Pathology of skin infections

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 microorganisms. The skin may function as a mirror of internal organs. In other words, the appropriate histopathological diagnosis of skin infections may lead patients to proper treatment and cure, and may contribute to human society by preventing infectious disorders.

Here, representative cases of sexually transmitted disease, rare skin infections and fulminant/lethal systemic infection manifesting in the skin are briefly demonstrated.

Ref.: Tsutsumi Y. Pathology of Skin Infections. 2013; 394 pages. Nova Science Publishers, NY, USA. ISBN: 978-1-62808-518-1

Features of skin infection

1) A variety of infections can be observed.
2) The frequency is high.
3) Skin manifestation may be a part of systemic infection.
4) Zoonosis is common.
5) Imported or opportunistic infections may be seen.
6) Lethal infection may occur.
7) It is important to realize the presence of the infectious diseases.

Sexually transmitted diseases

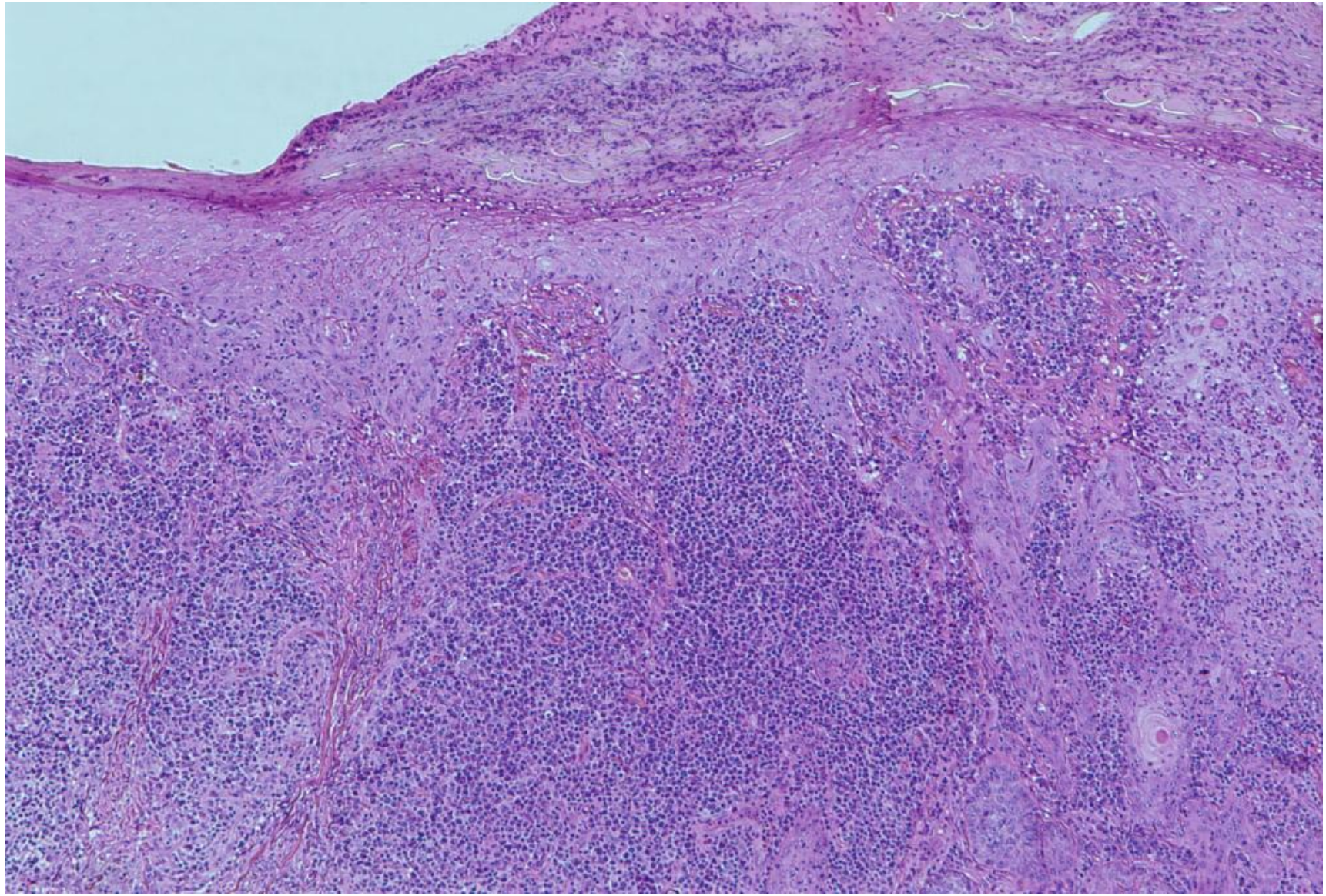
Pathological diagnosis of infectious diseases has a social responsibility to prevent the transmission of the pathogen.

Case. Skin rash in a remission stage of B-cell malignant lymphoma

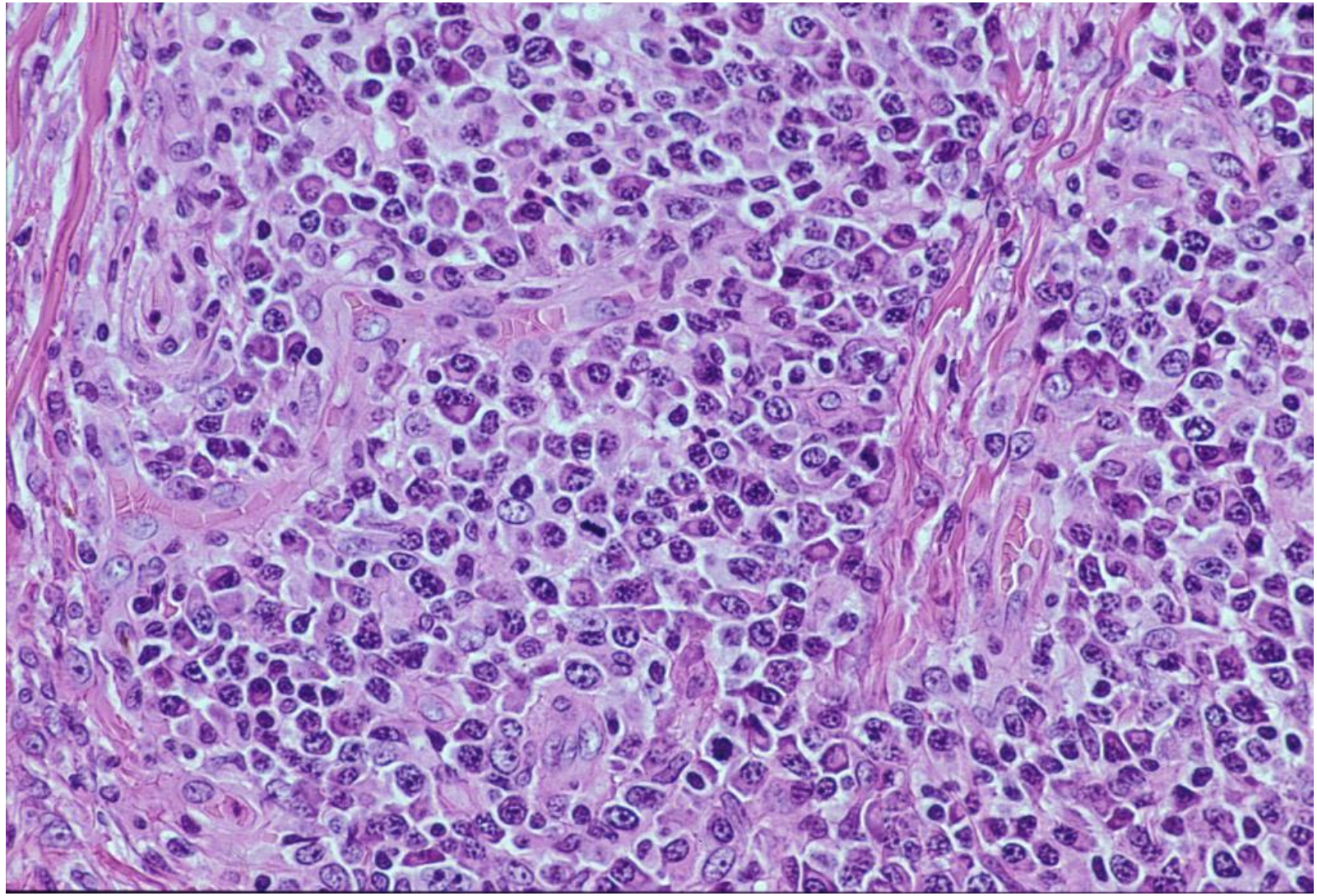
A 56 y-o male businessman:

The patient was treated for nodal large B-cell lymphoma 2 years before, and he kept a remission state. Skin papules appeared on the skin of the neck and scalp, in association with low fever. The clinician suspected of skin recurrence of malignant lymphoma, and biopsy was taken from the neck lesion. How is the diagnosis?

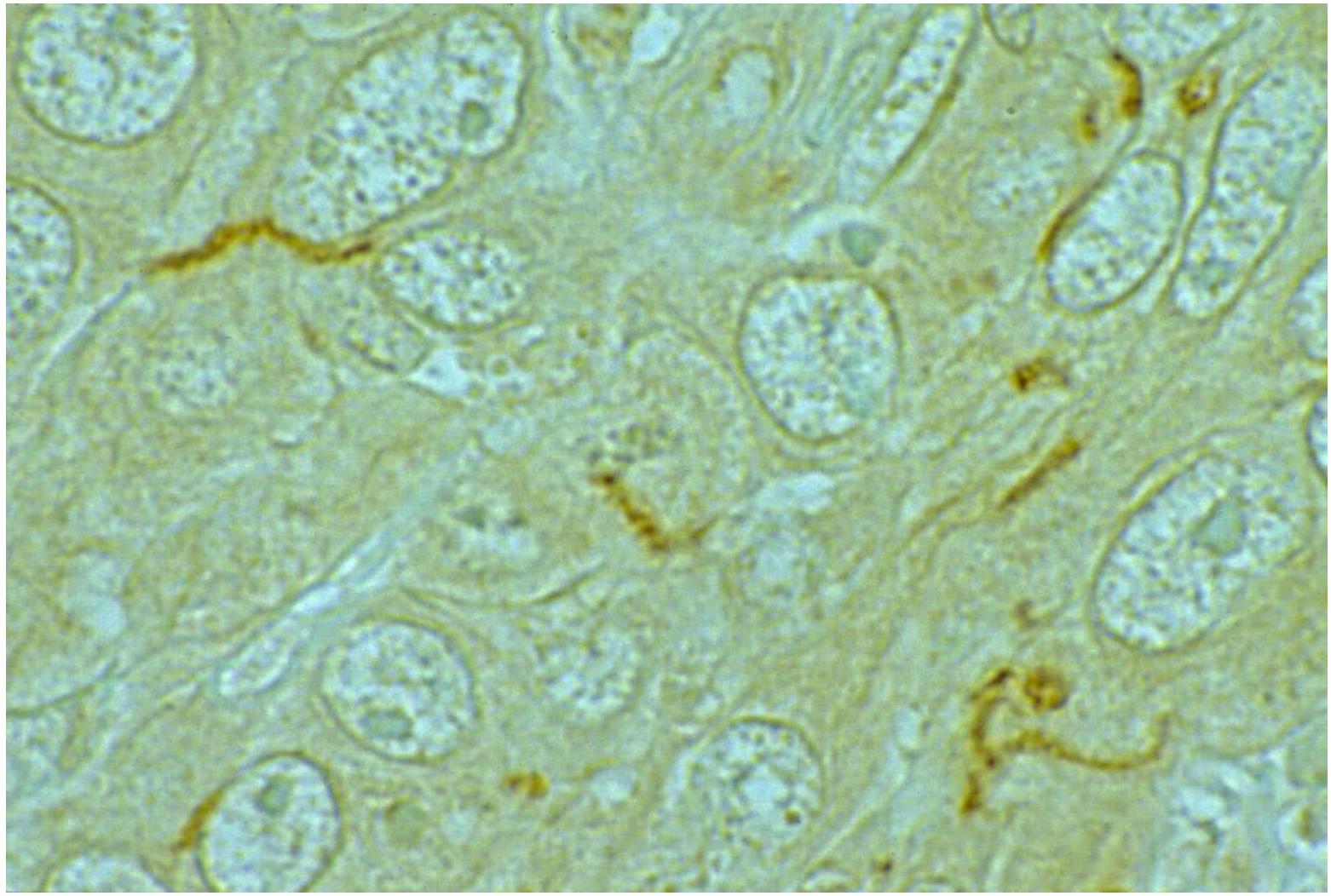
Skin papules with surface erosion on the neck of a 56 y-o male patient with a history of malignant lymphoma 2 years before. The left-sided papule was biopsied.



The neck skin lesion microscopically shows marked mononuclear infiltration with crust formation. Reactive elongation of the epidermal rete ridges is associated (H&E-1).

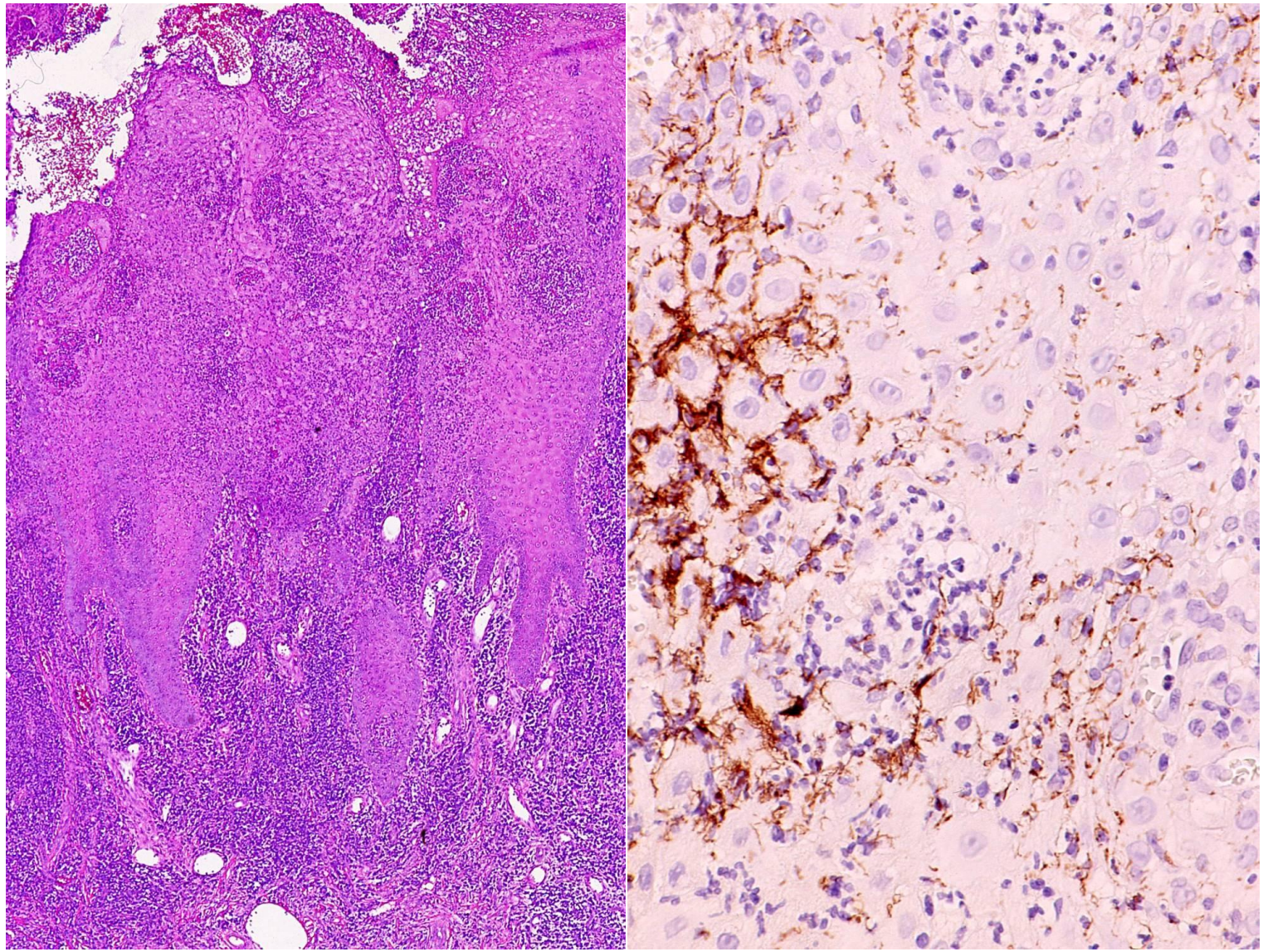


High-powered microscopic view of the neck skin lesion reveals dense infiltration of mature plasma cells. Endothelial swelling is associated. The recurrence of malignant lymphoma can be excluded, and the possibility of syphilis was suspected (H&E-2).

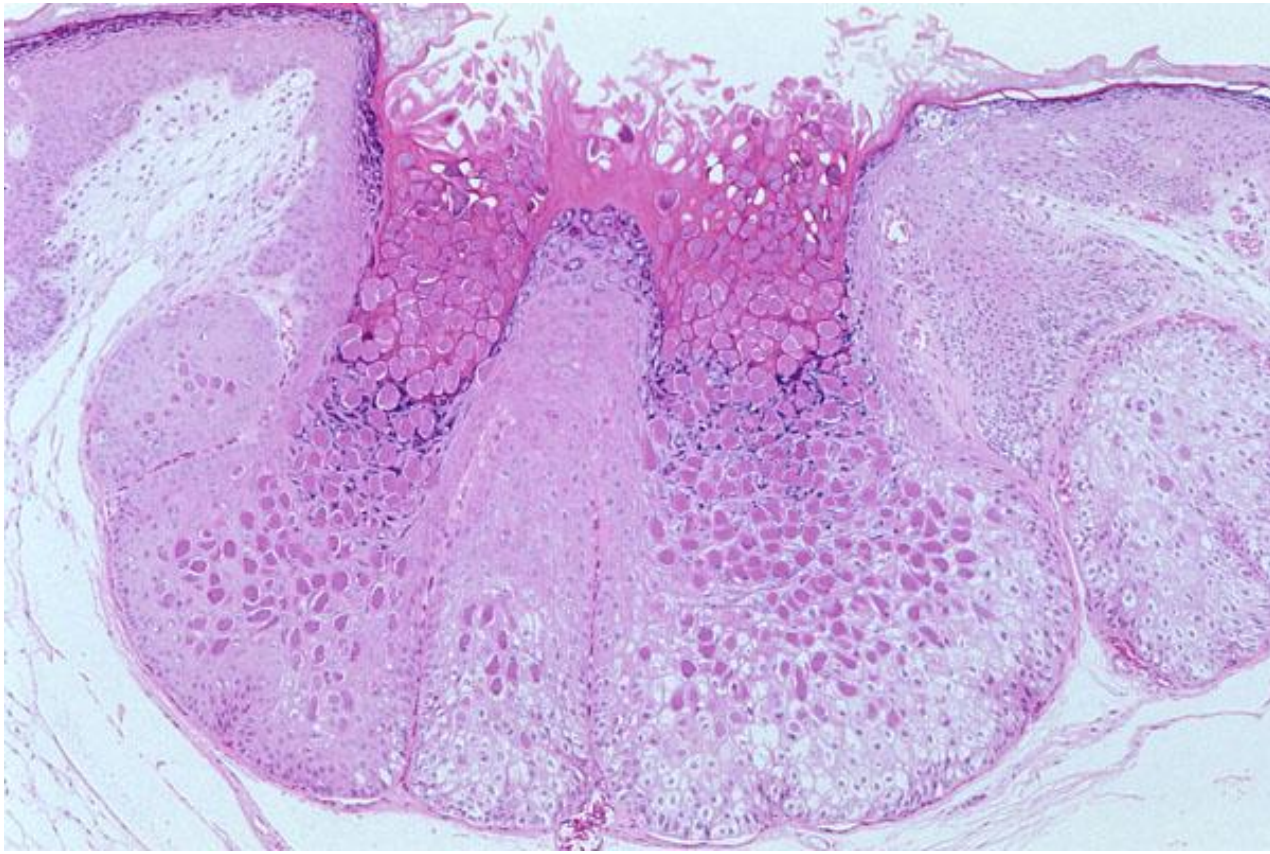


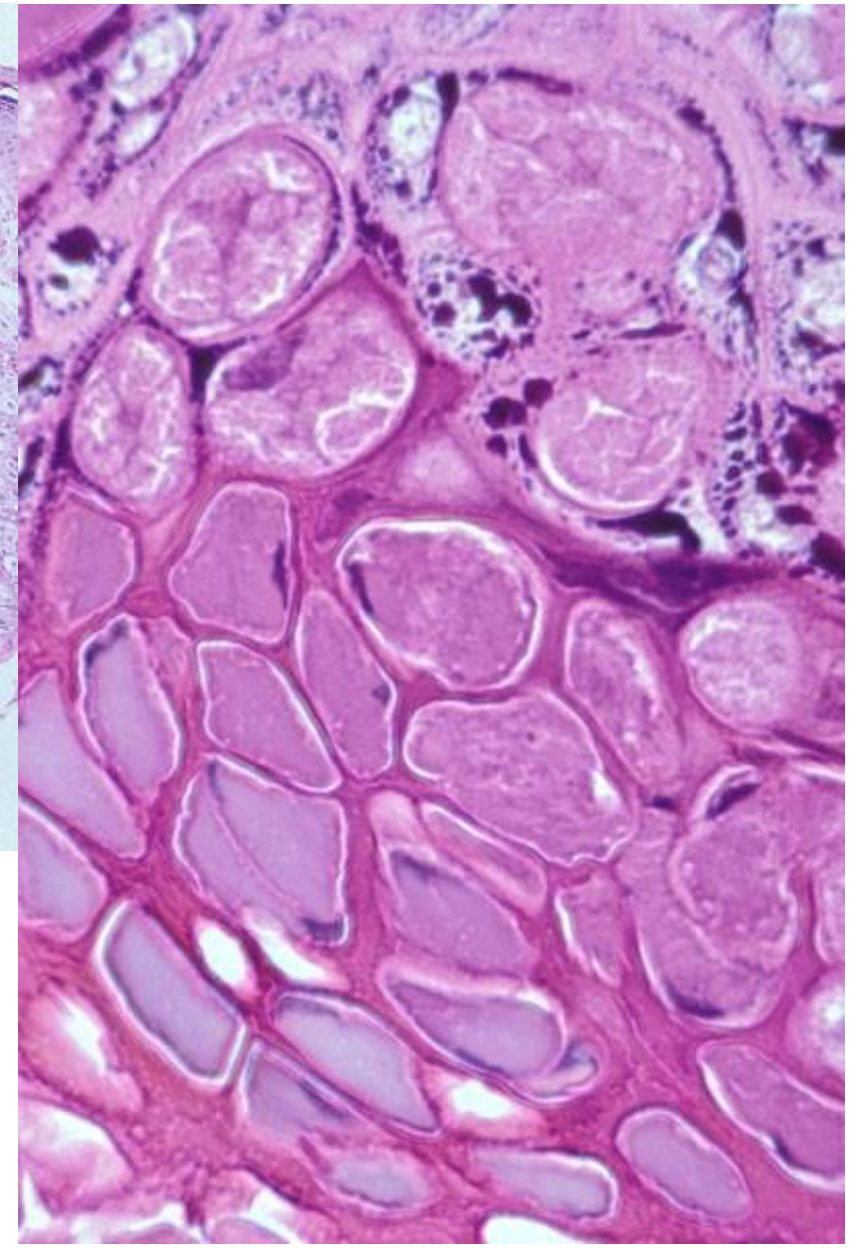
Immunostaining for *Treponema pallidum* antigens using a commercially available antiserum discloses the infection of brown-reacted spiral pathogens in the epidermis, confirming the diagnosis of secondary syphilides on the skin. Since the pathogens are invisible in the H&E preparation, the appropriate histopathological diagnosis of the clinically unsuspected syphilis is totally dependent upon whether or not the pathologist on duty can suspect of the possibility of syphilis in H&E preparation.

Secondary syphilides are typically seen as non-itchy rash (papules) on the palm and sole.



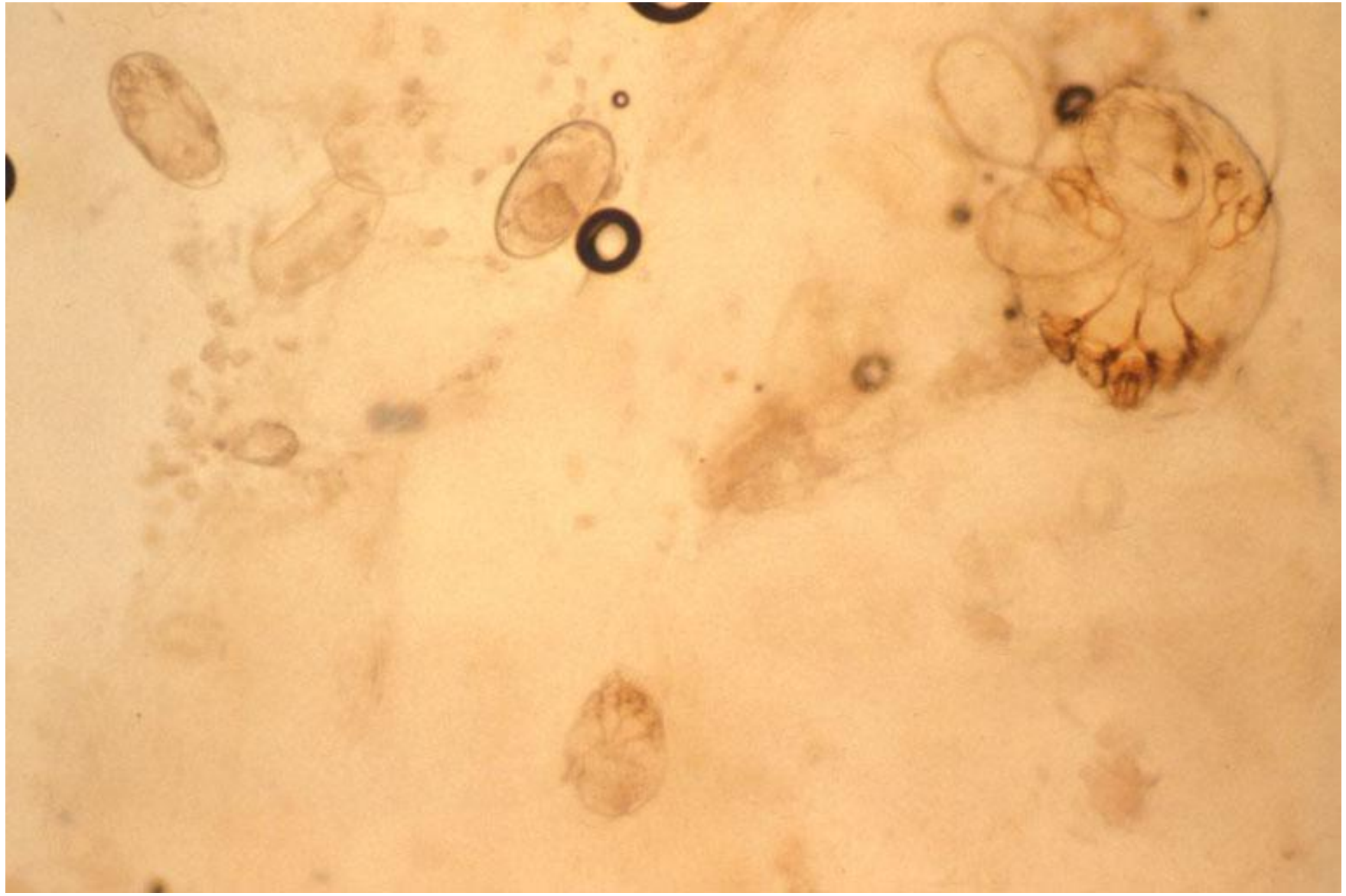
Secondary syphilide. Biopsy sampled from the skin rash on the palm shows inflammatory acanthosis (left: H&E). Immunostaining with *Treponema pallidum* antiserum discloses clustered spiral pathogens particularly among the acanthotic epidermis (right).



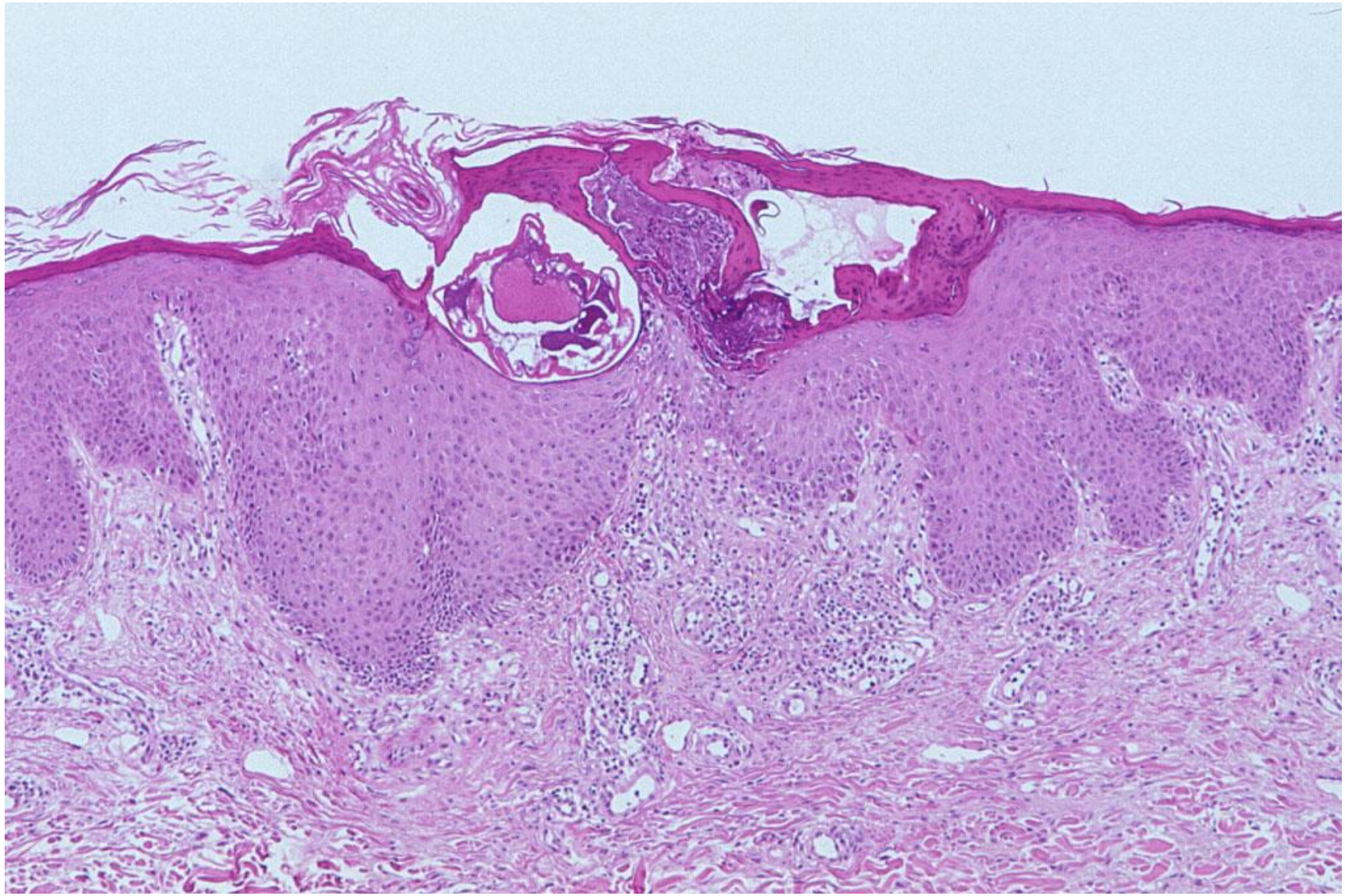


Molluscum contagiosum on the vulva of a female patent aged 40's. Molluscum lesions in adult patents may be a type of sexually transmitted disease (H&E). Characteristic large-sized intracytoplasmic inclusions are formed mainly in the hair follicle-associated sebaceous gland.

Scabies on the scrotum and penis in a male patient aged 20’s (gross appearance).
Scabies in healthy young adults may be a type of sexually transmitted disease.
Itchy papules are multifocally observed in the genitalia.



A scraping preparation from the scabies burrow (unstained material).
An adult mite, a larva, ova and pieces of feces are included.



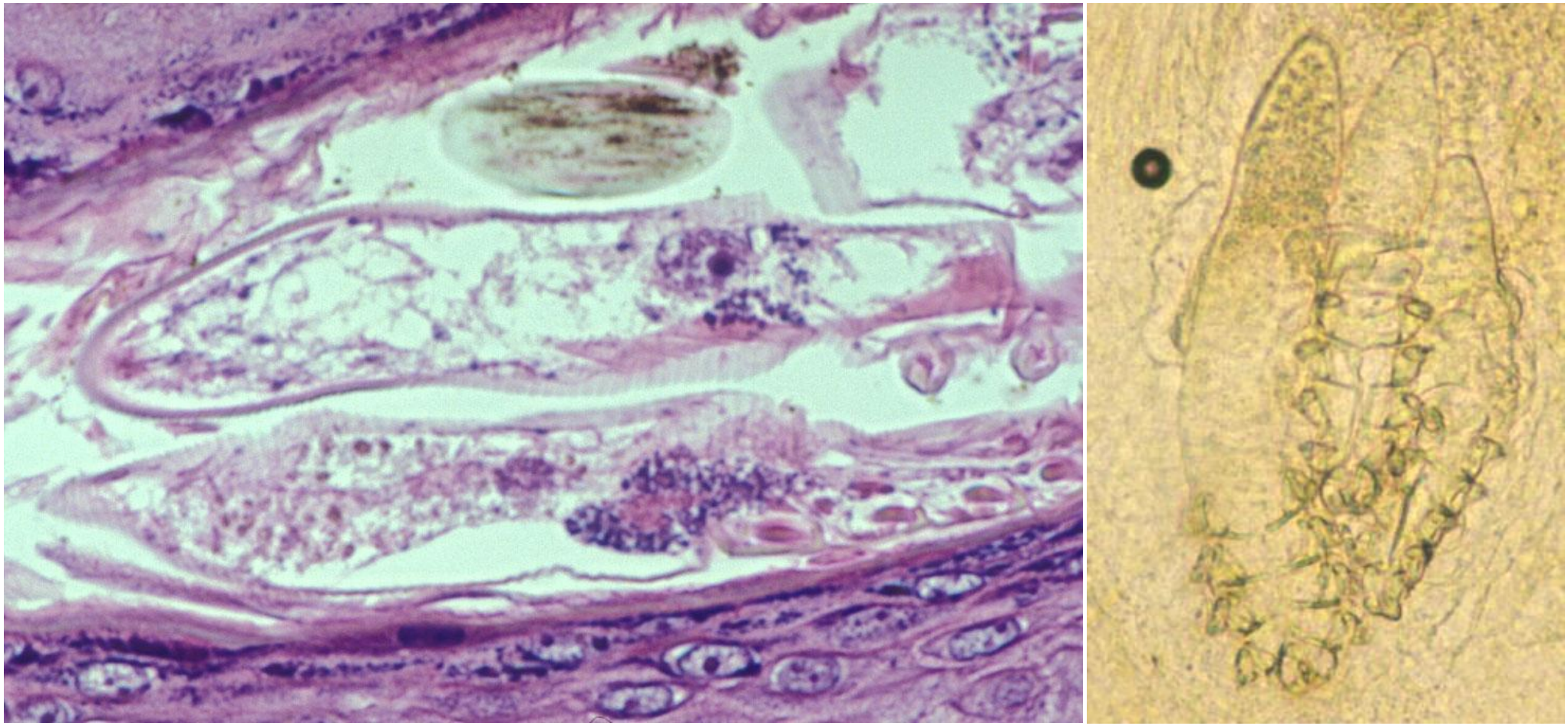
Scabies burrow in the biopsy specimen (H&E). An adult mite *Sarcoptes scabiei* is seen in the cornified layer of the epidermis. Mild chronic inflammation is seen in the upper dermis.

Infestation of **pubic louse** (crab louse), *Phthirius pubis*, on the eyelash. The pubic louse is 1-2 mm in size and crab-like in shape. It has a small head, a pair of antenna and three pairs of legs. The second and third legs are thicker than the front legs, and have curved claws. Sexually transmitted infestation usually occurs in the pubic hair. Eyelashes are another site of infestation. Microscopic appearance is shown in the right panel (H&E).

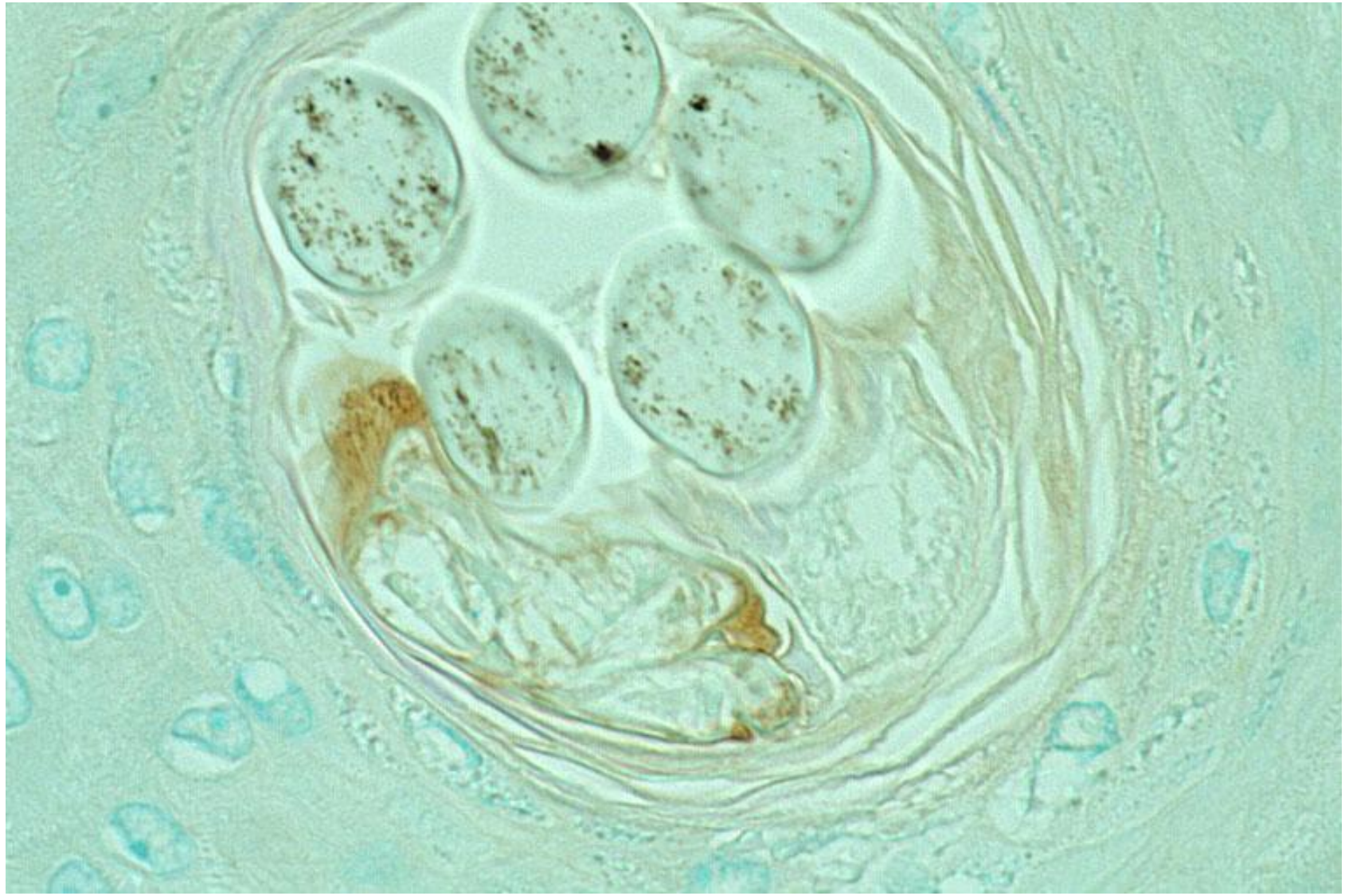
Cutaneous demodecosis and IgD immune response

Colonization of *Demodex folliculorum* or *D. brevis* in the hair follicles on the face is a universal and commensal phenomenon in most adult individuals. It is of note that IgD is specifically deposited on the non-pathogenic mites.

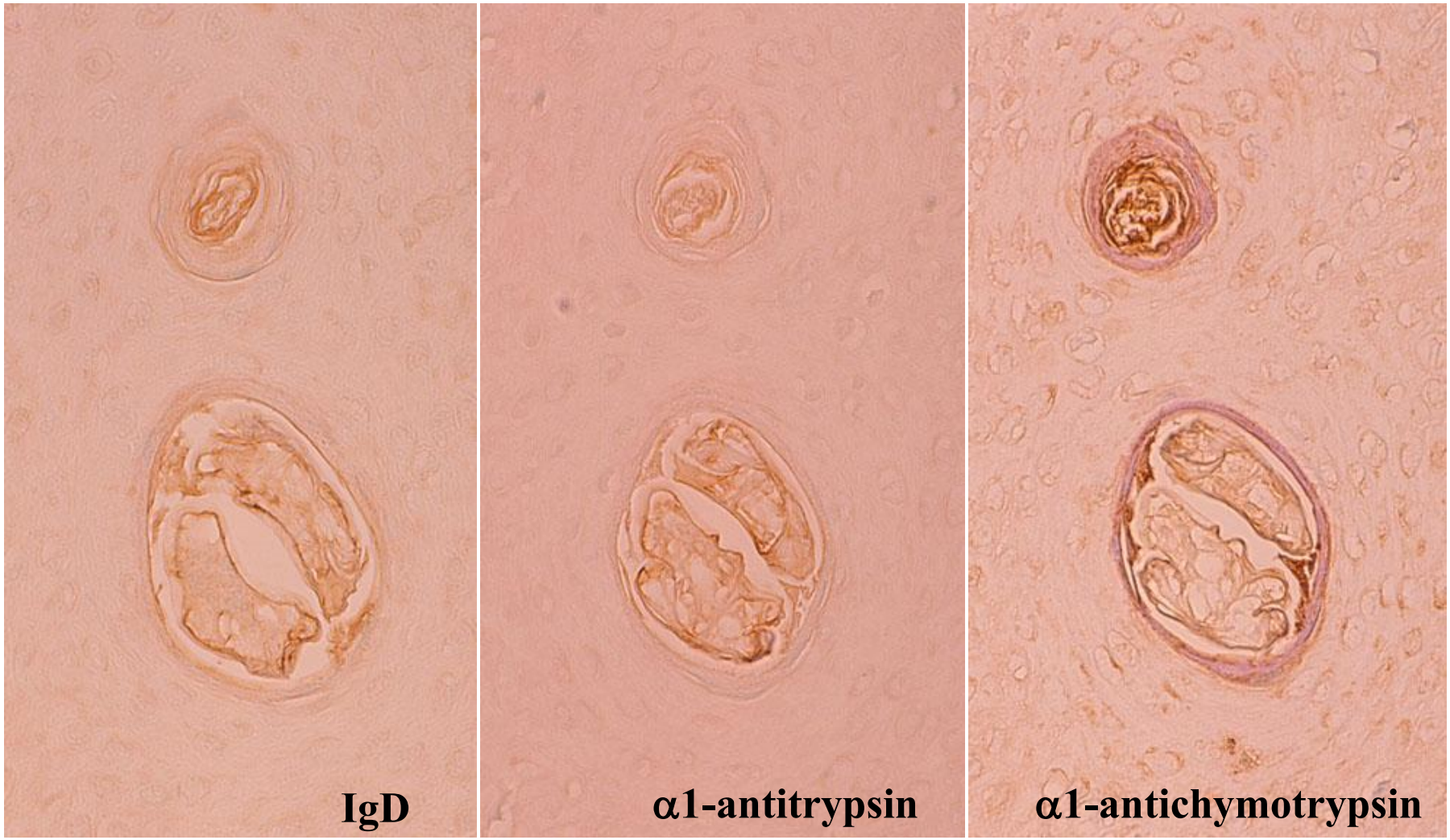
Ref.: Tsutsumi Y. Deposition of IgD, alpha-1-antitrypsin and alpha-1-antichymotrypsin on *Demodex folliculorum* and *D. brevis* infesting the pilosebaceous unit. Pathol Int 2004; 54(1): 32-34. doi: 10.1111/j.1440-1827.2004.01581.x



Demodex folliculorum in the air follicle of the facial skin. The mites with four legs are observed in the hair follicle in the specimen biopsied from nevocellular nevus (left: H&E). Scraping of the keratotic plug from the perinasal hair follicle shows a paired adult mites with four pairs of legs (right: unstained preparation). *D. folliculorum* is a normal resident of the seborrheic skin.



IgD deposition on the surface of *Demodex folliculorum* in the hair follicle seen adjacent to the hair shafts. IgD deposition is consistently seen as an immune response to the mite. In contrast, IgG, IgA, IgM and IgE are negative.



Immune response to *Demodex folliculorum* in the biopsy specimen. In addition to the IgD reaction (left), α1-antitrypsin (center) and α1-antichymotrypsin (right) are also deposited on the mites. **Ref.**: Tsutsumi Y. Deposition of IgD, alpha-1-antitrypsin and alpha-1-antichymotrypsin on *Demodex folliculorum* and *D. brevis* infesting the pilosebaceous unit. Pathol Int 2004; 54(1): 32-34. doi: 10.1111/j.1440-1827.2004.01581.x

Rare skin infections: Recognition of the infection is essential.

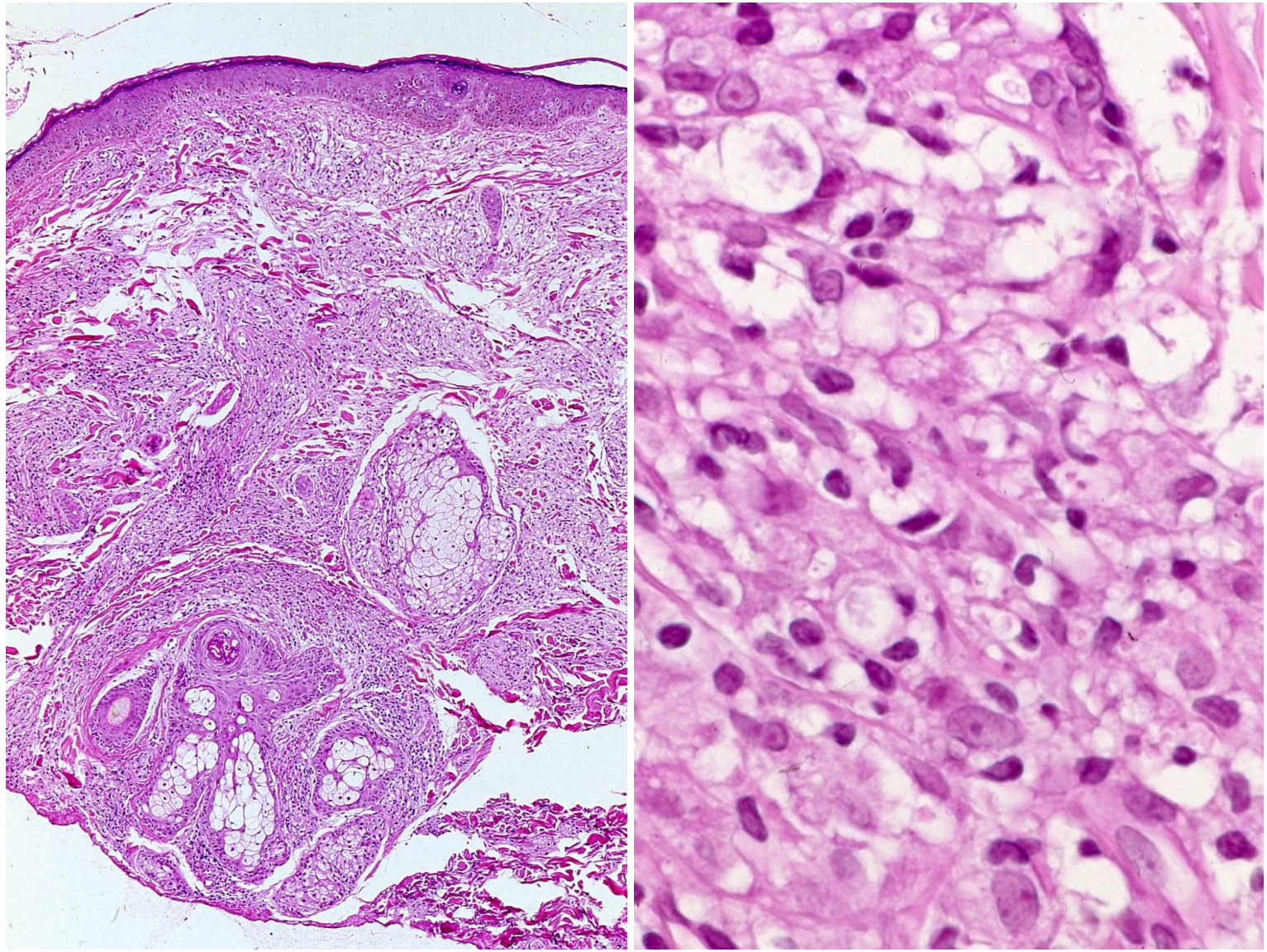
Hansen's disease

Pasteurellosis

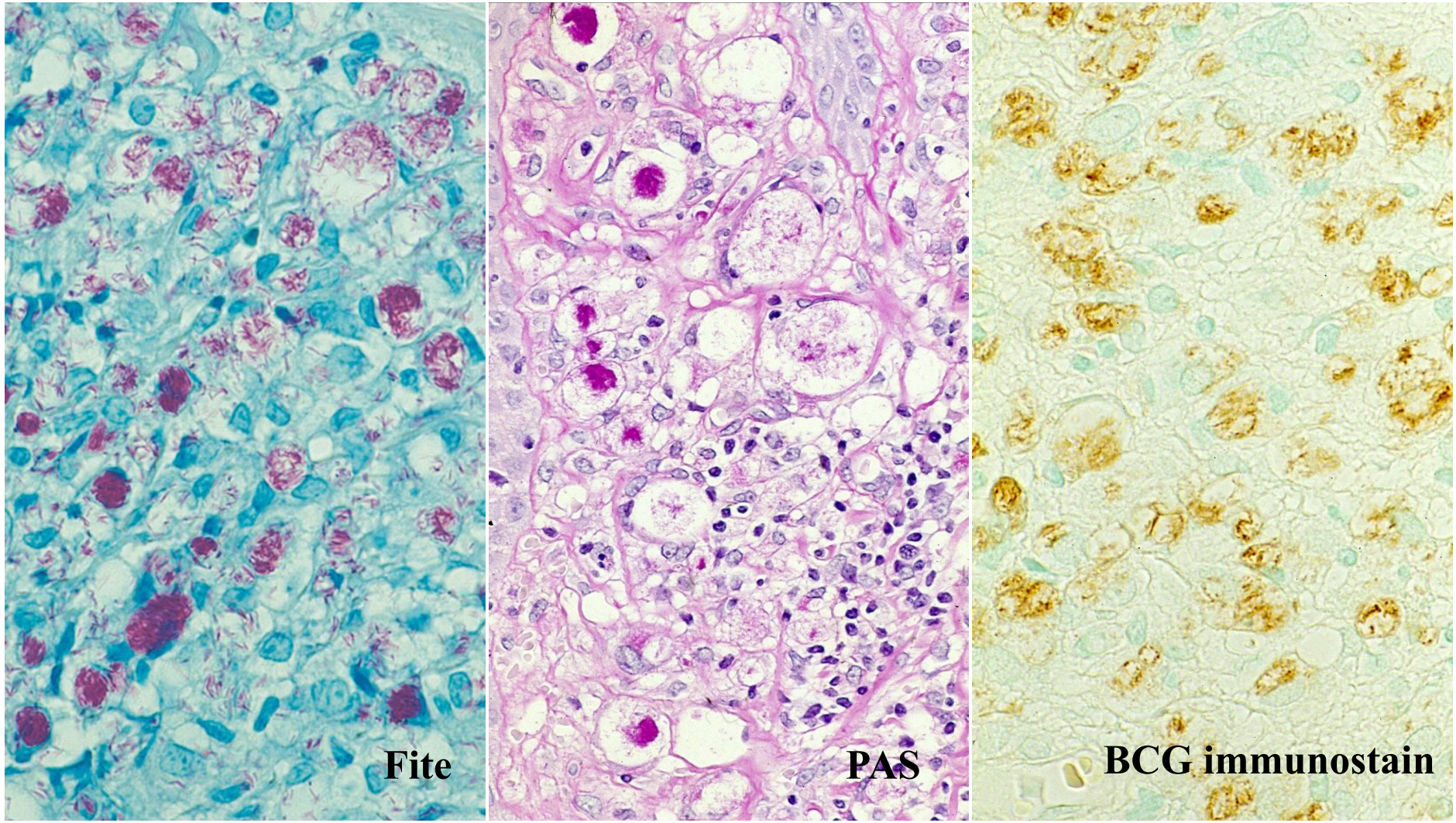
Protothecosis

Cutaneous leishmaniasis

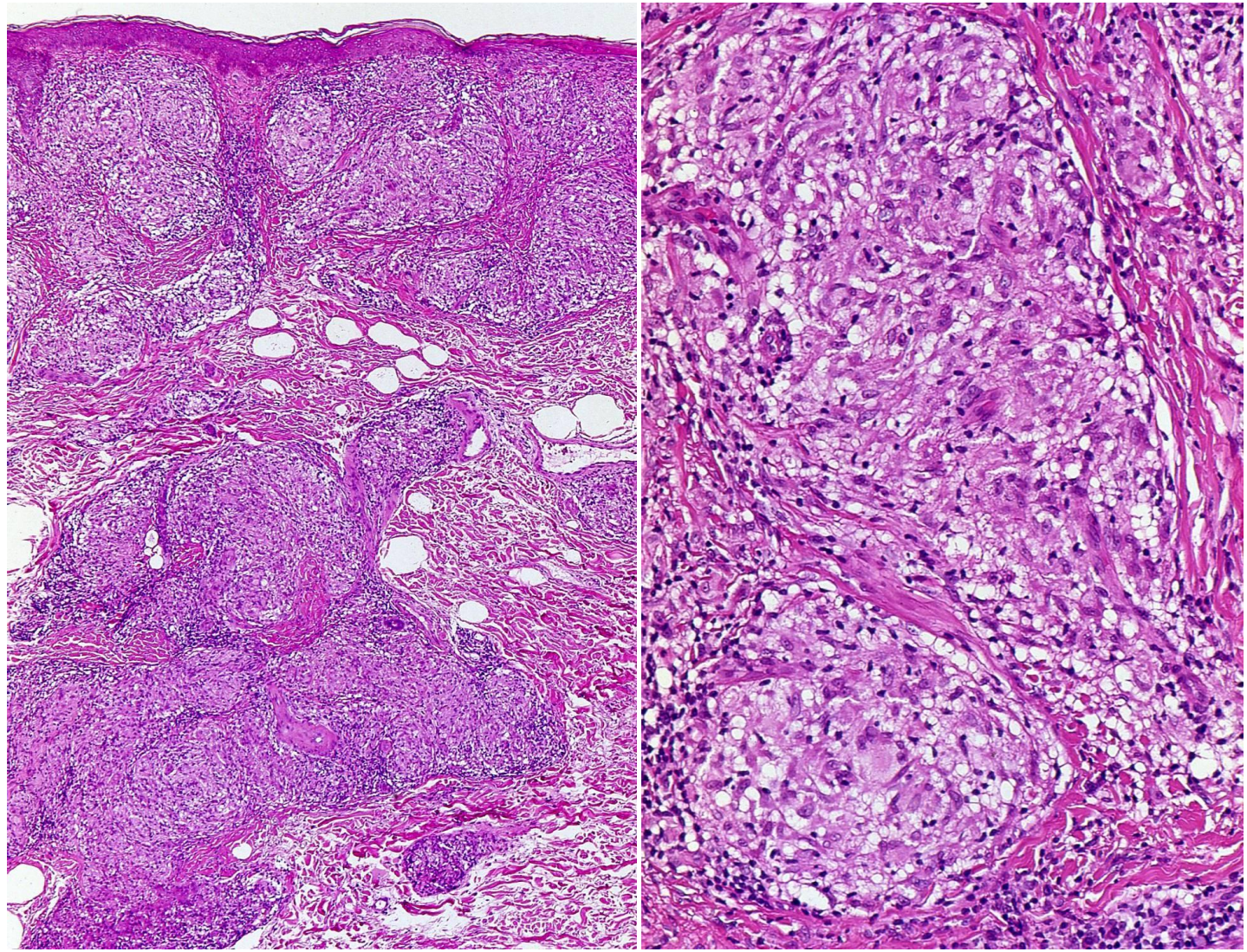
Cutaneous penicilliosis



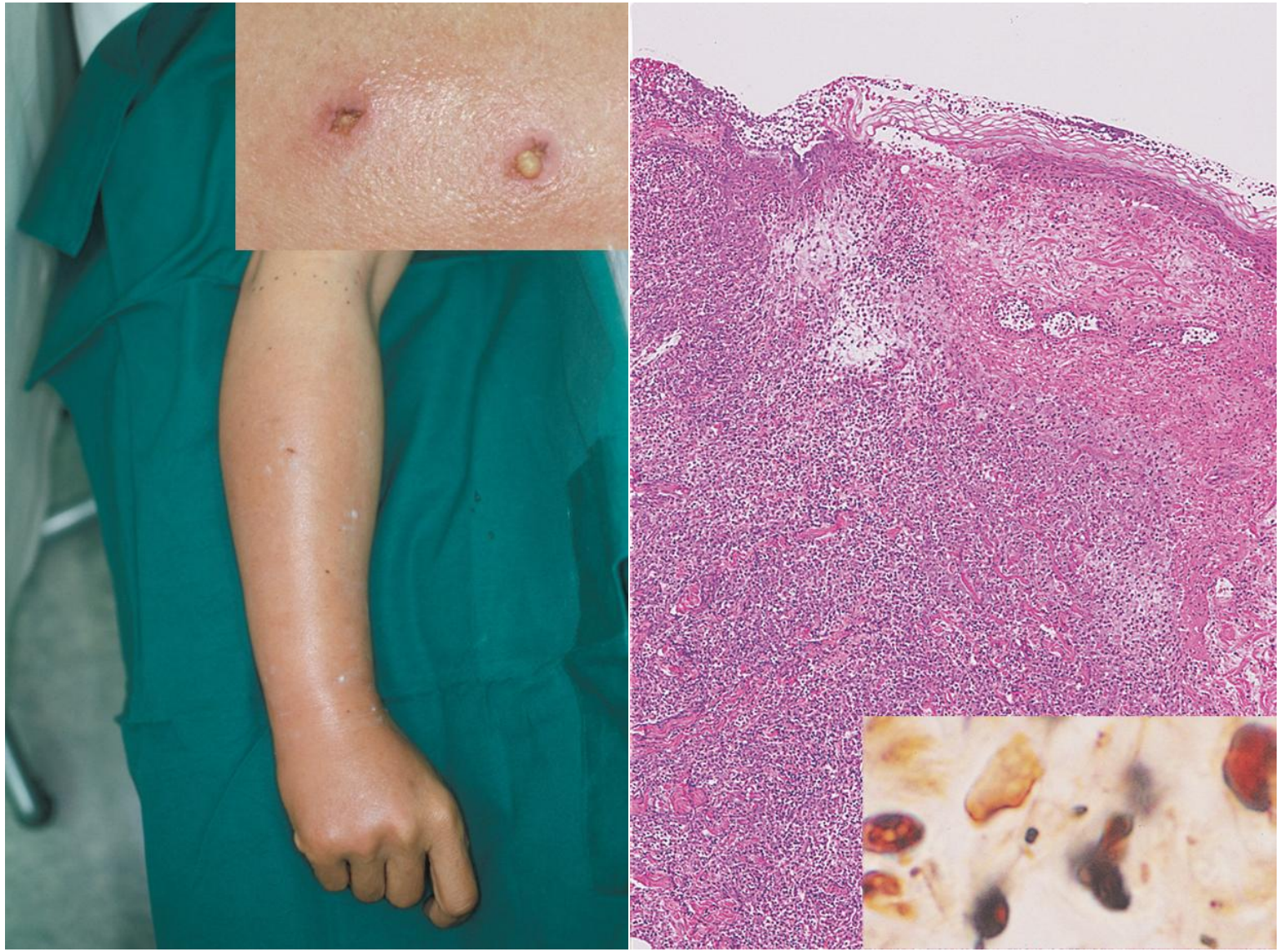
Lepromatous (multibacillary) leprosy (H&E). The dermis is occupied by foamy macrophages. The foamy macrophages frequently contain “globi” in the cytoplasm. Clusters of bacillary structures are discernible in the globi. Lymphocytic reaction is minimal.



Globi in lepromatous (multibacillary) leprosy (left: Fite method of Zihel-Neelsen's stain, center: PAS, right: immunostaining using BCG antiserum). The "globi" are occupied acid-fast bacilli (left). Fite method of Ziehl-Neelsen's staining is needed to demonstrate *Mycobacterium leprae* in paraffin sections. Globi are also reactive for PAS reaction and BCG antigens.

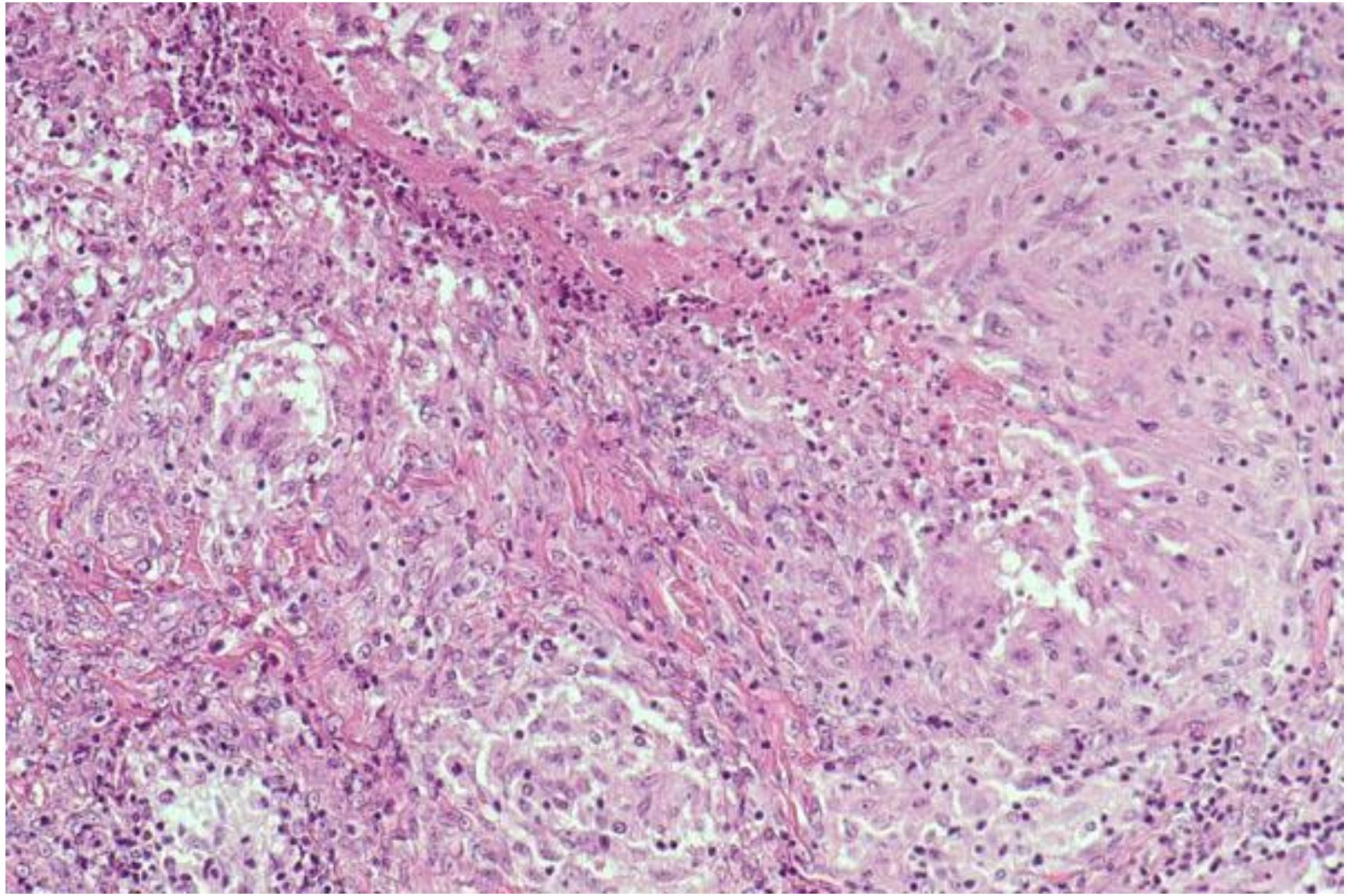


Tuberculoid (paucibacillary) leprosy (H&E). Non-caseous epithelioid granulomas are seen throughout the dermis. Multinucleated giant cells of Langhans type are scattered. Lymphocytic reaction is mildly observed. The Fite method fails to detect acid-fast bacilli in the lesion.

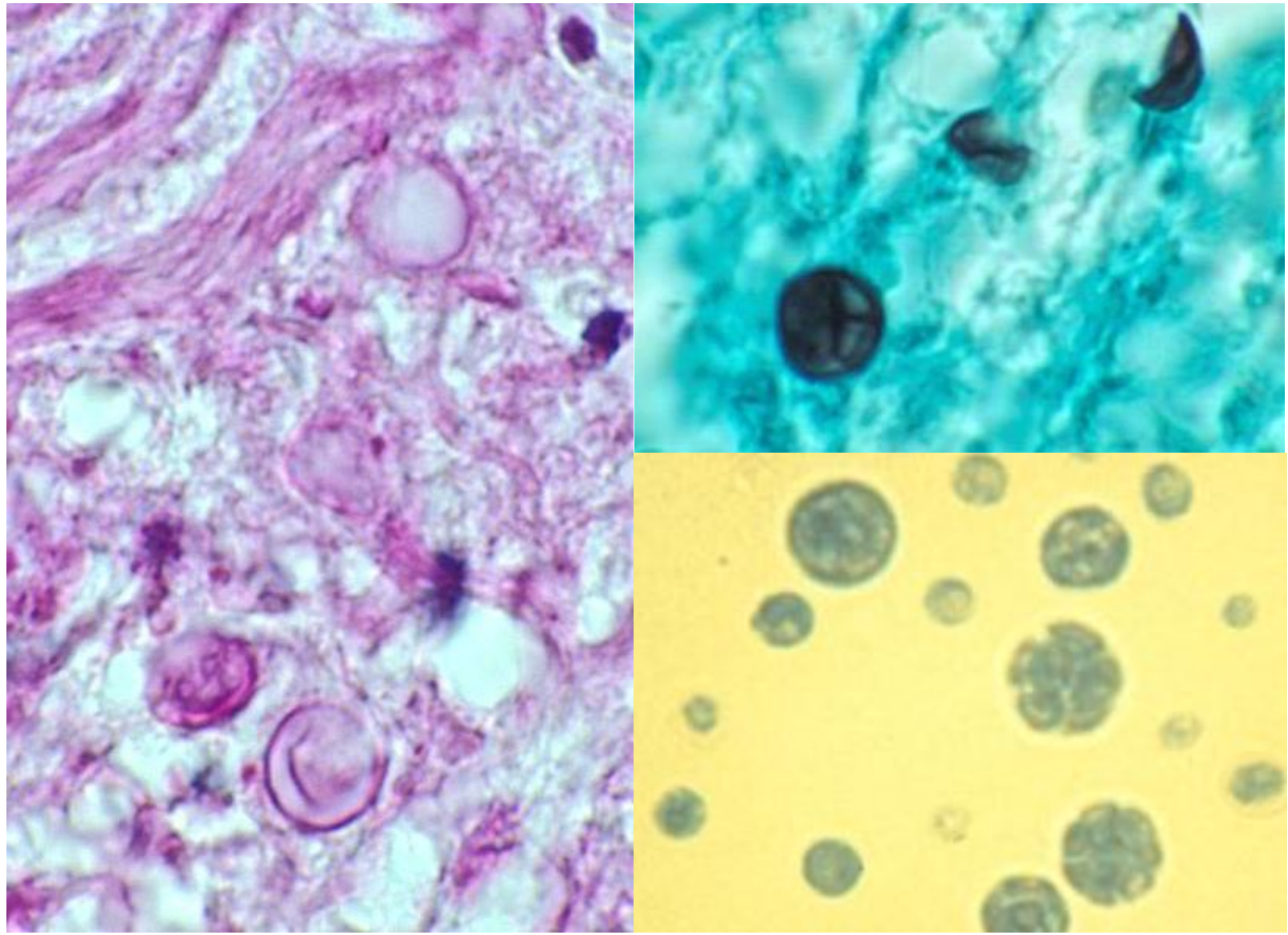


Cutaneous pasteurellosis. A 50 y-o female patient received a scratch wound by her domestic cat in the right forearm. The scratch wound is shown in the inset. High fever at 39C appeared next day, and phlegmonous inflammation with axillary lymphadenopathy was seen in the 3rd day (left). WBC 19,100, C-reactive protein 22.5 mg/dL. Biopsy reveals dense inflammatory (neutrophilic) infiltration in the dermis (right: H&E). Warthin-Starry silver demonstrates causative short rods in the cytoplasm of neutrophils (inset). Microbial culture identified *Pasteurella multocida*.

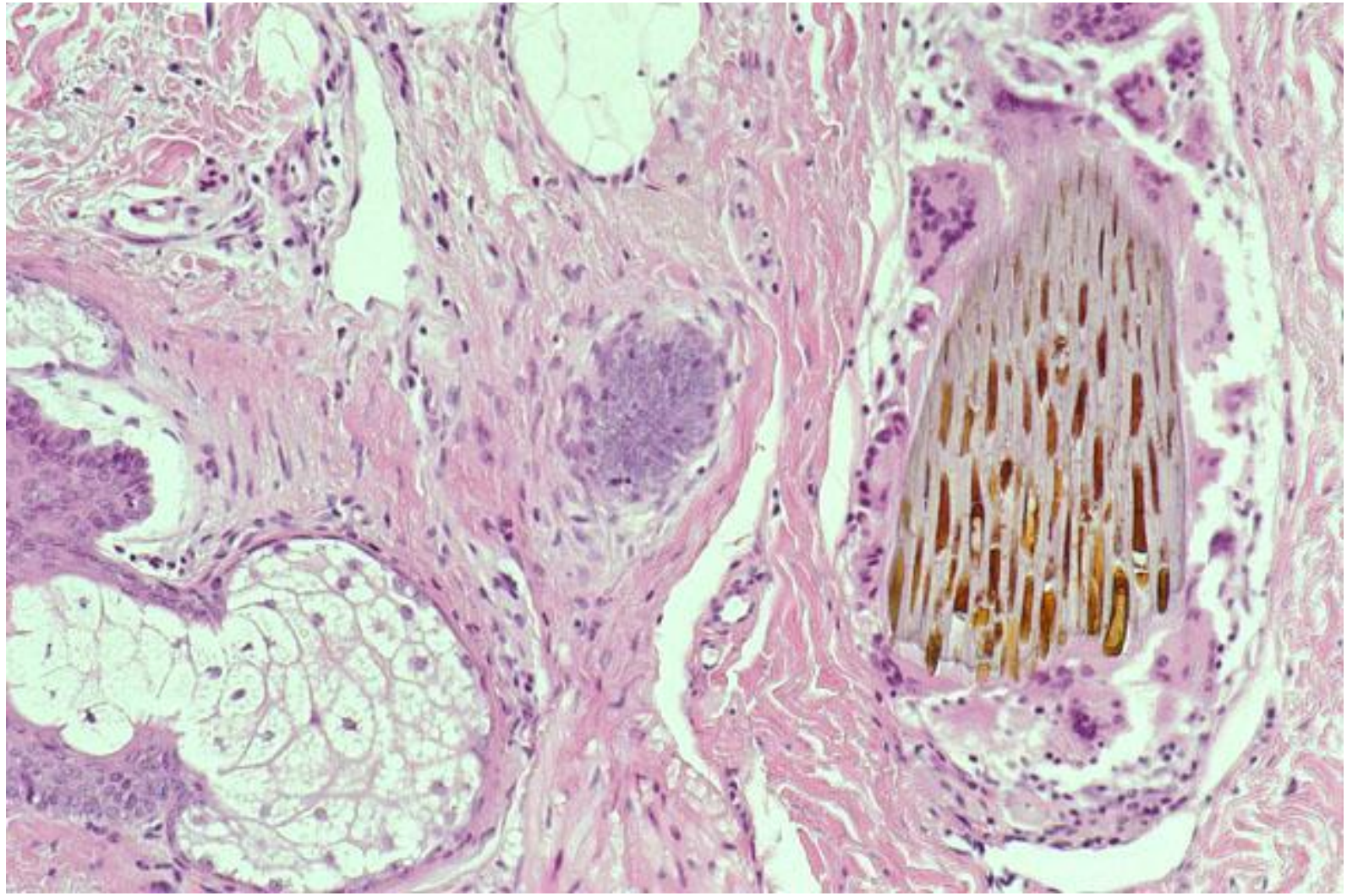
Gross appearance of protothecosis on the dorsum of the hand of a 66 y-o male farmer of mandarin orange. Fistulas draining from pustules are multifocally associated. *Prototheca wickerhamii* was cultured from the skin lesion.



Protothecosis on the dorsum of the hand of a 66 y-o male farmer of mandarin orange. Suppurative granulomas are formed in the dermis (H&E). *Prototheca wickerhamii* was cultured from the skin lesion.

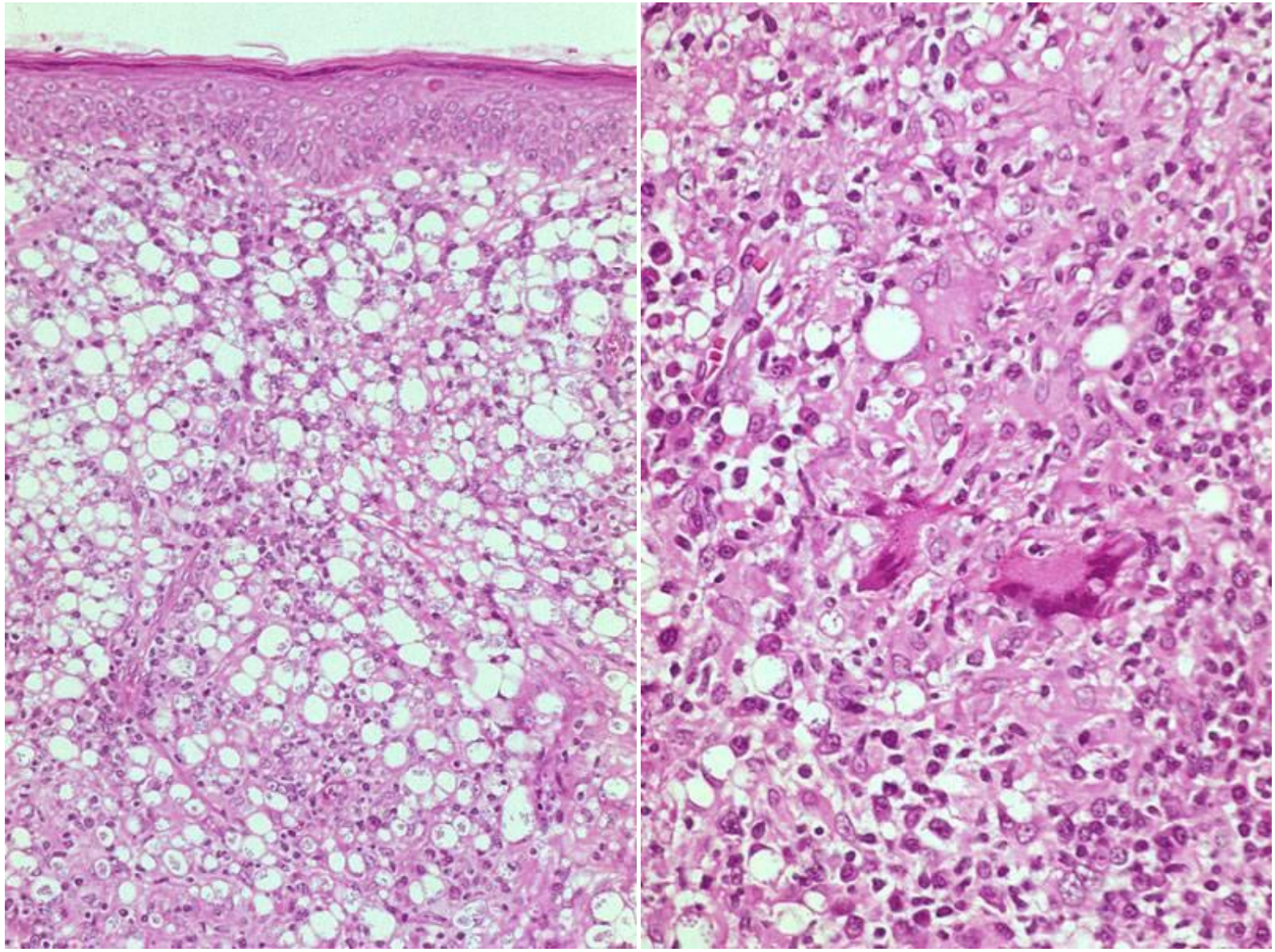


Protothecosis（*P. wickerhamii* infection*)*. Round-shaped pathogens are seen mainly in the necrotic zone (left: H&E). The pathogens are positively stained with Grocott (and PAS) (right top). Cultured pathogens forming sporandia are shown in the right bottom (Pap). The sporandia contain endospores to form morules.

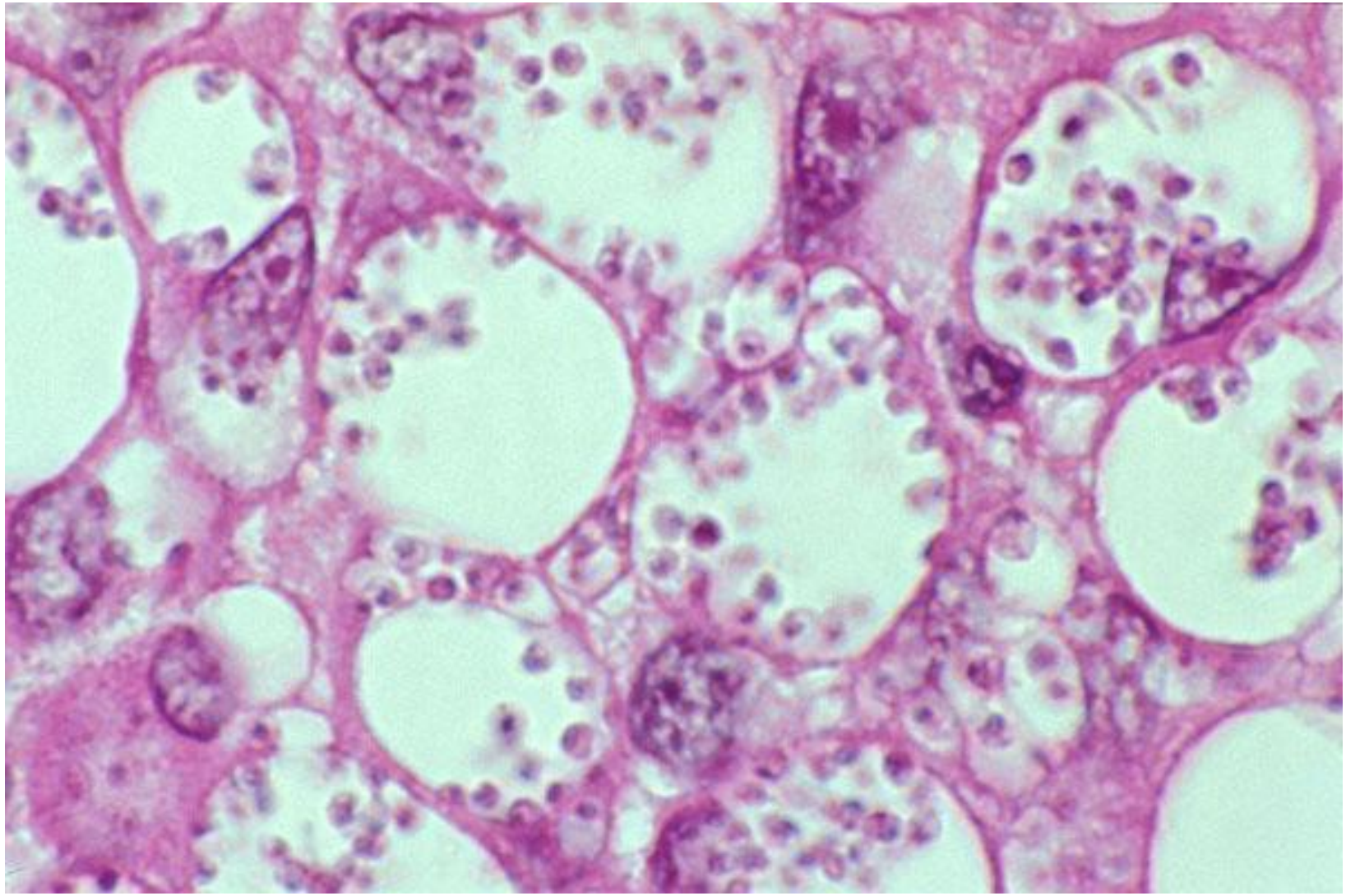


A splinter (a piece of wood) is observed in the dermis adjacent to the lesion of protothecosis (H&E). Protothecosis is mediated by trauma or splinter piercing. The pathogen *P. wickerhamii*, an algae without chlorophylls, is ubiquitously distributed in the environment, including the sap.

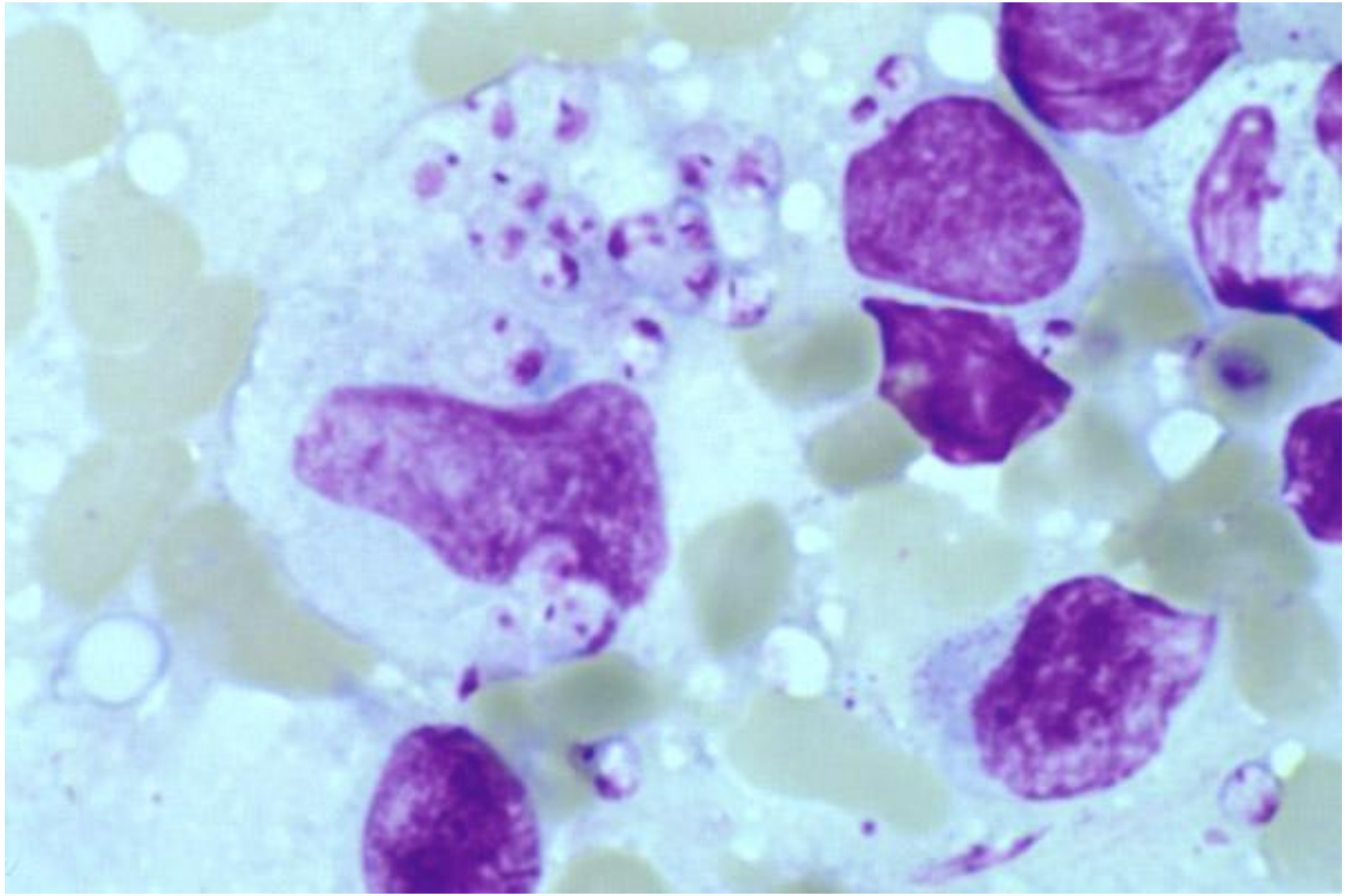
Leishmania tropica infection (Indian or urban type cutaneous leishmaniasis). A sandfly mediates the transmission of the protozoan pathogens, to form a single 4 x 2 cm-sized painless ulcerative lesion on the neck skin, months after the sandfly bite. Dogs function a reservoir host. The present male patient aged 60's stayed for business in India.



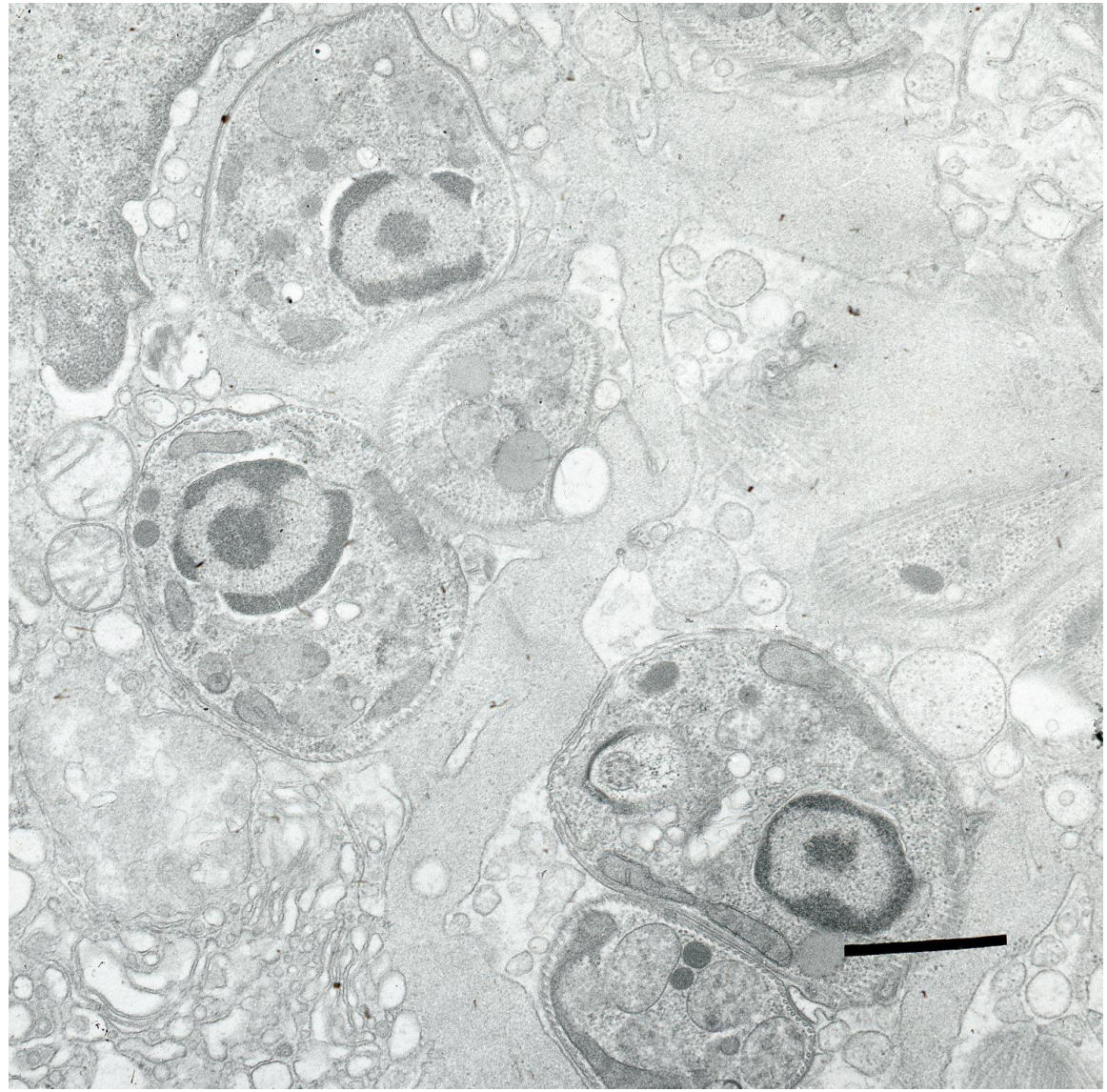
Leishmania tropica infection (Indian type cutaneous leishmaniasis) (H&E). Macrophages with vacuolated cytoplasm are clustered in the dermis. In the deep dermis, foreign body reactions with multinucleated giant cells are observed.



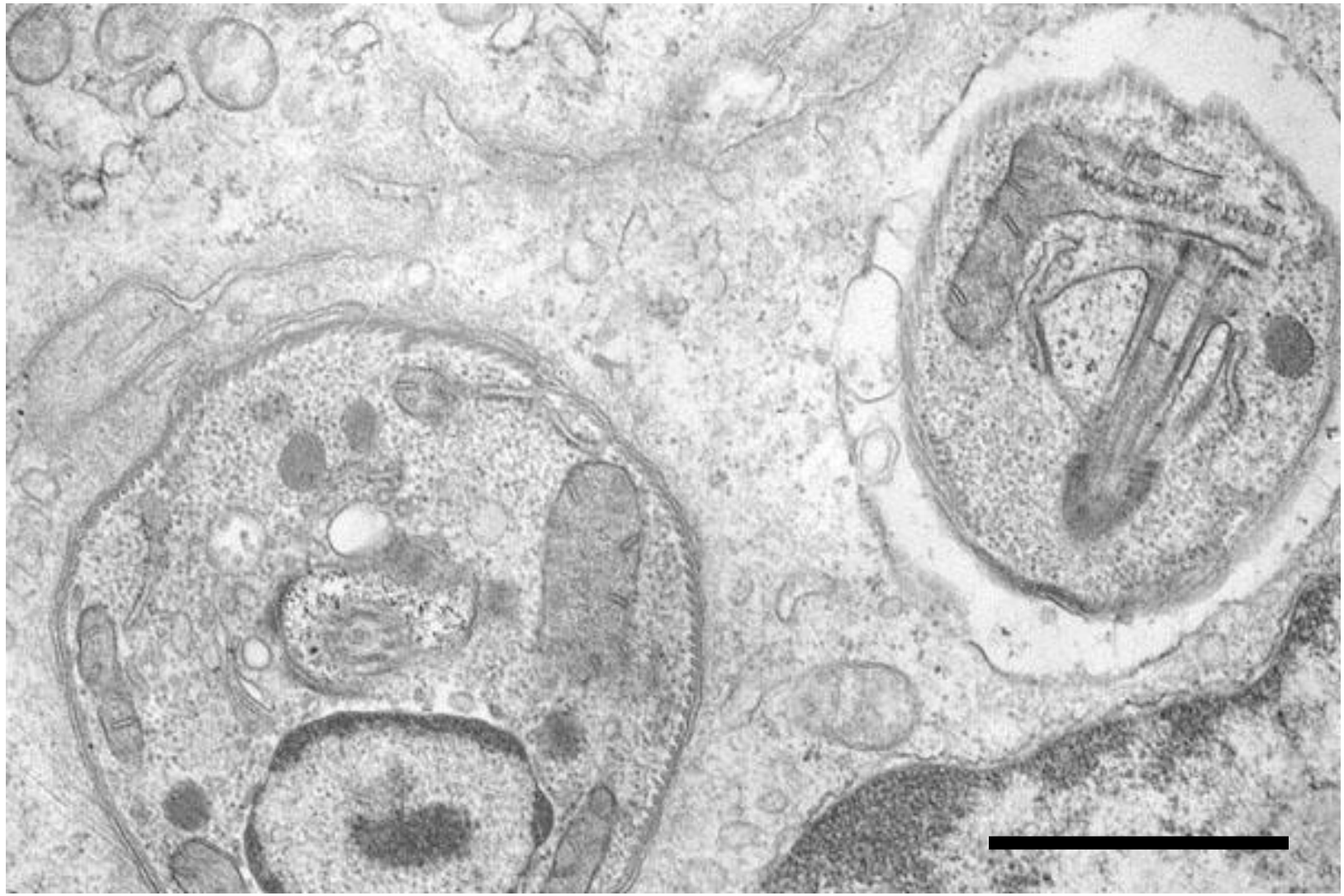
Leishmania tropica infection (Indian type cutaneous leishmaniasis) (H&E). High-powered view demonstrates round and mononucleated protozoan pathogens are clustered within the vacuoles of the macrophages.



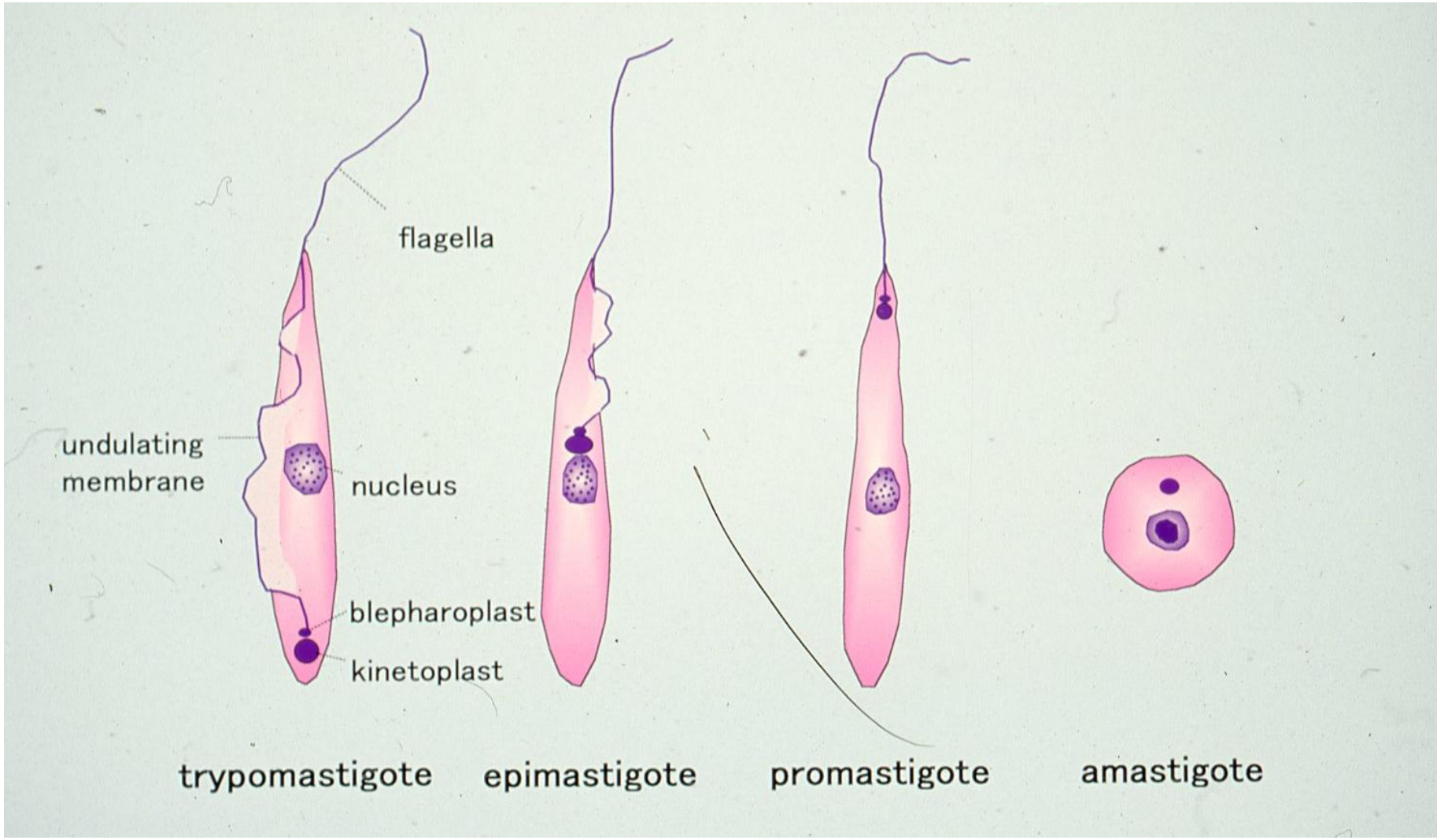
Leishmania tropica infection (Indian type cutaneous leishmaniasis) (stamp smear, Giemsa). Leishmanian pathogens in the cytoplasm of the macrophage possess a pair of a round to oval nucleus and a small-sized (dot-like) kinetoplast.



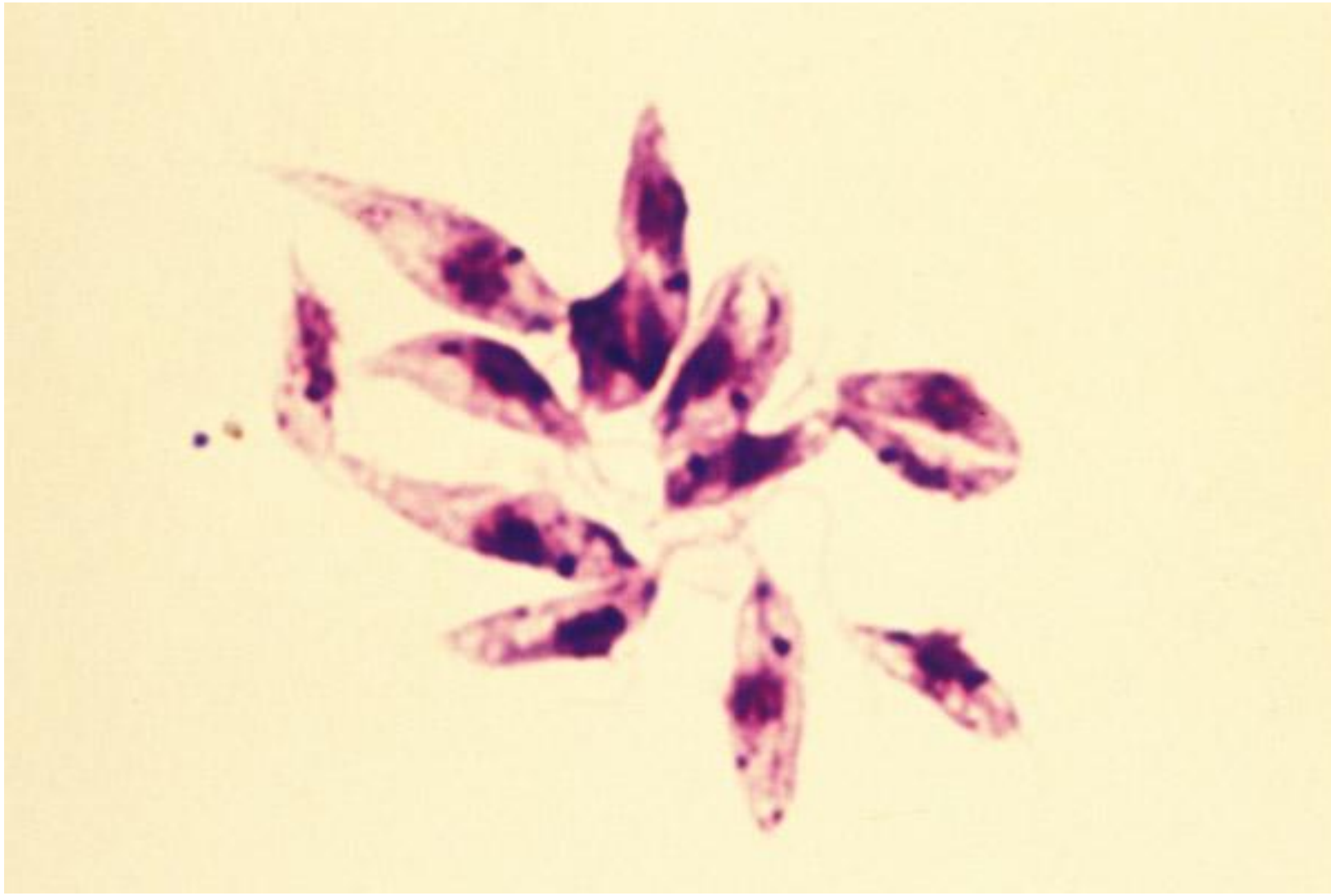
Leishmania tropica infection (Indian type cutaneous leishmaniasis) (ultrastructural features-1). Leishmanian amastigotes in the cytoplasm of the macrophage possess a round nucleus with a characteristic chromatin pattern (condensation along the nuclear membrane and centrally located rounded nucleosome). Bar indicates 1 μm.



Leishmania tropica infection (Indian type cutaneous leishmaniasis) (ultrastructural features-2). Leishmanian amastigotes in the cytoplasm of the macrophage possess a round nucleus with a characteristic chromatin pattern. Microtubules are regularly arranged beneath the plasma membrane. A kinetoplast (forming the base of the flagella) is seen. Bar indicates 1 μm.

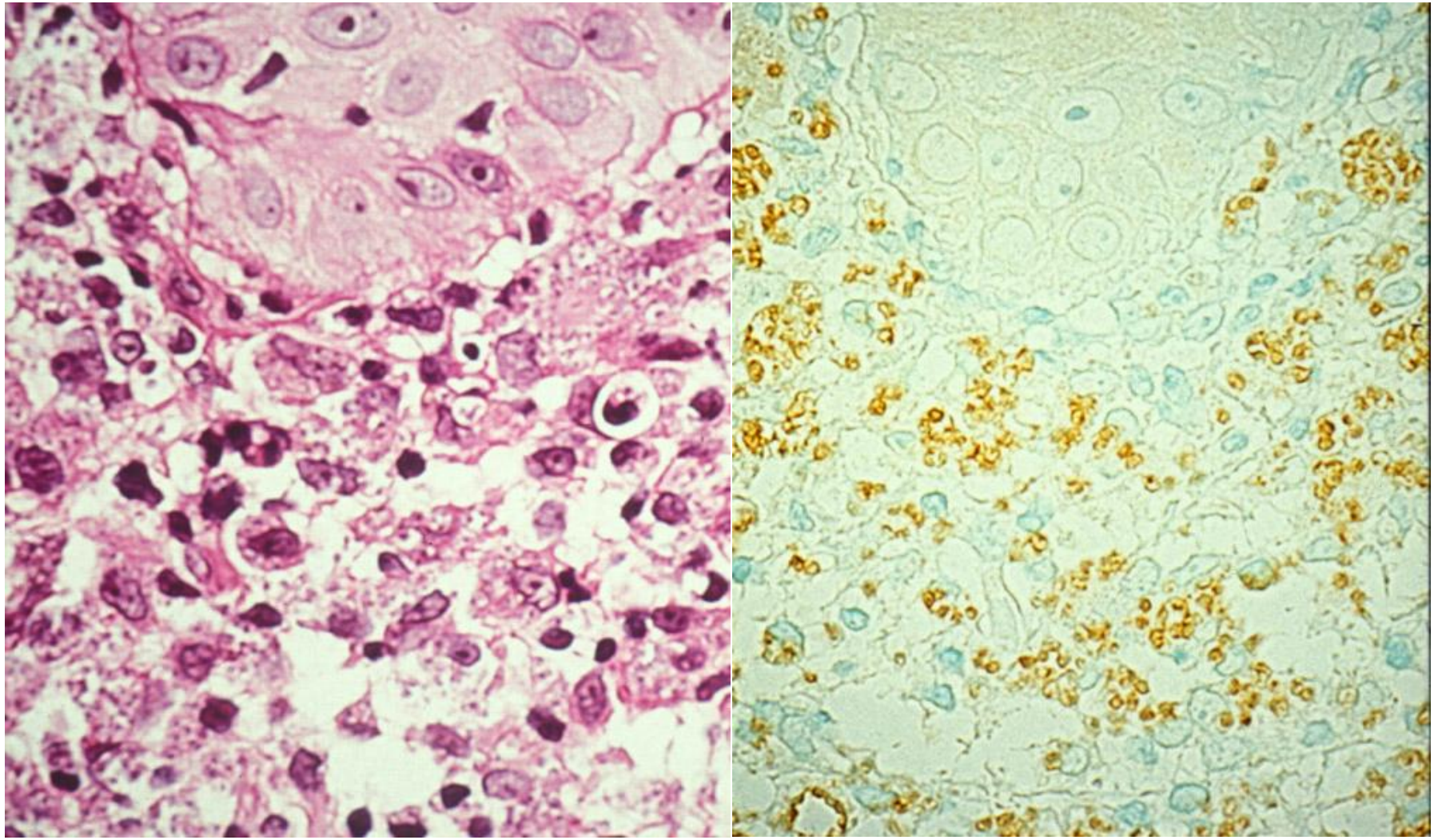


Schematic figures of morphological changes of Leishmania and Trypanosoma. Leishmania shows two phases amastigote and promastigote. The promastigote is seen within the mid-gut of the vector.



Cultured *Leishmania tropica*（May-Giemsa), showing a promastigote form. In the acellular culture media, the Leishmania transforms into the promastigote, a form in the mid-gut of the vector. An elongated oval shaped 25 μm-sized protozoa possess a long flagella beginning from the kinetoplast located at the front end of the protozoan body.

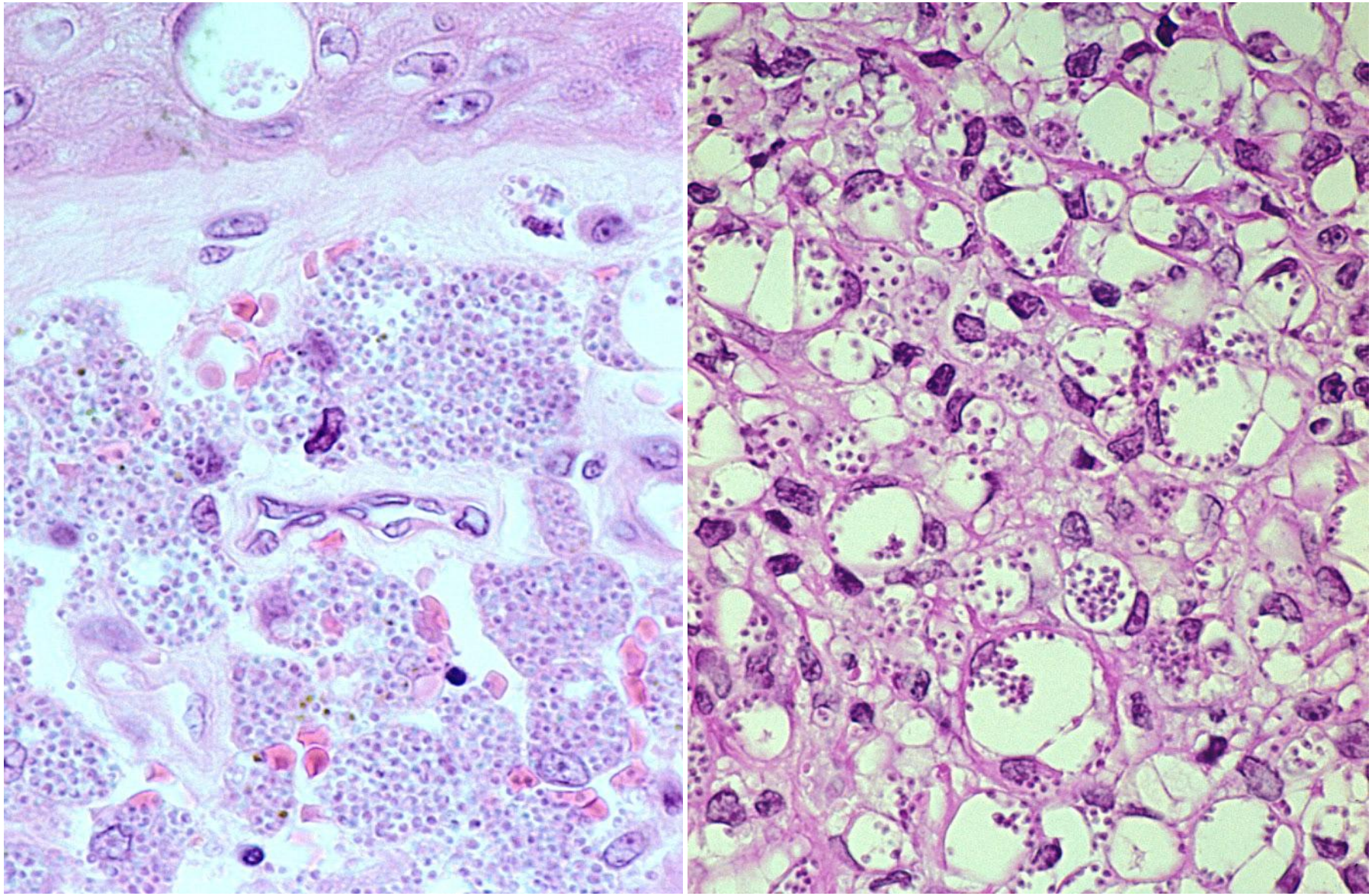
Leishmania major infection (African or rural type cutaneous leishmaniasis). A sandfly mediates the transmission of the protozoan pathogens, to form multiple painless ulcerative lesions on both forearms, months after the sandfly bite. Rodents function a reservoir host. The present Japanese male patient aged 36 years worked as a plantation volunteer in Mali, Africa.



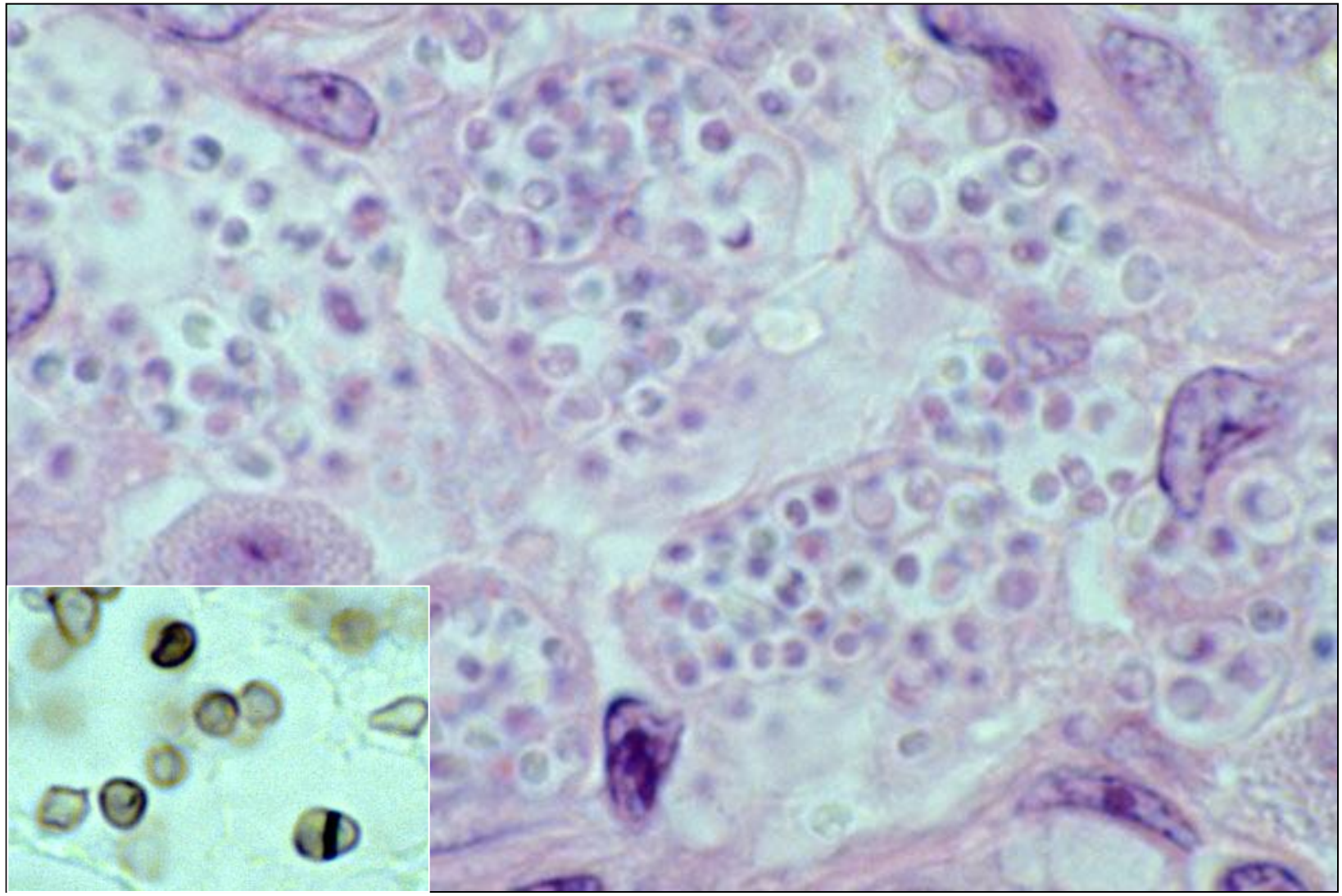
Leishmania major infection (African type cutaneous leishmaniasis) (left: H&E, right: immuno-staining using a diluted patient's own serum). The protozoan bodies actively grow within the macrophages in the dermis, Indirect immunoperoxidase staining using a 1:500 diluted patient's own serum clearly demonstrates the pathogens in formalin-fixed, paraffin-embedded sections.

Differential diagnosis

Opportunistic infection of *Penicillium marnefei* in AIDS patients in Thailand. Penicilliosis marnefei is endemic in the Southeastern Asia, and an important cause of death in this area. Microscopic features in H&E preparations are quite similar to leishmaniasis.



H&E microscopic features of the skin lesion in Penicilliosis marnefei (left) is indistinguishable from that of cutaneous leishmaniasis (right: *L. tropica* infection). P. marnefei is positively stained with PAS and Grocott, while the leishmanian bodies are negative for both.



Penicilliosis marneffei in an AIDS patient in Thailand (H&E, inset: Grocott). *P. marnefei* often involves the skin. The yeast form fungi grow in the cytoplasm of macrophages in the dermis. The fungi are positive with Grocott methenamine silver, and the formation of the septum (inset) is diagnostic of *P. marnefei*.

Fulminant/lethal infections involving the skin-1

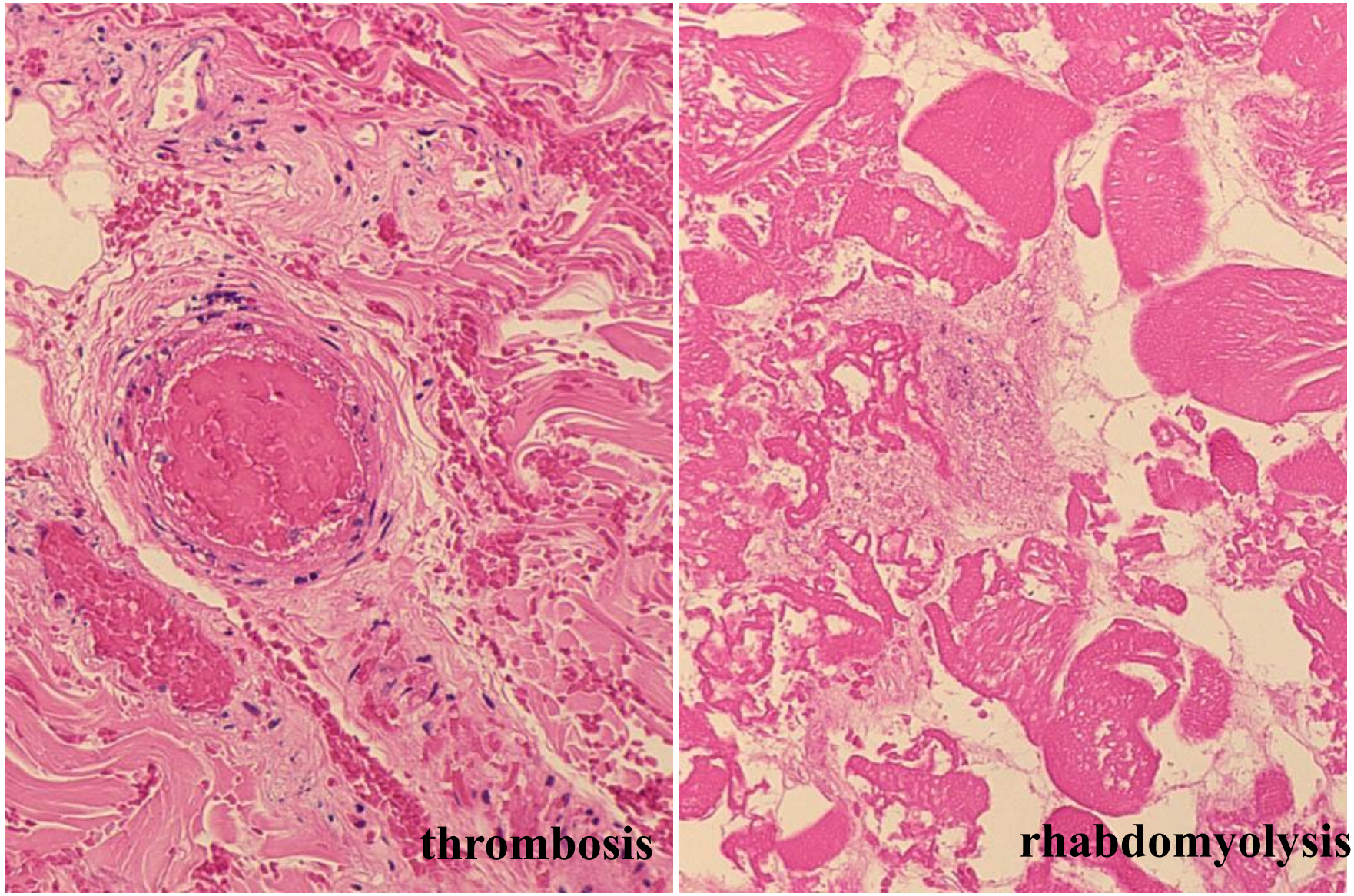
(flesh-eating bacterial infection)

1) Fulminant streptococcal infection

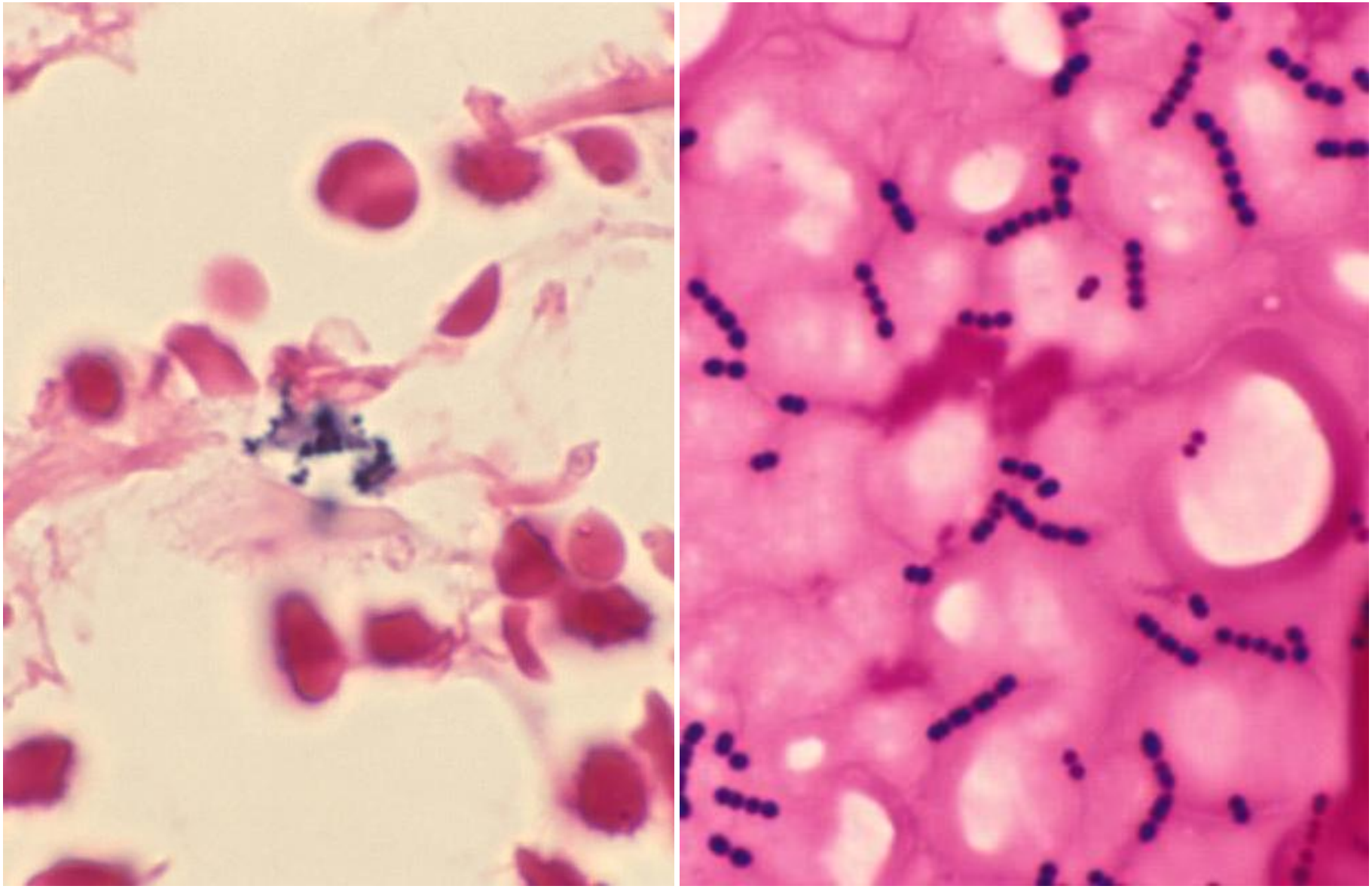
2) *Vibrio vulnificus* infection

Fulminant streptococcal infection with the primary focus in the scrotum (Fournier's gangrene). Progressive gangrene in the left leg necessitated emergency amputation, but the patient died soon.

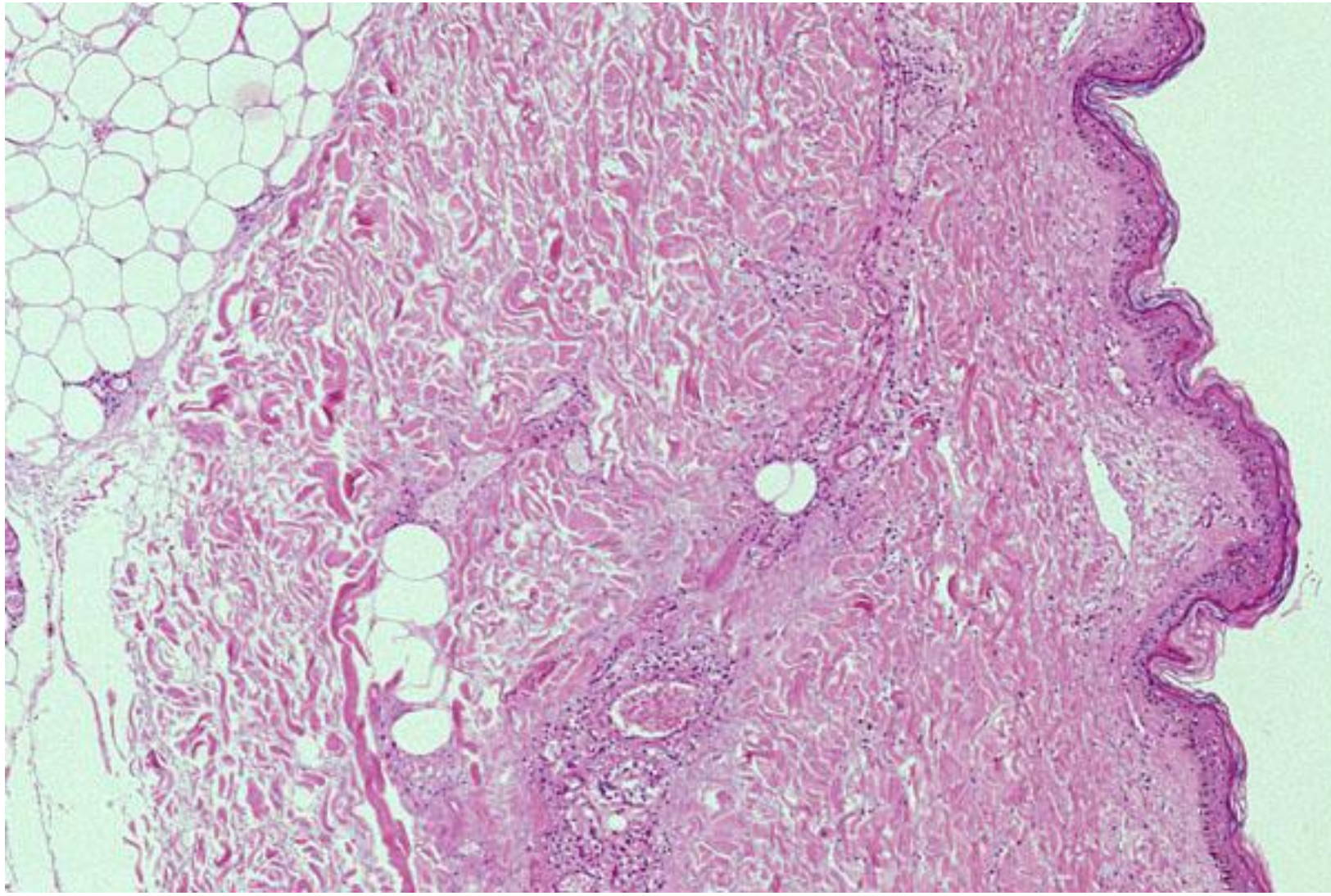
Fulminant streptococcal infection with the primary focus in the scrotum (Fournier's gangrene). Progressive gangrene extends through the left groin to the left leg. Erosive change is evident in the skin of the groin.



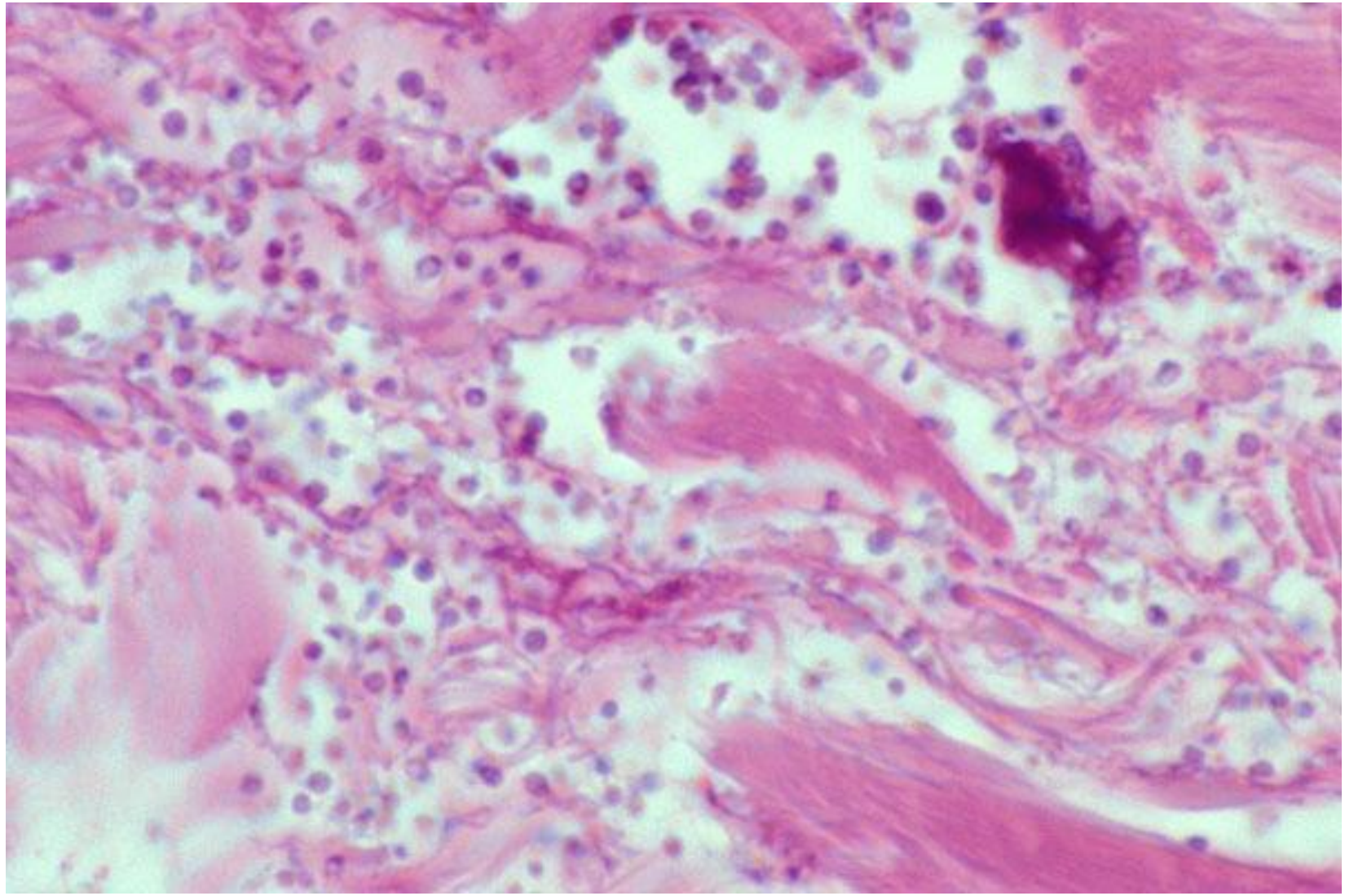
Fulminant streptococcal infection. The emergency amputated left leg shows massive ischemic changes: thrombosis (left) and myonecrosis (right) are observed (H&E).



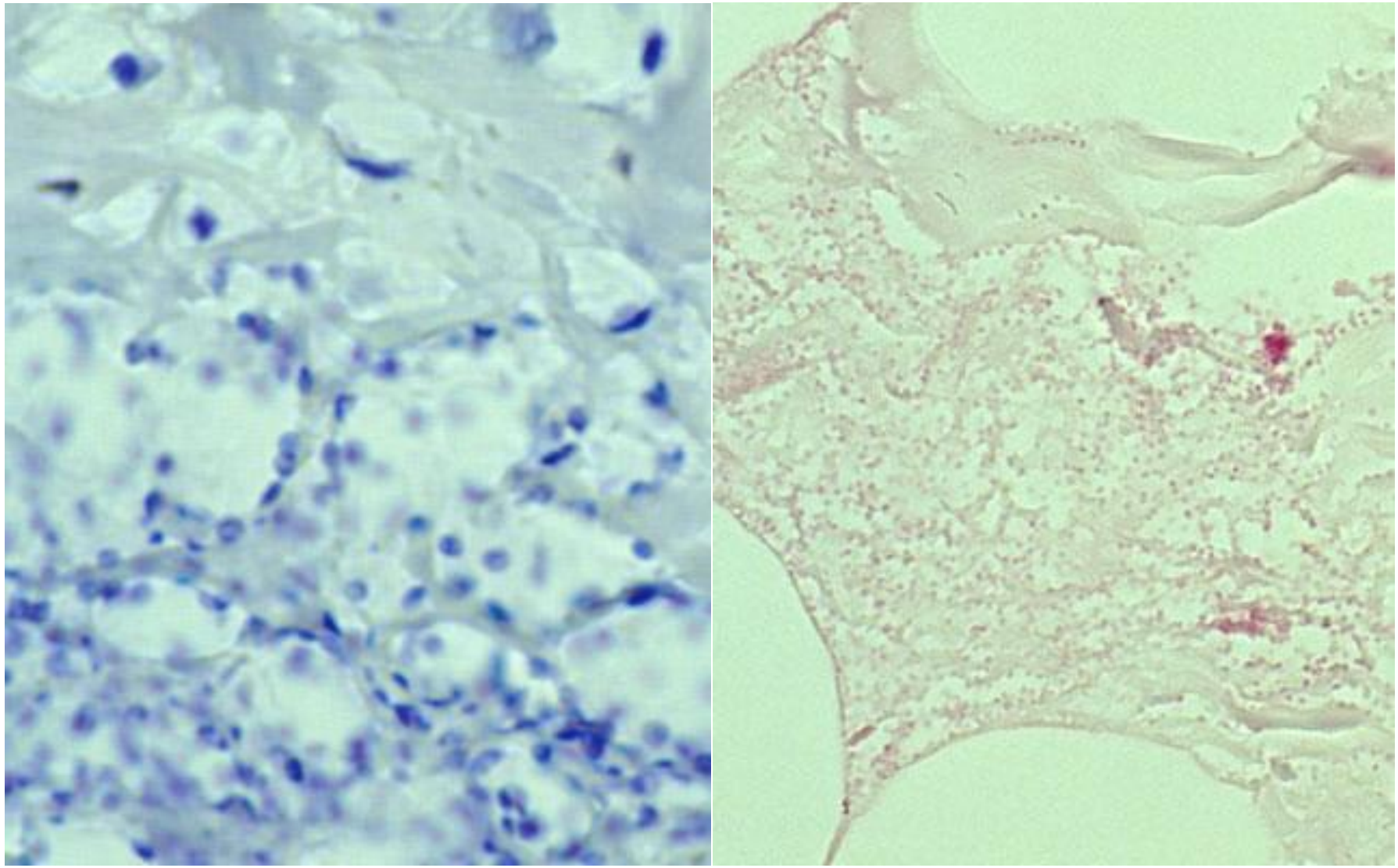
Fulminant streptococcal infection (Gram). Left: focal colonization of Gram-positive cocci in necrotic striated muscle tissue, right: the growth of chained Gram-positive cocci in the cultured peripheral blood.



Fulminant *Vibrio vulnificus* infection. A male patient aged 50's, suffering from type C liver cirrhosis, injured his finger while cooking fresh fish. Soon, progressive gangrene extended to the forearm. Biopsy from the gangrenous forearm discloses a cellular ischemic change of the deep-dermal sweat glands and subcutaneous fat tissue (H&E). This type of flesh-eating bacterial infection, relatively common in Japan, happens mostly in patients with liver cirrhosis. The source of infection is the fresh sea creature.



Fulminant *Vibrio vulnificus* infection. A male patient aged 50's, suffering from type C liver cirrhosis, injured his finger while cooking fresh fish. Soon, progressive gangrene extended to the forearm. High-powered view of the ischemic deep dermis around the sweat gland reveals growth of coccoid bacteria without inflammatory cellular reactions (H&E).



Fulminant *Vibrio vulnificus* infection. A male patient aged 50's, suffering from type C liver cirrhosis, injured his finger while cooking fresh fish. Soon, progressive gangrene extended to the forearm. High-powered view of the ischemic deep dermis around the sweat gland reveals growth of Gram-negative coccoid bacteria without inflammatory cellular reactions (left: Giemsa, right; Gram).

Fulminant/lethal infections involving the skin-2

1) Necrotizing fasciitis complicated with diabetic neuropathy and severe burn

2) Rhinocerebral mucormycosis complicated with diabetic coma

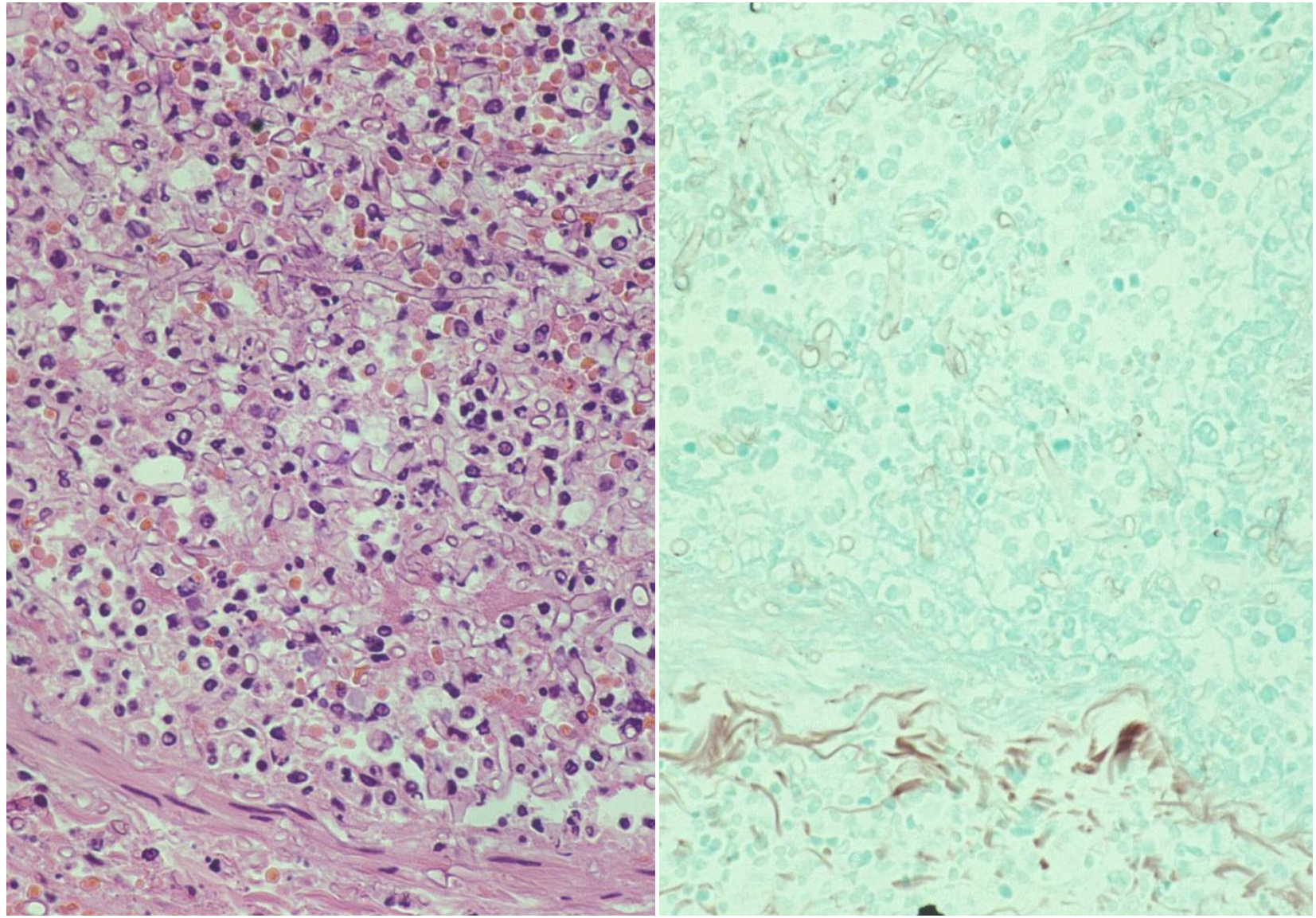
3) Hand foot and mouth disease encephalitis (enterovirus 71 infection)

4) Mosquito bite hypersensitivity in childhood

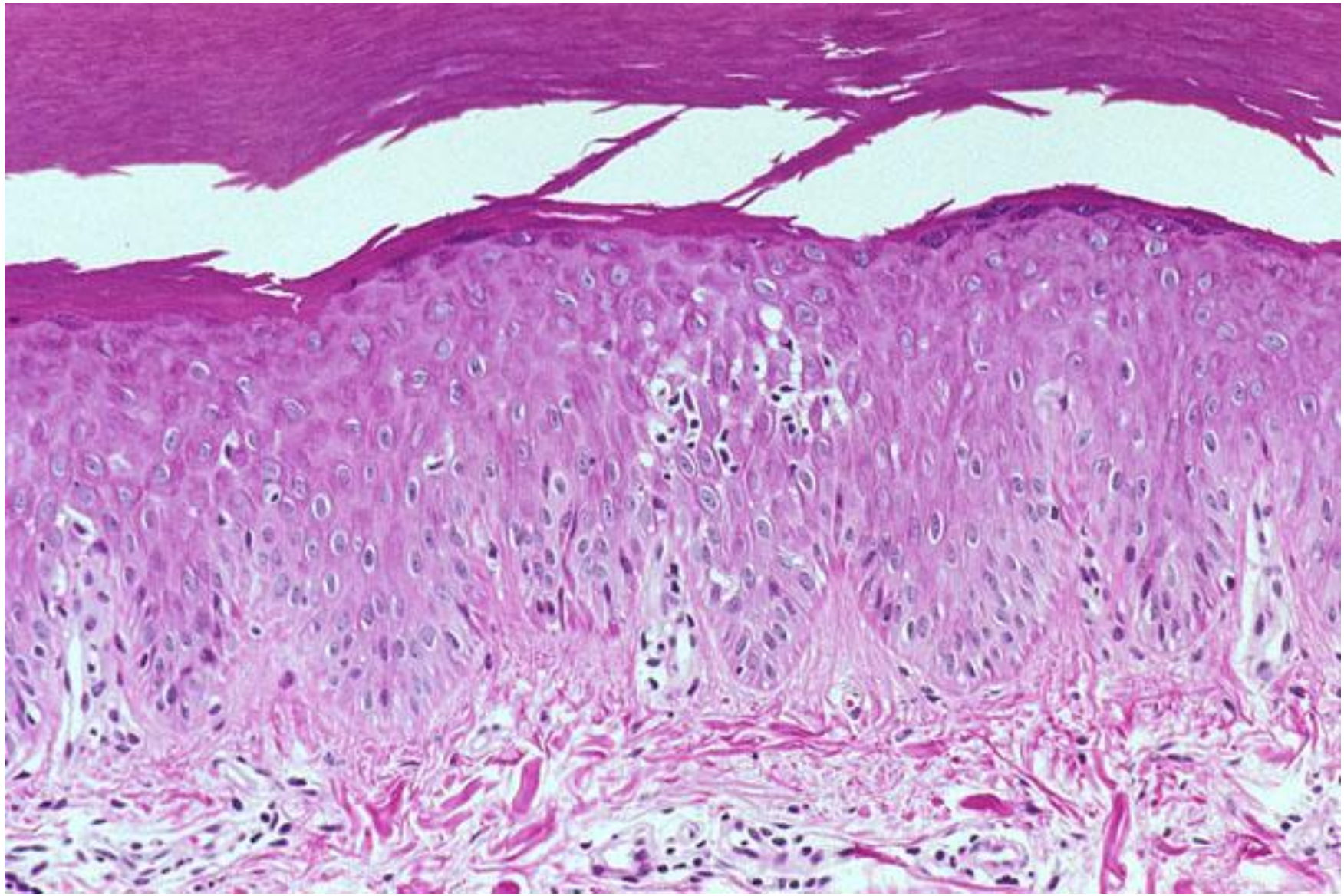
Necrotizing fasciitis seen in a male patient aged 70's with uncontrolled diabetic mellitus with diabetic neuropathy. He had lost the sensation of the foot. In the winter time, he warmed his feet with a fan heater. Severe burn with deep ulceration happened, resulting in necrotizing fasciitis with septicemia. Diabetes-induced dry gangrene is seen on the 1st and 2nd toes.

Necrotizing fasciitis seen in a male patient aged 70's with uncontrolled diabetic mellitus with diabetic neuropathy. Emergency amputation saved his life. He is now on rehabilitation for daily living. The sensation is retained at the site of amputation.

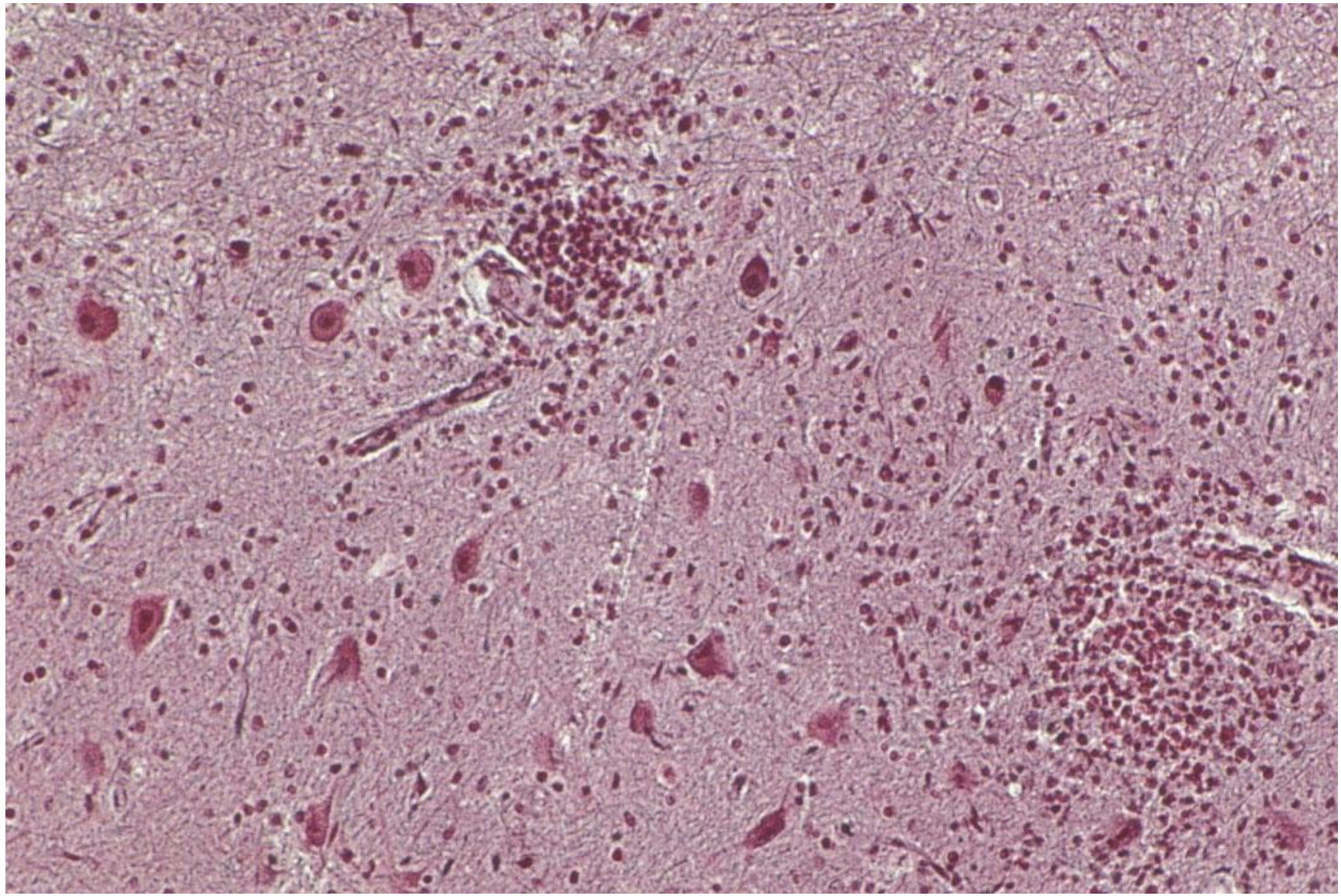
Gross appearance of lethal rhinocerebral mucormycosis seen in a male patient aged 50's with diabetic coma. Paranasal mucormycosis extends to the facial skin, eye balls and the basal part of the brain. The fungi extensively grow in the arterial vessels to form mycotic emboli. The susceptibility of infection was provoked by neutrophilic dysfunction due to severe diabetes mellitus.



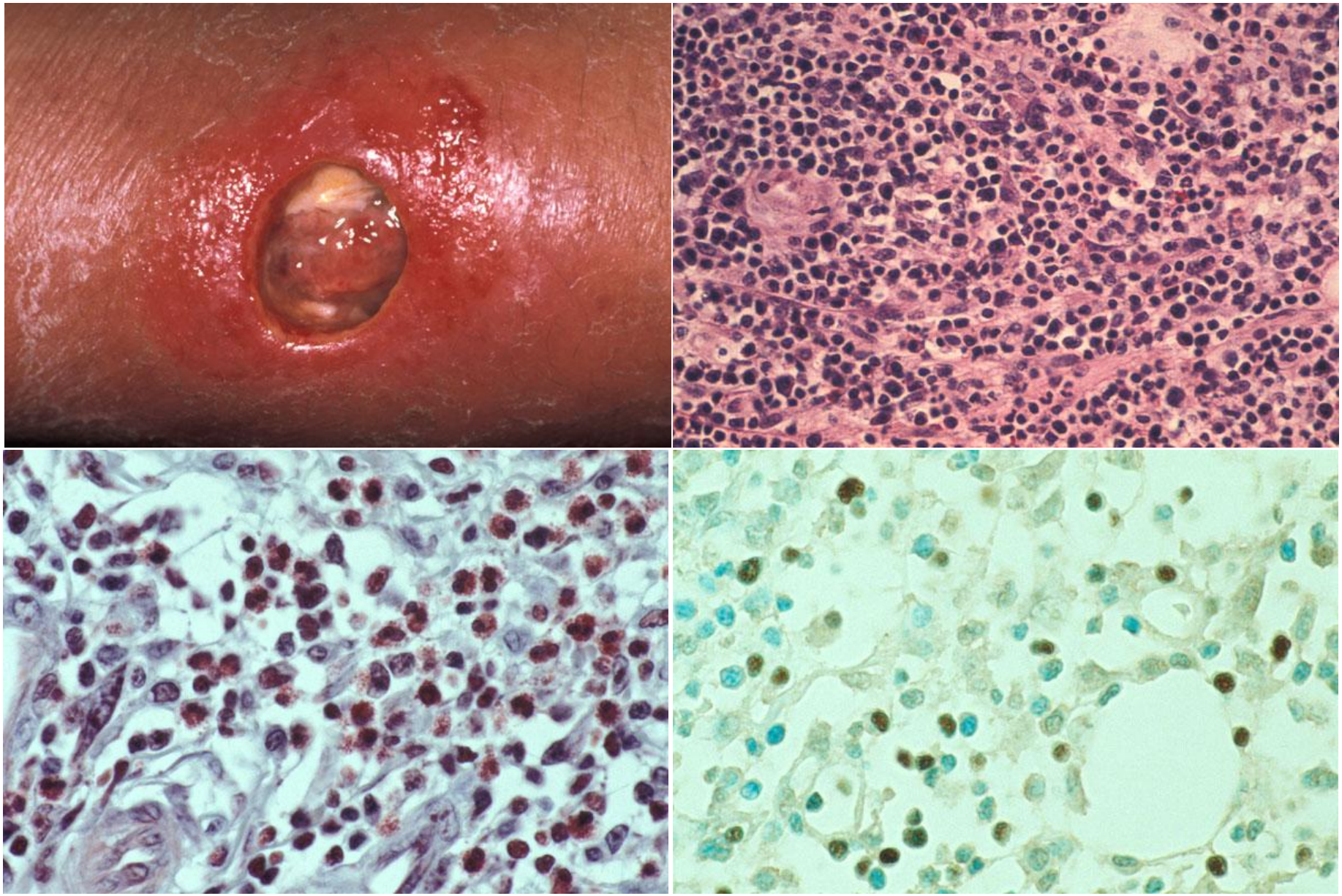
Lethal rhinocerebral mucormycosis seen in a male patient aged 50's with diabetic coma. Paranasal mucormycosis extends to the facial skin, eye balls and the basal part of the brain. The fungi extensively grow in the arterial vessels to form mycotic emboli (left: H&E, right: Grocott). The hyphae of zygomycetes are often poorly (faintly) stained with Grocott methenamine silver.



Skin biopsy of hand foot and mouth disease (H&E). Intraepidermal edema and lymphocytic infiltration are observed. Usually, the disease is caused by infection of Coxsackievirus A16.



Hand foot and mouth disease encephalitis (H&E). Lethal acute viral encephalitis may be seen in case of Enterovirus-71 infection. The dentate nucleus of the cerebellum reveals multifocal lymphocytic infiltration and neuronophagia.



Mosquito bite hypersensitivity seen in a 5 y-o girl. The site of mosquito bite was swollen with fever, and deep ulceration measuring 15 mm followed. Skin reaction against *Armigeres* was positive. Atypical lymphocytes appeared in the peripheral blood (17%). Microscopically, dense infiltration of small lymphocytes macrophages and eosinophils is observed (H&E). The nuclei of the lymphocytes are positive for EBER (right bottom). The lesion cured 4 weeks later.

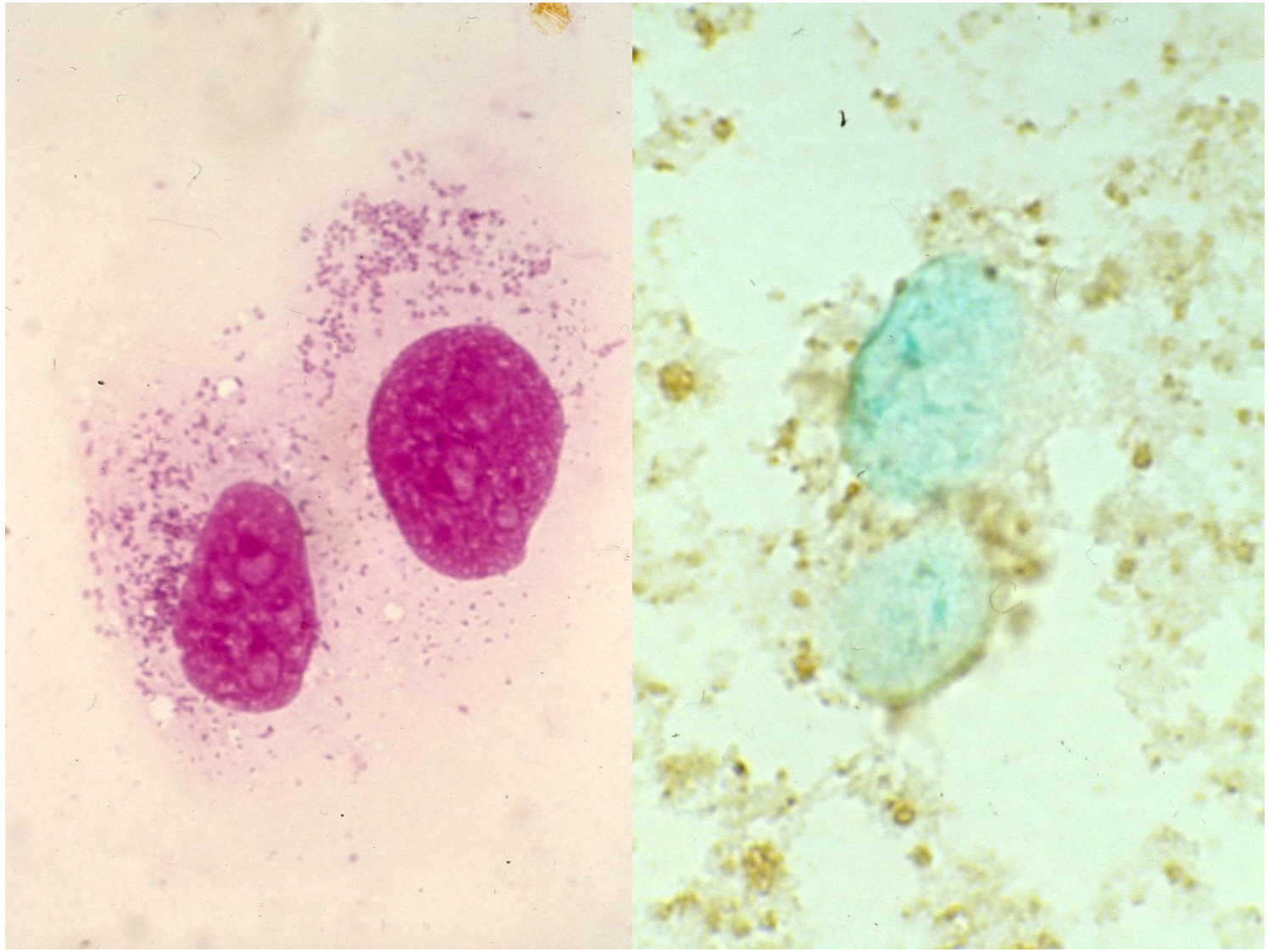
Rickettsiosis in Japan

1) Tsutsugamushi disease (scrub typhus) caused by *Orientia tsutsugamushi*

(Tsutsuga means a disease,

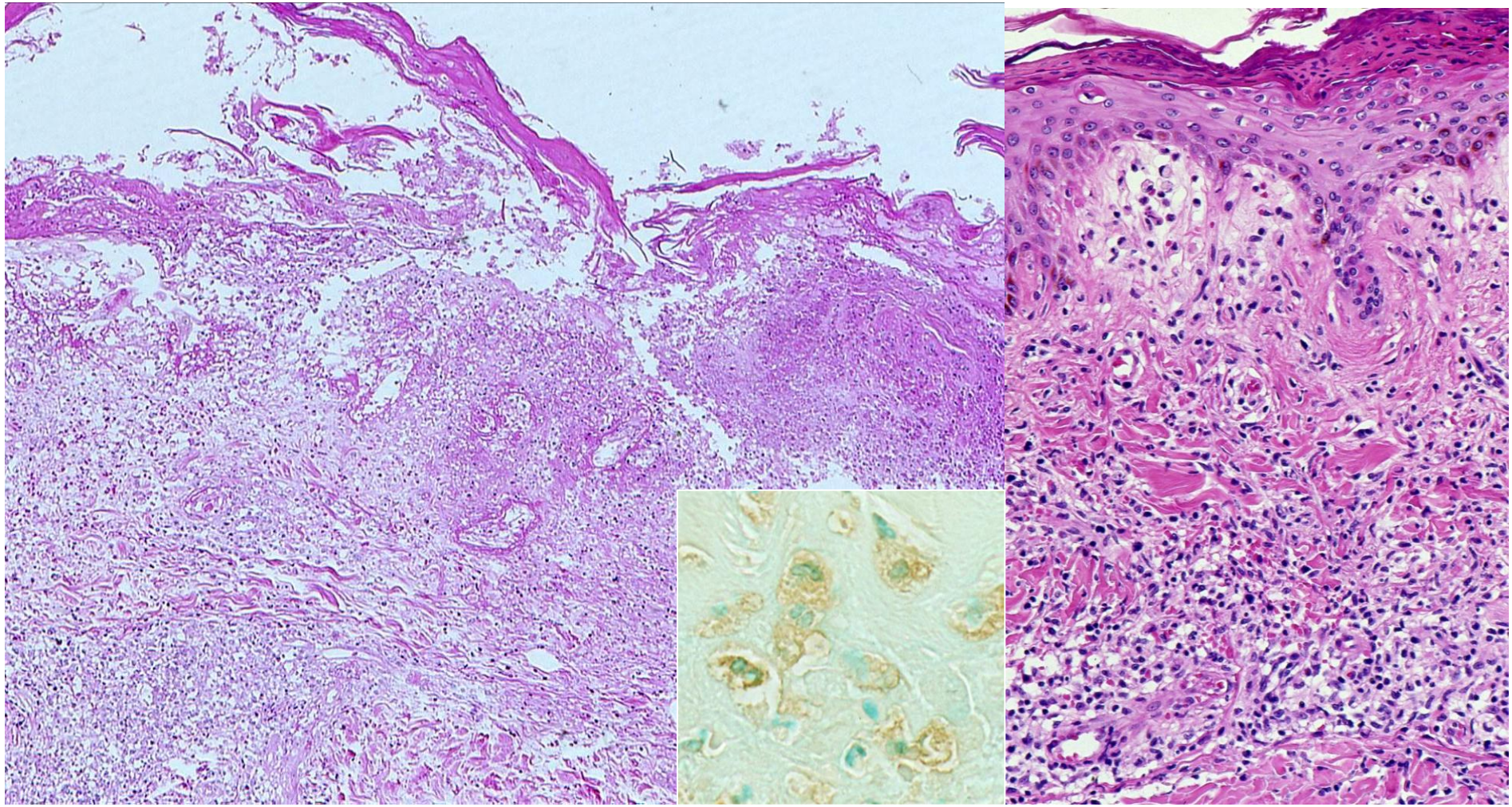
Mushi means a worm/bug in Japanese)

2) Japanese spotted fever caused by *Rickettsia japonica*



Cultured macrophages infected with *Orientia tsutsugamushi*, Kato strain. Left: Giemsa, right: immunostaining with antiserum against Kato strain of *O. tsutsugamush*i. Small-sized Gram-negative rods are demonstrated.

Tsutsugamushi disease (scrub typhus). Left: eschar, right: eruptions on the chest and upper arm. *Orientia tsutsugamushi* causing Tsutsugamushi disease is transmitted by the mite *Trombiculid* or *Leptotrombidium* (called tsutsugamushi in Japanese). The symptoms include high fever, eschar (left) and erupotions (right). Lymphadenopathy is common. DIC may be complicated in severe cases.



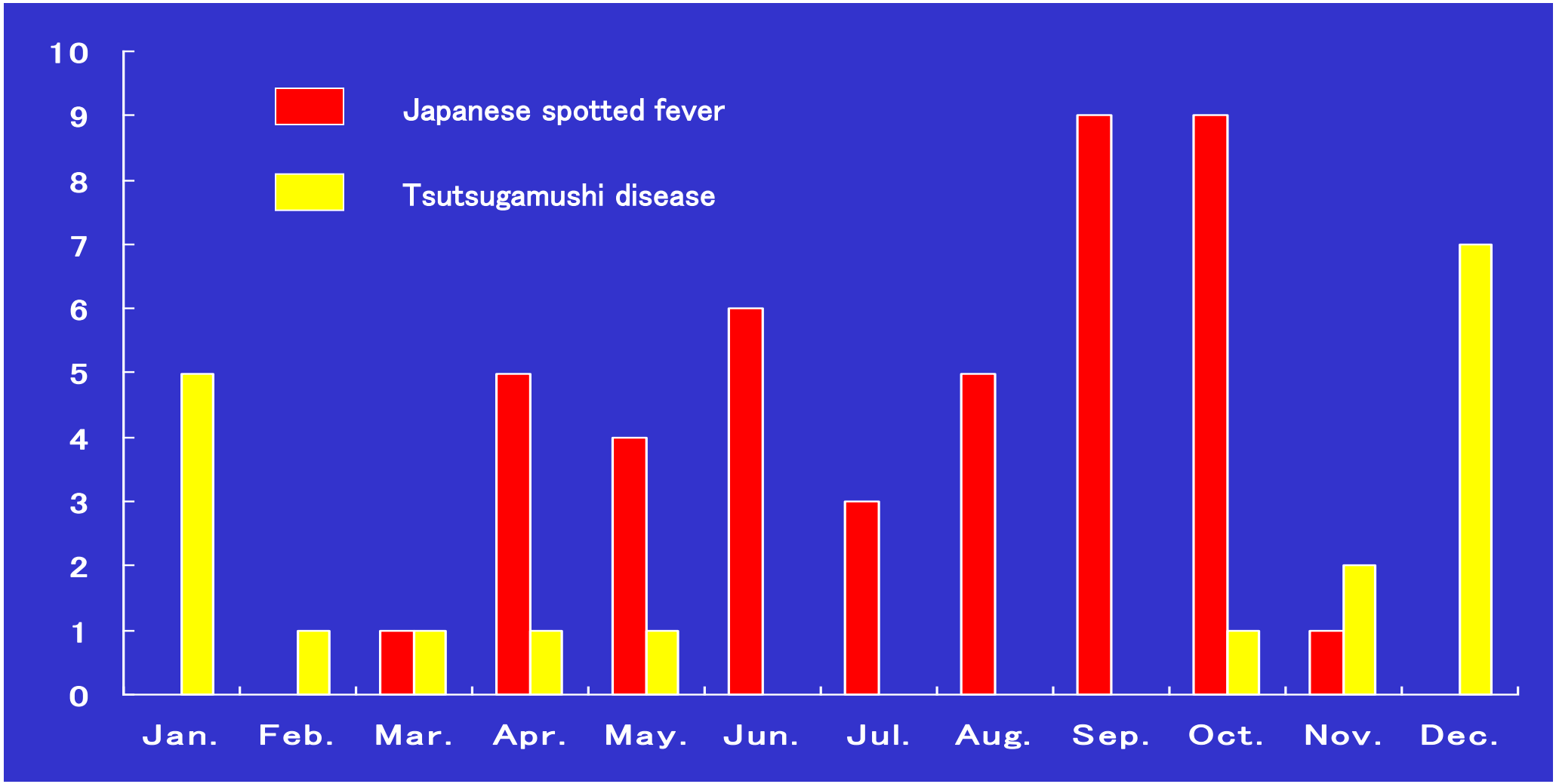
Tsutsugamushi disease (scrub typhus). Biopsy specimens from the eschar (left) and eruption (right) (H&E). Inset: immunostaining using patient's own serum diluted at 1:100. Inflammatory ulceration is seen in the eschar. Macrophages are infected with *O. tsutsugamushi* reactive with the patient's serum. The eruption reveals perivascular infiltration of mononuclear cells in the dermis.

Japanese spotted fever

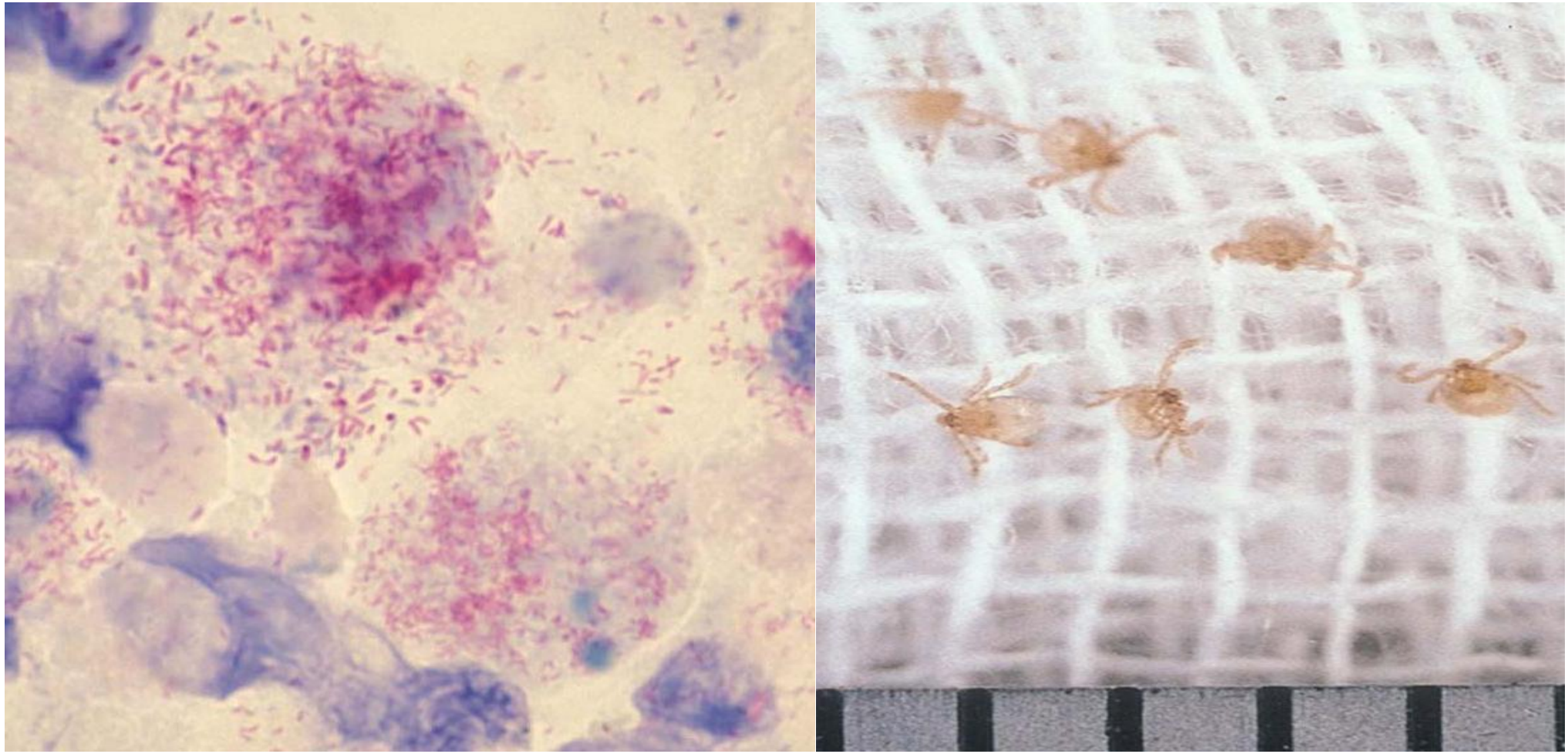
1) Japanese spotted fever (JSF) is caused by *Rickettsia japonica*, a unique spotted fever group rickettsia in Japan, endemic in southwestern parts of Japan, and seen in Spring through Autumn.

2) The trias of manifestation of JSF (high fever, eschar and eruptions) are common with those of Tsutsugamushi disease. The eruptions tend to be hemorrhagic. Lymphadenopathy, often seen in Tsutsugamushi disease, is infrequent in JSF. For the treatment, combination of Tetracycline/Minomycin and new quinolone is effective (new quinolone is ineffective for Tsutsugamushi disease).

3) JSF is principally transmitted by the larval tick Haemaphysalis flava, and at the onset, no adult tick is observed on the eschar.



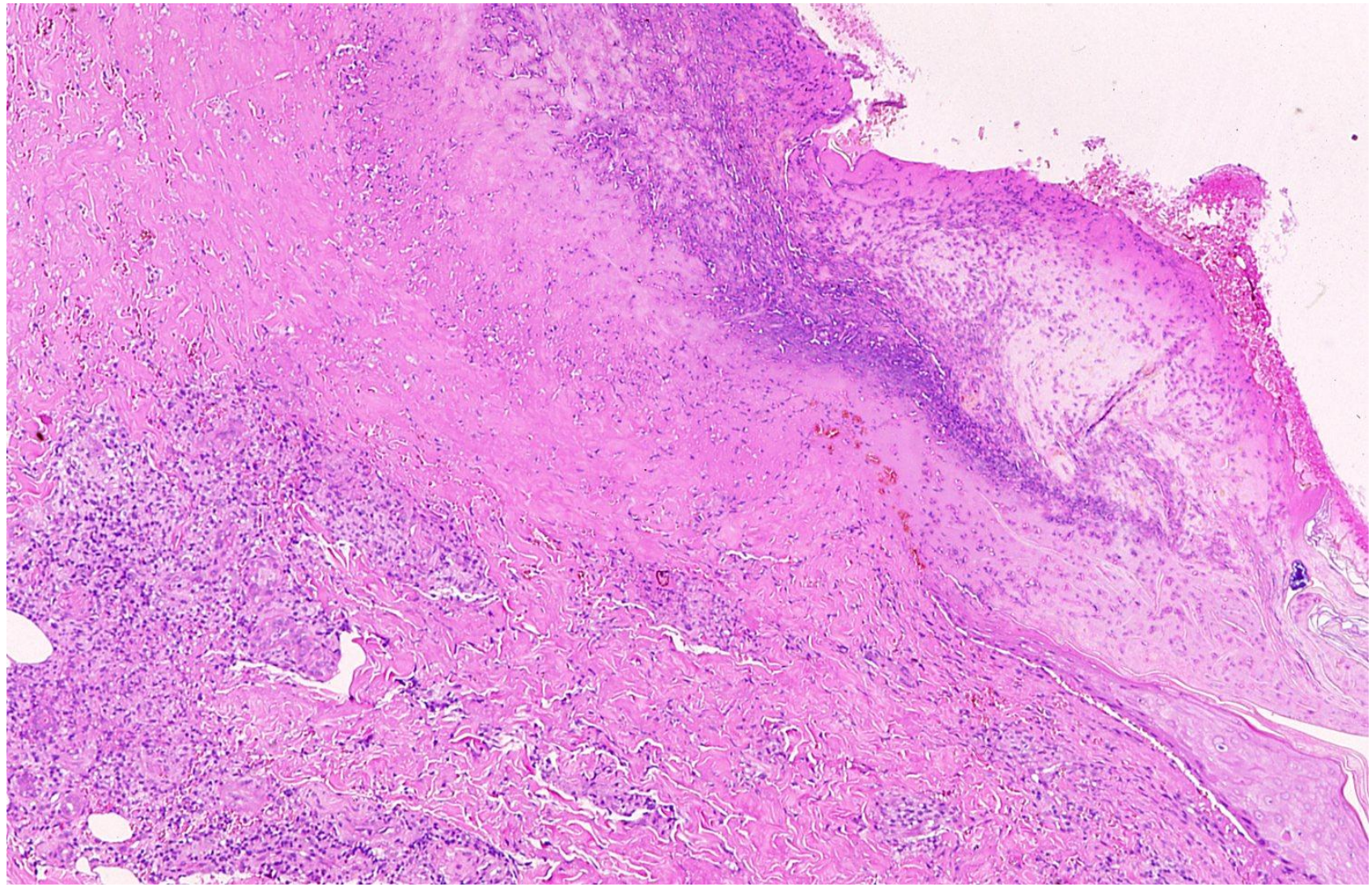
The incidence of Japanese spotted fever (JSF) and Tsutsugamushi disease by month in Tokushima Prefecture, Shikoku, Japan. JSF is mainly seen in Spring through Summer, while Tsutsugamushi disease is predominantly seen in Winter. Mahara Hospital, Tokushima, Japan.



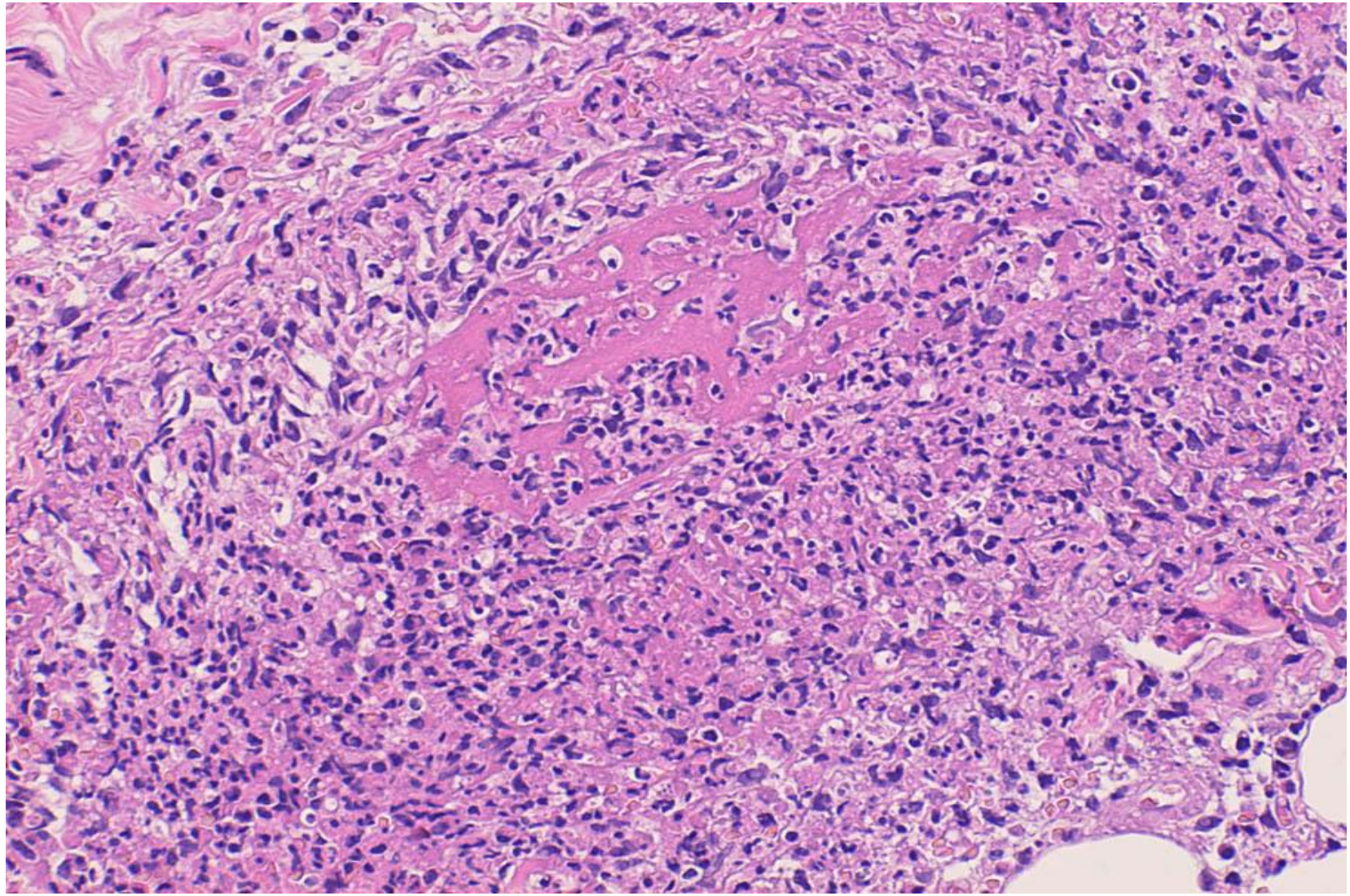
Rickettsia japonica infecting cultured macrophages (left: Gimenez stain) and 1 mm-sized larval ticks *Haemaphysalis flava* transmitting *R. japonica*.

Japanese spotted fever seen in a 76 y-o female patient living in Tokushima, Shikoku, Japan. An eschar and eruptions are shown. She went to the forest 1 week earlier. Leukopenia and thrombocytopenia were complicated, and the eruptions became hemorrhagic. By Minomycin treatment, she became afebrile in three days.

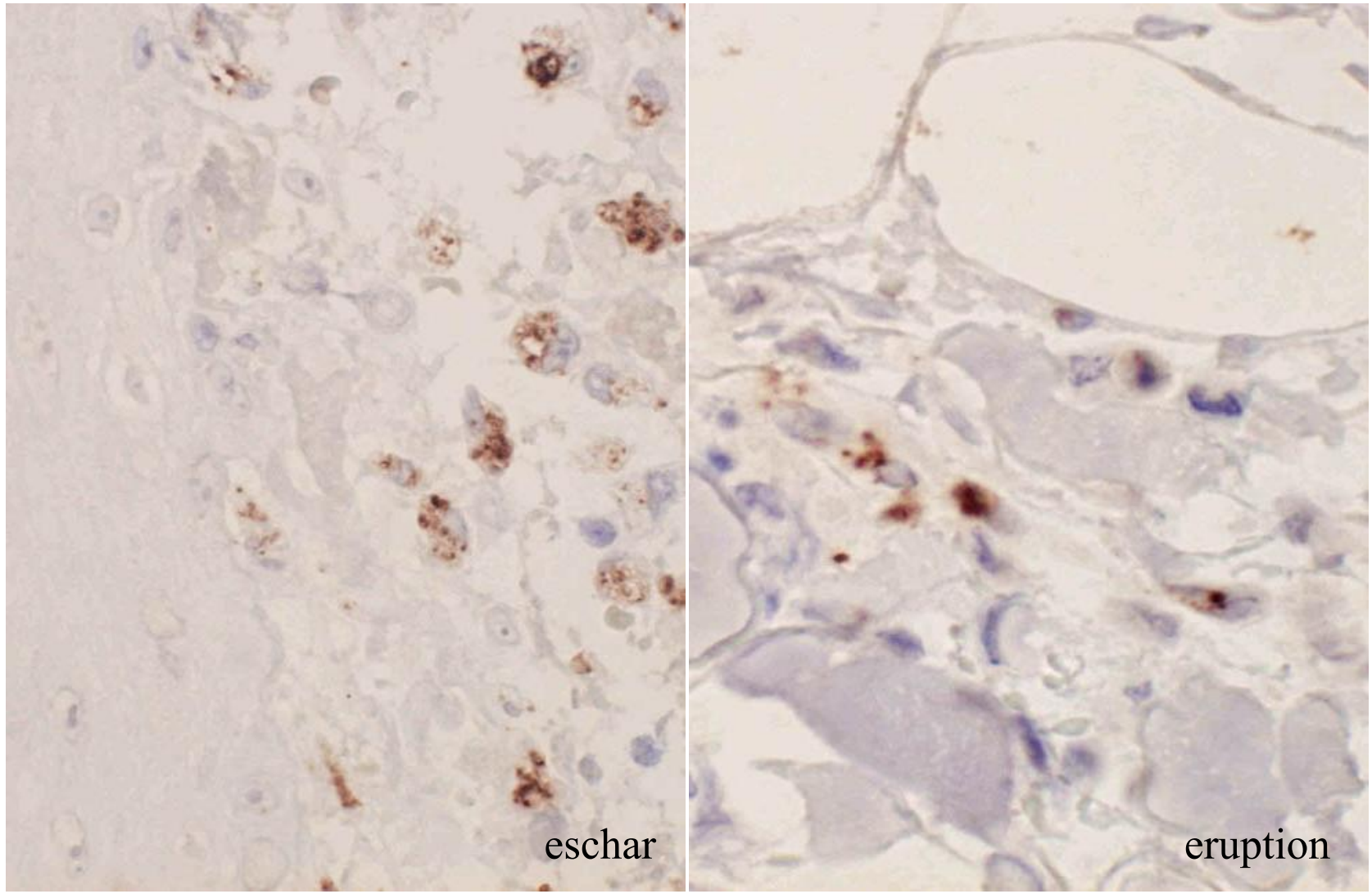
Japanese spotted fever seen in a 76 y-o female patient living in Tokushima, Shikoku, Japan. Eruptions on the back are shown. She went to the forest 1 week earlier. Leukopenia and thrombocytopenia were complicated, and the eruptions became hemorrhagic. By Minomycin treatment, she became afebrile in three days.



Biopsy taken from the eschar in JSF shows ulceration with inflammatory exudation and perivascular infiltration of mononuclear cells in the dermis (H&E).



Biopsy taken from the hemorrhagic eruption in JSF shows infection-associated necrotizing vasculitis with neutrophilic reactions (H&E).



Immunostaining for *R. japonica* antigen using a monoclonal antibody S3. In the eschar, the rickettsial antigen is seen in the cytoplasm of macrophages (left). In the eruption, the rickettsial antigen is seen mainly in capillary endothelial cells (right). **Ref.**: Tamakuma K, et al. Histopathological diagnosis of Japanese spotted fever using formalin fixed, paraffin-embedded skin biopsy specimens. Usefulness of immunohistochemistry and real-time PCR analysis. Clin Microbiol Infect 2012; 18: 260-267. doi: 10.1111/j.1469-0691.2011.03569.x

Summary of Immunoperoxidase Staining

	Days after Minomycin Ad	Skin Eschar	Skin rash
Case 1 (76F)	1 day	+	+ (NA)
Case 2 (77M)	4 days (afebrile)	+	+
Case 3 (51M)	1 day	+	～
Case 4 (65F)	4 days (afebrile)	+	－
Case 5 (52F)	7 days (afebrile)	+	+

(Same results obtained with Mabs S3 and X1.)

NA: necrotizing angiitis