A MODIFIED P.A.S. STAINING TECHNIC FOR FAVIFORM DERMATOPHYTES—ITS APPLICATION IN SLIDE CULTURES*

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The slide culture of fungi by the agar-block method, introduced by Duncan at the London School of Hygiene and Tropical Medicine, was described in 1950 by Riddell (1). This method yields good results in the culture of the common dermatophytes which—after inoculation at each side of the agar-block—tend to produce luxuriant growth of mycelium both on the slide and the coverslip. Riddell made use of lactophenol-cottonblue stain and claimed permanency of the wet-stained preparations sealed off with nail varnish.

Kligman and Lewis (3) denied this claim of permanency and recommended periodic-acid Schiff stain for permanent stained preparations. Procedures adopted differed slightly from those previously described for the demonstration of fungi in animal tissue (Kligman and Mescon (2)). We confirm that the periodic-acid Schiff stain—even in the usual laboratory procedure as employed in the staining of skin biopsy specimens—yields excellent results in staining dermatophytes, grown in slide culture according to the Duncan-Riddell technic. Its use, however, is restricted to the non-faviform dermatophytes, which grow luxuriantly outside the agar-block (Fig. 1.)

For several reasons this combined technic cannot be used for the culture and staining of *faviform* dermatophytes (*T. Schönleini, T. verrucosum, T. violaceum, T. ferrugineum, T. soudanense, T. concentricum*).

a. Riddell used agar-blocks of 2 mm. thickness. The slow growing faviform fungi, however, need a thicker layer of agar (±5 mm.) to prevent shrinking as appreciable growth is only obtained after several weeks.

b. Faviform fungi, scarcely yielding any mycelium outside the agar-block, grow predominantly submerged in the agar. Routine P.A.S. staining technic colors the entire agar-block deeply violet by oxidizing and subsequently staining the carbohydrate content of the substrate, thus obscuring the morphology of P.A.S. stained mycelium.

We have made use of a modified P.A.S. staining technic which results in selective staining of the faviform mycelium inside the agar-block without staining the agar substrate which is left completely transparent (Fig. 2). Modified P.A.S. staining procedures applied to the entire agar-block were as follows:

Procedures

1. Fix specimen embedded in agar-block in formaldehyde solution 4% for at least 24 hrs.
2. Rinse carefully in running tapwater for at least 1 hr.
3. Wash in distilled water.
4. Immerse specimen in solution A. (1 hr.)
5. Rinse in running tapwater (1 hr.)
6. Wash in distilled water.
7. Immerse specimen in modified sodium metabisulfite solution (3 hrs.) (10 drops Schiff's solution being added to 120 ml. sodium metabisulfite sol.)
8. Rinse in running tapwater for at least 3 hrs.
9. Immerse in alcohol 96% and allow specimen to stand during the night.
10. Next morning immerse specimen in anhydrous alcohol (1 hr.)
11. Immerse in 33⅓% phenol-xylene solution (1 hr.)
12. Immerse in xylene (1 hr.)
13. Place specimen in agar-block on a slide.
14. Seal off with malinol or caedax, proceeding step by step. (First pour out one drop on agar-block, cover with cover-slip and allow to stand for 1 hr.)
15. Add another drop of sealing fluid under cover-slip and allow to dry for 1 hr.

Repeat this procedure until agarblock is completely surrounded by sealing fluid.

Prescriptions

Solution A. 5 ml. sodium acetate ⅕ mol.; 10 ml. periodic acid 4%; 35 ml. ethyl alcohol 95%.

N.B.: mix shortly before use—usable once only.

Sodium metabisulfite solution. 6 ml. sodium metabisulfite solution 10%; 6 ml. hydrochloric acid; 120 ml. distilled water.

Schiff's solution. 900 mg. pararosaniline (Merck); 200 ml. distilled water (heated); 10 ml. hydrochloric acid 1 N. After being dissolved, add: 2 gm. potassium metabisulfite. Allow to stand for 24 hrs. Filter through coarse filtering paper.

We presume that this method can contribute to a closer study of the morphology of faviform dermatophytes. The observation of these fungi on a variety of enriched media and the possibility of keeping them growing for more than a month before staining, may disclose a richer morphology than hitherto known.

SUMMARY

Periodic-acid Schiff staining of dermatophytes grown by the agar-block method of Duncan-Riddell, cannot be used in case of faviform dermatophytes. A modified P.A.S. staining technic gives selective staining of the submerged mycelium of faviform fungi without coloring the agar-substrate.

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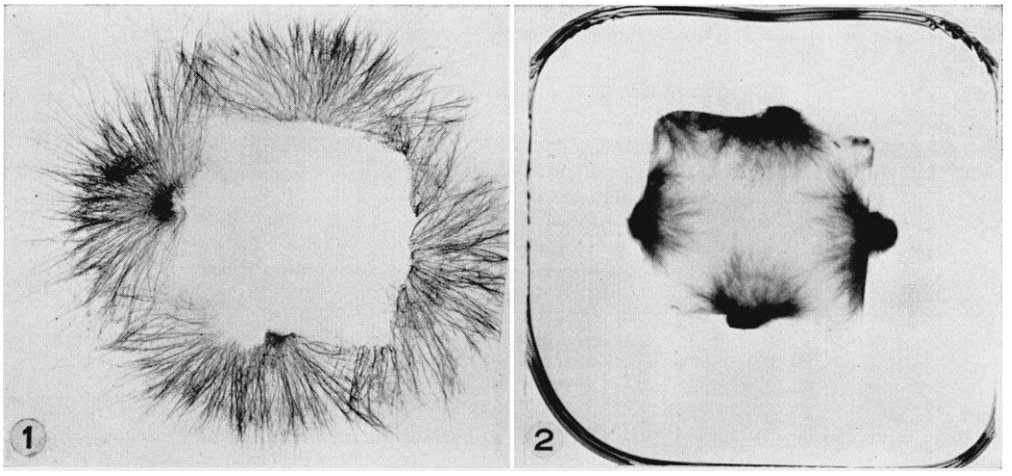

FIG. 1. *Non-faviform* dermatophyte (*M. canis*) growing luxuriantly outside agar-block. Routine P.A.S. staining of coverslip.

FIG. 2. *Faviform* dermatophyte (*T. ferrugineum*) growing predominantly inside agar-block. Modified P.A.S. stain of entire agar-block.

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