Cultural Characteristics and Life History of a new human Pathogen, Thailandia Candida, nov. gen. et nov. spec.

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With Plates III & IV.

Introduction.

Na Nagara, Jatinandan, and Vardhanabhuti (1956) reported a case of maduromycosis of finger from which an unidentified fungus was isolated. The pathogenicity and causative role of the isolate were verified by animal inoculations. This fungus is a true yeast-like organism (Mycelial yeast) but it produces sexual stage in human and animal tissues as well as in culture. It also forms spherules in the tissue. Investigations into its cultural characteristics and life history were made and the identification of the fungus was attempted.

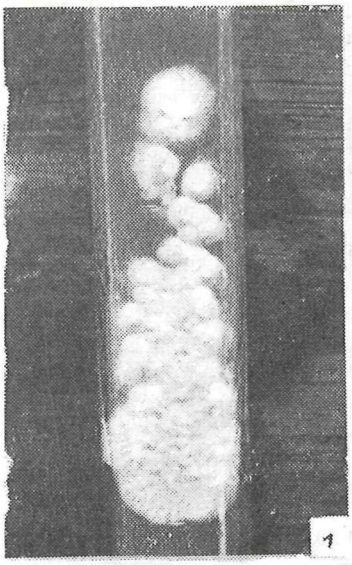
Materials and methods.

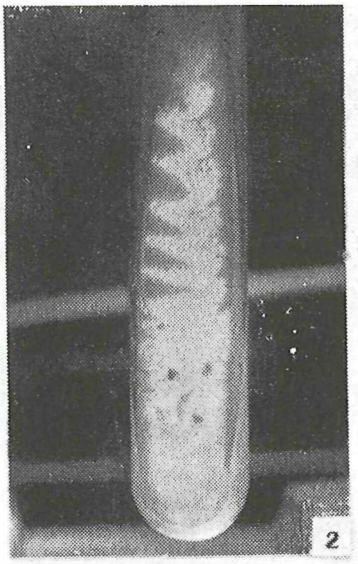
Cultural characteristics of the organism were observed on Sabouraud's dextrose agar, Difco corn-meal agar, carrot plug, Littman's ox-gall agar, nutrient agar, in gelatin, litmus milk, Sabouraud's dextrose broth, and nutrient broth, all at room temperature (about 30° C). Growth on brain-heart infusion glucose blood agar was also studied at 37° C.

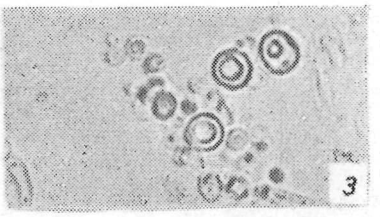
Microscopic morphology was studied by the examination of the growth from Sabouraud's dextrose agar slant at intervals upto sixty days. At the end of the period some slants were fixed in formal-saline solution and random histological sections were prepared and examined.

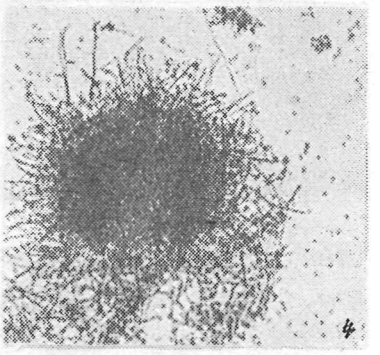
The tissue forms of the organism were studied by examining the pus obtained from lesions produced in white mice by the intra-peritoneal injection of a live fungal suspension and in guinea pigs by the injection of the suspension into the soft tissue at the sole of a

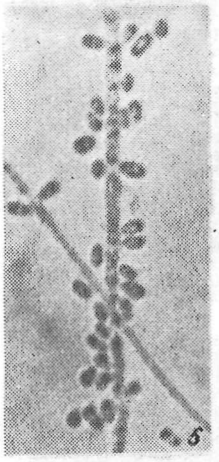
[1]) This work was carried out at the Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand. It was partly sponsored by the United States International Cooperation Administration, through the arrangements made by U.S.O.M. - Thailand, Dr. Charles E. Richards, Technical Advisor.

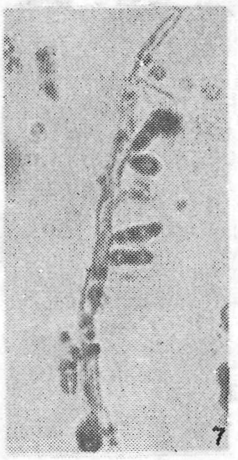


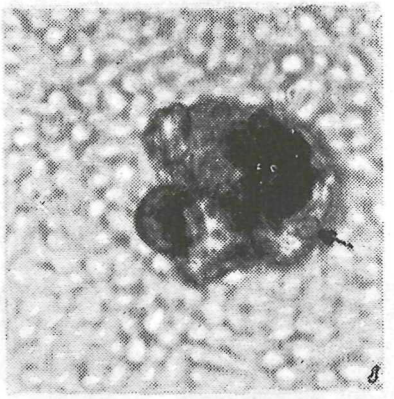


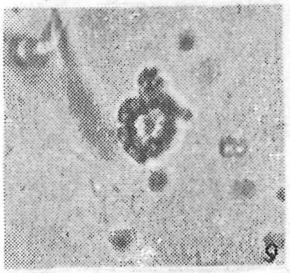


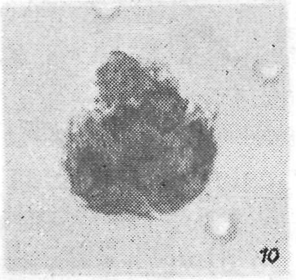


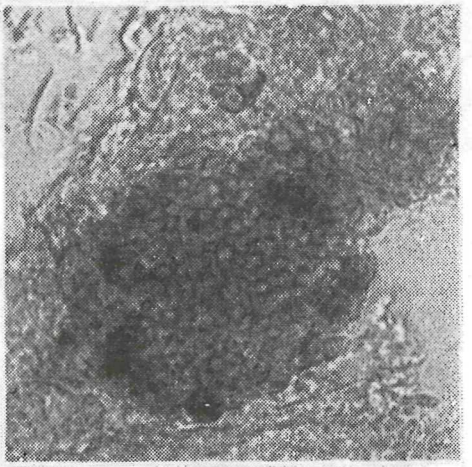


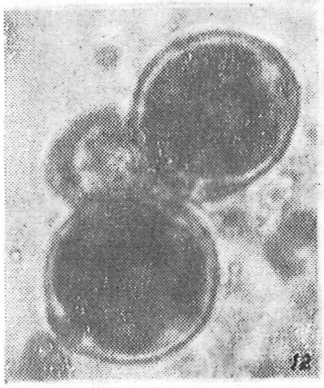


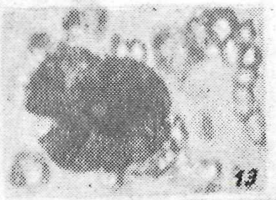


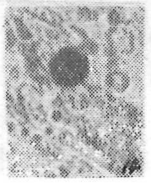


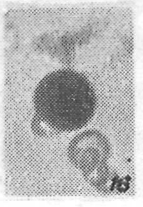


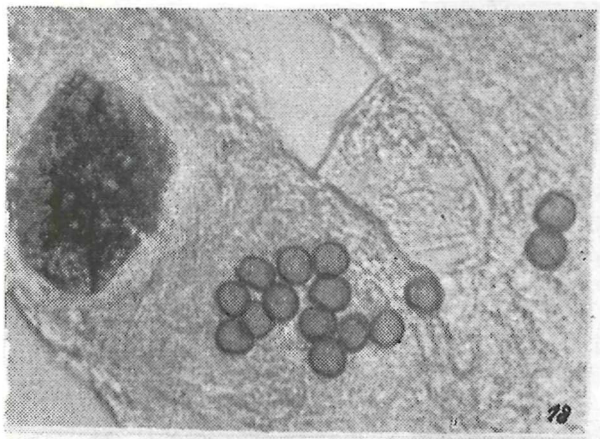


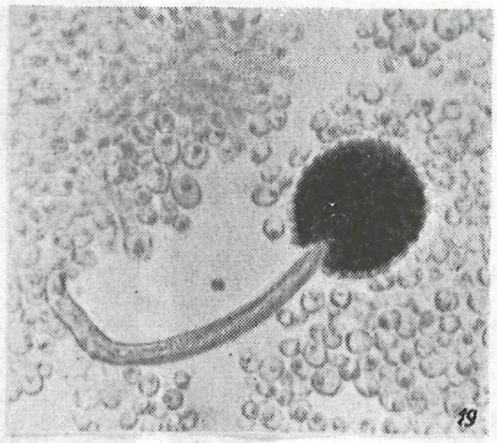


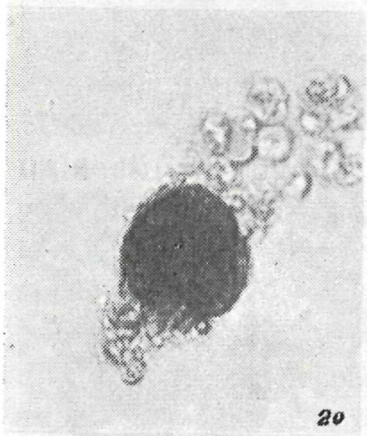


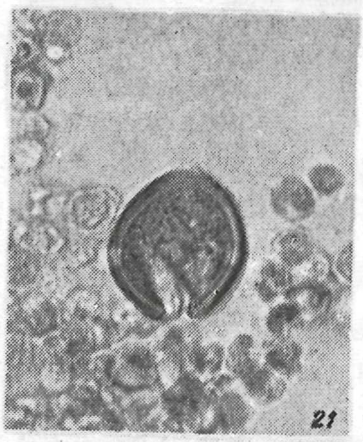


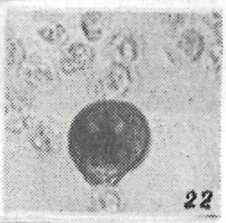


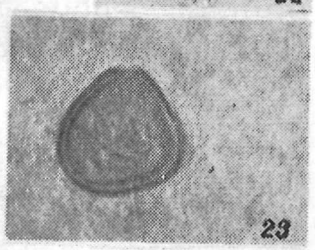


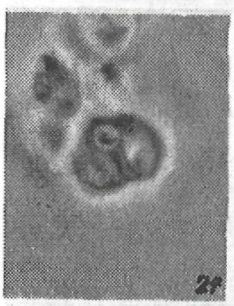


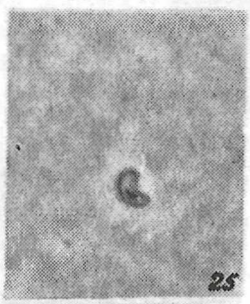


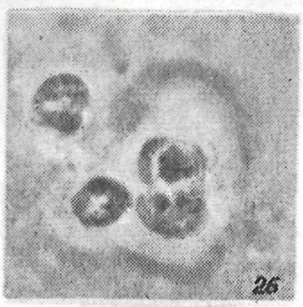


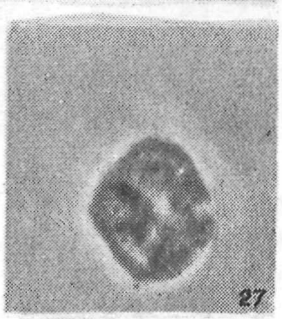


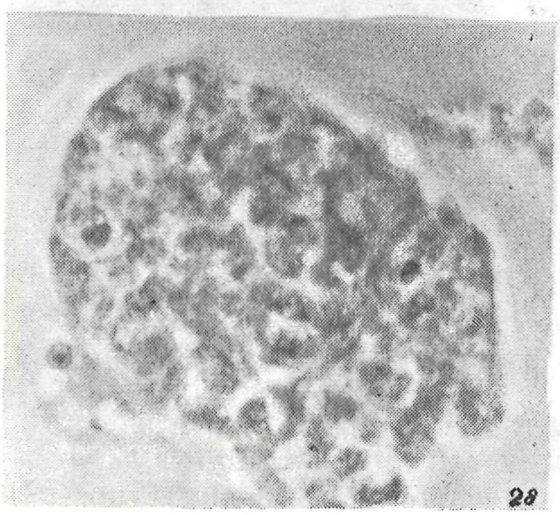


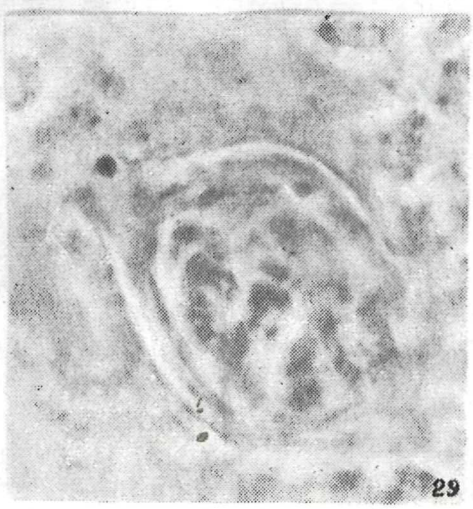












foot. Usually gastric mucin was not required, except with cultures kept for a long time under mineral oil, to produce frank suppuration. For the details of the development of the spherules examinations were made with the aid of a phase-contrast microscope under an oil immersion objective.

Biochemical reactions, according to the method described for species of *Candida* by Conant et al. (1954) were investigated, the sugars used being dextrose, sucrose, lactose, maltose, xylose, and mannitol.

Results.

Gross Morphology.

Sabouraud's dextrose Agar: Raised, round, smooth and creamy colonies, turning cerebriform, membranous and fawn in color with age (Fig. 1). Strong yeasty odor.

Difco corn-meal Agar: Yeast-like type of growth, more submerged than on the surface (Fig. 2).

Carrot plug: Growth luxuriant, prickly.

Littman's ox-gall Agar: Opaque, raised and round colonies. Growth is somewhat restricted.

Nutrient Agar: Yeast-like type of growth.

Brain-heart infusion Blood Agar, 37° C: On first few transfers the colonies were bacterial-like, later on they were yeasty with very few pseudomycelium in the agar substrate.

Litmus Milk: Not acid-forming nor coagulated.

Gelatin: Not liquefied.

Sabouraud's dextrose broth and nutrient broth: When freshly isolated the organism formed sediments at the bottom and strong surface pellicles which crept up the side of the tube at the end of forty-eight hours. Cultures kept under mineral oil for over one year lost the ability to form surface pellicles and form gas bubbles in liquid cultures.

Effect of aeration: No growth on all media under anaerobic condition.

Microscopic Morphology.

The thalli consist mainly of yeast cells of the sizes ranging from 3—8.5 μ in their largest diameter. These cells are either with single or with multiple budidngs. Thick-walled cells are found in old cultures (Fig. 3). Some of these cells bud. Pseudomycelia are formed on all media. The hyphae are septate but multinucleate, measuring 1.4—2.8 μ in diameter. Oidia-like cells are also found. Conidia-like structures (Figs. 5—7) are also present both in culture and in animal tissue. On closer examination these turn out to be blastospores that fail to

bud and develop multiple septae in order to elongate to form hyphal segments.

The perfect stage of the organism seems to consist basically of sexual primordia which develop from adjacent cells of the same hypha at the periphery of a subiculum-like mass (Fig. 4) which is formed by the end of the first week of cultivation on ordinary laboratory culture media. The sex initials apparently consist of an unicellular oogonium bearing a delicate and coiled trichogyne and a similar cell just underneath bearing ovoid antherides. These antherides discharge minute rod-shaped spermatia which are nonmotile. Fertilization has not been observed. The oogonium later enlarges and becomes multinucleate. It sends out a single ascogenous hypha at the tip of which rows of binucleate cells are formed, and the terminal ones develop into rows of asci. Surrounding hyphae arch over the oogonium to form a locule which sinks deeper in the mass of the subiculum as the latter enlarges. Ascocarp wall develops either from the original cell mass at the tip of the ascogenous hypha or from a nursing hypha growing in from the hyphal tissue at the wall of the locule, since each fruit-body is always anchored to the wall of the latter by at least two hyphal filaments, one of which is the remains of the ascogenous hypha. The ascocarp wall is distinct and definitely not formed from the surrounding hyphal tissue by dissolution. At first the fruit-body is heart-shaped with a pointed tip, but later it becomes more rounded. The wall is straw-colored, thin, membranous and with a granular appearance. Occasionally coiled appendages made up of highly refractile tissue are seen. Ascocarp - in - locules are usually found on the surface of the agar culture and are all sterile.

Fertile fruit-bodies develop in culture on common laboratory media by the end of a month. In smear preparations these consist of reddish brown microscopic bodies. The smallest and presumably youngest one observed has a cellular outer lining (Fig. 9) very much the same as the young ascocarp-in-locule, but rows of asci containing ascospores can be seen coming up from the basal portion when the fruit-body gets larger. Each ascocarp contains few to many asci (Figs. 8, 10). These are all lying free. Those seen in animal tissue (Figs. 11, 20) are apparently shield-shaped. The size varies greatly, from 25×27 μ upto 110×140 μ. In histological sections made from fixed agar cultures, the ascocarps, when seen in situ, are enclosed in dark brown hyphal tissue. Loculation is not evident. This specialized hyphal tissue develops from surrounding hyphae, because some of these, at the periphery, are partly hyaline and partly pigmented. Paraphyses are not evident at maturity. The ascocarps are without any ostiole.

The asci are reddish brown in color, round, or wide ovate with uniformly thick, transparent, colored outer membrane and a thin

veil-like inner membrane (Figs. 8, 12, 13, 17, 21, 22, 23). They usually open by a pore at the smaller end (Figs. 12, 21, 22, 23). The size of the ascus varies, on the average it is 22 × 28 μ. One ascus (Fig. 22) contains 8 ascospores. The ascospores are one-celled, round and brownish black. When inside the ascus they are usually small and light colored (Figs. 8 at arrow, 22), measuring 3—4 μ, but they are larger and darker when found outside, measuring upto 16 μ in diameter (Figs. 14, 15, 16, 18). The ascospore germinates by forming a germ tube. Before germination it appears much swollen, 3—4 times the usual size. After germination the spore wall rectracts and presents a tuberculate appearance (Fig. 19).

The essentially tissue form of the fungus found in human and animals is represented by the formation of spherical bodies of varying sizes depending on the stage of the development. These spherules consist of numerous spores enclosed in a thin hyaline membranous wall. The development of the spherule is illustrated in the Figures 24—29. Mature spores discharged from each spherule measure from 6—10 × 7—12 μ. Each spore is made up of a chain of highly refractile round or spindle-shaped bodies coiled up tightly inside the spore wall (Fig. 24). The wall ruptures, the chain is broken and each refractile unit is set free (Fig. 25). It enlarges and develops granular cytoplasm and a vesicular nucleus. The cell later divides forming a ball of two, four and more cells (Figs. 26, 27). Periclinal fissures develop and finally, through further cell divisions, a mature spherule is formed. The spherule ruptures (Figs. 28, 29) and individual spores are set free. The developmental cycle then starts all over again. Mature spherules measure 17—43 × 26—64 μ. They are found especially in places where there is frank suppuration.

Biochemical reactions.

When studied soon after its primary isolation before the organism has been put under mineral oil, dextrose was fermented with both acid and gas production. Only acid formation occurred with sucrose and lactose. Maltose, xylose, and mannitol were not fermented.

Detailed biochemical studies carried out by Dr. H. J. Scholer of the Abteilung für experimentelle Medizin, F. Hoffmann-La Roche & Co. A.-G., Basel, on a culture kept under mineral oil for more than one year and then passed back into a guinea pig and reisolated, showed that dextrose, sucrose, and maltose were fermented, while galactose and lactose were not. Carbon assimilation with dextrose, galactose, and sucrose were positive, while maltose was only slightly utilized, and lactose was not. For nitrogen assimilation test, there was no growth with potassium nitrate.

Taxonomic study.

This study was carried out under the direction of Dr. Emil Müller, Curator, the Institute for Special Botany, Swiss Federal Institute of Technology, Zürich, Switzerland.

There is no doubt that this fungus belongs to the *Plectascales* (*Ascomycetes*). The development of the ascocarp is similar to the *Gymnoascaceae* but our fungus does not agree with any of the genera described within this family, thouroughly investigated by Benjamin (1956). This author mentions in his key to the genera the new genus *Shanorella* Benj. which seems to be the nearest related taxon to the organism treated. But there are some differences which may justify to put it into a new genus. The asci are not dissolved at maturity, the ascospores are dark-brown and the fungus is growing yeastlike. Then it is a real parasite whereas the three isolates of *Shanorella spirotricha* Benj., the type species of *Shanorella,* grow either on animal hairs or feathers, as saprophytes. For this new type of *Gymnoascaceae* the name *Thailandia,* according to its origin (Thailand) is proposed. The type species is described as *Thailandia Candida.* The epitheton *„Candida"* indicates the similarity of this fungus, except its capability to form a sexual stage, with *Candida* species.

Thailandia nov. gen.

Cleistothecia globosa vel scutiforma, irregularia, singula; peridio tenui, hyphis fuscis, septatis, fragilis composito et rare appendiculato. Asci globosi, membranis distinctis circumdati, octospori. Sporae globosae, primo subhyaline demum fuscae.

Cleistothecia globoid or shield-shaped, somewhat irregular, single; peridium thin, composed of dark-brown septate hyphae, easily disarticulating at maturity, occasionally with coiled appendages when young; asci globose, persistent, with a distinct and relatively thick wall, which opens by a pore. Ascospores globose or slightly ellipsoid, small and light colored when in the ascus, becoming bigger and dark-brown outside the ascus.

Thailandia Candida nov. spec.

Cleistothecia globosa vel depressa, 110—140 µ diam., fusca; peridio hyphis septatis, fuscis formato et rare in cleistothecia parvula appendiculatis circumdata. Asci nun numerosi, globosi, 22—28 µ diam., 8-spori, membranis crassis et persistantis. Sporae globosae, 3—4 µ, hyaline, demum fuscae 10—16 µ diam.

Cleistothecia globoid or somewhat flattened, 110—140 µ diam., reddish brown to dark brown, with a thin peridium composed of dark-brown, septate hyphae and occasionally — when young — with coiled appendages around the peridium. Asci few to many in an

ascocarp, globose, 22—28 μ diam., thick walled, persistent at maturity with a pore-like opening through which the ascospores may leave the ascus, eight-spored. Ascospores globose to slightly ellipsoid, lightly colored, 3—4 μ diam. inside the ascus, dark-brown and upto 16 μ diam. outside the ascus.

In culture yeast-like cells with single or multiple buddings mixed with septate and coenocytic pseudomycelium. Blastospores and oidia present.

The fungus lives as a parasite in human and animal tissue but grows well on common culture media.

Cultures are kept by the author and in the Institute for Special Botany, Swiss Federal Institute of Technology, Zürich. The strain, after being kept in aritficial cultivation for more than three years, has lost most of its ability to produce sexual stage in culture, but this can still be obtained easily by animal inoculation.

Acknowledgements:

The author wishes to express his sincere gratitude to Dr. Emil Müller of the Institute for Special Botany, Swiss Federal Institute of Technology, Zürich, Switzerland. Dr. Müller has very kindly examined the various specimens sent to him and has read and helped to correct this paper and has given invaluable advices, without which the arrival at its actual systemic position and the recording of this fungus would not have been possible.

He also owes his thanks to several members of the staff of the Department of Medical Sciences, Thai Ministry of Public Health, notably Miss Sumana Suthiwatnaruput, who have helped in various ways in their spare times throughout the long period of investigation.

Zusammenfassung.

Thailandia Candida ist ein neuer, zu den *Gymnoascaceae* gehörender, humanpathogener Pilz mit *Candida*-artiger Wuchsform. Die Sexualstadien wurden sowohl in Kultur auf künstlichen Nährmedien, wie auch im Gewebe lebender Tiere nachgewiesen. Die Morphologie des Pilzes wird beschrieben und es werden auch ausführliche Angaben über die Reinkultur gemacht.

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Plate I.

Fig. 1. Surface of growth on *Sabouraud*'s dextrose agar. 2. Growth on Difco corn-meal agar. 3. Thick-walled cells in old culture, × 450. 4. A

subiculum-like mass, × 100. 5. Conidia-like structures in culture, × 450. 6—7. Same as 5, in animal pus, × 450. 8. A mature ascocarp in culture, × 450. 9—10. Developing ascocarp in culture, × 450. 11. An ascocarp in human pus, KOH-preparation and pressed, × 450. 12. A group of mature asci from *Littman*'s ox-gall agar, 45-day old, × 450. 13. An ascus cracked by pressure carefully applied to the cover slip, note cracked transparent outer membrane at left and intact inner layer, × 450. 14—16. Ascospores outside the ascus, × 450. 17. An immature ascus in culture, × 450.

Plate II.

Fig. 18. Ascospores in human pus, KOH-preparation, × 450. 19. A germinating ascospore from culture, × 450. 20. An ascocarp in guinea-pig pus, × 450. 21—23. Asci in animal pus, × 450. 24. A mature spore released from the spherule, × 450. 25. A free spindle-shaped body from a spore, × 450. 26. Two-celled stage, × 450. 27. A developing spherule, × 450. 28—29. Mature and rupturing spherules, × 450, note spherule membrane.