Reprinted from the Archives of Dermatology and Syphilology
May, 1929, Vol. 19, pp. 800-806

A NEW MONILIA-LIKE FUNGUS

CHARACTERISTICS OF AN ORGANISM ASSOCIATED WITH A DERMATOSIS PECULIAR TO WORKERS IN CANNERIES *

C. H. THIENES, M.D., PH.D.
PORTLAND, ORE.

In a previous report [1] was presented evidence of the mycotic origin of an occupational dermatosis occurring among workers in certain fruit canneries of the Pacific Northwest. The disease is known locally as "fruit poisoning." At the time the first paper was written, only three strains of the monilia-like yeast had been obtained. Since then, eight additional strains have been added to the collection. In a careful search of the medical literature a description of a similar organism could not be found. The following account of the characteristics of the new fungus will therefore be of interest.

SOURCE AND MORPHOLOGY [2]

The three original cultures were taken in January, 1924, from two patients with paronychia and from one with interdigital erosion, all of whom were infected the previous pear season at the same cannery. Six of the eight new strains were obtained from typical cases of paronychia or of interdigital erosion; a seventh was isolated from a box of pears just delivered to the cannery, and the eighth from a tray of pear parings at the workers' table. The organism was found in all typical lesions from which cultures were taken, but nontypical lesions yielded only bacteria or "wild yeasts." Cultures from conveying belts and vats were negative for the organism. The fact that one of the strains was isolated from a box of pears opens the possibility of the fruit being a source of the infection.

Cells from a twenty-four hour growth on solid mediums were round or oval budding forms, measuring from 5 to 8 microns in diameter.[3] An occasional elongated cell, 5 by 10 microns in diameter, was found. Rarely were true typhae found in young cultures on solid mediums. A definite cell membrane enclosed clear protoplasm con-

* From the Department of Pharmacology, University of Oregon Medical School.

1. Kingery, L. B., and Thienes, C. H.: Mycotic Paronychia and Dermatitis, Arch. Dermat. & Syph. **11**:186 (Feb.) 1925.

2. All the mediums used in these experiments were prepared in the Department of Bacteriology.

3. Thienes (footnote 1, figure 7).

taining an eccentric, highly refractile spherical body which was usually larger in cells grown on acid mediums. This refractile body did not stain with Giemsa's fluid but rather with sudan III and was considered to be a fat globule (figs. 1 and 2). Treatment with a diluted compound solution of iodine demonstrated glycogen granules throughout the cell. Within from forty-eight to seventy-two hours vacuoles filled with a glycogen-free fluid containing one or two chromophilic, coccoid granules in rapid brownian motion appeared in many of the

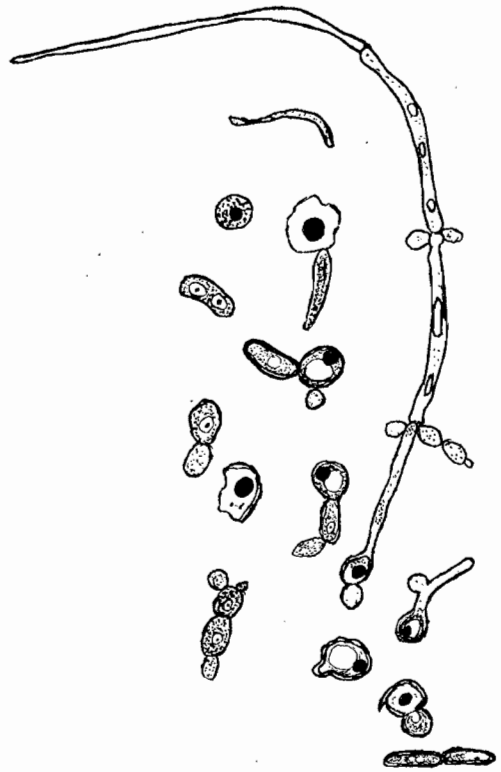

Fig. 1.—Camera lucida drawing of various forms from galactose broth, stained to show glycogen granules. The solid black bodies are fat globules. The vacuoles should be noted; × 1,000.

cells. Often such vacuoles nearly filled the cells of old cultures. Cells from old colonies were of many sizes and bizarre shapes. Some seemed to be shells containing only a fat globule, while others were elongated, containing one or more vacuoles and several fat globules; still others were cystlike, either clear or granular like chlamydospores, and measuring as much as 15 microns in diameter. Small greenish bodies, difficult to distinguish from fat globules, were found floating free in hanging

drop preparations from old colonies; these were at first thought to be ascospores, but they never were seen to germinate. Tortuous hyphal forms of from one to six segments, some of which were branched, were abundant in the gnarled masses of giant colonies.

In liquid mediums, twenty-four hour cultures consisted of septate hyphae, some of which were branched, together with the round or oval budding forms similar to those on solid mediums. At one end of a segmented hyphal thread the enlarged, round mother cell was

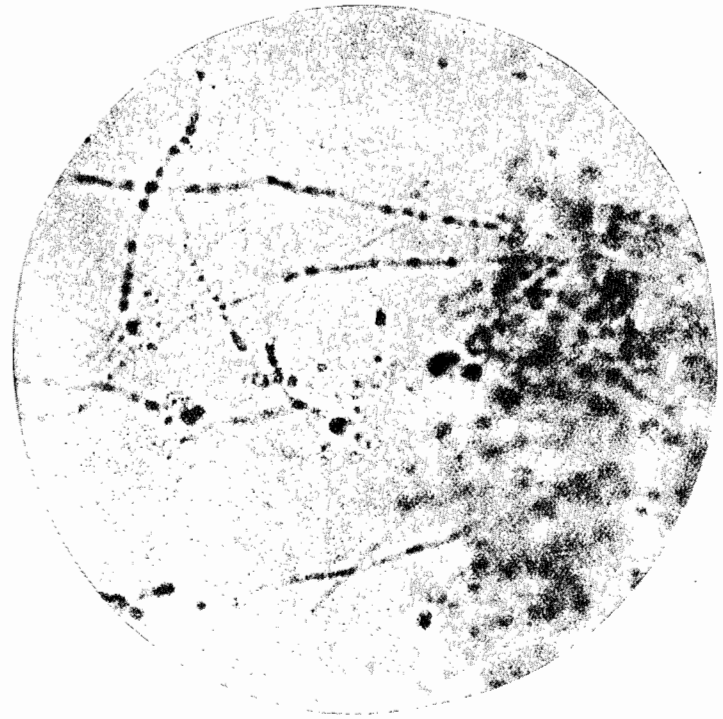

Fig. 2.—Photomicrograph of old hanging drop. The beaded appearance of hyphae due to fat globules should be noted; × 800.

frequently found, sometimes budding and usually containing a vacuole and fat globule, as shown in figure 1. Segments of the hyphae varied from 10 to 60 microns in length and from 2 to 3 microns in diameter, except for the tapering terminal segments which measured as much as 100 microns in length. Side-buds or conidia without sterigmas occurred, occasionally pleurogenous, but usually at the ends of segments; the conidia themselves often bore buds, and these, in turn, others. The end of the conidium-bearing segment was enlarged, like the joint of an insect's leg. Terminal hyphal segments, placed on a hanging drop of solid medium, were seen to become septate, the new terminal segment

elongating and again septating or giving rise to a bud, which either elongated or budded further. Such a preparation rapidly became overgrown by the budding conidia, obscuring the threads. Ascitic fluid was an especially good medium for the development of conidia-bearing hyphae. Hyphae of old hanging drop cultures in sugar broth were given a beaded appearance by numerous fat droplets, shown in figure 2.

In superficial skin lesions, the organism developed both budding forms and hyphae, the latter predominating. In the deeper tissues, such as the lung, liver and kidney of experimental animals, the round cells predominated, and hyphae present were composed of only from two to four short segments. The "double contour" was not a constant feature in stained tissue sections. Figures showing the forms obtained from tissues are to be found in a previous report.[1]

Reproduction was always by gemmation, attempts to develop asci on gypsum blocks, on Gorodkowa's medium and in old cultures having been unsuccessful.

The fungus was gram-positive, but not acid-fast. Young cells took the ordinary stains, but older cells stained poorly, except with aniline gentian violet or carbolfuchsin. A nucleus was not demonstrable by the iron-hematoxylin method. In thin tissue sections, the Gram-Weigert method was fairly efficient in demonstrating the organism.

Dilute suspensions of a twenty-four hour broth culture in extract broth were killed in ten minutes at 54 C.

CULTURAL CHARACTERISTICS

Twenty-four hours at 37 C. or three days at room temperature were usually sufficient for an abundant growth. The appearance on solid mediums varied with the medium, being most abundant on alkaline dextrose agar (*p*H 7.2), and decreasing in the order named: acid dextrose agar (*p*H 6.4), autoclaved carrot, autoclaved pear, autoclaved potato, plain agar, plain gelatin, Löffler's serum medium, blood agar, raw carrot, raw liver and clotted blood. On solid mediums, the surface of the growth was slightly moist, dull white and raised; during the first week, colonies coalesced, becoming light cream colored, later showing a faint tinge of green. The consistency was that of thick top cream. On fruit, the growth was spreading and more moist; on potato it was dry, with the development of minute, chalklike pillars. In liquid mediums, the organism settled to the bottom or adhered to the lower side of leaning tubes. A ring usually developed at the surface, amounting to a collar in fermenting sugar mediums.

In gelatin stabs, hyphae grew out from the line of inoculation. There was no characteristic appearance, such as the pine-tree growth

of *Monilia psilosis* of Ashford,[4] but different tubes of the same strain in the same batch of gelatin exhibited different arrangements of outgrowth. Gelatin was not liquefied.

A loopful of a twenty-four hour broth culture of each strain was inoculated on 75 cc. each of alkaline dextrose agar (4 per cent dextrose, *p*H 7.2) and acid dextrose agar (4 per cent dextrose, *p*H 6.4) in 250 cc.

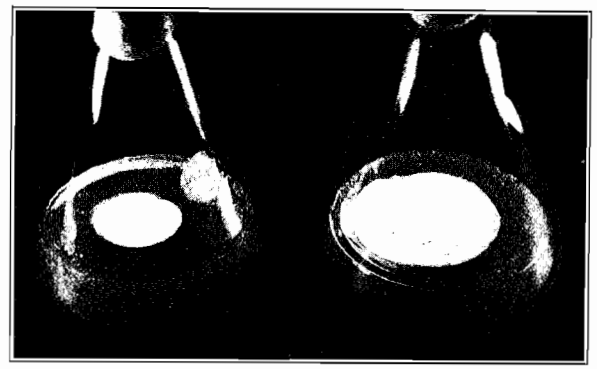

Fig. 3.—Giant colonies from three months' growth at room temperature; left, acid dextrose agar; right, alkaline dextrose agar.

Fig. 4.—Giant colonies from five months' growth at room temperature, during warm weather; left, alkaline dextrose agar; right, acid dextrose agar.

Erlenmeyer flasks, and incubated at room temperature. A smooth, light cream colored plaque developed on the surface, mycelial processes growing into the medium by the third month (fig. 3). During the third month, circular ridges developed on the surface, and the color was

4. Ashford, B. K.: Studies in Moniliasis of the Digestive Tract in Porto Rico, Am. J. M. Sc. **150**:680, 1915; The Etiology of Sprue, ibid. **154**:157, 1917.

tinged with green. Later, bubbles formed and ruptured, causing a gnarled, crumpled-paper-like mass, as in figure 4, especially on those colonies grown during the warm months. The piled-up cells were of both round and thread forms.

Dry aerial hyphae were not developed under any conditions tried, either aerobic or anaerobic, although cultures were kept for as long as one year.

Whether protected from drying or not, most of the cultures were alive at the end of six months, but not at the end of one year. Cells placed on gypsum blocks and kept moist with sterile distilled water lived for two months, but were apparently dead at the end of four months.

FERMENTATION TESTS

The tube-within-a-tube method, with 0.5 per cent solutions of various sugars in extract broth, was found to be satisfactory. Tests were run at both room and incubator temperature. Dextrose, levulose and maltose were fermented, with the production of alcohol, acetic acid and carbon dioxide; galactose was fermented with the production of alcohol and acetic acid but not with carbon dioxide.[5] Fermentation was practically complete in forty-eight hours in the incubator or in one week at room temperature, except in the case of maltose, which required a day longer in the incubator and from two to four days at room temperature. Saccharose, lactose, levo-arabinose, raffinose, mannite, inulin, salicin, xylose, glycerol and dextrin were not fermented. All sugar broths not fermented were made more alkaline than the control. There was no change in the *p*H of dextrin broth. After two or three weeks, the sugar broths which had been fermented became alkaline, with the absorption of carbon dioxide.

Litmus milk was rendered alkaline and clotted within seventy-two hours at 37 C. or within ten days at room temperature.

Neither nitrite nor indole was formed. Calcium oxalate crystals were found in old liquid cultures.

COMMENT

Morphologically and culturally, the organism described in this paper had many characteristics in common with *Monilia psilosis* of Ashford, although the fact that it failed to develop the "inverted fir tree" growth in gelatin definitely differentiates it from the sprue fungus. There are also other reported fungi which it resembles; yet it differs from each

5. One of the strains on gelatin was sent to Dr. B. K. Ashford in Porto Rico. This strain had been shown previously to ferment maltose, but Dr. Ashford reported that it had not fermented maltose in his laboratory.

in some important feature.[6] The morphologic characteristics of the organism and its failure to produce ascospores suggest a close relationship of the fruit canners' fungus to the "parasaccharomyces" of Anderson [7] and to *Moniliae* as listed by Clements,[8] but the present confusion in the taxonomy of "blastomycetes" does not permit of a definite classification.

SUMMARY

1. A new fungus is described. It is considered to be the etiologic agent in an occupational dermatosis among workers in canneries.

2. Morphologically, the fungus occurred both as a round yeastlike form, and as branched, septate hyphae with conidia. Dry, aerial hyphae were not observed.

3. Reproduction was by budding. Asci were not observed.

4. Gelatin was not liquefied. Litmus milk was clotted and rendered alkaline, and dextrose, levulose and maltose were fermented, with the production of alcohol, acetic acid and carbon dioxide. Galactose was fermented, with the production of alcohol and acetic acid only.

5. Although closely resembling *Monilia* or parasaccharomyces, the organism cannot yet be definitely classified.

6. Engman, M. F.: A Peculiar Fungus Infection of the Skin, Arch. Dermat. & Syph. **1**:370 (April) 1920. Castellani, A.: Medical Mycology, J. Trop. Med. **27**:49 and 61, 1924.

7. Anderson, H. W.: Yeast-Like Fungi of the Human Intestinal Tract, J. Infect. Dis. **21**:341, 1917.

8. Clements, F. E.: The Genera of Fungi, Minneapolis, H. W. Wilson, 1909.

ADDITIONAL BIBLIOGRAPHY

Greenbaum, S. S., and Klauder, J. V.: Yeast Infections of the Skin, Arch. Dermat. & Syph. **5**:332 (March) 1922.

Weidman, F. D.: Resemblance of Yeasts in Cutaneous Scrapings to Hyphomycetes, ibid. **5**:325 (March) 1922.

Mitchell, J. H.: Erosio Interdigitalis Blastomycetica, ibid **6**:675 (Dec.) 1922.

Boggs, T. R., and Pincoffs, M. C.: A Case of Pulmonary Moniliasis in the United States, Bull. Johns Hopkins Hosp. **26**:407, 1915.

Simon, C. E.: A Case of Yeast (Monilia) Infection of the Lung, Am. J. M. Sc. **153**:231, 1917.

Dubreuilh and Joulia: De l'intertrigo mycosique, Bull. Soc. franç. de dermat. et syph. **6**:192, 1921.

Stickel, J.: Zur Frage der Erosio Interdigitalis Blastomycetica, Dermat. Wchnschr. **72**:257, 1921.

Guggenheim, R.: Ueber Onychomykosis Oidiomycetica, Arch. f. Dermat. u. Syph. **142**:305, 1923.

Englehardt, W.: Ein Beitrag zur Aetiologie oberflächlicher Hautblastomykosen und Hautsoormykosen, ibid. **146**:313, 1924.

Steinfield, E.: A Study of Yeasts Found in the Sputum of Patients with Asthma and Chronic Bronchitis, J. Lab. & Clin. Med. **8**:744, 1923.

Braxton-Hicks, J. A.: A Case of Peri-Onychia Due to a Blastomyces, Lancet, **206**:128, 1924.

Shaw, F. W.: A Monilia from the Respiratory Tract, J. Lab. & Clin. Med. **12**:968, 1927.

Zeisler, E. P.: Monilia Infection of the Tongue, Arch. Dermat. & Syph. **15**:171 (Feb.) 1927.