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Studies on Pityriasis Versicolor in Egypt III. Laboratory Diagnosis and Experimental Infection

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Pityriasis versicolor is diagnosed mostly clinically and by the presence of short hyphae and clusters of spores seen in direct microscopical examinations of skin scrapings. Numerous attempts have been made to isolate the causative organism. All cultures yielded an organism related to the genus *Pityrosporum*, (BENHAM, 1939; EMMONS, 1940; GORDON, 1951; and others). The same organism could be isolated also from normal persons (GORDON, 1951; ALEXANDER, 1967, ROBERTS, 1969). A number of early workers considered the organism seen in scales of P. V. as the cause of the lesion in which it was found (COURREGES HARDY, 1876, HODARA, 1894). Others (HORAND, 1874, 1875, and NYSTROM, 1875) considered that it was a harmless saprophyte. Many authors tried to prove that *Pityrosporum orbiculare*, which is commonly isolated from cases of P. V. is the same organism seen in direct microscopical examination of scales of P. V. lesions, and hence the cause of the disease. STERNBERG and KEDDIE (1961) and KEDDIE et al. (1963) demonstrated through immunofluorescent studies a common antigen between the two organisms.

In this work, comparative studies of the organisms seen in direct microscopic examination and cultures from 200 P. V. were carried out, as well as histopathological findings of 18 patients were reported. Different media were tried, and with the isolated organisms experimental infections were done.

Materials and Methods

Two hundred patients suffering from Pityriasis versicolor (P. V.) were examined. At first scrapings were treated with 20% KOH solution. Later this method was substituted by the Cellophane stripping technique, in which the sticky side of a piece of cellophane tape was gently pressed down on the suspected lesion, then removed and immediately pressed on a clean, oil free slide on which a drop of Parker blue-black ink was placed. The preparation was then examined directly under low, high and oil immersion lens of the microscope.

For cultural examination, 3 different media: Sabouraud's dextrose agar, mycological agar and Wort agar were used to which olive oil, cotton seed oil and oleic acid were added on the surface of the media.

The cultures were incubated at 37° C aerobically. Some were incubated in anaerobic atmosphere. The isolated strains were diagnosed by direct microscopical examination of lactophenol cotton blue preparation, and by fermentation and assimilation tests.

For histopathological examination, biopsies were taken from 18 patients with P. V. lesions and from 6 patients with hypopigmented patches following successful treatment, as proved by negative microscopical examination and negative fluorescence.

For experimental infection, 10 male and 10 female volunteers were chosen (25—35 years old). They were completely healthy and free from any skin lesions. P. V. scales as well as cultures of *P. orbiculare*, were suspended in sterile olive oil and rubbed across the upper back and shoulders to the sternoclavicular area. On each patient the scale suspension was applied on the right side and the culture suspension on the left. Scrapings from the inoculated areas were collected at the end of the first, second, third and fourth weeks and thereafter every month for one year. These were examined both microscopically and culturally.

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Results

1. *Direct microscopical examination*

Malassezia furfur could be easily demonstrated by direct microscopical examination of KOH preparations (Fig. 1).

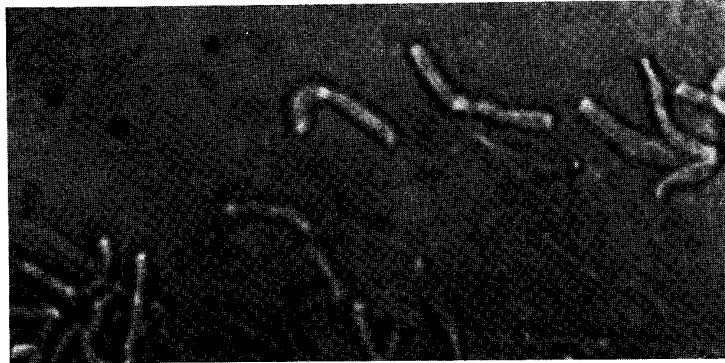


Fig. 1: KOH preparation of P. V. scales showing short thick hyphae ($\times 925$)

Staining preparation with Giemsa's stain was time consuming. The cellophane tape method was much easier and more practical, especially when a drop of parker ink was placed on the slide. In such preparations, short, thick, hyphae, $1,7-5,1 \times 4,2-10,2$ microns, were seen. Spores were arranged mostly in grape-like clusters. They varied in diameter from 3,4 to 4,2 microns and had thick walls. The number of spores and hyphae varied from lesion to lesion and in different patients. In some preparations they were very scanty and difficult to detect; in others the scrapings seemed to consist completely of hyphae and spore collections (Fig. 2).



Fig. 2: Cellophane tape preparation of P. V. scales showing spores and hyphae

2. Isolation

Cultural examination of P. V. scales on different media yielded only *Pityrosporum orbiculare*. The isolation of the organism succeeded only when oil was added on the surface of the media. Olive oil and cotton seed oil gave good results. Oleic acid was not satisfactory and paraffin oil had no effect. Incorporation of oil into the medium was useless. The organism did not grow in anaerobic atmosphere. Mycological agar with olive oil was found to be the best medium that gave the highest percentage of positive cultures (table 1).

Table 1: Results of cultural examinations of skin scrapings from 200 patients with P. V. lesions on different media

Medium	No of isolations of <i>Pityrosporum orbiculare</i>
Sabouraud dextrose agar	0
+ olive oil	138
+ cotton seed oil	140
+ oleic acid	60
+ oleic acid*	0
+ stearic acid*	0
+ oleic and stearic acid	98
+ paraffin oil	0
+ anaerobic incubation	0
Mycological agar	0
+ olive oil	142
+ cotton seed oil	140
+ oleic acid	54
Wort agar	0
+ olive oil	128
+ cotton seed oil	126
+ oleic acid	60

On all the media used, the organism grew slowly. At the end of a week, a pin-point, smooth colonies, much like *Candida albicans* were seen. Microscopical examination of culture mounts revealed the presence of thick-walled and spherical budding cells, which closely resembled the spores that appeared in direct microscopical examination of skin scrapings (see fig. 3). They were slightly smaller in diameter, about 1,7 to 3,4 microns. The isolated organisms did not ferment or assimilate glucose, galactose, maltose, sucrose or lactose. Subculturing on different media was successful.

3. Histopathology

In all sections stained with H. & E., the epidermis was normal in appearance, with a mild degree of hyperkeratosis and follicular plugging. The dermis contained minimal

* incorporated in the medium

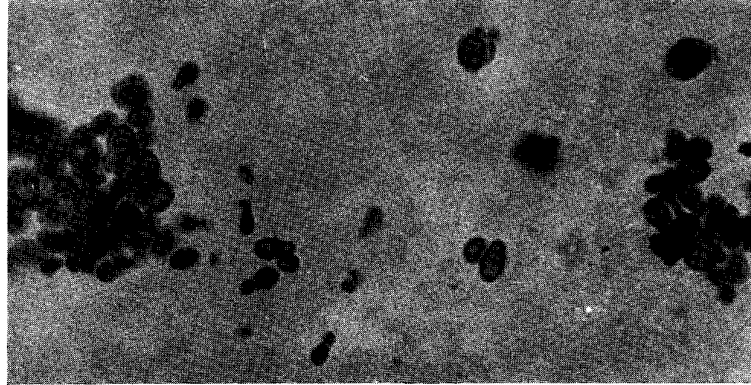


Fig. 3: Culture mount of *Pityrosporum orbiculare*
(Gram's stain, $\times 970$)

inflammatory infiltrate mainly of lymphocytes, plasma cells and histiocytes. Perifollicular reaction was noted in 2 cases. In sections stained with PAS stain, the organism was found to be abundant in the horny layer of the skin and did not penetrate the other layers. It was also seen in hair follicles (Fig. 4). The organism appeared the same as seen in direct

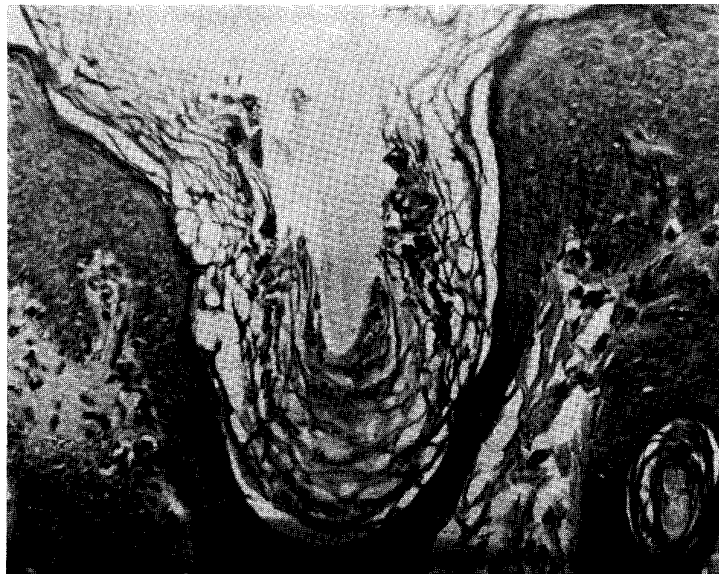


Fig. 4: Histopathological sections from a case of P. V. showing spores
and hyphae in a hair follicle (PAS stain, $\times 220$ and $\times 450$)

microscopical examination of P. V. scales. The spores were 2.5 to 3.4 microns in diameter and the hyphae were $1.7-2.5 \times 10.2-14$ microns.

No organisms or pathological changes could be demonstrated in the hypopigmented patches of treated persons.

4. *Experimental infection*

No lesions typical of P. V. were observed by the 20 inoculated persons. Direct microscopical and cultural examinations gave negative results.

Discussion

The causative organism of P. V. has been and is still a matter of discussion. This controversy could be attributed to the difficulty of culturing the organism. In cellotape preparations, the organism was found to be present in two forms: hyphae-like structures and groups of spores with some of which showing budding. This was also confirmed by histopathological studies on 18 cases, as the organism showed the same picture as seen in direct microscopical examination. Histopathological examinations of 6 cases with hypopigmented patches of treated P. V. areas and other 6 biopsies taken from clinically normal persons, from sites commonly affected with P. V., revealed the presence of neither hyphae nor spores. Thus we conclude that the organism seen in direct microscopic examination and histopathological sections is the causative organism of P. V. Comparing the organism seen in direct microscopic examination of scrapings from lesions of P. V. with that obtained in culture from the same lesions and that seen in histopathological sections from patients with P. V., we can conclude that the organism isolated by us resembled that found in direct microscopic examination and histologic sections, both in appearance and in measurements. This is also proved by the ultrastructural studies carried out by KEDDIE (1966) with the help of the electron microscope. The fact that the organism rarely develops hyphae in cultures may be explained as a diphasic character, i. e. the organism has 2 phases: a budding phase and a hyphal one, and the organism in its pathogenic form in the tissues is able to produce mycelia and blastospores while in cultures only blastospores and rarely hyphae. VANBREUSEGHEM (1954) reported transitional forms between the mycelium and clusters of spores, in stained tape preparations from P. V. lesions.

Experimental infections using skin scrapings or cultures of *P. orbiculare* were unsuccessful. This agreed with the findings of GORDON (1951). BURKE (1961) claimed that she was able to produce typical scaly lesions with the appearance of the organism on microscopy in two patients suffering from Cushing's syndrome and in one with malnutrition. She was however unable to convert colonies of *P. orbiculare* on the skin to the organism seen in direct microscopy.

Summary

Mycological and histopathological studies were done on 200 pityriasis versicolor patients chosen at random.

Pityrosporum spores and hyphae have been demonstrated in all cases through direct microscopical examination as well as in histopathological sections. Culturing succeeded in 71.2% of the cases on mycological agar with olive oil pipetted over the surface of the medium. Negative results were obtained in anaerobic atmosphere and when mineral oil was used. Subculture of the organism was successful in a fluid medium containing Czapek-Dox, yeast extract and Tween-20.

Zusammenfassung

Mykologische und histopathologische Untersuchungen wurden bei 200 nicht ausgesuchten Patienten mit Pityriasis versicolor durchgeführt.

Sporen und Hyphen von *Pityrosporum* wurden in allen Fällen sowohl direkt mikroskopisch als auch in histopathologischen Schnitten nachgewiesen. Die Kultur gelang in 71,2 % der Fälle auf mykologischem Agar, auf dessen Oberfläche Olivenöl pipettiert worden war. Negative Ergebnisse wurden erzielt, wenn Mineralöl verwendet wurde und in anaerober Atmosphäre. Subkulturen gelangen in flüssigem Medium aus Czapek-Dox-Lösung, Hefeextrakt und Tween 80.

Die Tatsache, daß die gezüchteten Organismen in Kultur nur selten Hyphen bilden, kann durch den diphasischen Charakter erklärt werden, d. h. der Organismus hat 2 Phasen: eine Hefeform und eine Mycelform; in der pathogenen Form im Gewebe werden sowohl Mycelien als auch Blastosporen gebildet, in Kultur jedoch im allgemeinen nur Blastosporen und selten Hyphen.

Experimentelle Infektionen bei 20 Personen mit Hautgeschabsel oder Kulturmaterial von *Pityrosporum orbiculare* hatten keinen Erfolg. Dies stimmt mit den Befunden von GORDON überein. Die direkten mikroskopischen Befunde und die kulturellen Untersuchungen waren negativ.

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