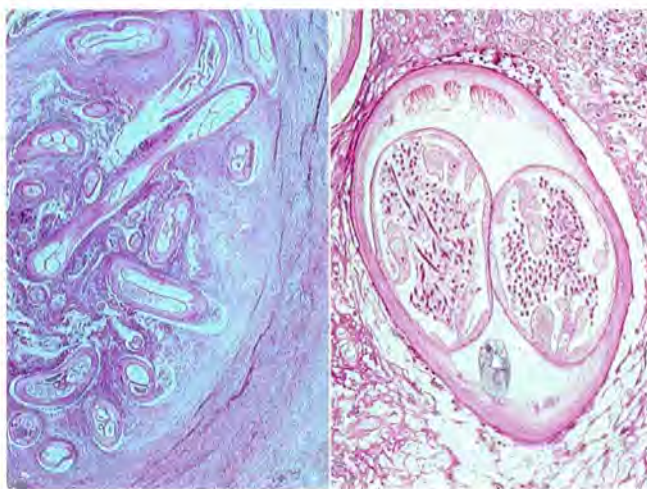


Figure VI-B-2-1. Onchocerciasis in a Mexican. Female adults of *Onchocerca volvulus* are clustered in the subcutaneous nodule. The uterus is filled with microfilariae. The cuticles are thick, and the muscle layer is thin (H&E). The courtesy by Prof. Kennichi Kakudo, Kobe Tokiwa University, Faculty of Health Science, Kobe, Japan.

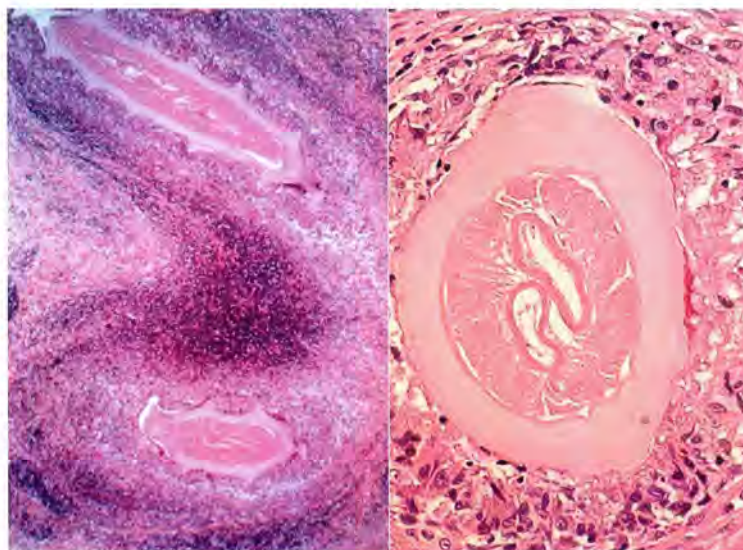


Figure VI-B-2-2. *Onchocerca gutturosa* infection in Kyushu Island, Japan. In the skin nodule, an eosinophilic granulomatous reaction has been provoked against a filariform helminth with a ridge-forming thick cuticle and well-developed muscle layer. No microfilariae are noted (H&E). The courtesy by Prof. Hiroyuki Takaoka, Oita University Faculty of Medicine, Yufu, Japan.

Onchocerca gutturosa infection, bovine onchocerciasis, is occasionally encountered. This zoonotic onchocerciasis provoking a localized skin nodule is a very rare occasion. The final host of *O. gutturosa* is the cow, and it is transmitted by gnat. A total of six kinds of zoonotic onchocerciasis are seen in Japan, particularly among farmers in Kyushu Island. Within eosinophilic granulomatous reaction, a filariform helminth is observed (Figure VI-B-2-2). The worm is featured by the ridge-forming thick cuticle and well-developed muscle layer. No ova or microfilariae are formed, because the worm does not become mature in the human. Paravertebral (extradural) mass formation by zoonotic *O. lupi* has recently been reported.

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Note 47. Africans and blindness

Blindness prevalence rates in Africa vary widely but the evidence suggests that approximately 1% African people is blind. The major causes are cataract, trachoma and glaucoma. The bulk of blindness in the region is preventable or curable. Efforts should focus on universal eye problems, for which there are cost effective remedies, such as cataract and refractive problems. Those problems which occur focally and can be prevented by primary healthcare measures, such as trachoma, leprosy, measles, onchocerciasis and vitamin A deficiency, are also of critical importance.

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C) Gnathostomiasis

Zoonotic parasitosis by *Gnathostoma* species, gnathostomiasis, causes a creeping disease, namely cutaneous larva migrans (Figure VI-C-1). The larval worm is found in the dermis or subcutaneous tissue. The migration speed of *Gnathostoma* spp. is high, so that superficial echography is powerful to identify the location of the larval worm. The reddish skin no longer has the parasite because of a delay in host inflammatory reaction. At the migration front, cellular reactions are scarcely observed. With time, a deeply situated nematode provokes eosinophilic cellulitis and granulation tissue reaction around the worm-provoked tunnel.

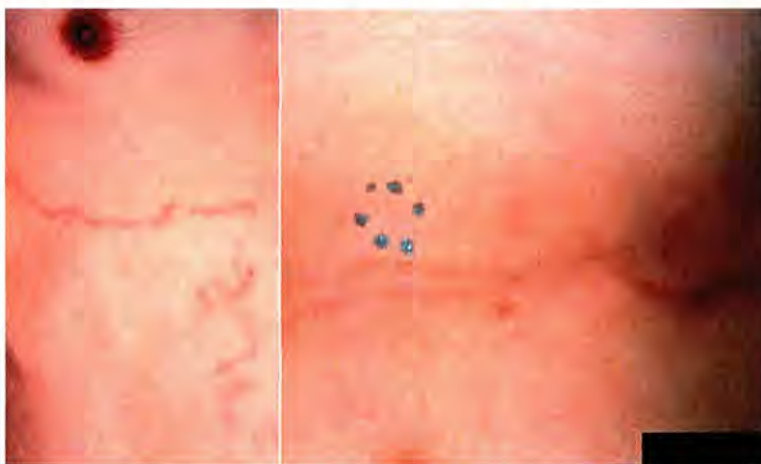


Figure VI-C-1. Creeping disease caused by gnathostomiasis. On the chest (left) or abdominal (right) wall, linear red streaks are observed along the way of larval migration in the dermis and subcutis. Cutaneous echography is effective to identify the location of the parasite as indicated by a circle in the right panel. The courtesy by Dr. Koichi Nagakura, Department of Parasitology, Tokai University School of Medicine, Isehara (left), and by Dr. Yuichiro Yamasaki, Nakagawa Nanachome Dermatology Clinic, Yokohama, Japan (right).

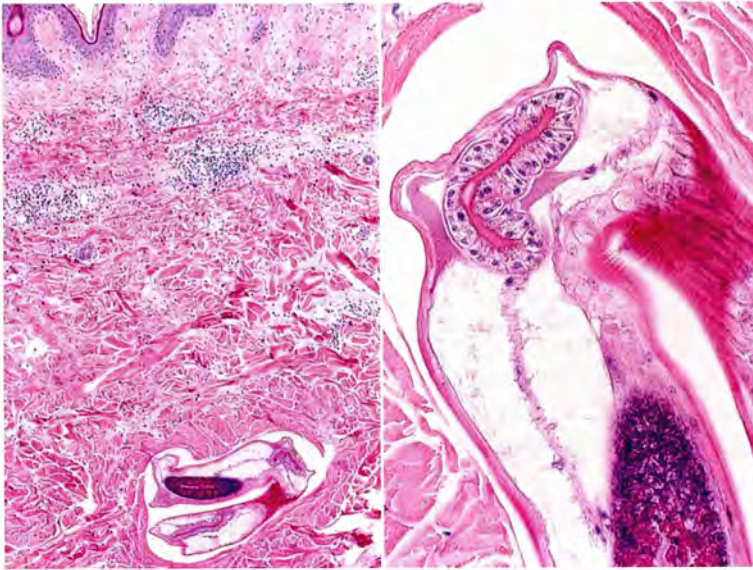


Figure VI-C-2. Histology of gnathostomiasis (I). The nematode larva (*Gnathostoma hispidum*) is located in the deep dermis. Inflammatory reaction has not yet been induced at the migration front. Cross-striated cuticles, polymyarian/coelomyarian type muscle coat, and monolayered gut are recognized. No gonads are identified (H&E).

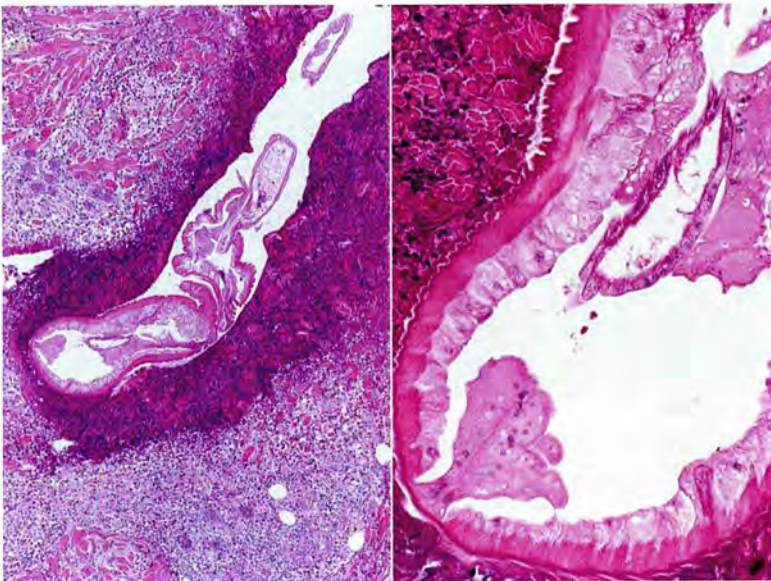


Figure VI-C-3. Histology of gnathostomiasis (II). Marked eosinophilic reaction has been provoked. Cut surfaces of the migrating nematode larva of *Gnathostoma hispidum* show wrinkled cuticles, the muscle layer of polymyarian/coelomyarian type and well-developed lateral chords. The gut is monolayered (H&E). The courtesy by Dr. Yuichiro Yamasaki, Yokohama, Japan.

Histologically, cut surfaces of the nematode show spiculated cuticles, the muscle layer of polymyarian/coelomyarian type (see Note 49) and well-developed lateral chords. On cross section of *Gnathostoma hispidum* at the level of the esophagus, four tubules (ballonets) surround the esophagus. The gut is monolayered and lined by single-layered microvillous

cells in *G. hispidum* (Figure VI-C-2). No gonads are observed. The species of *Gnathostoma* can be distinguished microscopically by the number of the nuclear layer in the gut epithelium: *G. hispidum* and *G. nipponicum* 1, *G. doloresi* 2 and *G. spinigerum* 3-7. With the indirect immunoperoxidase staining using 1:500 diluted patient's serum is reactive to the gut component of the nematode (Figure VI-C-3). It seems that the gut component is much more immunogenic than the cuticles.

The final hosts of *G. hispidum* are the pig and wild pig. The first intermediate host is the cyclops (water flea), and the 2nd intermediate host is freshwater fish (loach). Eating of raw loach imported from China, Taiwan, Hong Kong and Korea has a risk of this parasitosis. Mexican cuisine Cebiche and Thai cuisine Som phak may also cause gnathostomiasis. The incidence of gnathostomiasis is highest in Southeastern Asia.

The final host and the 2nd intermediate host of *G. nipponicum* are the weasel and Japanese loach, respectively. Those of *G. doloresi* are the pig/wild pig and landlocked salmon, while those of *G. spinigerum* are the dog/cat and snakehead mullet. Until 1960's, creeping disease by *G. spinigerum* was common in Japan.

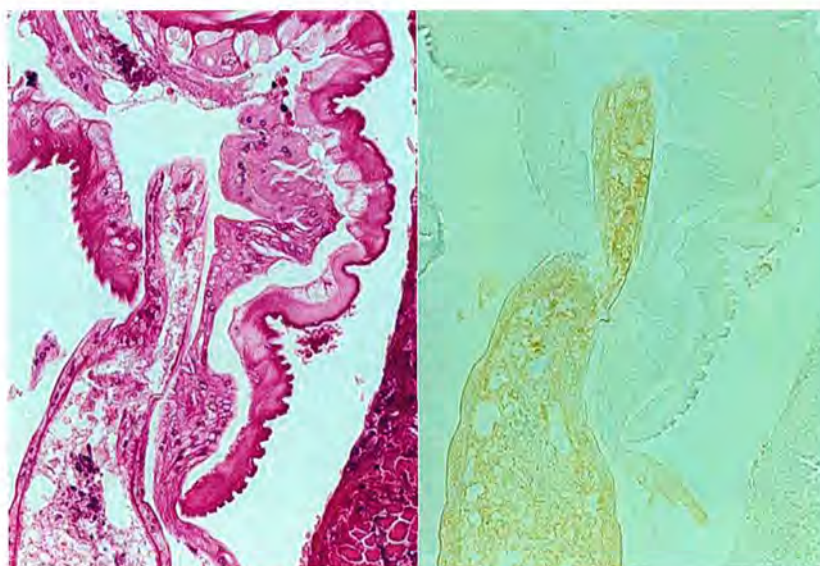


Figure VI-C-4. Reactivity of the *Gnathostoma* larva with patient's own serum. The indirect immunoperoxidase staining using 1:500 diluted patient's own serum mainly demonstrates the gut component of the parasite (left: H&E, right: immunostaining).

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Note 48. Creeping disease (cutaneous larva migrans)

Creeping disease represents parasitic larva migrans in the skin. Linear and migrating swelling of the skin is caused by intradermal or subcutaneous migration of the larva of helminth. Typical examples of creeping disease include gnathostomiasis. Spiruroid nematode larva migrans is another nematode causing creeping disease in Japan. A creeping disease caused by larvae of the canine hookworm, *Ancylostoma brasiliense*, is popular in Southeast Asia and Central and South America, but rare in Japan. Sparganosis and cysticercosis are caused by skin migration of cestode larva. The moving speed is fast in case of the nematode larvae, in association with marked eosinophilic reaction. In contrast, the cestode larvae move slowly, provoking little or mild eosinophilia. The nematode larva contains the gut, but no gonad or ova. The cestoda lacks the gut tissue and is histologically characterized by calcareous corpuscles in the myxoid matrix. Microvillous cuticles function to absorb the nutrients from the surrounding environment. Creeping disease may also result from trematode infestation, *Paragonimus westermani*.

Visceral larva migrans is seen in the lung, lymph node, liver and spleen. Typical examples include anisakiasis, dirofilariasis and toxocariasis (*Toxocara canis* infection). Cat round worm, *Toxocara cati*, and pig roundworm, *Ascaris suum*, may also provoke visceral larva migrans. Migration into the eye ball and brain is especially problematic. Migration of cestode larvae into the eye ball and brain is especially serious in sparganosis and cysticercosis.

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Note 49. Microscopic structures of the nematode body in transverse sections

According to the structure of the muscular coat, three types of body wall can be recognized: a) polymyarian type, b) meromyarian type, and c) holomyarian type (Figure VI-C-5).

The body wall is composed, from the outside to inside direction, of the cuticle, hypodermis and muscle layer. The hypodermis is clearly seen in the larva, but in the adult worm it is not apparent. The larval hypodermis focally protrudes toward inside (among the muscle cells) to form dorsal and ventral median lines and a pair of lateral chords. In the muscle layer, a row of muscle cells are seen. The muscle layer is segmented by the lateral lines and chords into four groups. In the polymyarian type, numbers of muscle cells are noted. In the meromyarian type, 2 to 4 cells are seen in one segment. In the holomyarian type, the muscle cells are fused (not grouped).

According to the shape of the muscle cells, platymyarian type (consisting of flat muscle cells) and coelomyarian type (consisting of columnar-shaped muscle cells) can further be divided. Polymyarian type muscle coat commonly contains coelomyarian type muscle cells.

Ascaris shows the polymyarian/coelomyarian type muscle coat. *Enterobius* and *Ancylostoma* show the meromyarian/platymyarian type muscle coat. *Filaria* reveals the meromyarian/coelomyarian type muscle coat. The trichurid (*Trichuris*, *Capillaria* and *Trichinella*) exhibits the holomyarian/coelomyarian type muscle coat. In the trichurid, the hypodermis is focally thickened to form a bacillary band.

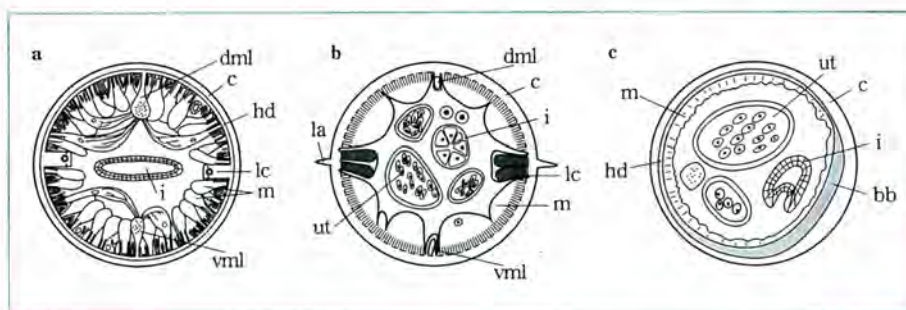


Figure VI-C-5. Schematic microscopic structures of the nematode body in transverse sections. a, polymyarian/coelomyarian type (*Ascaris*), b, meromyarian/ platymyarian type (*Enterobius*, *Ancylostoma*), c, holomyarian/coelomyarian type (*Trichuris*). bb: bacillary band, c: cuticle, dml: dorsal median line, hd: hypodermis, i: intestine, la: lateral ala, lc: lateral chord, m: muscle cell, ut: uterus, vml: ventral median line.

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D) Spiruroid Nematode Larva Migrans

Bullous creeping disease is characteristic of spiruroid nematode larva migrans. Creeping disease occurs 2-4 weeks after eating 7 cm-long fresh firefly squid (*Watasenia scintillans*) caught in Toyama Bay facing Japan Sea. Commonly, finely linear vesicular eruptions are seen on the abdominal skin (Figure VI-D-1). Deep dermal or subcutaneous involvement is

also noted (Figure VI-D-2). The moving speed of the 6-10 mm-long filamentous larva is as high as 2-7 cm/day. The adult form, *Crassicauda giliakiana*, infests the ureter of the giant beaked whale (*Berardius arnuxii*).

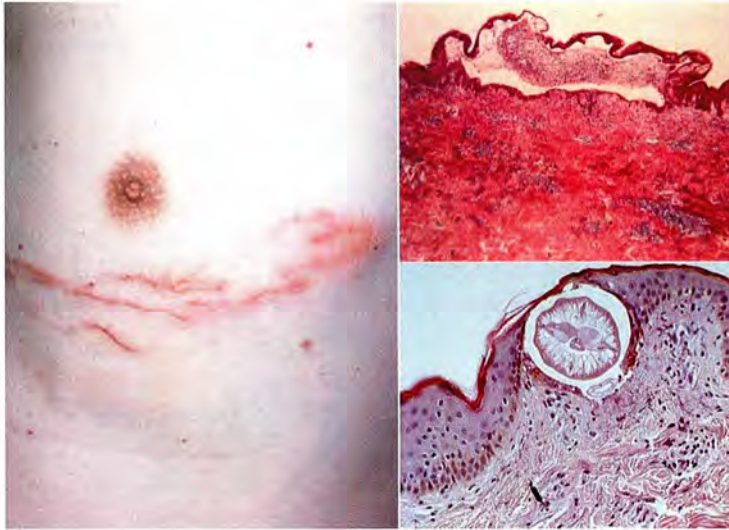


Figure VI-D-1. Creeping disease caused by a spiruroid nematode larva. Finely linear vesicular eruptions are seen on the abdominal skin (left). At the site of vesiculation, a tunnel-like space is formed within the epidermis. Eosinophilic reaction is associated (right top: H&E). The larva, 100 μ m in width, is seen just beneath the epidermis, but without cellular reaction. Basophilic clover-shaped lateral chords, thick muscle coat and gut lumen are noted (right bottom: H&E). The courtesy by Late Dr. Noboru Kagei at National Institute of Infectious Diseases, Tokyo, Japan.

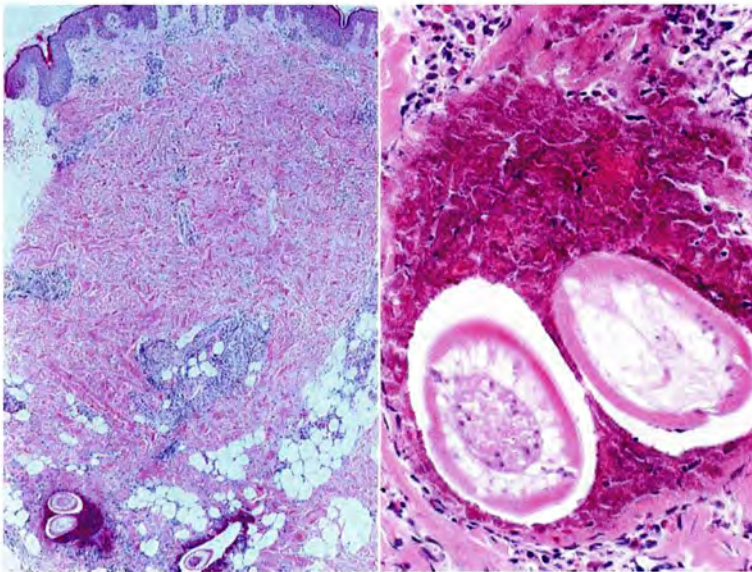


Figure VI-D-2. Deep dermal involvement by a spiruroid nematode larva. Cut surfaces of small-sized nematode larva are seen in the deep dermis. Tissue eosinophilia is observed around the larva (H&E). The courtesy by Dr. Noriko Otaki, a dermatologist at Kudanzaka Hospital, Tokyo, Japan.

Microscopically, the larva, 100 μm in width, is seen just beneath the epidermis. Paired basophilic clover-shaped lateral chords are observed. Cross section of spiruroid nematode larva shows multinucleated gut epithelium, thick muscle coat of polymyarian/coelomyarian type and cross-striated cuticles. Eosinophilic infiltration is evident in the surrounding dermis. The migrating larva may be seen in the subcutaneous tissue or in the eye ball.

Firefly squid from Toyama Bay is on the market in March through August. The larva occasionally causes acute obstructive ileus due to larval migration into the gut wall, where marked eosinophilic cellulitis is provoked (Figure VI-D-3). This serious clinical manifestation of acute abdomen type occurs 36 hours after eating raw squid (much earlier than the creeping disease).

The transmission can be avoided by freezing of the squid or preservation under high salt concentration. The incidence of this disease is thus decreasing in Japan. Many residents along Toyama Bay say that raw squid just after landing is very delicious, and a bunch of the fresh (non-frozen) squid is quickly sent to luxurious Japanese-style restaurants in Tokyo. This makes the reason for the persistence of this cutaneous larva migrans in Japan.

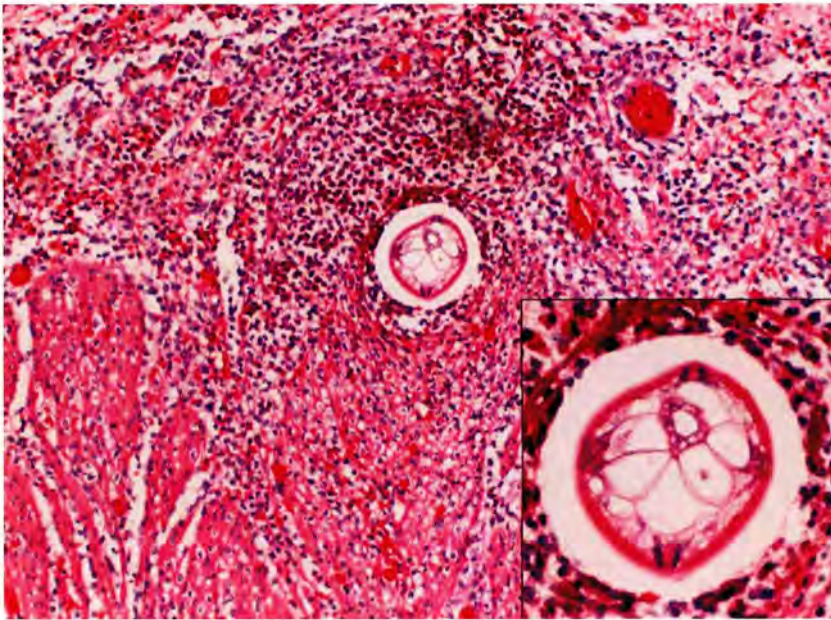


Figure VI-D-3. Mechanical ileus caused by a spiruroid nematode larva. In the small bowel wall, marked eosinophilic infiltration against the migrated nematode larva is observed, resulting in intestinal obstruction. Inset demonstrates a close-up view of the parasitic larva (H&E). The courtesy by Late Dr. Noboru Kagei, Tokyo, Japan.

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E) Dirofilariasis

Dirofilariasis is commonly seen in the lung, causing a solitary pulmonary nodule (coin lesion). A microfilaria embolizes in a branch of pulmonary artery. *Dirofilaria immitis* is the dog heartworm, and microfilariae are transmitted to human by mosquito bite. This filarial nematode enters into the subcutaneous tissue, travels to the right ventricle where it dies, and embolizes the pulmonary arteries, causing small pulmonary infarction, which subsequently appears as a coin lesion. The nodule is usually identified incidentally by chest radiography in asymptomatic patients, and distinction from lung cancer is thus needed. Eosinophilia is associated. The diagnosis is commonly made after surgical excision.

Extrapulmonary dirofilariasis occurs mainly in the subcutaneous tissue, as a creeping disease. Cut surfaces of the microfilaria are seen in eosinophilic abscess. Thick cuticular layer with internal ridges, well-developed thick muscle layer of meromyarian/coelomyarian type and small-sized gut are observed. The internal cuticular ridges are characteristic of *Dirofilaria* (Figure VI-E).

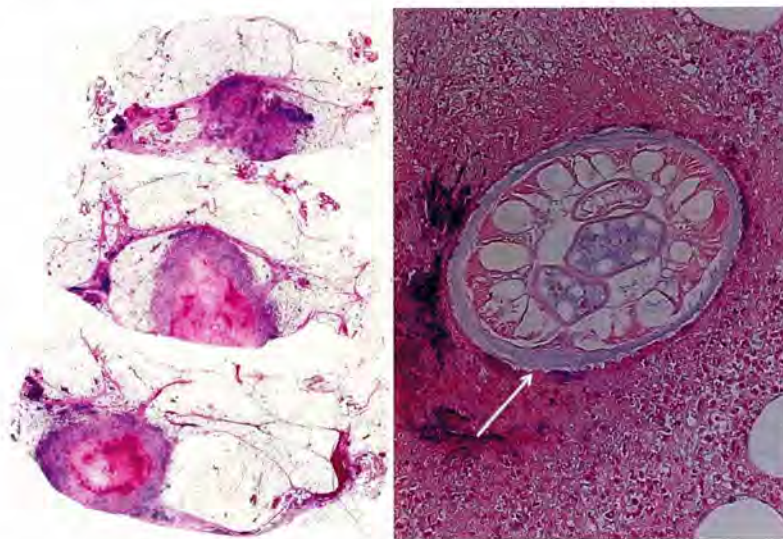


Figure VI-E. Cutaneous dirofilariasis. Extrapulmonary dirofilariasis occurs mainly in the subcutaneous tissue, as a creeping disease. In eosinophilic abscess, one observes cut surfaces of the microfilaria with thick cuticular layer, internal ridges (arrow), well-developed thick, meromyarian/coelomyarian type muscle layer and small-sized gut. No gonads are seen (H&E).

D. tenuis and *D. ursi* are the heartworms of the raccoon and black bear, respectively. Once injected into the human skin, the microfilariae stay there to form subcutaneous nodules.

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F) Ancylostomiasis

Ancylostomiasis (ankylostomiasis) caused by infection of *Ancylostoma* hookworms. The name is derived from Greek ancylos "crooked or bent" and stoma "mouth." *Ancylostoma duodenale* and *Necator americanus* infest the duodenal mucosa and suck blood, resulting in iron-deficiency anemia of the host. When persons walk over larva-contaminated soil, the larva of filarial form penetrates through the intact barefoot, and causes an allergic skin reaction. Similar to the case of filariform larvae of *Strongyloides stercoralis*, when the larva of the human hookworm infests the human, the skin reaction is mild and no creeping eruptions occur. If humans come into contact with the larva of dog or cat hookworms, *Ancylostoma brasiliense* or *A. caninum*, the larva is unable to complete their migratory cycle to settle in the gut. The zoonotic hookworm larva migrates in the skin, producing snake-like markings and severe itch near the site of entry (mostly on the foot, leg or buttock) (Figure VI-F-1). It is believed that the larva penetrates through the hair follicle or tiny skin cracks. Eruption develops a few days after the larval irritation, and the larva itself is usually located 1-2 cm ahead of the eruption. It is usually very difficult to find the larval body in the biopsy samples, because of its small size (600 μ m in length) and high moving speed (5-20 mm/day). Eosinophilic reaction is evident around the tunnel (larval trail).

Creeping eruption of this type, so-called "ground itch", is common in tropical and subtropical countries and the southeastern states of the United States, but very rare in Japan. All the Japanese patients suffered from the disease abroad. In the endemic areas, direct contact with damp, sandy soil contaminated with infected cat or dog feces makes a risk factor. The nickname of the larva is thus "sand worm". Children have more chances of infestation than adults. Ivermectin treatment is known to be effective. Guimarães, et al. reported the

invasion of a small ancylostomid larva into the hair follicle-associated sebaceous gland, clearly showing double lateral cuticular alae and long esophagus microscopically (Figure VI-F-2). The filariform larva of the sand worm microscopically resembles that of *Strongyloides stercoralis*.

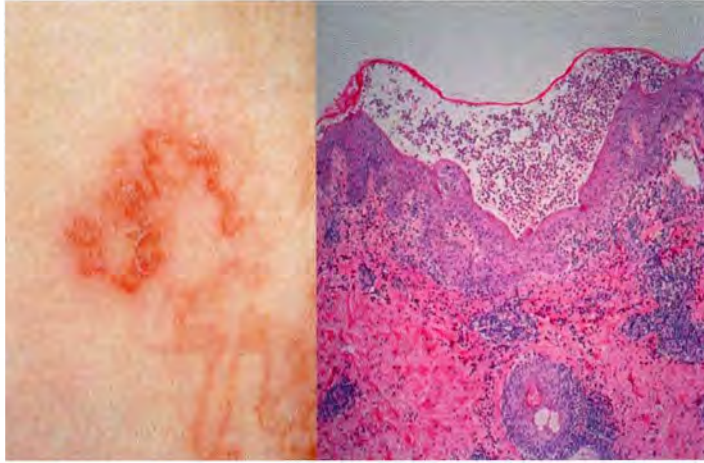


Figure VI-F-1. Creeping disease in imported ancylostomiasis. Skin migration of a zoonotic hookworm larva in Thailand produces snake-like markings on the leg with severe itch (left). Biopsy reveals an intracorneal tract space with active infiltration of eosinophils. It is very difficult to identify the infested larva under a microscope (right: H&E). The courtesy by Dr. Yuko Taniguchi at Kudanzaka Hospital, Tokyo, Japan.

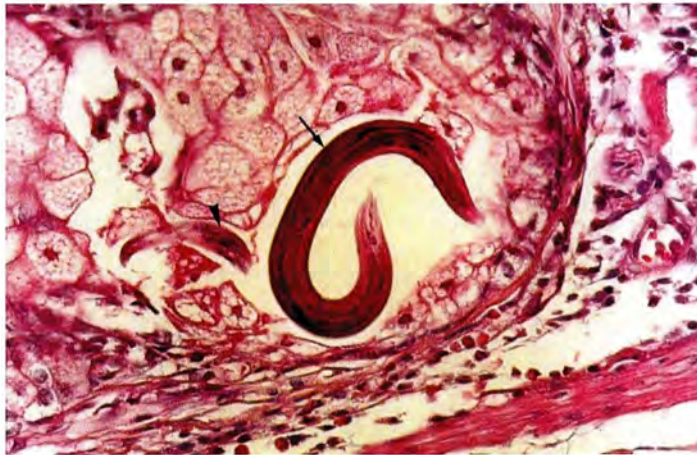


Figure VI-F-2. A small ancylostomid larva in the sebaceous gland on the scalp. Double lateral cuticular alae and long esophagus are observed in the 600 μm -long larva (H&E). The courtesy by Prof. Lucinda C. Guimarães, Universidade Federal do Triângulo Mineiro, Uberaba, Minas Gerais, Brazil.

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G) Paragonimiasis

Paragonimus westermani (lung fluke) parasitizes in a lung cavity to form a worm nodule, lined by an eroded bronchial wall. A coin lesion is seen on a chest X-ray film. In the human, a single parthenogenetic adult worm or paired hermaphrodite worms (7-16 mm long and 4-8 mm wide) reside within a cavity formed by a dilated bronchus. Numerous viable ova are seen in the bronchial wall, in association with marked inflammatory reaction, including eosinophils and Charcot-Leiden's crystals. Sputum cytology demonstrates golden yellow-colored 85 x 50 μ m asymmetric ova of *P. westermani*. An operculum and yolk cells are seen (Figure VI-G-1).

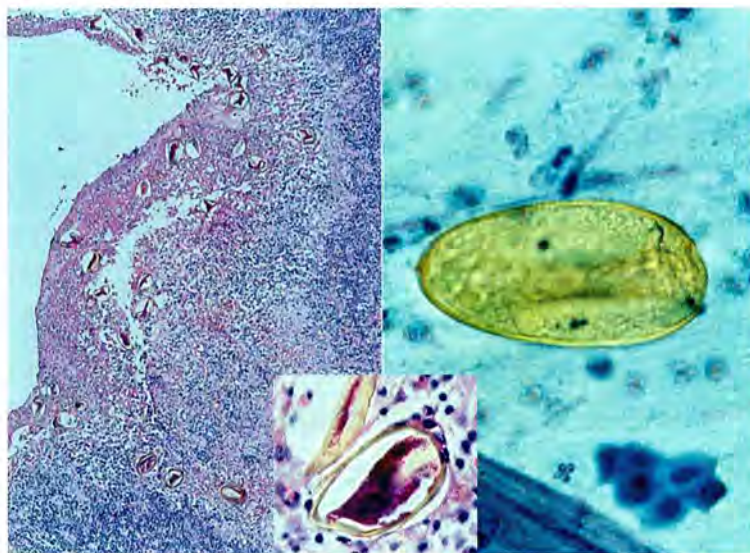


Figure VI-G-1. Pulmonary paragonimiasis. The eroded bronchial wall shows marked inflammatory infiltration. Numbers of ova are seen within the wall (left and inset = high-powered view of viable ova, H&E). Sputum cytology shows a golden yellow-colored asymmetric ovum of *P. westermani*. An operculum and yolk cells are seen in the egg (right: Papanicolaou). Eosinophilic background is noted. The patient ate Japanese freshwater crab half year earlier. The courtesy by Dr. Hideki Yokoo, a pathologist at Kitasato University Medical Center, Kitamoto, Japan (cytology specimen).

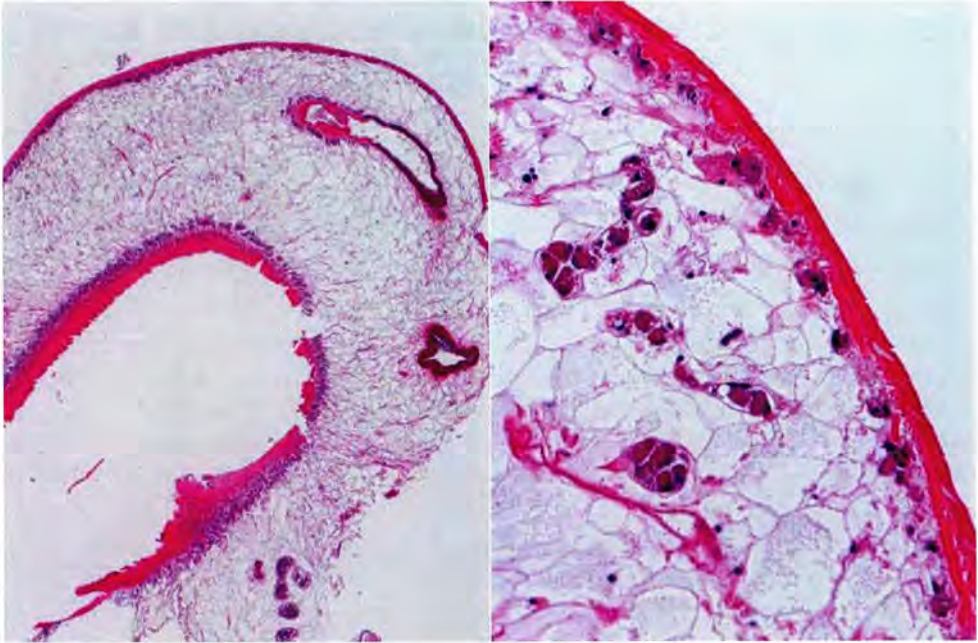


Figure VI-G-2. Cutaneous paragonimiasis. The worm was fragmented by the biopsy procedure. The spiked cuticles, subcuticular muscle cells and vitelline follicles in the spongy matrix are noted. No ova are identified (H&E). The final diagnosis was made by PCR analysis. The courtesy by Dr. Akiko Sumi, a pathologist at Kurume University School of Medicine, Kurume, Japan.

Of importance is to take a history that the patient ate Japanese freshwater crab (*Geothelphusa dehaani*) about half year earlier. It is known that wild pigs may act as a paratenic host, so that history taking for eating fresh meat of wild pigs is also needed. Histologically, the body wall of the fluke consists of the spiked cuticles, subcuticular muscle cells and vitelline follicles in the spongy matrix.

The ingested metacercariae migrate from the small bowel to the lung, through the diaphragm and pleura. Occasionally, visceral or cutaneous larva migrans may occur. Cutaneous larva migrans (creeping disease) is mainly seen on the chest wall.

A representative case of paragonimal cutaneous larva migrans is presented in Figure VI-G-2. In this case, the worm had been fragmented by the biopsy procedure, so that the final diagnosis was confirmed by PCR analysis using DNA extracted from formalin-fixed, paraffin-embedded sections.

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Note 50. Author's advice when you eat sushi or sashimi in Japan

In order for enjoying Japanese cuisine safely, you should know something important, the author believes.

In Japanese "sushi" restaurants, river or freshwater crabs, about 2 cm in size, may be placed next to sliced raw fish or fresh shellfish. In most instances, this merely aims at decoration. Occasionally raw crabs are served. Even when they were already boiled, you should better not eat them, since the shell (carapace) may have been stuck with live metacercariae of *Paragonimus westermani* or *P. miyazakii*, which were spilled out from fresh crab organs onto the cooking table or contaminated knives.

Anisakis simplex larva may be found in any sea fish or squid. The main site of infestation in the sea creature is such internal organs as peritoneum, gills and ovaries of the marine creature, and the larvae are observed within muscles when infestation is heavy in degree. In sushi and sashimi simply slicing the muscles, a thread-like 2 mm-long white-colored larva may be buried. In 2-12 hours after eating sushi and sashimi, acute stomachache happens (gastric anisakiasis). Endoscopic removal of the larva inserted into the gastric mucosa makes the simple and effective treatment. In gastrectomized patients, intestinal anisakiasis provoking obstructive ileus may occur in 24 hours. Adult roundworms parasitize the stomach of the dolphins and whales.

Veteran cooks in Japanese sushi restaurants are well trained not to serve the larva-containing muscles or secretly remove the contaminated larva. In order to avoid infestation, you should select sushi restaurants employing experienced cooks. Or you can destroy the larval body with your teeth by chewing sushi and sashimi well. Many Japanese people do not chew many times as a custom, in order to keep the fresh taste of the material.

Regarding the raw firefly squid from Toyama Bay facing to Japan Sea, occasionally contaminated with spiruroid nematode larva, refer to Chapter VI-D. The risk of infestation of this nematode larva can be avoided when the sea food is once frozen before service. The Japanese people often hesitate to do so, because they believe freezing kills the best taste. Tuna and other fish caught on the open sea far from land are very safe, since they must be kept frozen on the boat.

Sperm bags of mature male squid (cuttlefish) may be stuck to the oral cavity and tongue when squid sashimi is contaminated with the 2 cm-long sperm bag, as is described in Chapter VII-N.

Another risk of the squid includes the larva of *Tentacularea*, *Nybelinia surmenicola* Okada, a tentacled 6-7 mm-long sea creature residing in the internal organs of the squid. This is a plerocercoid of the Cestoda parasitizing the *Squalus* shark and ray. The larva protrudes four horns (suckers) when it parasitizes the host. Numbers of fish and squid are parasitized with the larva of *Tentacularea*, so that sushi and sashimi of horse mackerel and bonito may contain the moving larvae. The traditional Japanese pickled sea food, Shiokara, is made of the salted and chopped whole body of the squid. This Japanese dish may be contaminated with the salt-tolerant, horn-bearing *Tentacularea* larvae. The larvae are not harmful to the human, but there are rare records in which the larvae stuck onto the throat mucosa, causing pain or foreign sensation.

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H) Sparganosis

Sparganosis (*Sparganum mansoni* infestation) is a creeping disease (cutaneous larva migrans) caused by a slowly moving larva (plerocercoid) of *Spirometra erinacei*. Because of its slow movement, linear eruptions are infrequently formed. Instead, subcutaneous nodule formation is common. The long, soft and unsegmented white worm (5 mm to 60 cm long depending upon the stage of development) does not show a cystic change (Figure VI-H-1). The larva survives for years.

The final hosts of *S. erinacei* are the dogs and cats, the first intermediate host is the cyclops (water flea), and a wide range of the vertebrates from the amphibians to mammals, including the human, serve as the second intermediate host. The patients usually have a history of taking the poorly cooked snake (or squeezed viper blood), frog, or wild chicken. An endemic of sparganosis has been recorded in male medical doctors' group in Tokyo, who enjoyed drinking squeezed viper blood for having vigor or vitality.



Figure VI-H-1. Cutaneous sparganosis (I). Formation of the creeping trail is a rare event in sparganosis (left). Instead, a slowly moving subcutaneous nodule is often formed. The excised soft and unsegmented white worm is as long as 5 cm (right). The courtesy by Prof. Hiroyuki Takaoka, Department of Parasitology, Oita University Faculty of Medicine, Yufu, Japan.



Figure VI-H-2. Cutaneous sparganosis (II). Skin incision easily visualizes a migrating white-colored plerocercoid (left). Cut surfaces of the larva located in the dermis through subcutis are characterized by monomorphous internal myxoid matrix surrounded and septated by microvillous tegument. Inflammatory reactions are active (right: H&E). The courtesy by Dr. Koichi Nagakura, Department of Parasitology, Tokai University School of Medicine, Isehara, Japan.

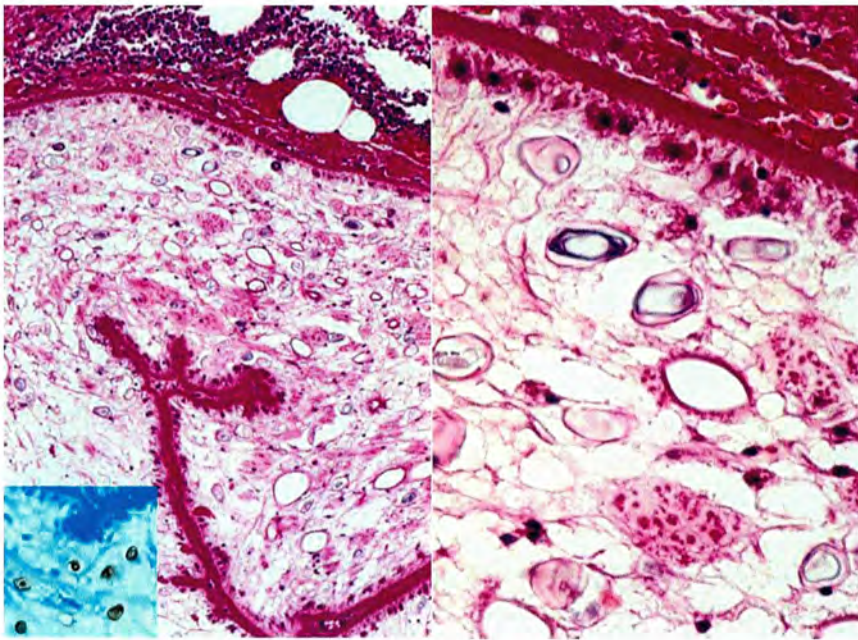


Figure VI-H-3. Cutaneous sparganosis (III). The plerocercoid consists of the folded microvillous tegument, subtegumental spindle cell layer and internal myxoid matrix. Neither gut, gonad nor protoscolex is found. In the myxoid matrix, excretory vesicles, muscular fascicles and calcareous corpuscles are identified. Inset indicates Kossa stain demonstrating calcified substances (calcareous corpuscles).

Moving speed of the plerocercoid is slow, so that the larva can easily be found in the biopsy sample (Figure VI-H-2). The plerocercoid is seen in the dermis or subcutaneous tissue. Lymphocytic reaction and granuloma formation are consistent with the slow moving speed of the larva. Tunnel-like tissue defects (dead spaces) filled with inflammatory exudates represent a trail of plerocercoid movement (larval trail). Eosinophilic reaction is usually mild.

The larva histologically consists of the microvillous tegument, subtegumental spindle cell layer and internal myxoid matrix (Figure VI-H-3). The tegument is folded. Neither gut nor gonad is seen. In contrast to cysticercosis, no protoscolex is found and no cystic change. In the myxoid matrix of the plerocercoid body, excretory vesicles, muscular fascicles and calcareous corpuscles (calcified bodies) are seen (refer to Note 51). The calcareous corpuscles are the hallmark of the *Cestoda* (tapeworm). Kossa stain clearly discloses the calcified material. The matrix is rich in glycogen. The tegument is also PAS-reactive.

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Note 51. Calcareous corpuscles and the Cestoda

Calcareous corpuscles (calcified bodies), spheroid or ovoid layered mineral concretions, are the histologic hallmark of the *Cestoda* species of both adult and larva. They contain abundant calcium carbonate structures, and composed of organic bases coupled with inorganic substances. Their size ranges from several to 50 μm , and heterogeneous layered structure suggest a cyclic process of biomineralization. The function is unclear, but they may buffer anaerobically produced acids and serve as a reservoir for inorganic irons.

References

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I) Cysticercosis

Taenia solium is a cestode (tapeworm) parasitizing the human, and pigs act as an intermediate host. The larva termed cysticercus infects in swine striated muscles. When the human eats poorly cooked pork contaminated with the cystic larva measuring 4-15 mm, it grows within the bowel lumen into the adult tapeworm 2-4 m long, consisting of one scolex, strobila (intervening narrow portion) and a chain of proglottids. The proglottid of *T. solium* lacks the uterine pore, so that the ova are infrequently detected with the stool examination.

Not infrequently, however, the cysticercus invades the human body to form a cystic inclusion(s) in the subcutaneous tissue, brain, eye ball and striated muscle. Such a phenomenon is called paratenesis, and the individual patient serves as a paratenic host. Human cysticercosis is also caused by ingesting raw or unheated vegetables (for example, Chinese cabbage) contaminated with fertilized eggs (containing hexacanth embryo), and in this case multiple cysticercus tend to be formed mainly in the brain (neurocysticercosis). In the brain, a racemose form of cysticercus is seen, showing grape-like clusters with digitiform exogenous buds but without scolex formation. Against the dead worm, an inflammatory reaction is provoked. Korean "kimchi" (hot salted Chinese cabbage) is one of the candidates for the causative food. Cysticercosis is common not only in Asia, but also in Africa, Central America, Mexico, Spain, Portugal and Eastern Europe.

Grossly, a solitary cystic lesion with white-colored, solid mural nodules is seen in the subcutaneous tissue (Figure VI-I-1). The patients are regarded as the paratenic host of *Cysticercus cellulosae* (the second stage larva). The patient has eaten a cysticercus embedded within pork.

Histological examination reveals features of cestode (tapeworm). The body wall is lined by eosinophilic microvillous tegument (cuticular villi or microtrichia) and single-layered subcuticular cells, whereas the myxoid body matrix (parenchyma) contains numerous small vesicles, calcareous corpuscles and excretory ductules. Neither digestive tract nor gonadal structure is seen. In the white-colored mural nodule, a protoscolex, accompanying paired suckers and a rostellum armed with 6 hooklets, is identified (Figure VI-I-2). Outside the cyst, host connective tissue is scarcely inflamed. Host reaction is hardly demonstrated while the cyst remains unruptured.

Distinction from sparganosis (*Sparganum mansoni* infestation), a creeping disease caused by a slowly moving larva (plerocercoid) of *Spirometra erinacei*, is needed. Formation of hooklets and cystic body cavities is characteristic of *Cysticercus cellulosae*. This makes the point different from *Sparganum mansoni*.

Taenia saginata is a 4-10 m long tapeworm of the human. Cows act as the intermediate host. Transmission is mediated by ingesting cysticercus-containing poorly cooked beef. No cysticercosis is provoked in the human body.

In Japan, most cases of adult tapeworm parasitosis show infestation of *Diphyllobothrium* species (particularly, *D. nihonkaiense*), which is mediated by eating raw salmon and trout. *D. nihonkaiense* (meaning the Japan Sea in Japanese) does not provoke malnutrition, in contrast to *D. latum* common in the Scandinavian countries. It is well known that Ms. Maria Callas (1923-1977), a famous American opera (soprano) singer, used a plerocercoid of *D. latum* as the slimming pill.

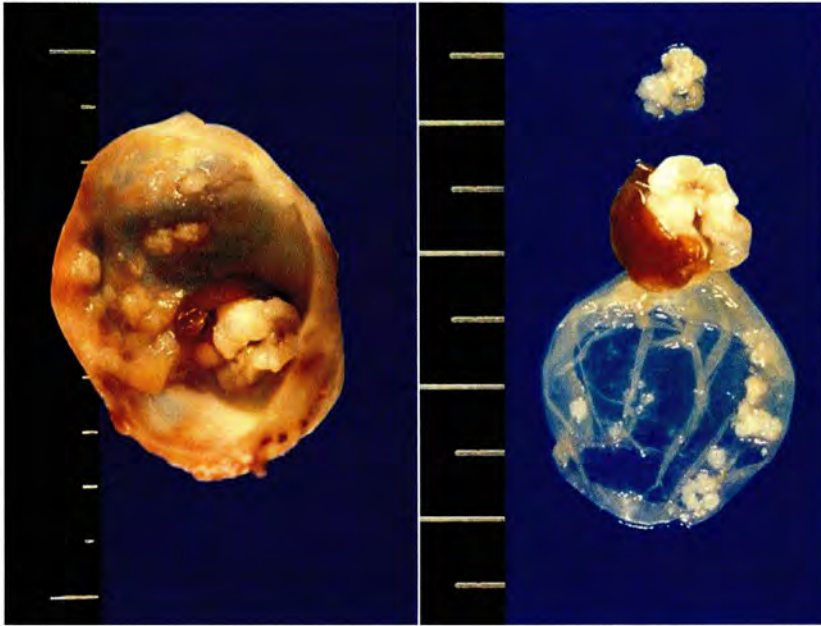


Figure VI-I-1. Cutaneous cysticercosis. A solitary cystic lesion measuring 2.5 cm in diameter was surgically removed from the subcutaneous tissue. White-colored, solid mural nodules are formed on the cyst wall. The cyst-lining content is shown in the right panel.

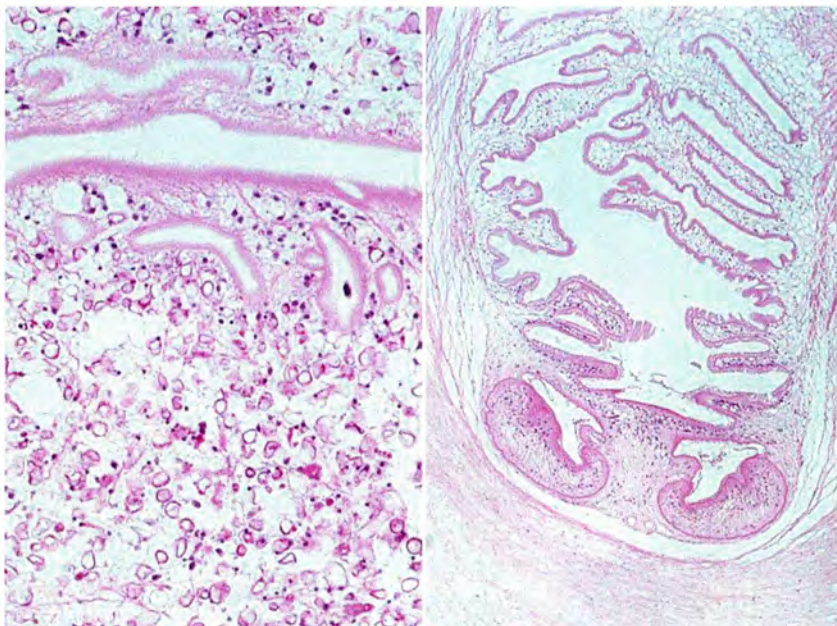


Figure VI-I-2. Histology of cutaneous cysticercosis. The body wall is lined by the microvillous tegument and single-layered subcuticular cells. The myxoid body matrix contains numerous small vesicles, calcareous corpuscles and excretory ductules. Neither digestive tract nor gonadal structure is seen (left: H&E). In the white-colored mural nodule, a protoscolex accompanying paired suckers is identified. On this plane, a rostellum armed with 6 hooklets is not observed (right: H&E).

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J) Dracunculiasis

Dracunculiasis, or guinea worm disease, is caused by a large female of the nematode *Dracunculus medinensis*, which emerges painfully and slowly from the skin of the lower limb. In the 17th century, Europeans saw the disease on the Guinea Coast of West Africa. This is the reason why *Dracunculus* is also called as "Guinea worm". The worm can infect animals, and sustainable animal cycles occur in North America and Central Asia but do not act as reservoirs of human infection.

Dracunculiasis is solely caused by drinking water containing the cyclops, usually from ponds. Just 1 year later, a mature female worm, up to 1 m in size (the biggest nematode), gradually appear out of the ulcerated leg skin at a speed of 2-3 cm/day to provoke severe pain. Grossly, a coiled subcutaneous worm was observed in the leg of a Nigerian boy suffering from dracunculiasis (Figure VI-J-1). Severe pain restricts the labor and results in a big economic damage to the society of the endemic area. Patients enter the water to relieve the pain, when the Guinea worm releases microfilariae into the water. The life cycle between the cyclops and human is very simple.

It has a significant socioeconomic impact because of the temporary disability that it causes. A campaign to eradicate the disease was launched in the 1980s and has made significant progress under the leadership of the Carter Center held by Jimmy Carter, the previous president of the USA. The Carter Center struggles with eradication of this preventable disorder in West Africa. The Carter Center's activities include water supply, health education, well-boring and distribution of the filtration mesh and straw for the people (Figure VI-J-2). Mesh and straw filtrate 1 mm-sized cyclops off, to prevent transmission mechanically.

The World Health Organization regards dracunculiasis as the second target of infectious disease eradication. By the end of 1998, Asia became free of dracunculiasis. Transmission was also interrupted in Cameroon and Senegal during 1997. In 2007, dracunculiasis is confined to 5 countries in Africa (South Sudan, Guiana, Mali, Niger and Nigeria). South Sudan (independent from Sudan in 2011) remains the most endemic country. Only 542 cases were recorded in 2012 and global eradication is within reach.



Figure VI-J-1. Dracunculiasis. A Nigerian boy manifests a coiled and long worm in the subcutaneous tissue of the leg. The worm slowly moves out of his foot. Severe pain restricts the labor and results in a big economic damage to the society of the endemic area. The courtesy by Late Dr. Masamichi Aikawa, Tokai University Medical Research Institute, Isehara, Japan.

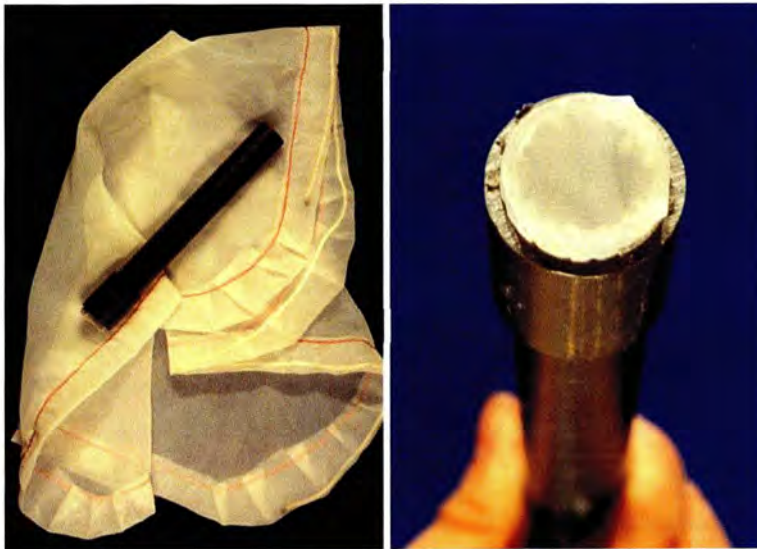


Figure VI-J-2. Mesh and straw used for the prevention of dracunculiasis. The Carter Center supplies mesh and straw to filtrate 1 mm-sized cyclops off the drinking water. These mechanically prevent transmission. The courtesy by Late Dr. Masamichi Aikawa, Isehara.

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Note 52. Asclepius and fiery snake

The logo mark of the World Health Organization represents Asclepius, a god of medicine, and a snake around the stick he holds (Figure VI-J-3). Dracunculiasis was endemic along the Red Sea in the era of the Old Testament. The Guinea worm, *Dracunculus medinensis*, was called "fiery snake". Actually, the snake around the Asclepius' stick is the Guinea worm! It can be supposed that in the Greek era, Mr. Asclepius was especially good at completely removing the whole body of the fiery snake from the infested skin by using a stick. Once the body of the fiery snake was destroyed, the dead parasite would provoke active and serious inflammation.



Figure VI-J-3. The logo mark of the WHO representing Asclepius, a god of medicine, and a snake around the stick he holds. In the Greek era, Mr. Asclepius was supposedly very good at completely removing the whole body of the "fiery snake" by using a stick.

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K) Cercarial Dermatitis

Cercarial dermatitis ("swimmer's itch") is an itchy inflammatory response to the skin penetration of non-human parasitic larvae (cercariae). Avian schistosomes cause cercarial dermatitis but without systemic complications, reflecting the host skin reaction to the destroyed component of the cercariae. It is contracted while swimming or wading in lakes. Dermatitis schistosomica is a transient, local irritant or hypersensitivity reaction during the penetration phase of the cercariae. Prevention of the disease is difficult.

The water snail is the intermediate host of the *Schistosoma*. Cercariae are released from the snail. Cercariae of the avian *Schistosoma*, *Gigantobilharzia sturniae*, which parasitizes the bird "grey starling" provoke dermatitis in human at the site of skin penetration. Another type of cercarial dermatitis is caused by the cercariae of *Trichobilharzia brevis*, which parasitizes mallard. Transmission was mediated by small snail, *Austropeplea ollula*. Itchy hemorrhagic vesicles are formed on both legs along the water line of the rice field, and are recurrent for years in the season of rice planting.

Histologically, biopsy specimen shows allergic skin reactions such as reactive acanthosis with erosions and inflammatory edema with tissue eosinophilia. In cases of repeated infection, hemorrhagic blisters of allergic nature are formed, and a large subepidermal bulla is recognized. It is difficult to identify the cercariae within the biopsied lesion. A representative case is demonstrated in Figure VI-K. A cercaria isolated from small freshwater snail, *Polypylis nitidella*, living in the rice field is shown in the inset. It is of note that skin lesions are hardly seen when cercariae of *Schistosoma* that parasitizes the human invade the skin. Cercarial dermatitis is seen worldwide. The first description was from the state of Michigan, USA. Regarding marine dermatitis or "sea bather's eruption", see Chapter VII-L.



Figure VI-K. Cercarial dermatitis. Itchy hemorrhagic vesicles are formed on both legs along the water line of the rice field (left). Histologically, allergic skin reactions include reactive acanthosis with erosions and inflammatory edema with tissue eosinophilia (right). Inset shows a cercaria isolated from small freshwater snail, *Polypylis nitidella*. The courtesy by Dr. Terutaka Kadosaka, Department of Parasitology, Aichi Medical University School of Medicine, Nagakute, Japan.

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L) Nematode Larva Contamination

Nematode larvae may be incidentally contaminated in the cytologic smear specimens. Numbers of free-living and non-pathogenic small nematode (roundworms) live and grow in the soil. When fertilized eggs in dusty air happen to be inhaled into the airway of healthy individuals, they soon hatch into small larvae. They appear in the sputum cytology, and are morphologically indistinguishable from *Strongyloides stercoralis*, which may be seen in serious and often lethal disseminated strongyloidiasis in immunocompromised hosts, particularly in AIDS patients. Such an incidental contamination is of little clinical significance. Similar incidental findings may also be encountered in urine samples.

Nematodes should not be confused with oligochaetes ("earthworms" or segmented worms). Oligochaetes possess both circular and longitudinal body wall muscles, while nematodes only have longitudinal body wall muscles.

Figure VI-L demonstrates the incidental occurrence of several nematode larvae on the smear preparation sampled from the athlete foot (tinea pedis). In addition to PAS-positive hyphae of fungi, several filariform worms are identified in Giemsa-stained preparations. The specimen might have sampled from soil-contaminated toe lesions.

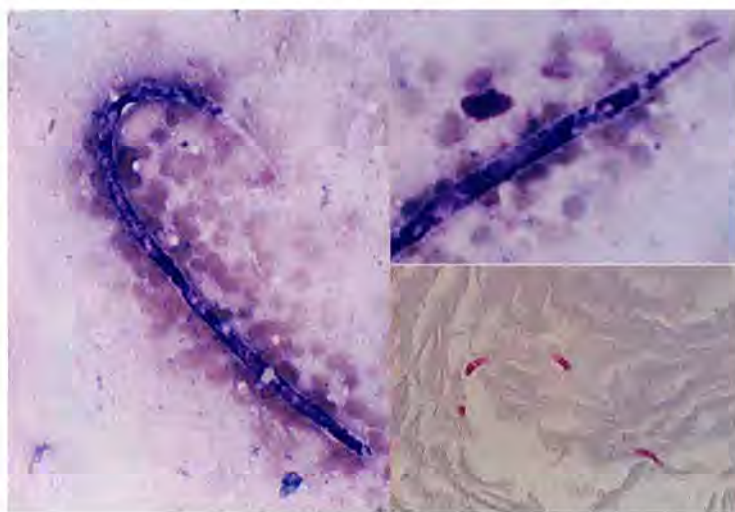


Figure VI-L. Nematode larvae contaminated on a smear prepared from athlete foot. Grossly invisible (300 μ m-long), non-pathogenic nematode larvae are incidentally found on the smear preparation (left and right top: Giemsa). PAS stain identifies fungal hyphae (right bottom). The courtesy by Dr. Keisuke Kikuchi, a pathologist at Obihiro Kosei General Hospital, Obihiro, Japan.

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Arthropod Infestations

There are five major classes of arthropods: a) the Insecta (insects), b) Arachnida (ticks and mites), c) Crustacea (lobsters, crabs and shrimps), d) Dipropoda (millipedes) and e) Chilopoda (centipedes). They may provoke a local reaction at the site of contact or portal of entry. They also act as vectors of bacteria, rickettsiae, chlamydiae, spirochaetae, viruses, protozoa and helminths. Immunological reactions to the parasite or its parts may result in disseminated skin lesions.

A) Scabies

1) Scabies as Nosocomial Infection

Scabies, infection of human itch mite, *Sarcoptes scabiei hominis*, is highly contagious. The female adult mite, measuring 0.3-0.45 mm, has four pairs of legs. It is characterized by severe pruritus, in association with papules, pustules, burrows, nodules and, occasionally, urticarial lesions. Lesions are commonly found on the wrists, finger webs, antecubital region, axillary fossa, areola, periumbilical region, lower abdomen, genitals and buttocks. Itchy papules are most frequently seen on the interphalangeal area, with burrow formation. The mite burrow formation is pathognomonic of scabies. Diagnosis is based on the history taking, physical examination, and demonstration of mites, eggs or scybala (feces) on microscopic examination. The female adult mite, larvae, eggs and pigmented feces are observed in the unstained skin scratch preparation after 20% potassium hydroxide treatment (Figure VII-A-1-1). The mite eats keratinocytes, so that the feces are pigmented by melanosomes and mitochondria.

Histologically, within the cornified layer of the biopsy specimen, the cut surface of the female *Sarcoptes scabiei hominis* with the spiked cuticle and immature and mature eggs is observed. The epidermis is moderately acanthotic and the dermis is actively inflamed. IgE immune response to the mite is demonstrated by immunostaining (Figure VII-A-1-2). Other immunoglobulins, including IgG, IgA, IgM and IgD, are negative on the mite.

Several topical scabicides are available, but permethrin cream appears to be less toxic and more effective in cases that are resistant to other agents. Systemic Ivermectin administration

is quite effective to eradicate the mites. Successful management further requires evaluation of individuals with a close contact with the patients.

Nosocomial infection of scabies is problematic, particularly in the ward of neurosurgery and in the care homes/stations for the aged. Appropriate cleaning is needed for the linen to avoid unnecessary transmission. In-house transmission is also problematic worldwide in nurseries, kindergartens, schools, orphan houses and those places where many children assemble and stay.

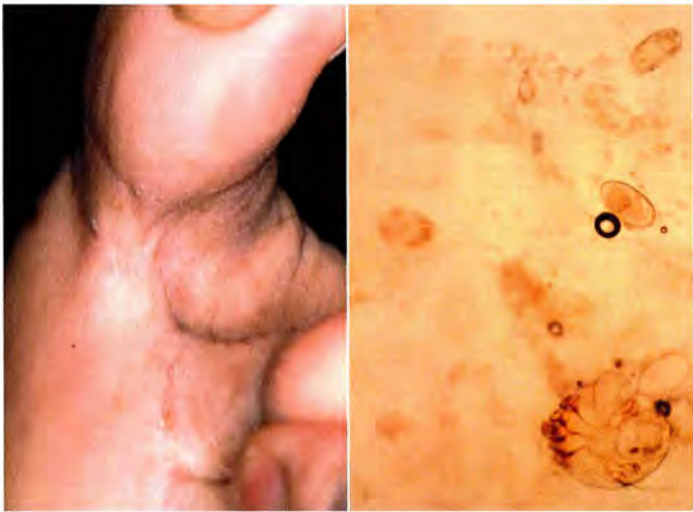


Figure VII-A-1-1. Scabies burrow and 20% KOH-treated scratch preparation. The scabies burrow is prone to be formed in the interphalangeal area (left). On the unstained smear preparation, an adult female mite with four-paired legs, larvae, eggs and brown-colored feces are observed (right). The courtesy by Prof. Emeritus Muneo Ohkido, Department of Dermatology, Tokai University School of Medicine, Isehara, Japan.

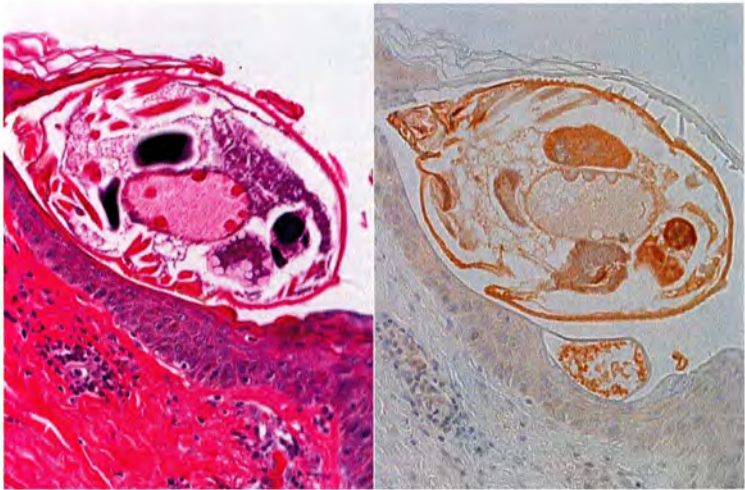


Figure VII-A-1-2. Histology of scabies. A tunnel (burrow) is formed within the cornified layer. A cut surface of female *Sarcoptes scabiei hominis* with the spiked cuticle and immature and mature eggs is observed. The dermis is actively inflamed (left: H&E). IgE deposition is demonstrated on the surface of the mite (right: immunostaining).

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2) Scabies as Sexually Transmitted Infection

Scabies in the young adult may belong to one of the sexually transmitted infections. The scrotal skin is the major site of infection. Multiple itchy papules and vesicles are seen on the scrotum (Figure VII-A-2).



Figure VII-A-2. Scabies as a sexually transmitted infection: presentation of 2 cases. Multiple itchy papules and vesicles are observed on the penile shaft and scrotum. Scabies in the young adults should be regarded as an STI.

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3) Crusted Scabies (Norwegian Scabies)

Crusted scabies or Norwegian scabies is a severe form of the mite infestation. Dirty gray-colored, oyster shell-like scaled skin lesions are seen, particularly on the head, ears, extremities, joint surfaces and trunk (Figure VII-A-3-1). Crusted scabies is highly contagious, and overwhelming numbers (millions) of mites can be proven in the hyperkeratotic skin lesions (Figure VII-A-3-2). In the unstained skin scratch preparation after potassium hydroxide treatment, numbers of adult female mites with four paired legs are observed, together with larvae, eggs and feces. A delay of the diagnosis inevitably results in nosocomial infection of numbers of nurses and residents in the hospital. Cleaning of the used linen in hot water ($>50^{\circ}\text{C}$) is important to avoid unnecessary transmission. The dermis is moderately inflamed. Immunostaining for IgE shows deposition of IgE on the surface of the mite. An allergic disease nature is suspected.

A special form of crusted scabies is nail scabies, clinically resembling onychomycosis. The distal subungual area can represent a reservoir of mites. The involved dystrophic nails are hyperkeratotic and accompanied by psoriasis-like accumulations of the scales under the nails (Figure VII-A-3-3). Even after successful treatment of the hyperkeratotic lesions on the other parts of the skin, the dystrophic nails persist. The mites survive in the subungual material and are a source of reinfestation.

Crusted scabies should be suspected in any atypical itching (or non-itching) rash in AIDS patients. Attempt must be taken to make a diagnosis by skin scraping, or if negative, the skin biopsy. Therapy requires the sequential use of scabicides (ivermectin), usually over a longer period than required to clear an ordinary case of scabies.



Figure VII-A-3-1. Crusted scabies. Gray-colored, oyster shell-like scaled skin is characteristic. In this case (a Japanese old female), the delay of the diagnosis resulted in nosocomial infections among nurses and residents.

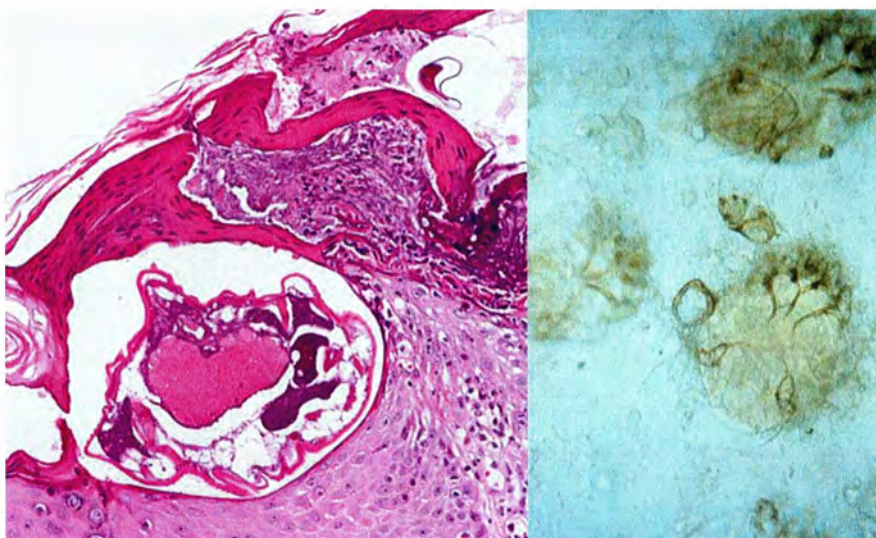


Figure VII-A-3-2. Biopsy and scratch specimens from crusted scabies. Adult female mites and ova are easily observed in the hyperparakeratotic layer (left: H&E). KOH-treated unstained scratch preparation demonstrates numerous mites under the microscope (right). The lesion is highly contagious.



Figure VII-A-3-3. Nail scabies. The distal subungual area is heavily infested with mites. The involved dystrophic nails are hyperkeratotic and accompanied by psoriasis-like accumulations of scales under the nails. The courtesy by Dr. Noriko Ohtaki, Department of Dermatology, Kudanshita Hospital, Tokyo.

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B) Demodicosis

Demodex folliculorum and *D. brevis* are normal flora (common inhabitants) in the hair follicle associated with well-developed sebaceous glands, particularly on the facial skin. Horizontal contact transmission of the mites happens in infancy, when his/her parents or grandparents hug the baby to make a close face-to-face contact. The mites may provoke acne demodecica or worsen rosacea when secondary bacterial infection occurs. After the use of steroid ointment, the number of mites increases significantly. Granulomatous reaction may be associated with *Demodex* infestation.

D. folliculorum, 0.3 mm in length, resides mainly in the outlet (infundibulum) of the hair follicle distal to the sebaceous gland junction, while *D. brevis*, 0.2 mm in size, is seen mainly within the sebaceous gland lumen. They are rich in glycogen, and possess four pairs of small legs and basophilic granular material in the body. The mites are frequently found in the pilosebaceous unit in the biopsy specimen of the facial skin (Figures VII-B-1 and VII-B-2).

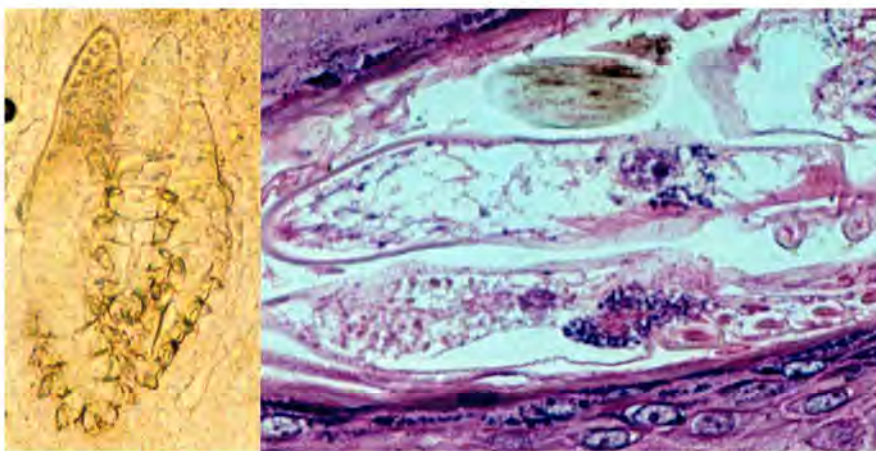


Figure VII-B-1. *Demodex* as a normal flora in the pilosebaceous unit on the face. Unstained scratch preparation demonstrates three mites with four-paired legs (left). Mites with four-paired legs are also observed in H&E preparation (right).

The author happened to observe specific binding of IgD and serum protease inhibitors, such as α -1 antitrypsin (AAT) and α -1 antichymotrypsin (AACT), on the mites residing within the hair follicle (Figure VII-B-3). IgG, IgA, IgM and IgE are negative. Allegedly, IgD is susceptible to trypsin-like proteases. The consistent demonstration of AAT and AACT on the mites may suggest a protective function of these serum protease inhibitors to avoid proteolytic degradation of IgD molecules. On the other hand, it has been clarified that trypsin-like proteases are produced by a variety of mites. The mite-derived proteases are highly immunogenic and become asthmatic allergens in case of house dust mites. The protease-mediated activation of the kallikrein-kinin system is inhibited by soybean trypsin inhibitors.

Therefore, AAT and AACT on the mites may function as a protective host response to the mite infestation. Although the pathophysiologic roles of such plasma proteins remain unsettled, the findings may suggest a novel functional aspect of IgD and the serum protease inhibitors.

Demodex folliculorum folliculitis may lead to a pruritic papular eruption on the face and the upper part of the trunk in patients with AIDS.

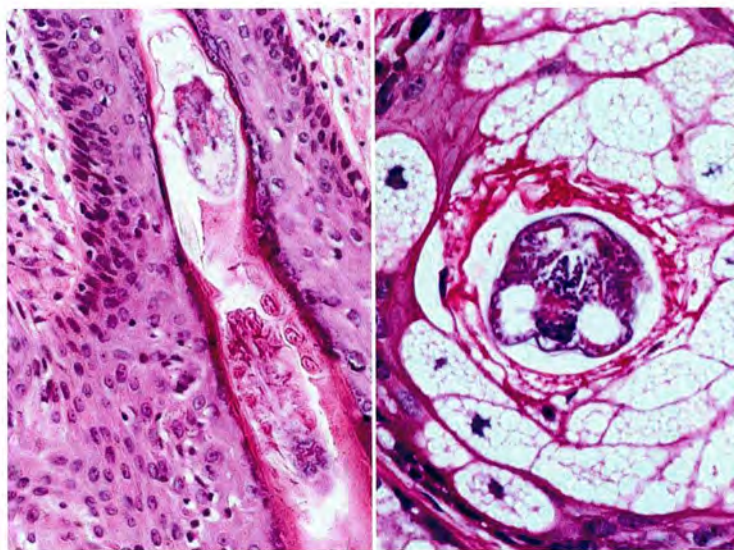


Figure VII-B-2. Two types of *Demodex*: *D. folliculorum* versus *D. brevis*. In the outlet of the hair follicle, *D. folliculorum* often resides (left), while the deeply situated mites among the sebaceous gland belong to *D. brevis*.

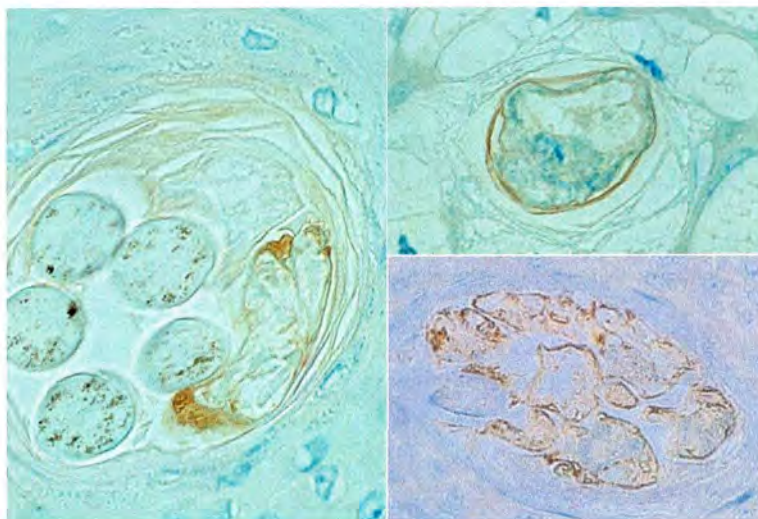


Figure VII-B-3. IgD and alpha-1 antitrypsin on *Demodex* mites in the hair follicle. IgD immunoreactivity is consistently deposited on the surface of the mites (left: *D. folliculorum*, right top: *D. brevis*). Immunoreactive alpha-1 antitrypsin and alpha-1 antichymotrypsin, representative serum protease inhibitors, co-localize with IgD on the mite surface (right bottom: immunostaining for alpha-1 antitrypsin). The findings suggest a unique host response to the mites.

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C) Tick Bite

Mites and ticks, a group of invertebrates (arachnid) related to spiders and collectively called as Acari or Acarina, are not categorized in the insect (without separation of the head, thorax and abdomen). More than 20,000 kinds of mites and ticks are recorded. Mites are 0.4-3 mm in length, while ticks are 3-30 mm. Mites are seen both on the land and in the water, while ticks, bloodsucking parasites, live on the land, primarily in woods and pastures. Some carry disease-producing microorganisms, which they transmit to the mammals, birds and reptiles. Most of them are harmless to the human.

Ticks are divided into two families: hard ticks (*Ixodidae*) and soft ticks (*Argasidae*). The presence of the scutum or hard shield characterizes the hard ticks. Direct injuries to humans and animals may be serious, and are mainly observed in infestations with ixodid hard ticks but also in those with some argasid soft ticks as *Ornithodoros* species. In both, 3 stages of development are known: the larvae, nymphs and adults. The larvae have 3 pairs of legs, while the nymphs and adults have 4 pairs.

Ixodid ticks require 3 hosts (small animals/birds for the larva, medium-sized animals for the nymph and large animals for the adults), and their life cycle takes at least one year to complete. In contrast, nymphs of the argasid soft ticks go through as many as seven instars, each requiring a blood meal. The adult female of the soft tick feed several times with the duration of a few hours, and this feeding style is in sharp contrast to the hard tick feeding just once for a long period up to 6-10 days. During feeding of the soft ticks, excess fluid is excreted by the coxal glands.

Ticks need energy from the blood in order to grow, develop and lay eggs. Ticks find the hosts by detecting animals' breath and body odor, or by sensing body heat, moisture and vibration. They can not fly or jump, and thus ticks must wait in a "questing" position, holding onto leaves and grass by their 3rd and 4th pair of legs. Piercing mouthparts are adapted for sucking juices from plants and for penetrating the animal skin. When a tick or mite bites, an

anchoring structure in the head holds firmly to the wound. In the mite this structure is smooth, and the mite is easily brushed off. In contrast, the tick has a toothed structure, so that the mechanical removal of the entire tick may be difficult.

Unlike hard ticks, adult soft ticks (*Ornithodoros*) live for many years, and they can survive for long periods in a fasting state. In fact, *Ornithodoros turicata* ticks are known to transmit *Borrelia* spirochetes in the laboratory setting after 7 years without a blood meal!

The ticks, particularly hard ticks, transmit the following tick-borne disorders: Spotted fevers (Rocky Mountain spotted fever, Japanese spotted fever, etc), ehrlichiosis, Q fever, Russian tick encephalitis, Crimean-Congo hemorrhagic fever, tularemia, relapsing fever, Lyme borreliosis, and babesiosis.

Recently, a novel and severe tick-mediated viral infection named severe fever with thrombocytopenia syndrome (SFTS) has been reported in China, Korea and western Japan. SFTS virus belongs to phlebovirus, a single-stranded RNA virus, in the family of bunyavirus (Crimian-Congo hemorrhagic fever virus and Rift Valley fever virus are also categorized in the bunyavirus). The disease is transmitted by the tick, likely *Haemaphysalis* spp.

Regional lymph node swelling with massive necrosis is observed in this lethal condition. Hypercytokinemia with hemophagocytic syndrome plays an important role in the lethal pathogenesis. Severe suppression of neutrophils may provoke *Aspergillus* infection in the lung. The endemic areas in Japan are similar to those of Japanese spotted fever, since the vectors are common. Treatment strategy for Japanese spotted fever, Minomycin/doxycycline plus new quinolone, is effective against SFTS, because the combined use of the drugs effectively suppresses hypercytokinemia.

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Note 53. Ticks and Oral Sex

Tick investigators commonly collect many ticks with the “tick dragging” method using cotton-made white flags and keep them to carry in a small glass tube. Paired adult *Ixodes* ticks may soon mate in the tube. A male tick inserts the sperm bag (spermatophore) into the female genital aperture with his mouth (hypostome).

The male tick does not have a penis, so that he should enjoy oral sex. Since the female genital aperture is open on the ventral surface, so that the pair mates in a face-to-face position (Figure VII-C-0). In non-*Ixodes* species, the adult male tick must suck the blood of an animal before the copulation, but the male of *Ixodes* tick challenges the sexual behavior without sucking blood. The coitus will last for 1 hour. The male tick soon dies after mating. The female *Ixodes* tick sucks blood after or during the copulation, and the maximal blood-sucking for a week is necessary for the female to lay eggs for up to 20 days period. The female dies after laying thousands of eggs.



Figure VII-C-0. Mating paired ticks. Just after collection of the paired adult ticks *Ixodes persulcatus* on the field in Wakkanai, the northernmost part of Hokkaido, they soon mate within a small glass tube. The smaller-sized male tick (the top one) uses his mouth to insert the sperm bag into the female genital aperture. The paired face-to-face position is kept for 1 hour.

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1) Gross and Microscopic Findings of the Tick

Tick bites usually provoke no pain or itchy sensation. After full blood-sucking in 6-10 days, the adult tick becomes swollen up to 2 cm in size and reddish-colored. Figure VII-C-1-1

illustrates gross features of ticks sucking the patients or after the completion of blood-sucking. *Ixodes ovatus* often parasitizes the eye lid. A blood-sucking nymph of *Haemaphysalis flava* and an adult tick of *Ixodes turdus* are seen on the abdominal skin. *Amblyomma testudinarium* spontaneously detached from the perineal skin because of the completion of blood-sucking is also illustrated. Adult *Amblyomma testudinarium* likes the external genitalia and perianal region to be stuck. Some say *A. testudinarium* as an erotic tick. The patients often regard the tick as a “tumor”, because there is little foreign sensation.

Microscopic appearance is illustrated in Figures VII-C-1-2 through VII-C-1-5. The paired hypostomes of the gnathosoma (jaw portion of the tick) are inserted into the dermis, with formation of cement-like material at the ulcer base. In the gnathosoma, well-developed striated muscles and salivary gland are observed. The gut lumen is filled with sucked blood. The exoskeleton is birefringent and poorly stained. After forced removal of the tick, ulceration with purulent exudation is formed. A remaining hypostome is embedded in the eosinophilic cement material at the ulcer base. The cement material is Luxol fast blue-positive (rich in phospholipid component).

Around the cement material at the base of ulcer, coccal infection is frequently observed. The cocci are Gram-positive, and secondary staphylococcal infection is indicated. Inflammatory reaction remains mild. It has been reported that ticks secrete salp 15 and salp 16 to control and govern the host cellular response. Dr. Satoru Konnai, Department of Disease Control, Hokkaido University Graduate School of Veterinary Medicine, Sapporo, Japan, found that salp 16 suppresses neutrophil functions (personal communication).



Figure VII-C-1-1. Gross appearance of ticks and tick bites. Nymph of *Haemaphysalis flava* on the abdominal skin (left top), moving adult of *Amblyomma testudinarium* spontaneously detached from the anal skin (right top), adult of *Ixodes turdus* on the abdominal wall (left bottom) and adult of *Ixodes ovatus* on the eye lid (right bottom). The courtesy by Dr. Fumihiko Mahara, Mahara Clinic, Anan, Tokushima, Japan.

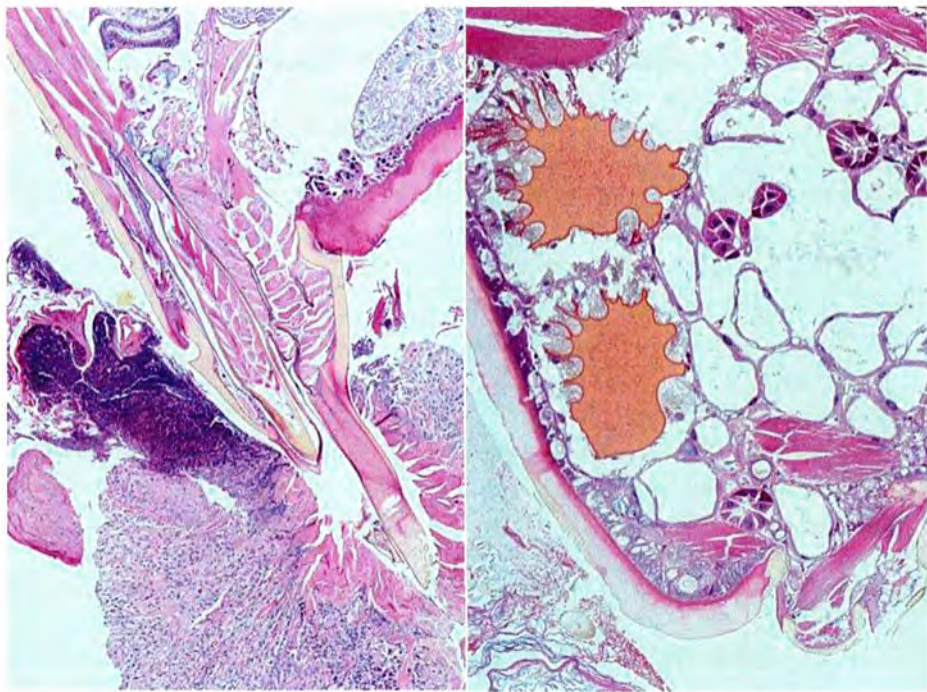


Figure VII-C-1-2. Histology of tick bite (I). The paired hypostomes of the gnathosoma (jaw portion of the tick), inserted into the dermis, are supported by cement material at the ulcer base (left: H&E). In the gnathosoma, well-developed striated muscles and salivary gland are observed. The gut lumen is filled with the host blood. Spiracles, consisting of a respiratory system of the insects and arachnids, are dispersed. The exoskeleton is birefringent and poorly stained (right: H&E).

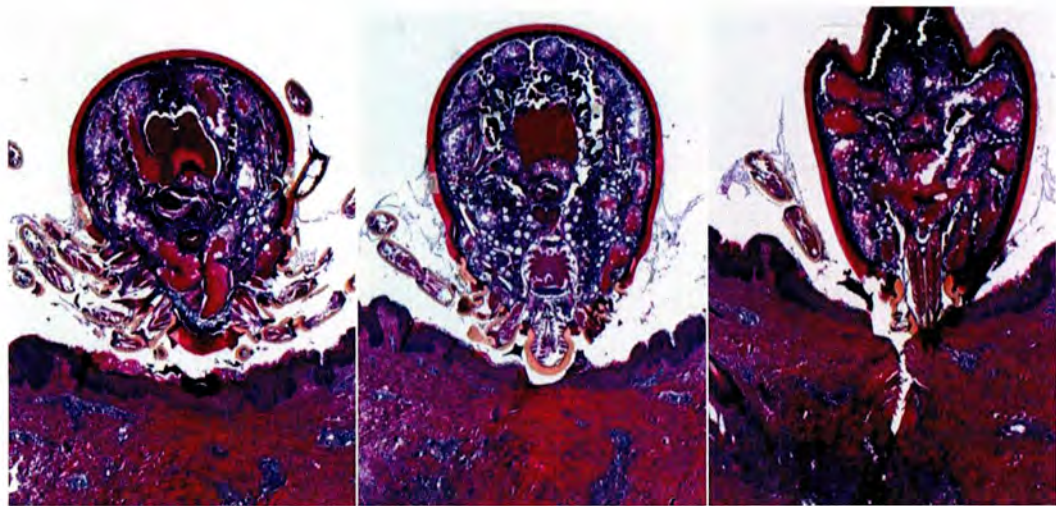


Figure VII-C-1-3. Histology of tick bite (II). Step sections of an adult *Ixodes* tick on the back reveal four-paired legs (left), well-developed striated muscles in the gnathosoma (center) and paired hypostomes stuck into the dermis (right: H&E). Note that the brain directly governs a) the striated muscles of the legs by radiating peripheral nerve fibers and b) the hypostomes by direct special continuity (center panel).

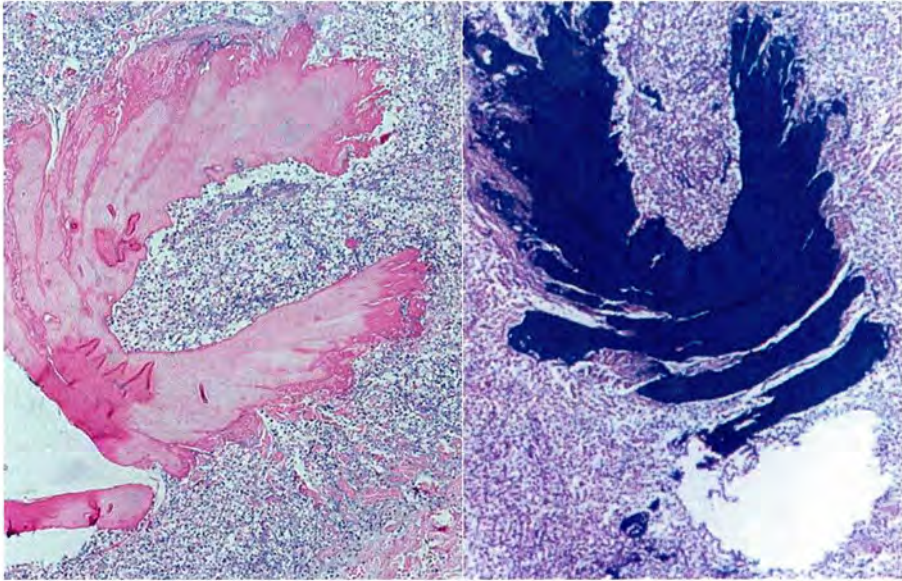


Figure VII-C-1-4. Intradermal cement material at the base of tick bite. Eosinophilic, lamellated cement material is a secretion from tick salivary gland to fix the hypostomes of the tick in the dermis (left: H&E). It contains phospholipid so as to be stained with Luxol-fast blue dye (right: LFB-H&E stain).

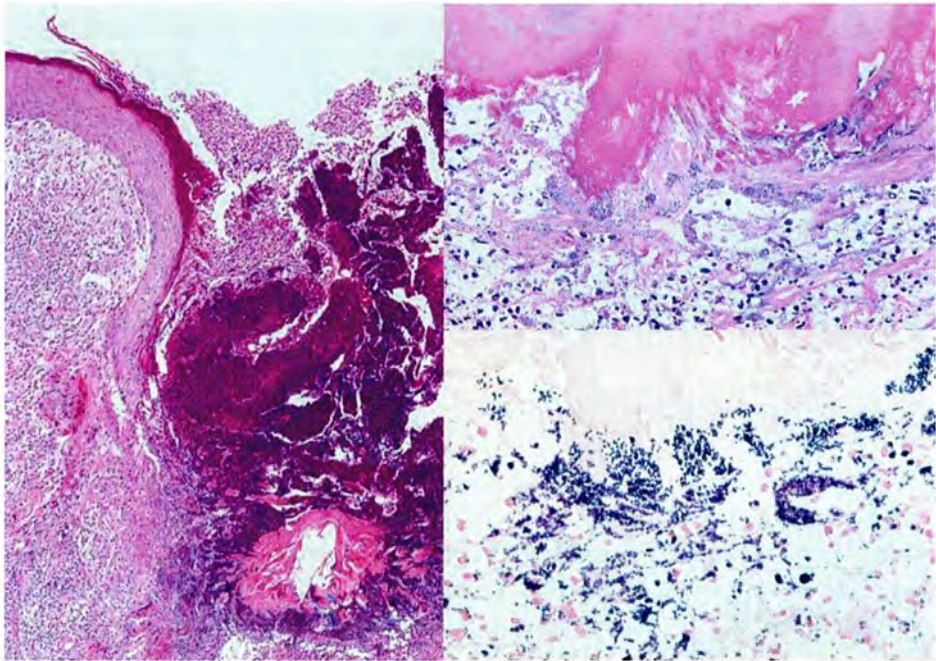


Figure VII-C-1-5. Secondary staphylococcal colonization around the cement material. When the tick was forcibly removed, part of the hypostome embedded and fixed in the cement material remains at the ulcer base. The hypostome appears transparent and birefringent (left: H&E). Staphylococci secondarily colonize around the cement material, but with minimal neutrophilic reactions (right top: H&E, right bottom: Gram).

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Note 54. Madanins, tick-derived anticoagulants

It is known that ticks produce anti-inflammatory substances and anticoagulants, in order to continue blood sucking for up to 10 days. Animals or humans should never be aware of tick biting, and blood must not be coagulated. In 2003, novel antithrombin molecules, madanin 1 and 2, were identified from the salivary gland of the ixodidae tick, by a Japanese research group. Madanins are 7 kDa proteins, and named after a Japanese word “Madani” which means tick. Madanins are actively secreted from the salivary gland of the tick. Importantly, antibodies against madanins are not detected in the patients’ sera. Namely, madanins escape the host immune response. This fact may allow us to use madanins as a clinically applicable anticoagulant.

Regarding hirudin, an anticoagulant isolated from the leech, refer to Note 55.

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2) Tick-Associated Rash Illness (TARI)

Tick bite occasionally provokes tick-centered skin eruption (Figure VII-C-2-1), but without other signs and symptoms. Repeated tick bites may provoke itchy local skin reactions with vesicle formation. Allergic nature has been suspected as an etiological factor, like in other types of insect bite or sting. It is known that the bite by soft ticks, *Ornithodoros savignyi* and *O. coriaceus*, may cause active and painful local skin reactions, including vesicles, pustules and ulceration (see Chapter VII-C-3).

Occasionally, we encounter a centrifugally spreading erythematous lesion, pseudo-Lyme dermatosis, at the site of tick bite, clinically resembling Lyme borreliosis. Lyme disease is provoked by *Borrelia burgdorferi*, which grows in and transmitted by *Ixodes scapularis* (refer to Chapter II-CC-2). In 2008, Blanton et al. proposed the concept of “southern tick-associated

rash illness (STARI)", in which *Borrelia*-unrelated Lyme-like erythema migrans is observed at the site of *Amblyomma* tick bite. No pathogens have been identified in the STARI lesion.

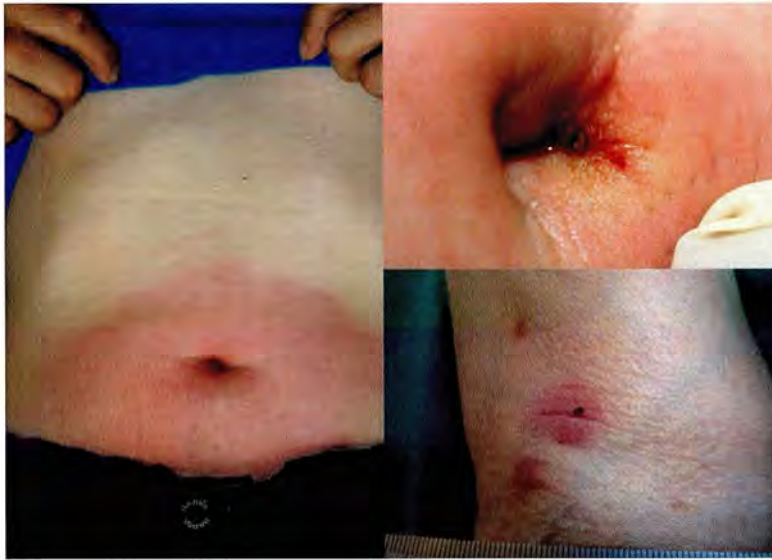


Figure VII-C-2-1. Tick-centered skin eruptions: presentation of 3 cases. Tick bites may provoke tick-centered skin eruptions. On the left panel, pseudo-Lyme dermatosis or TARI showing centrifugally spreading eruption is presented. Allergic nature has been suspected, but infectious etiology should also be investigated. The courtesy by Dr. Yuji Morita, Myojin Clinic, Kozagawa, Wakayama (left) and by Dr. Fumihiko Mahara, Mahara Clinic, Anan, Tokushima, Japan (right).

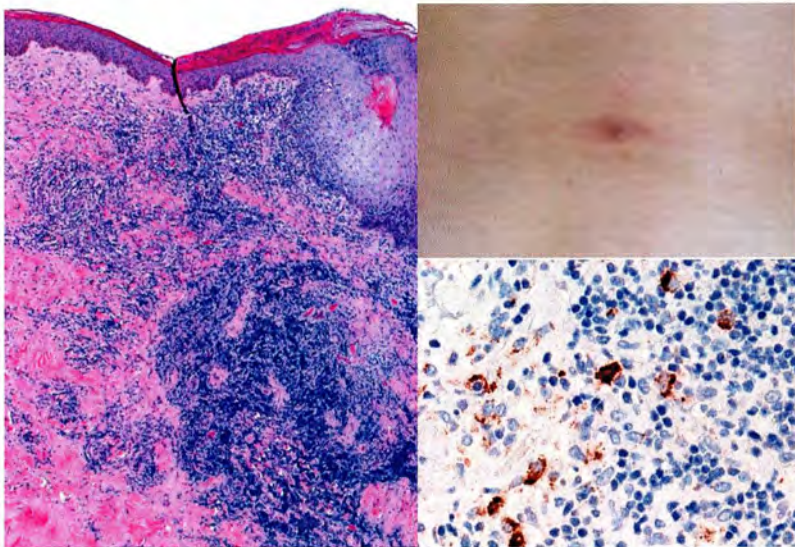


Figure VII-C-2-2. Probable infectious etiology of the tick-centered eruption. Biopsy from the tick bite lesion (right top) shows reactive acanthosis and dense infiltration of lymphocytes throughout the dermis (left: H&E). Immunostaining using a monoclonal antibody S3 against the antigen common to spotted fever group rickettsiae (or the alkali-extracted APS antigen) reveals granular positivity in the cytoplasm of endothelial cells and macrophages (right bottom). The patient accompanied fever but no systemic eruptions. The possibility of Japanese spotted fever was serologically and clinically denied in this case.

In 2013, Natsuaki et al. described the revised concept TARI (“tick-associated rash illness”), since they found similar conditions in Japanese cases, in which the causative ticks belong to *Amblyomma testudinarium* and even *Ixodes persulcatus*. They propose an allergic mechanism for the centrifugally migrating erythema.

Under the suspicion of a microbial (infectious) etiology, particularly in case of pseudo-Lyme dermatosis or TARI, we have tried to identify rickettsial antigens and DNA in the skin biopsy specimens by using both the immunoperoxidase staining and real-time PCR technique.

Some showed positivity (Tsutsumi, unpublished observation), so that further investigations are needed to clarify the possibility of localized skin infection of spotted fever group rickettsia of low virulence, including *Rickettsia tamurae*, as the mechanism of the tick bite eruptions (Figure VII-C-2-2). Refer to Chapter II-EE-2. The infectious lesion may be associated with fever, but without systemic rash (Dr. Yuji Morita at Myojin Clinic, Kozagawa, Japan: personal communication).

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3) Australian Tick Bite Paralysis and Venomous Tick Bites

Australian tick bite paralysis is caused by *Ixodes holocyclus* (Figure VII-C-3). In a Japanese lady presented here, a tick was stuck on the scalp during a trip in Queensland State, Australia. *Ixodes holocyclus* is known to secrete strong paralytic neurotoxin termed holocyclotoxin. Two days after surgical resection of the tick, erythematous edema around the bite site and right facial palsy appeared. The patient completely recovered in one month. Removal of the tick allegedly induces and accelerates the paralytic symptoms in 1-2 days.

It is known that more than 40 tick species may produce neurotoxins and hard ticks such as *Dermacentor* and *Amblyomma* may cause tick paralysis to humans. Actually, cattle, sheep and pet animals are the main victims of these venomous ticks. In severe cases, respiratory muscle paralysis may occur. Bite periods for several days are needed for the ticks to secrete neurotoxins into the saliva.

Ornithodoros savignyi ("sand tampan") is a soft tick species common in the semi-desert regions of North and East Africa, the Middle East, India and Sri Lanka. When adult ticks attack deer, cattle and humans, an irritating and sometimes toxic bite happens, accompanying painful local swelling with formation of bullae, pustules and ulcers. Tampans paralyze and kill the livestock, especially young calves and lambs, through secretion of toxic agents during feeding, and the condition is called sand tampan toxicosis.

O. coriaceus ("pajaroello tick") is observed in deer beds under trees and near large rocks in northern California, Nevada and Mexico. The bite irritates deer, cattle and humans, accompanying a severe skin reaction. *O. guernei* shelters in tree-shaded soil in arid zones of Australia where kangaroos and humans rest.

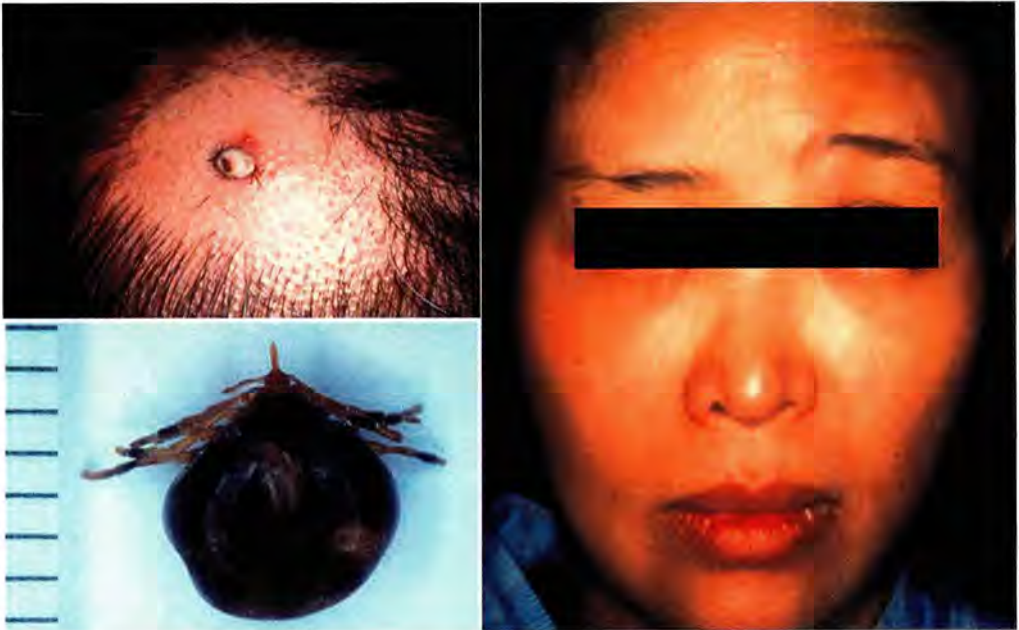


Figure VII-C-3. Australian tick bite paralysis. An adult tick was stuck on the scalp during a trip in Queensland State, Australia (left top). The tick was identified as *Ixodes holocyclus* (left bottom). Two days after surgical removal of the tick, erythematous edema around the bite site and right facial palsy appeared (right). The courtesy by Prof. Kayoko Matsunaga, Department of Dermatology, Fujita Health University School of Medicine, Toyooka, Japan.

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D) Mites and Disorders

Representative mite-borne infections are Tsutsugamushi disease and scrub typhus (Chapter II-EE-1). Scabies is an infective disease caused by the pathogenic mite (Chapter VII-A). *Demodex* is a normal skin inhabitant (Chapter VII-B). It should be cautious that the incidence of pruritic rat mite dermatitis is increasing in western countries and in Japan. Bites by tropical rat mites, *Ornithonyssus bacoti*, provoke severe itching and papular urticaria, lasting for 2 weeks. It is of note that pet rats may suffer from the zoonotic mites.

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1) Mite Contamination in Cytology and Pathology Specimens

Mites may be contaminated in cytology and pathology specimens. In Papanicolaou-stained cytology specimens, house dust mites, 0.1-0.2 mm in length, are occasionally demonstrated. They come from the air dust. Short legs can be observed. When one drops a biopsy or surgical specimen onto the house dust at the corner of a drawer, the specimen surface would be fully attached with dust mites. The contamination is clearly and surprisingly revealed in H&E-stained histologic sections. Representative features are shown in Figure VII-D-1.

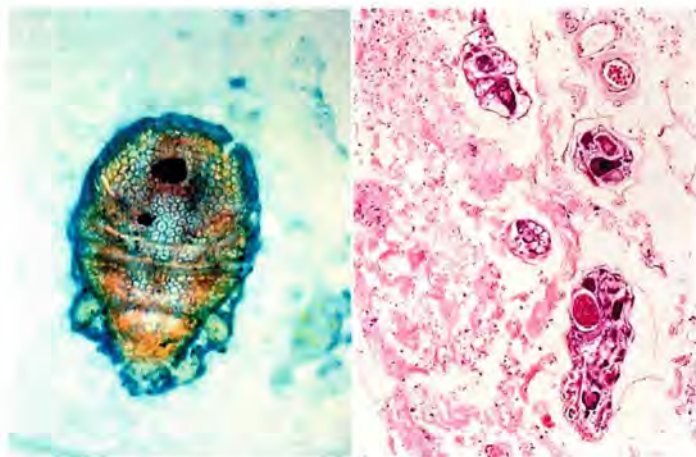


Figure VII-D-1. Mite contamination in cytology and pathology specimens. A four pair-legged mite is contaminated on a Papanicolaou-stained cervical smear preparation (left). House dust mites are heavily attached onto the gastric biopsy specimen after incidental drop into the house dust at the corner of a drawer (right: H&E).

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2) House Dust Allergens

Dust mites (or bed mites) are the most common allergens in the house dust. The 8-legged creatures, around 140 μm in size, multiply easily in warm and humid places. They are most frequently found in pillows, mattresses, carpets and upholstered furniture. The European house dust mite (*Dermatophagoides pteronyssinus*) and the American house dust mite (*Dermatophagoides farinae*) comprise two major causative species. Patients allergic to these dust mites react to proteins within the bodies and feces of the mites. The major allergens are called Der-P1 and Der-P2, as well as Der-F1 and Der-F2. Allergic rhinoconjunctivitis caused by dust exposure provokes a runny or stuffy nose, itchy and watery eyes and sneezing. House dust exposure can also trigger asthma symptoms. Scanning electron micrographs of *D. farinae* are shown in Figure VII-D-2.



Figure VII-D-2. Scanning electron micrographs of *Dermatophagoides farinae*. The mite's body is covered with thick cuticles, and consists of three parts: gnathosoma (head region), propodosma (carrying the 1st and 2nd legs) and hysterosoma (locating the 3rd and 4th legs). Dust mites produced 10-20 particles of feces. Mature female mites lay 1-2 eggs per day. It takes 1 month to become an adult, and the adult mites can live up to 2 months. The dead bodies and fecal pellets can trigger allergic responses.

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E) Myiasis

1) Furuncular Myiasis

Myiasis, infestation of fly larvae (maggots) to humans or other vertebrates, may appear as furuncular nodules. The maggot of fly (myia in Greek) causes furuncular myiasis, and the nodule is called "warble". Furuncular myiasis occurring in the Western hemisphere, especially in Central and South America, is caused by *Dermatobia hominis* infestation. In Africa, *Cordylobia anthropophaga* is a causative agent, and in the Middle East, *Wohlfahrtia magnifica* is pathogenic. Travel history, gross examination, and biologic evaluation of the removed fly larvae lead to identifying the causative organism. Presentation of "flesh-eating maggot" infestation is called "specific myiasis". The fly causing "specific myiasis" such as *D. hominis* and *C. anthropophaga*, is not distributed in Japan. Therefore, furuncular myiasis is categorized in an imported infectious disease.

A painful, cratered skin nodule is noted on the elbow of a Japanese male who stayed in Brazil. Marked inflammatory reaction is seen around the intradermal cavity. Histology of the head portion of the maggot obtained from the cavity shows the gnathosoma with birefringent hypostome, well-developed striated muscles and spiculated cuticles. The body portion of the maggot shows the hyaline cuticles, striated muscles, fat bodies, food channel and spiracles (Figure VII-E-1-1).

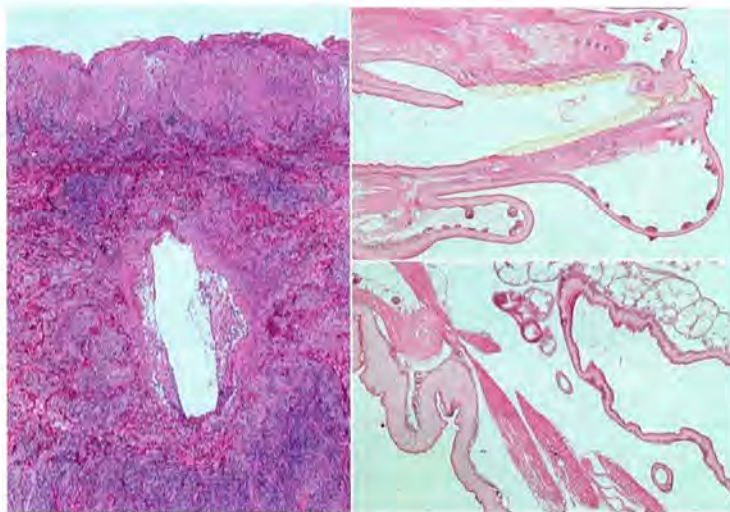


Figure VII-E-1-1. Furuncular myiasis caused by *Dermatobia hominis* in Brazil. Marked inflammatory reaction has been provoked around the intradermal cavity (left). Histologically, the gnathosoma consists of birefringent hypostome, striated muscles and spiculated cuticles (right top). The body portion of the maggot shows the hyaline cuticles, striated muscles, fat bodies, food channel and spiracles (right bottom: H&E). The courtesy by Dr. Masami Imakita, a pathologist at Rinku General Medical Center, Izumisano, Japan.

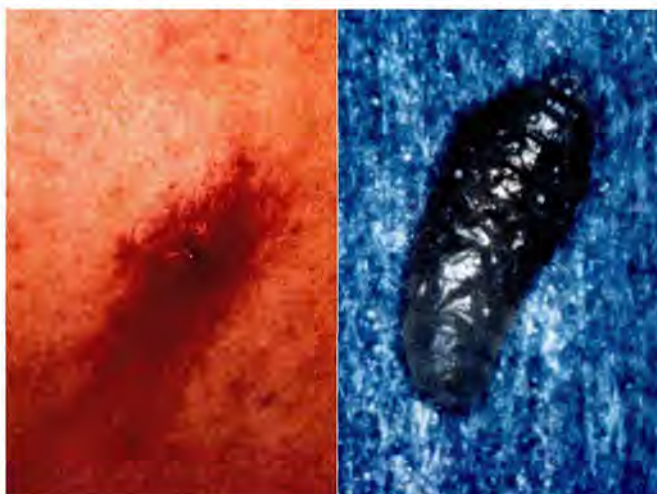


Figure VII-E-1-2. Furuncular myiasis caused by *Cordylobia anthropophaga* on the back of a Japanese male who stayed in Zambia. The warble with a central crater is painless (left). The second-instar maggot of *C. anthropophaga* obtained from the crater was a 7 mm grayish white maggot possessing 13 body segments. Black dots are seen around the head (right). The courtesy by Dr. Yuko Sato, Department of Dermatology, Jikei University School of Medicine, Tokyo, Japan.

Furuncular myiasis caused by *Cordylobia anthropophaga* is formed on the back of a Japanese male who stayed in Zambia. The warble with a central crater is painless. Two maggots were obtained from the lesion. The second-instar maggot of *C. anthropophaga* is 7 mm in length and grayish white in color, and possesses 13 body segments. Black dots are seen around the head (Figure VII-E-1-2).

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2) Semispecific and Accidental Myiasis

Semispecific myiasis is featured by secondary infestation of the maggot in the necrotic portion of the body, such as decubitus and ulcerated cancer. In semispecific myiasis, eggs are

incidentally blown onto the necrotic lesions of the body surface. Accidental myiasis means accidental contamination of fly maggots in the specimen.



Figure VII-E-2-1. Semispecific myiasis. Squamous cell carcinoma of the facial skin in the aged with dementia was badly smelled (left). From the ulcer base, a total of 54 maggots were obtained (right top). The maggots were identified as third-instar larvae of *Lucilia caesar*, a common fly throughout Japan (right bottom). The courtesy by Dr. Toshiyuki Sugi, a dermatologist at Saitama Municipal Hospital, Saitama, Japan.

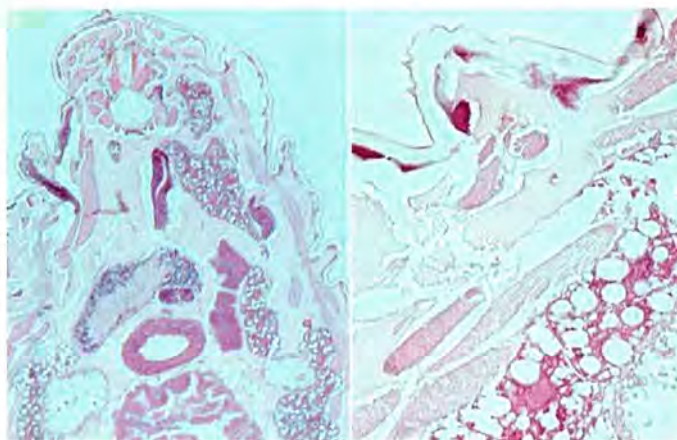


Figure VII-E-2-2. Accidental myiasis. A maggot of *Sarcophagidae*, a common fly in Japan, was accidentally contaminated in the stool. Histologically, the worm consists of the spiculated cuticle, striated muscles, fat bodies, food channels and spiracles (H&E).

In Figure VII-E-2-1, semispecific myiasis occurred in the lesion of squamous cell carcinoma of the facial skin in the aged with dementia. The ulcerated 3 cm skin cancer was badly smelled, and numerous maggots were obtained from the ulcer base. A total of 54 maggots were obtained from the necrotic ulceration of cancer. The maggots are thus not primarily pathogenic. The maggots were identified as third-instar larvae of *Lucilia caesar*, a common fly throughout Japan.

The maggot of *Sarcophagidae* was accidentally obtained from the stool (Figure VII-E-2-2). Two possibilities should be considered: ingestion of egg-blown meat (the maggot was laid

in the bowel) and blowing eggs by the fly just after defecation (the maggot was laid outside the bowel). In this case of accidental myiasis, the maggot was found in the non-diarrheal stool. The spiculated cuticle, striated muscles, fat bodies, food channels and spiracles are seen.

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F) Sand Flea Bite

Sand flea bite, or tungiasis, is caused by penetration of a fertilized female sand flea, *Tunga penetrans*. Sand flea bite commonly affects the feet of the pig. This ectoparasitosis is seen in Latin America, Caribbean islands, Africa and south Asia, but not in Japan. Lesions are confined to the feet. Because of the increase of international/intercontinental traveling, imported diseases are to be seen more often (Figure VII-F).



Figure VII-F. Sand flea bite (tungiasis) in Venezuela. A painful heel nodule with a central hole (left). The toe is involved in another Japanese male who stayed in Venezuela (right top). A brown-colored 5 mm-sized adult female flea, *Tunga penetrans*, is burrowed just beneath the epidermis with central black hole. Histologically, the gut, ovary, ova and striated muscles are recognized (right bottom: H&E). The courtesy by Dr. Satoshi Kurihara, Kurihara Dermatology Clinic, Tokyo, Japan.

A painful heel nodule with a central hole is seen in a Japanese male who stayed in Venezuela. A brown-colored 5 mm-sized adult flea was burrowed just beneath the involved epidermis with central black hole. The histology of the sand flea demonstrates components of the gut, ovary, ova and striated muscles.

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G) Louse Infestation (Pediculosis)

Louse is the common name of wingless and 6-legged insects, *Phthiraptera*. Of more than 3,000 species of lice, colored pale beige to dark gray, three are known to cause human diseases, pediculosis: namely, pediculosis capitis, pediculosis corporis and pediculosis pubis, caused by *Pediculus humanus humanus*, *P. humanus corporis* and *Phthirus pubis*, respectively. The former two (the head and body lice) are similar in shape, and 2-4 mm (sesame seed) in size. Body lice are notorious to transmit and spread epidemic typhus, trench fever and louse-borne relapsing fever. They lay eggs in the seams of clothing, and blood sucking causes intense itching. Pubic lice are 1 mm in size, and sexual transmission occurs (phthiriasis). Eggs (nits) hatch into nymphs that finally develop to the adults. Lice do not burrow into the skin, while they pierce the skin with their tiny needle-like mouthparts. Pediculosis is treated with a gamma isomer of hexachlorobenzene, but various mixtures of pyrethrins seem more effective.



Figure VII-G-1. Head louse infestation. Scalp hair of a long-haired girl is attached by multiple small white dots (left). Microscopically, a thick cuticle-covered body of a louse, *Pediculus humanus humanus*, attaches onto the hair shaft (right).



Figure VII-G-2. Pubic louse infestation as sexually transmitted infection. The pubic lice, *Phthirus pubis*, commonly infest at the narrow angle between the orifice of hair and the skin. Pubic hair (left top) and eyelashes (right top) are affected. Microscopy demonstrates a wingless, 6-legged and finely haired body, 1 mm in size, which is connected to the hair shaft via 2nd and 3rd legs (left bottom). Histological section of the louse infested on the eyelash shows the head, body and legs (right bottom: H&E).

The head and pubic lice parasitizing on the hard hair produce equivalent disorders, with itching, bites, and eggs on the hairs. The head lice cause a disease of the scalp, usually seen in children, particularly long-haired girls (Figure VII-G-1). Head-to-head contact during play at school or at home is the main cause of transmission.

The pubic lice live on the short hair in the pubic regions, the body, the axilla, the eyebrows and the eyelashes (phthiriasis palpebrarum) (Figure VII-G-2). Pubic lice infestation may be predictive of concurrent *Chlamydia trachomatis* infection. Adolescents infested with the pubic lice should be screened for other sexually transmitted infections, including chlamydia and gonorrhea.

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H) Honey Bee Sting

Many different types of insects inflict a poisonous bite or sting. The venomous insects causing medical problems include a) bees (the domestic honey bee, the Africanized "killer bee" race and the bumble bee), b) vespids (paper wasps, hornets, and yellow jackets), and c)

ants (the fire ant). These insects all belong to the *Hymenoptera* order. The notorious wasp/vespid venom is neutral in pH, and contains up to 13 different protein antigens (allergens), as well as various amines and peptide toxins such as mastoparan, which induces degranulation (histamine release) from mast cells. The venom of the honey bee (*Apis mellifera*) is acidic because of the presence of formic acid, and induces immediate-type hypersensitivity (the sting reaction). Beekeepers may have a joint disease related to bee stings. The etiopathogenesis remains unknown. This condition should be called as "beekeepers' arthropathy. In Japan, only limited kinds of stinging ants are distributed.

The representative potent allergens in the *Hymenoptera* group are shown below.

- (a) A low-molecular-weight honey-bee allergen called as Api m6
- (b) Phospholipase A2, as a potent venom allergens in North American bumble-bee (*Bombus pennsylvanicus*)
- (c) The vespid venom Ves v5 allergen showing IgE-binding activity
- (d) The carbohydrate antigens likely causing cross-reactivity among honey-bee and vespid venoms
- (e) The venom allergens of *Pachycondyla chinensis*, an ant commonly seen in Japan and Korea. Anaphylactic reactions have been reported.

Histologically, at the site of honey bee sting, eosinophilic cellulitis is formed, in association with epidermal vesiculation and exudation. A yellow-colored birefringent material (stick needle) is seen in the upper dermis. High-powered view discloses the yellow-colored stick needle of a honey bee embedded in the dermis (Figure VII-H).

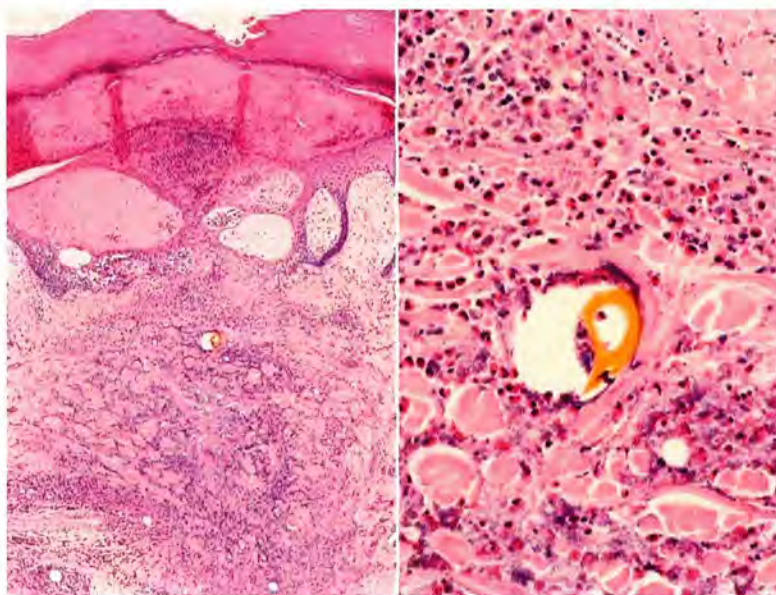


Figure VII-H. Honey bee sting. Epidermal vesiculation and exudation are seen at the site of honey bee sting. A yellow-colored birefringent material is seen in the upper dermis showing eosinophilic cellulitis (left). High-powered view discloses the yellow-colored stick needle of a honey bee *Apis mellifera* (right: H&E). The courtesy by Dr. Takashi Tsuchida, Diagnostic Pathology Division, Hamamatsu Medical University Hospital, Hamamatsu, Japan.

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I) Poisonous Moth-Induced Dermatitis

The larvae (caterpillars) of the poisonous moth (tussock moth) have a distinctive appearance of alternating bristles and haired projections. More than 2,500 known species are distributed all over the world, and some of them are poisonous. Tussock moth caterpillars have 0.1–0.2 mm urticating or poisonous hairs (often hidden among longer, softer hairs) which can cause itchy and painful reactions, when they come into contact with the exposed skin of the head, neck and upper extremities. It should be noted that the urticating hair is seen on the second-instar larvae through chrysalides (pupae) and adults, and the dried shed skin of the larva is still irritable. The larvae shed several times. The hairs break off very easily and are extremely irritable to the skin. Urticating hairs on the smear preparation aspirated from the blisters of the skin are illustrated (Figure VII-I-1).



Figure VII-I-1. Poisonous moth, *Euprocis pseudoconspersa*, and the urticating hairs. The Japanese tussock moth with haired skin grows on the tea plant (left). The aspirate from the blister formed on the irritated skin demonstrates numbers of poisonous needle-like hairs, 0.1–0.2 mm in length (right). The courtesy by Prof. Emeritus Muneo Ohkido, Department of Dermatology, Tokai University School of Medicine, Isehara, Japan.

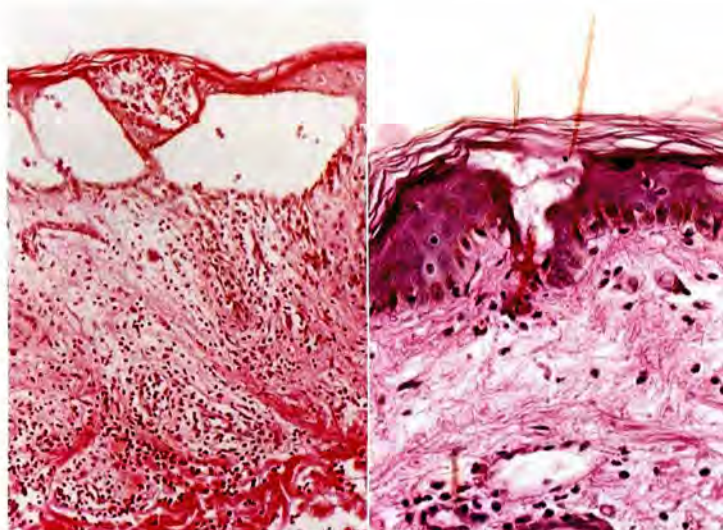


Figure VII-1-2. Experimentally challenged vesiculated skin lesion caused by poisonous moth hair stick. Small papules with central blisters are penetrated by poisonous needles. Eosinophilic reaction is associated (H&E). The courtesy by Dr. Yoji Kubo, Kubo Dermatology Clinic, Nagasaki, Japan.

The urticating hairs contain proteases, phospholipase A and histamine. Japanese tussock moth, *Euprocis pseudoconspersa*, hatches on the leaves of tea plant, camellia and sasanqua. Clinically, more than half of the patients are unaware of the touch with the poisonous moth caterpillars.

In Figure VII-1-2, biopsy histology of the blister experimentally challenged to the arm of a volunteer is demonstrated. Small papules with central blisters are associated with eosinophilic infiltration in the upper dermis, and penetrating poisonous needles are noted.

Another type of poisonous moth includes slug (or oriental) moth, *Monema flavescens*, in the family of *Limacodidae*. In the summer time, toxins in urticating needles induce immediate allergic reactions. Japanese people call this small adult moth as the “electric bug”.

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J) Hirudiniasis

Hirudiniasis represents infestation of leeches on the skin. The leech belongs to the phylum Annelida. The leeches infesting the human to suck blood include a) *Hirudo nipponia*/*Hirudo medicinalis*, b) *Dinobdella ferox* and c) *Haemadipsa zeylanica japonica*.

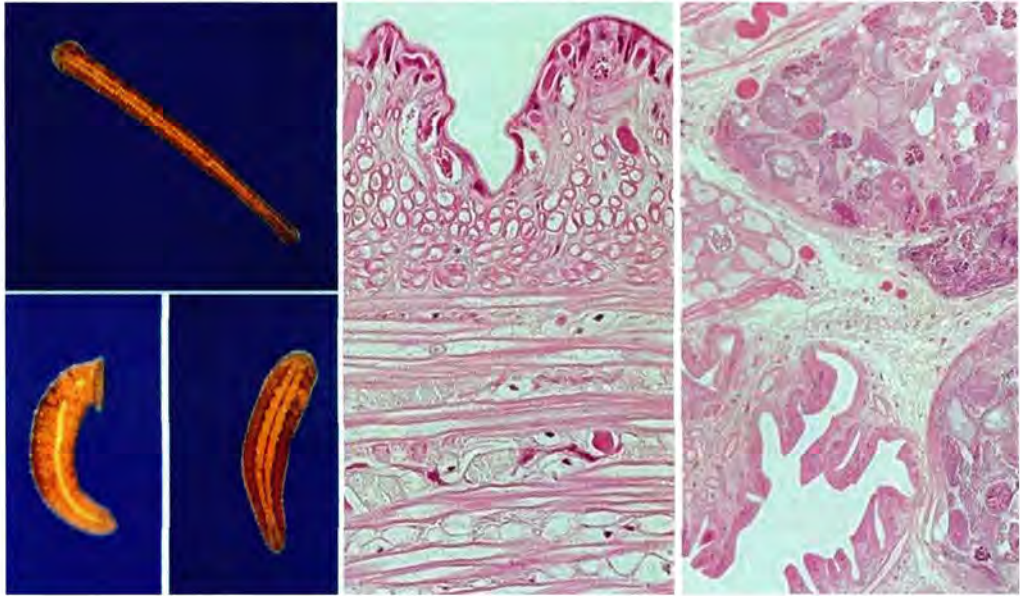


Figure VII-J. Japanese land leech, *Haemadipsa zeylanica japonica*. The land leech has a brown-colored and striped body, which is flexible in length (left three panels). Histologically, the body is covered with the cuticles and subcuticular spindled cell layer, and is rich in both circumferential and longitudinal smooth muscle layers (center: H&E). The gut and gonads of both sexes are identified in the body lumen (right: H&E).

The former two live in the water, while the latter is seen on the land (land leech). *D. ferox* is called a nose leech, because it infests in the nose of animals such as deer. A human case of nose leech infestation has been reported in Kyushu Island in Japan. Infestation in the vagina has also been recorded.

Hirudo- means leech in Latin, and, interestingly enough, “Hiru” being the leech in Japanese. Fully mature bisexual (hermaphrodite) adults are green, brown or greenish-brown with a thin red stripe on the dorsal side. They have two suckers (anterior and posterior). The anterior sucker is used for feeding. After piercing the skin and injecting strong anticoagulant, named hirudin, and anesthetics, they suck out blood as much as 5-15 ml. Gross and microscopic appearance of Japanese land leeches attached onto the arm skin is demonstrated in Figure VII-J.

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Note 55. Leeches and treatment

The leech of European species, *Hirudo medicinalis*, living in the water consumes 5-15 ml of blood (4-6 times their body weight in a single feeding). Leeches have been historically used in medicine to remove bad blood from patients suffering from various diseases, including gastric ulcer. Leech therapy was popular in ancient India and Greece, and continued into the 18th and 19th centuries in both Europe and North America. Leech therapists used up to 10 leeches, so that the patient lost up to 150 ml of blood during the treatment. Contemporary uses of leeches include the reattachment of body parts and reconstructive and plastic surgeries and, in Germany, treating osteoarthritis.

In China and also in Japan, the dried leech powder of *H. medicinalis* or *Hirudo nipponia*, "Suitetsu" or "Shuizhi", has long been used as a traditional medicine for removing blood stasis and improving circulation. It contains "hirudin", a 65-amino acid active and clinically applied anticoagulant.

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K) General Comments on Arthropod Bites and Stings

Insect bites and stings cause an immediate skin reaction often resulting in redness and swelling in the attached area (Figure VII-K-1). Stinging insects encompasses in the wasp family include honey bee, bumble bees, vespids/hornets (yellow jackets) and fire and harvester ants. Biting insects include cone-nose bugs (*Triatoma*), mosquitoes, fleas, midges (horseflies, deerflies, gnats/blackflies and sandflies), spiders, centipedes, acari (mites and ticks), lice and bedbugs. Stings of bees, vespids/hornets, ants and spiders are painful and may lead to an anaphylactic shock state. The vespids/hornets are most dangerous. In Japan, 20-30 persons are killed by the vespids/hornets annually. Representative stinging ants in Japan include *Pachycondyla chinensis*. *P. chinensis* is widely distributed in Japan and Korea, and has a toxic needle. Allergic reactions to the ant venom are clinically important (see Chapter VII-H). Bites of mosquitos and fleas cause itching rather than pain.

The skin reactions to insect bites and stings usually disappear within a few days. Occasionally, the local reactions happen to last for up to two years, and these bites may be misdiagnosed as benign or cancerous lesions. The severity of bites and stings varies depending on the type of insect and sensitivity of the person. Mosquito bites show both the immediate type and delayed type hypersensitivity reactions. In the delayed type commonly seen in the infancy period, itchy eruptions are seen on the next day. Midges and horseflies

provoke hemorrhagic bites. Gnat bite often causes prolonged allergic skin reactions (Figure VII-K-2). Allergic reactions to *Triatoma* bites have been reported in the United States. Regarding mosquito bite hypersensitivity with Epstein-Barr virus activation, refer to Chapter IV-G-5.

There are two types of arthropod bites and stings.

- a) The protective bites
- b) The feeding bites.

The protective bites occur when the arthropod is agitated and seeks to defend itself. In the feeding bites, the blood is used for developing fertile eggs. The arthropods in the feeding bite group include: mosquitos, midges, fleas, biting flies, bedbugs, lice, mites and ticks. In the former four groups, the bites are felt immediately, while the bites by the latter four are not sensible.

The microscopic appearance of the insect bites and stings is generally featured by a wedge-shaped superficial perivascular infiltrate in the dermis, consisting of abundant lymphocytes and some eosinophils. The features are non-specific and common in other dermatoses including drug reactions and urticaria (Figure VII-K-3). The likely causes of unknown insect bites include bedbug (*Cimex lectularius*), rat mite (*Ornithonyssus bacoti*) and cat flea (*Ctenocephalides felis*). Recently, the incidence of bedbug bite is increasing in Japan.

Spiders are active hunters to paralyze and kill their victims. Spider bites may happen when humans unintentionally press up against spiders. Only spiders of fairly large species possess chelicera (a fang-like appendage near the mouth) long enough to penetrate human skin. The spider venoms include necrotic toxins, neurotoxins and active amines.

In Japan, two species of venomous spiders are known: "the yellow sac spider" (*Chiracanthium japonicum*) and "the redback spider" (*Latrodectus hasseltii*). The 5-10 mm-sized yellow sacs, carrying yellow to beige-colored abdomen, are widely distributed in Japan except in Okinawa. *Chiracanthium* spp. is seen all over the world. The bites provoke severe pain, but are not lethal. The redbacks are a dangerous spider endemic in Australia, and were incidentally exported to Japan in 1995. Now, the redbacks are widely distributed in the southern half areas in Japan. The 1 cm-sized female is featured by the black body with a prominent red stripe on the back of her abdomen and long slender legs. The Redbacks prey on insects, but can capture small lizards. The bites cause severe pain lasting for over 24 hours. An antivenom antiserum is available.

Scorpions are 8-legged predatory arthropod in the class Arachnida. A venomous stinger is seen in the narrow, segmented and forward-curved tail. Scorpions are found worldwide, and only a limited number of scorpions have venoms capable of killing a human being. The venom stored in a pair of glandular sacs is a mixture of neurotoxins, enzyme inhibitors, etc. Non-venomous species are also able to penetrate the human skin and deliver sharp, unpleasant stings with localized redness. It should be noted that the venoms of a polymorphic scorpion, *Mesobuthus eupeus*, (meucin-13 and meucin-18) exhibit anti-microbial effects on bacteria, fungi and yeasts, and medical application is possible. In contrast to spiders, the scorpions show fluorescence when exposed to ultraviolet light (or Wood lamp). The fluorescent component, β -carboline, may have a role in scorpion light detection,

In Japan, two kinds of weakly venomous scorpions are distributed in the southernmost islands of Okinawa prefecture. They are: 3 cm-sized “dwarf wood scorpion” (*Liocheiles australasiae*) and 6 cm-sized “lesser brown scorpion” (*Isometrus maculatus*).

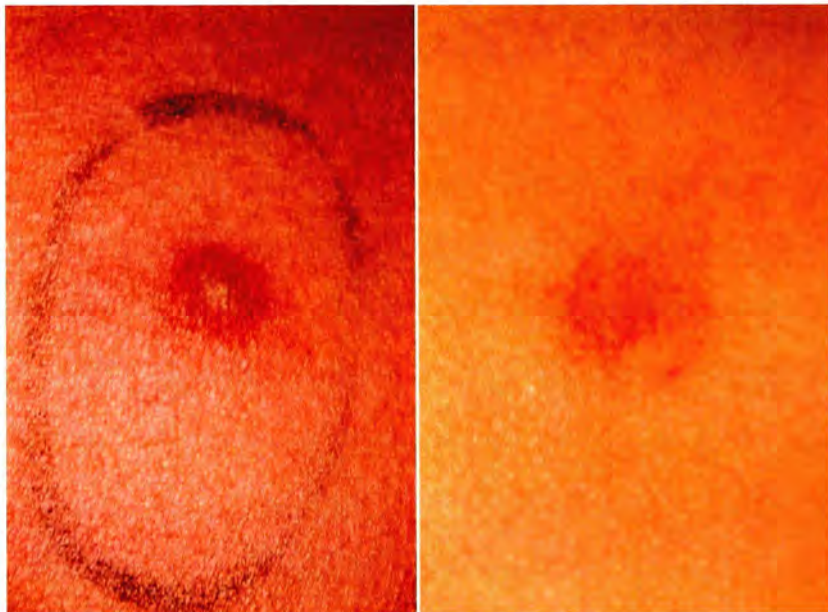


Figure VII-K-1. Gross features of representative insect bites. Wasp bite provokes painful local swelling (left). Centipede bite causing local pain and swelling is characterized by chevron-shaped or V-shaped marks (right). Large paired forcipules of the centipede pierce the skin and inject venom into the wound.

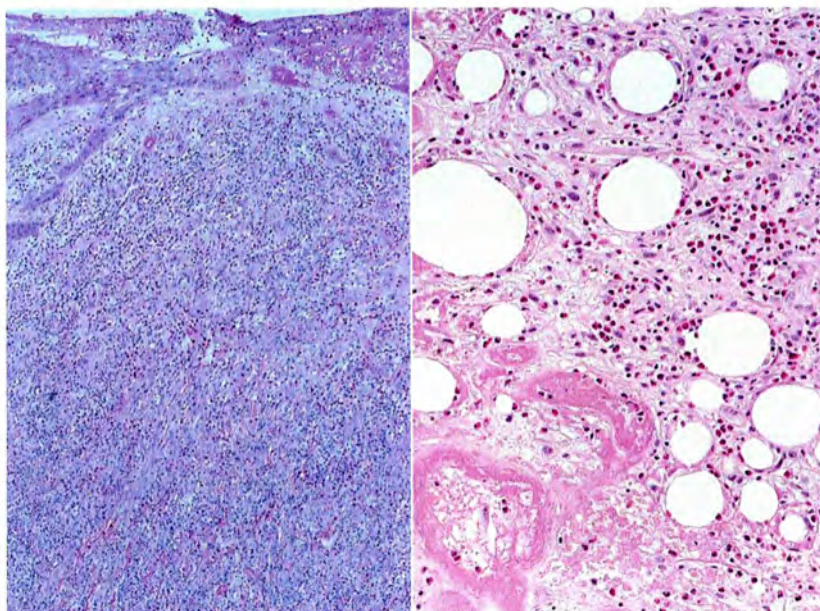


Figure VII-K-2. Histology of gnat bite. Gnat bite often causes prolonged allergic skin reactions. The epidermis is ulcerated and the dermis reveals dense and severe infiltration of inflammatory cells (left). Angioneurotic edema with eosinophilic response is seen in the subcutaneous tissue (right: H&E).

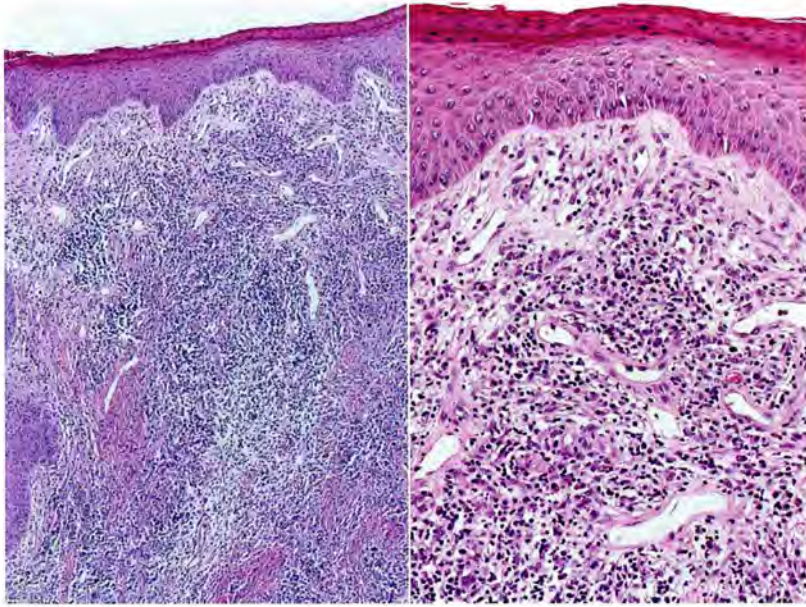


Figure VII-K-3. Histology of insect bite on the nose. The local reaction against insect bite may last for months. Biopsy reveals pseudolymphomatous dense lymphocytic infiltration throughout the dermis, in association with angiogenesis and eosinophilic reaction (H&E).

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L) Seabather's Eruption (Plankton Dermatitis)

Marine dermatitis or "seabather's eruption" is caused by stings of zooplankton, zoea, a 1.5 mm-long larval stage of crabs or shrimps. Zoea has a needle. This incidental irritation by the zoea is also called as plankton dermatitis. After sea bathing, multiple small and itchy papules are formed on the skin covered with bathing wears (Figure VII-L). Eruptions occur soon after one returns to the coast, when the salt concentration is elevated by drying. The frequency is high when the sea water is turbid or the red tide outbreaks. Those who wear wool-made bathing suits are susceptible to the plankton sting. In Japan, they say "erotic worms" because the eruptions are confined to the private parts and female breast.

Similar pruritic dermatitis (hypersensitivity reaction) is also caused by stings of the immature nematocysts of larval-stage thimble jellyfish (*Linuche unguiculata*), sea anemones (*Edwardsiella lineata*) and other larval cnidarians.

So-called swimmer's itch represents cercarial dermatitis (see Chapter VI-K).



Figure VII-L. Seabather's eruption. After sea bathing, multiple small and itchy papules are formed on the skin covered with bathing wears. The eruptions are caused by the needle stick of zooplankton, zoea, a 1.5 mm-long larval stage of crabs or shrimps (inset indicates zoea). The courtesy by Dr. Masao Nakano, Nakano Dermatology Clinic, Hiratsuka, Japan.

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Note 56. Dogger Bank itch

"Dogger Bank itch" is allergic contact eczematous dermatitis caused by a metabolite produced by a marine organism. The disease is common in fishermen who work in the Dogger Bank (an important fishing bank) in the North Sea. It is caused by the sea chervil, *Alcyonidium diaphanum*, a seaweed-like animal colony, abundant in this area. The sea chervil frequently came up with the fishing nets. Itching red eruptions appear on the flexor aspects of the elbows and forearms, and spread to involve the hands, fingers and arms, extensively. The rash is caused by delayed-type hypersensitivity, and the specific allergen was determined to be the chemical, (2-hydroxyethyl) dimethylsulfoxonium ion. The causative agent, *A. diaphanum*, is a member of the phylum *Bryozoa*, consisting of minute, sessile, filter-feeding animals that live in colonies. *A. diaphanum*, apparently resembling seaweed, is a gelatinous, smooth, sponge-like colony up to 15-30 cm long, growing on the rocks and shells from lower shore down to approximately 100 m.

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M) Stings by Crown-of-Thorns Starfish and Other Marine Creatures

Crown-of-thorns starfish (*Acanthaster planci*), 25-35 cm-sized multiarmed starfish, are notorious as a coral reef destroyer, distributed in the tropical and subtropical regions of the Pacific and Indian Oceans. They are usually of subdued colors, pale brown to grey-green but may be more brightly colored. They have long and sharp, venomous thorn-like spines on the upper surface of the body. The toxins include saponins (asterosaponins) and other chemicals such as plancitoxins I and II. In the human in touch with the starfish, the spines perforate the skin tissue, immediately causing a sharp stinging pain that lasts for several hours, persistent bleeding due to the hemolytic effect of saponins, and nausea/vomiting and tissue swelling that may persist for a week or more. As the most severe reactions or envenomations, numbness,

tingling, weakness, joint pains, headache, cough and, in rare cases, paralysis occurs. The spines of the starfish become embedded in the tissue, so that they must be removed by surgery.

Other venomous marine creatures venomous include a) venomous jellyfish such as Portuguese man-of-war (*Physalia physalis*) and box jellyfish (*Chironex fleckeri*) (in the family of cnidarians), b) corals (in the order *Anthozoa*) c) cone shell snail and blue-ringed octopus (in the family of molluscs), d) sea urchins, sea cucumber and starfish of other species (in the family of echinoderms), e) fire sponge, f) seaweed (refer to **Note 56**), and g) venomous fish, such as stonefish and stingray.

Figure VII-M illustrates gross appearance of the puncture wounds by crown-of-thorns starfish and Portuguese man-of-war jellyfish in Okinawa, Japan.



Figure VII-M. Puncture wounds caused by crown-of-thorns starfish and Portuguese man-of-war jellyfish in Okinawa, Japan. Touch with the crown-of-thorns starfish provokes painful and severe skin eruptions with systemic manifestations (left). Portuguese man-of-war is a representative venomous jellyfish, causing linear vesiculated eruptions (right). The courtesy by Dr. Masao Nakano, Nakano Dermatology Clinic, Hiratsuka, Japan.

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N) Incidental Sting of Squid Sperm Bag into Oral Mucosa

This must be a very unique illness in Japan.

When one eats raw sexually mature squid (cuttlefish), one or more white non-motile substances may sting into the oral cavity and cause pain. The material represents the sperm bag (spermatophore) of a mature male squid. The 2 cm-long sperm bag contains clustered actin filaments at one end as a shooting machine. The sexual intercourse of the squid is supposed to be painful (see Note 57). Biopsy reveals an encapsulated bag stuck into the oral squamous mucosa. Numerous sperms are seen inside the encapsulated bag, and thus grossly appear to be white (Figure VII-N-1). Unshot sperm bags may be admixed in the package of squid Sashimi sold in a Japanese supermarket.

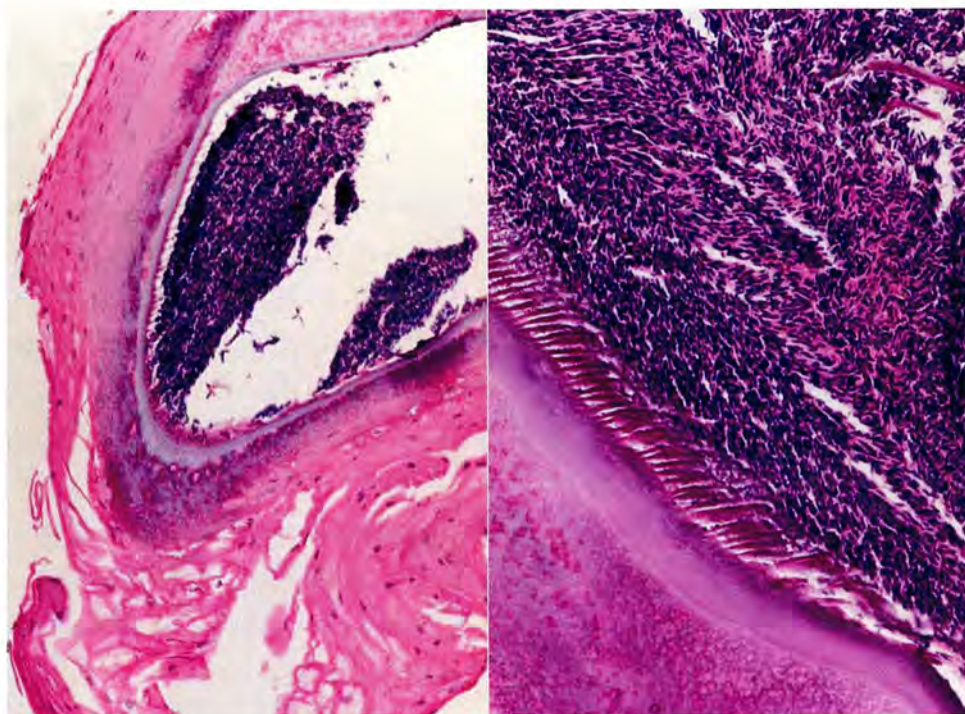


Figure VII-N-1. Incidental sting of squid sperm bag into oral mucosa. Biopsy reveals an encapsulated bag stuck into the oral squamous mucosa. Numerous sperms are seen inside the encapsulated bag (H&E).

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Note 57. Sexual intercourse of the squid is painful!

In order to understand the pathogenesis of the incidental sting of a squid sperm bag into the oral mucosa, we should realize the mode of sexual behavior of the squid. Two of the ten legs of the squid are for sexual activity, and male squid inserts his sperm bags into the vagina of the female squid by using these two specially differentiated legs. The 2 cm-long sperm bag (spermatophore) contains actin filament-rich structures (as a shooting device) at its end. The external sheath is transparent. The internal bag is full of sperms, and thus white in color. By a contact with the female vagina, they are shot to sting into the internal body wall of the female (Figure VII-N-2). The sperms are gradually leaked into the body cavity to maintain fertilization of the ova for a long period of time.

For dinner, a man ate squid sashimi which his wife just bought in a supermarket. He suddenly felt pain in the oral cavity and he found a small white material stuck into his tongue. An emergency surgeon removed the white, non-moving material to submit histological evaluation. What this unlucky guy presented here ate was the unshot sperm bag, intermingled with sliced body wall of the female squid in a mating season. The author hopes your luck that the squid “sashimi” you eat now is not contaminated with “shootable” sperm bags.

In Sagami Bay in Kanagawa prefecture and also in Mikawa Bay in Aichi prefecture, fishing of squid on the boat is popular. The mania for squid fishing often says that it's delicious to eat the fresh squid on the boat. One brave guy ate the whole body of squid on the boat just after fishing-up. It was just a season of wedding of the squid couple. His mouth was full of painful white material. The pitiful squid he devoured was of no doubt a mature male!



Figure VII-N-2. Sperm bag of the squid. The 2 cm-long sperm bag (spermatophore) contains actin filament-rich shooting device at its end. The external sheath is transparent. The internal bag is full of sperms, and thus white in color (left). By a contact with the female vagina, they are shot to stick into the internal body wall of the female squid (right).

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O) Venomous Animals and Poisonous Plants

A variety of reptiles and mammals are venomous to the human. Venomous snakes possess venom glands and specialized teeth for the injection of venom. Neurotoxins or hemorrhagic toxins are noted. India's Big Four of the venomous snake species, responsible for causing the most snake bite cases in India, include king cobra, common krait, Russell's viper and saw-scaled viper. Polyvalent serum effectively neutralizing the venom of all of the Big Four snakes is available in India. Taipans in Australia, *Vipera berus* and Pallas' viper in Europe, and rattlesnakes, copperheads, cottonmouths and coral snakes in the United States are other notorious examples. In Japan, three kind of venomous snakes are observed: Mamushi pit viper, ringed grass snake (Glosbe, Yamakagashi) and Habu (*Protobothrops flavoviridis*). Pit viper bites with painful swelling are most common, and in severe cases thrombocytopenia is associated. Two parallel holes of poison fangs are characteristically observed at the site of injury (Figure VII-O-1). Among 2,000-3,000 bites, 10-20 persons are killed by the snakes annually in Japan. Specific antiserum treatment (passive immunization) is effective.

Venomous lizard, Gila monster, a giant, slow-moving lizard, is distributed in the southwestern United States through northwestern Mexico. The venom in the saliva of Gila monster contains neurotoxins. Of note is that exenatide, a glucagon-like peptide-1 analogue resistant to endopeptidase, was identified within the venomous saliva, and is now used as an "incretin" for the treatment of type 2 diabetes mellitus. Exendin-4, a synthetic analogue of exenatide, stimulates insulin secretion from β -cells of Langerhans islets.

A few kinds of mammals have venoms in their saliva. The venomous mammals include vampire bats, European moles and shrews. Male platypus has a venomous spur on the hind legs. Poisonous brown secretions are observed in the brachial gland of the slow loris. The spines of hedgehogs are also poisonous. Few amphibians are venomous or poisonous, and no venomous or poisonous birds are noted.

Numbers of poisonous plants can be listed up. In addition to toxicosis by ingestion of the plant as a food, the irritation of the skin presents a major problem in daily living. Four types of defense mechanisms are used by the plant world: a) mechanical (by thorns and barbs), b) chemical (by irritable and toxic saps or ingredients), c) phototoxic (by chemicals activated after sunlight exposure), and d) mechanical-chemical (poisonous fine needles on the leaves and stems sting the skin to give microscopic hollow for poisoning).

One typical example is described here. Poison ivy, poison oak and poison sumac are the plants which contain an irritating, oily sap called urushiol. By the oxidation and polymerization of this phenol derivative, a hard lacquer for painting is obtained. Urushiol triggers allergic contact dermatitis after its direct contact with skin, resulting in an itchy rash (Figure VII-O-2). The allergic rash appears within hours of exposure or up to several days later. The exposed to urushiol also occurs by touching objects coated by the sap of the poison

plants. The name of urushiol comes from the Japanese word of the tree *Toxicodendrom vernicifluum* “Urushi”.



Figure VII-O-1. Bite by a venomous pit viper Mamushi. Mamushi pit viper bite causes painful and hemorrhagic swelling around two parallel holes of poison fangs at the site of injury (left). The 45-80 cm-long viper has two large and long poison fangs in the mouth, injecting hemorrhagic toxins to the victims (right). The courtesy by Dr. Fumihko Mahara, Mahara Clinic, Anan, Tokushima, Japan.



Figure VII-O-2. Urushi allergy seen in a Japanese boy. Itchy swelling of both hands is seen in a boy who played in the park. Contact dermatitis is caused by allergic reactions against urushiol. The courtesy by Prof. Kayoko Matsunaga, Department of Dermatology, Fujita Health University School of Medicine, Toyoake, Japan.

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Greetings

The author is a general diagnostic pathologist and a certified cytologist, particularly interested in the field of infectious diseases. In 2000, he published a color textbook "Atlas of Infectious Disease Pathology" (Bunkodo, Tokyo) written in Japanese, and in 2004, a web site entitled "Pathology of Infectious Diseases" written in English was open for public at: <http://info.fujita-hu.ac.jp/~tsutsumi/>.

This book is focused on pathology of skin infections. Among others, the skin is the organ with the highest variety of infections. The author sincerely hopes that the readers enjoy both the macro- and microscopic varieties of the lesions, pathologists routinely use the information for the differential diagnosis, and patients receive an appropriate and timely treatment based upon the proper histopathological diagnosis.

The author has tried to include episodes, history and events closely related to infections very unique in Japan, as much as he can. He sincerely hopes that the readers would also enjoy such descriptions.

The author cordially thanks many of his colleagues and friends and around 100 pathologists and clinicians who kindly supplied him valuable cases, samples, photographs, antibodies and probes.

The motto of the author is a pathologist whose face is open to patients. He accepts the second opinion of the histopathological diagnosis, especially for breast cancer ladies, and enjoys playing oboe with patients, the handicapped and students. The author believes that the music is one of the best tools for communication. The present publication is thus dedicated to all the patients who stimulated the author as a professional diagnostic pathologist.

June, 2013

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Profile of the Author



Yutaka Tsutsumi, M.D., was born in Yokohama, Japan in 1951. He graduated from Keio University School of Medicine, Tokyo, in 1976, and the Graduate School of Medicine in 1980. Then, he worked for Tokai University School of Medicine, Isehara, Kanagawa, for 21 years as a researcher, teaching staff and diagnostic pathologist. In 2001, he moved to Fujita Health University School of Medicine, Toyoake, Aichi, as a pathology professor.

He is a certified pathologist approved by the Japanese Society of Pathology, a certified cytologist approved by the Japanese Society of Clinical Cytology, and an Infection Control Doctor certified by the Japanese Society of Infectious Diseases. He is proud of being a pioneer pathologist introducing immunostaining for routine histopathological diagnosis in Japan. As the leader of the Japanese Pathologists Philharmonic, he enjoys playing oboe.

His motto is being a pathologist whose face is open to the patients. He often receives second opinion for the histopathological diagnosis of cancer, particularly breast cancer, and dedicates to a cancer self-help group, Wakabakai, as a consultant, facilitator and cooperator.

He hopes to help cancer patients have a positive mind and thinking and reset their life and living. He believes that music is a valuable tool for intimate communication between patients and medical staff.

As a general pathologist, he published in 2007 a single-authored and Japanese-written textbook (Volumes 1 through 12) entitled “The Perfect Pathology”, covering all the field of pathology, and released its DVD version in 2009. In the field of infectious disease pathology, he published a Japanese-written textbook entitled “Atlas of Infectious Disease Pathology” in 2000. The web site “Pathology of Infectious Diseases” (English version) is open for public since 2004 at: <http://info.fujita-hu.ac.jp/~tsutsumi/index.html>.

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