LIPOPHILIC YEASTLIKE ORGANISMS ASSOCIATED WITH TINEA VERSICOLOR*

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Tinea versicolor is a common, usually mild disease of the superficial layers of the skin, readily diagnosed by the finding of characteristic fungoid elements in scrapings from the lesions (Fig. 1). Culture of the causative agent has not been accomplished with certainty and, although it is not a diagnostic necessity, its isolation would be desirable in order to determine more precisely the taxonomic relationships and physiological properties of the fungus and to establish unequivocally its role in the disease process. In a series of attempts to culture the agent involved, the results of which are reported herein, there have been isolated at least three lipophilic yeastlike organisms, one of which is considered a new species.

Malassezia furfur, the presumed etiologic agent of tinea versicolor, is known with certainty only from its appearance in the diseased skin. According to Robin (9), who in 1853 named the organism *Microsporon furfur*, Eichstedt first associated this fungus with the disease in 1846. The name was changed to *Malassezia furfur* in 1889, by Baillon (1).

Numerous attempts have been made to isolate this fungus but none of the reportedly successful procedures has yielded consistent and indisputable findings, despite the regularity and abundance with which the typical structures appear in scales from the lesions. Moore (6) presented an extensive review and evaluation of previous cultural attempts and reported culture in one instance, on relatively simple media, of an organism identified by him as *M. furfur*, which produced typical tinea versicolor lesions in one of 8 human volunteers. Panja (7) cultured several strains of an organism, also considered to be *M. furfur*, which was nutritionally fastidious on primary isolation but grew "on all laboratory media in subculture". Panja (8) later found that addition of various oils to his media caused a great increase in the growth of the fungi, which were characterized as oval, yeastlike, and producing mycelium.

EXPERIMENTAL

Cultural studies were undertaken on a series of cases of tinea versicolor, proved by the finding of typical structures of *M. furfur* in lesions. The media chosen for primary isolation were Sabouraud dextrose agar (Difco) overlaid with olive oil, and the same with penicillin (20 units per ml.) and streptomycin (40 units per ml.) added to the agar. Moore's peptone-dextrose medium also was used in some of the early attempts at isolation as were several other miscellaneous media.

Lesions were cleansed thoroughly with an alcohol sponge and scrapings made with a sterile scalpel. In most cases the scales were transferred to small, sterile Petri dishes for transport to the laboratory. Occasionally the scales were placed directly onto the isolation media. Liquid media were shaken well following inoculation with the scrapings. On solid media, the inoculum was introduced below

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the surface, at several points on the slant, and oil was added when indicated. All media were incubated at 37°C., with the tubes containing oil in a slanting position so that the entire surface of the medium was kept constantly bathed in the oil. Best growth was obtained when, in addition, these tubes were tilted manually each day for the first week or so of incubation, causing a daily redistribution of the oil.

Results of Primary Cultures

In a series of 22 consecutive cases of tinea versicolor, 16 yielded one or more isolates of fungi, all of them yeastlike organisms. All were classified as "lipophilic" since, on subculture, they grew little or not at all on ordinary media such as Sabouraud dextrose agar unless an oil or fatty acid was added. Although no non-lipophilic fungi were obtained on the primary isolation media, some of the organisms cultured were more fastidious than others with respect to their oil requirement. Bacteria were found frequently on media not containing the antibiotics, but they were not studied further.

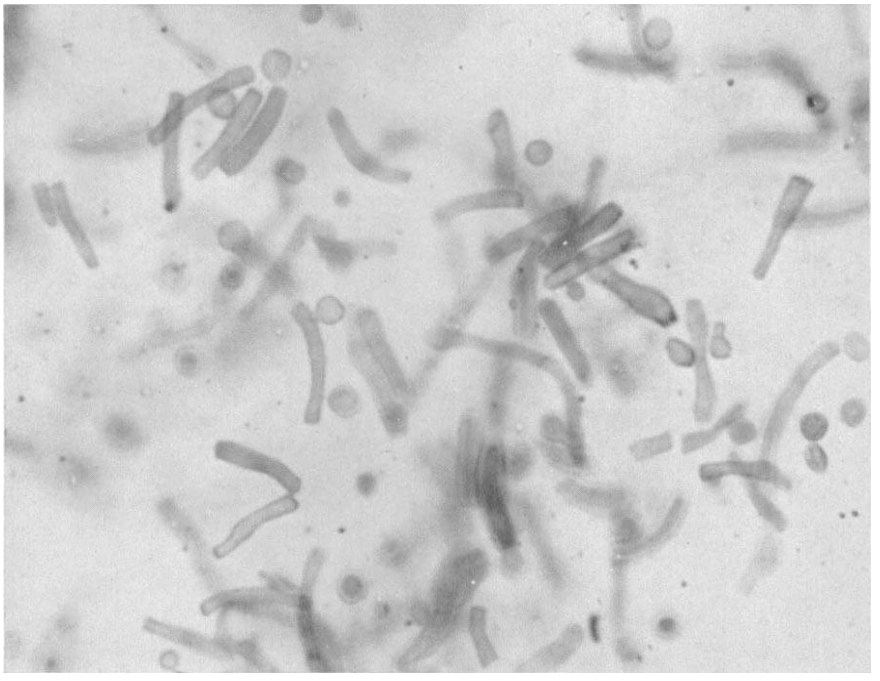

FIG. 1. *Malassezia furfur* in scales from a human lesion. Periodic acid-Schiff stain. ×1470.

The most frequently cultured fungus was a thick-walled, spherical, budding organism, 2.1–4.8μ in diameter, very closely resembling the spherical elements of *M. furfur* that appear in lesions. It was recovered only from young, actively spreading lesions, in 13 of 18 cases, and produced no growth on Sabouraud dextrose agar (Difco) without oil. Since it was so consistently associated with

lesions of tinea versicolor and so highly suggestive, morphologically, of the round forms of *M. furfur*, it was subjected to intensive cultural studies as well as experimental inoculations in human subjects. The results of these studies, to be detailed later in this paper, have failed to establish a probable identity between this organism and *M. furfur*, and since it does not fit the published descriptions of any other yeastlike fungus it is considered a new species. It has been named *Pityrosporum orbiculare* on account of the spherical appearance of its cells (Fig. 2a) and its obvious relationship to *Pityrosporum ovale* (2). A more complete account of *P. orbiculare* is given elsewhere (4).

In 3 of the 13 cases from which *P. orbiculare* was obtained, other lipophilic forms, varying in morphology from oval (Fig. 2c) to almost bacillary ("bottle bacilli", Fig. 2d) also were recovered, and from 3 additional cases such forms alone were obtained. After several transfers, 6 of the strains originally thought to be pure cultures of *P. orbiculare* became overgrown by such oval organisms. All of these are being investigated further, particularly with respect to a comparison with *P. ovale*, which several resemble, and to the possibility of a causative role in tinea versicolor.

The medium found most successful in the primary culture of *P. orbiculare*, as well as the other lipophilic yeasts, was Sabouraud dextrose agar (Difco) with added penicillin and streptomycin, plus oil. The same medium without antibiotics yielded yeasts on several occasions but they were usually mixed with or overgrown by bacteria. Nine attempts at isolation from active lesions on Moore's medium yielded bacteria alone or no growth at all. In 6 cases in which these three media were used in parallel, *P. orbiculare* was recovered in pure culture on the first medium on 4 occasions and on the second only once, being mixed with bacteria another time.

Biological Characteristics of P. orbiculare

P. orbiculare can be maintained indefinitely in culture on Sabouraud agar with oil, with or without the antibiotics, when incubated at 37°C. and transferred every 3 weeks. The oldest strain now viable has survived more than 27 transfers over a period of 16 months. The organism grows almost as well at 30° as at 37°, producing a heavy yeastlike growth after 2 weeks, but development at 25° is very poor even after 5 weeks.

When olive oil was replaced, on the above media, by other vegetable oils or by fatty acids the following results were observed. Good growth occurred with corn, cottonseed, linseed, soybean and peanut oils and with lauric, myristic, palmitic and stearic acids; no growth with castor oil or with the unsaturated acids, oleic and linoleic. One strain grew, but poorly, with glycerine as the additive, and none was able to utilize mineral oil. Corn meal agar (3) or wort agar with olive oil permitted good growth, somewhat poorer than on the Sabouraud media. No growth was obtained on washed, vitamin-free agar with olive oil. In contrast, Benham (2) found that *P. ovale* gave excellent growth with oleic acid but much less with the saturated acids or soybean or linseed oil. All of her strains grew to some extent with glycerine.

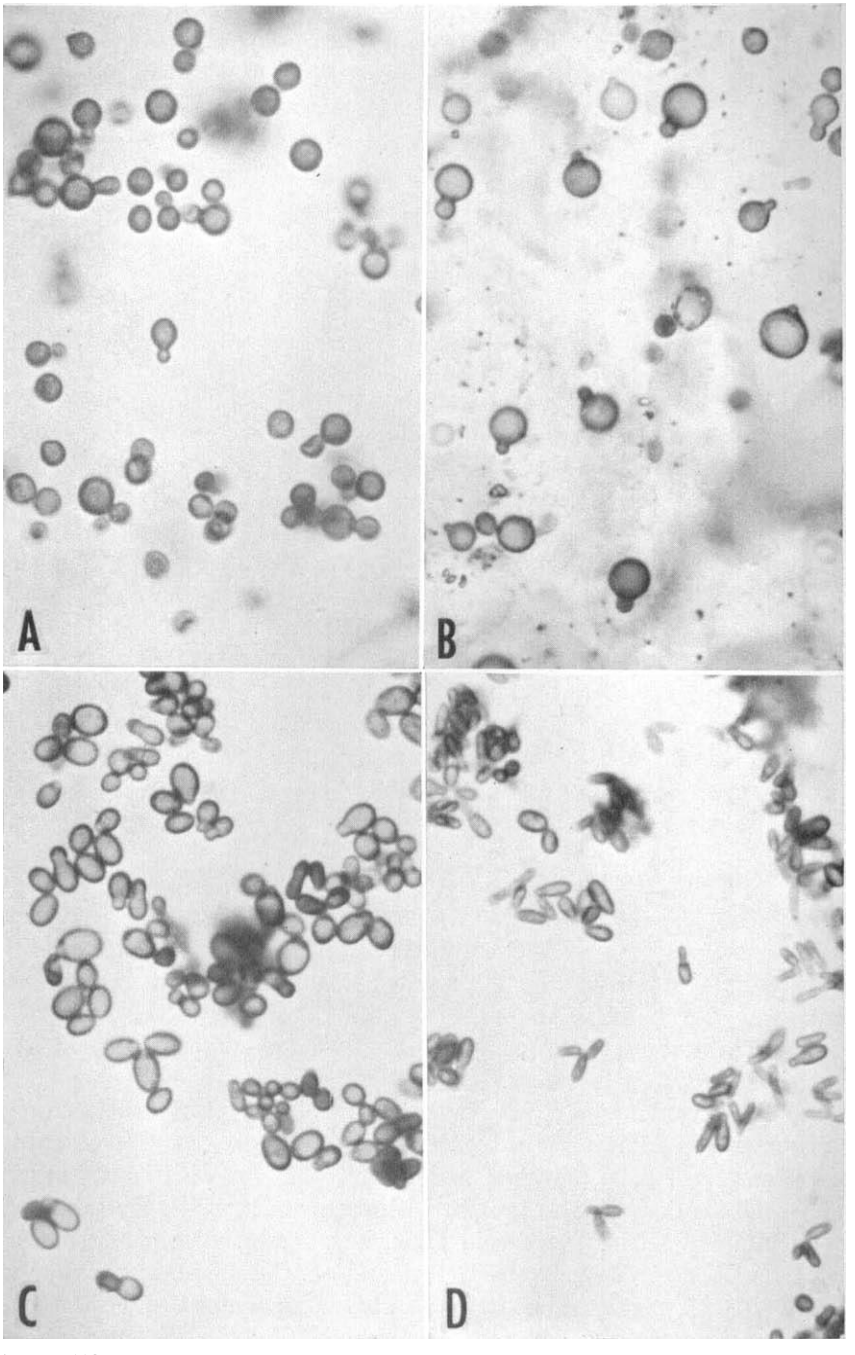

FIG. 2. (All specimens stained by Periodic acid-Schiff method. × 1470) *a. Pityrosporum orbiculare* in culture. *b.* Spherical, budding cells in normal skin. *c.* Lipophilic oval budding forms in culture. *d.* Lipophilic "bottle" forms in culture.

Subculture to the following media, without added fatty substances, was unsuccessful; MacLeod's glycerine agar (5), wort agar (Difco), Panja's modified Petroff agar (7), Sabouraud maltose agar (Difco), Czapek's agar, potato-dextrose agar, nutrient agar (Difco), corn meal agar, Moore's peptone broth (6), glucose-peptone broth plus one per cent saponin, and MacLeod's broth.

Experimental Inoculations

Over a period of a year 9 human volunteers were subjected to a total of 22 cutaneous inoculations with 6 strains of *P. orbiculare* and 2 other lipophilic organisms isolated from lesions of tinea versicolor. In addition, 2 volunteers were inoculated with a culture of *Malassezia tropica* (isolated from a case of tinea flava in Nigeria) obtained from the London School of Hygiene and Tropical Medicine. All inoculations were to the neck, shoulders or upper part of the arms. The site selected in each instance was cleansed with ethanol and then either rubbed to erythema or scarified by scraping with a blunt knife, following which an oil suspension of the yeastlike cells from culture was rubbed in. A number of the inoculation sites were subsequently kept covered with sterile gauze or rubber sheeting, while others remained exposed.

Despite repeated examination of the experimental areas for periods ranging from 1 to 7½ months, no lesions typical of tinea versicolor were ever observed, nor were the characteristic hyphal elements of *M. furfur* found in scrapings. However, cells resembling *P. orbiculare* were still discernible microscopically after 3 or 4 weeks.

Attempts to reproduce the lesions on shaved and scarified skin of rabbits and guinea pigs likewise resulted in failure.

Occurrence of Lipophils on Normal Skin

A limited series of studies on normal skin was undertaken in order to determine whether the various lipophilic yeastlike organisms mentioned above are necessarily associated with tinea versicolor. Scrapings from the neck, shoulders or arms of 8 individuals ostensibly free of the disease were subjected in each case to direct microscopic examination and to culture. Microscopic mounts were stained with methylene blue and the scales were implanted in triplicate on tubes of Sabouraud agar with antibiotics and olive oil. Upon direct examination seven of the 8 skin samples revealed spherical, budding cells similar to those of *P. orbiculare*. These were quite numerous in 4 instances (Fig. 2b). Three specimens showed, in addition, a number of oval, budding forms. All 8 yielded lipophilic yeasts in culture, usually on all 3 tubes. From 2 individuals an organism was obtained which resembled *P. orbiculare* morphologically and physiologically, while in all other cases only oval forms were cultured. The latter were less exactingly lipophilic than is *P. orbiculare*, and grew to a limited extent on ordinary Sabouraud agar, producing streptococcus-like colonies on streaked plates after 4 or 5 days.

These results would suggest that the organisms cultured from tinea versicolor

lesions are normal inhabitants of the skin, but it is apparent that much work remains to be done in order to determine their significance. It is possible that any of them may, under certain predisposing circumstances, be the cause of pathological conditions, including tinea versicolor.

SUMMARY

1. Attempts to culture *Malassezia furfur* by the use of agar media overlaid with oils have resulted in isolation of a series of lipophilic yeastlike organisms from lesions of tinea versicolor. No fungi were recovered in several trials with Moore's peptone—dextrose medium.

2. In a high percentage of cases there was recovered a spherical organism, exacting in its nutritive requirements, which apparently represents a new species. It has been given the name *Pityrosporum orbiculare*.

3. Some biological characteristics of *P. orbiculare* are presented.

4. Experimental inoculation with cultures of *P. orbiculare* has failed to produce tinea versicolor in a number of human volunteers and laboratory animals.

5. *P. orbiculare* has been cultured from 2 of 8 apparently normal skins. The remaining 6 yielded other lipophilic yeastlike organisms.

REFERENCES

1. Baillon, H.: Traite de Botanique Medicale Cryptogamique. Paris, Octave Doin, P. 234, 1889.
2. Benham, R. W.: The cultural characteristics of *Pityrosporum ovale*—a lipophylic fungus. J. Invest. Dermat. **2:** 189, 1939.
3. Conant, N. F. et al: Manual of Clinical Mycology, Philadelphia, W. B. Saunders Co., P. 319, 1944.
4. Gordon, M. A.: The lipophilic mycoflora of the skin. I. *In vitro* culture of *Pityrosporum orbiculare*, n. sp. Mycologia, in Press.
5. MacLeod, J. M. H. and Dowling, G. B.: An experimental study of the pityrosporum of Malassez. Brit. J. Dermat. **40:** 139, 1928.
6. Moore, M.: Cultivation of *Malassezia furfur*, etiological agent of pityriasis (tinea) versicolor. Mycopathologia **1:** 53, 1938.
7. Panja, G.: The malassezia of the skin, their cultivation, morphology and species. Trans. 7th Cong. Far Eastern Assn. Trop. Med. **2:** 442, 1927.
8. Panja, G.: A new oil medium for enhancement of growth of the malassezia. Indian M. Gaz. **81:** 171, 1946.
9. Robin, C.: Histoire Naturelle des Vegetaux Parasites. Paris, J. -B. Bailliere, P. 438, 1853.