A SIMPLE FLUORESCENT METHOD FOR THE DETECTION OF SUPERFICIAL FUNGI IN SKIN AND HAIR

A Combined Stain with Acridine Orange and Potassium Hydroxide*

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The classical method for the detection of superficial fungi in skin scrapings and hair is the 10–20% potassium hydroxide preparation studied by means of the white light microscope. In experienced hands, the above method is a simple but highly effective tool. Such preparations are, however, subject to misinterpretation by the similar appearance of various artifacts to that of hyphae. More specifically, the artifacts of prime importance are vegetable fibers, either in the clinical specimen or on the slide and the appearance of superimposed cell walls in thick preparations. A method is, therefore, needed which would be as simple as the KOH preparation but in addition would provide an adequate means for ruling out false positives due to artifact. A method is presented which fulfills these criteria, by combining acridine orange, a fluorescent stain for fungi, with the KOH.

MATERIALS AND METHODS

Samples of hair and skin scrapings were obtained from patients and personnel with clinical dermatomycosis, culturally proven and suspect.

A 20% solution of KOH was prepared by dissolving 10 grams of KOH pellets in 50 ml. of distilled water.

A 1:1000 stock solution of acridine orange was prepared from the powdered form of the material and kept refrigerated for use as required. For these studies, a Reichert Zetopan microscope with fluorescent attachments was used. Observations were also made using an ordinary compound microscope and light source adapted for blue-violet light by the addition of filters (1, 2).

The working stain was prepared by adding 1 ml. of 1:1000 acridine orange solution slowly to 9 ml. of the 20% KOH solution to prevent precipitation, giving a final dilution of acridine orange of 1:10,000. The preparation of small amounts of the stain, 10 ml., is recommended as the solution tends to cloud and precipitate when standing more than 12 hours.

An alternative method which may be used is to add 1 ml. of the 1:1000 acridine orange solution to 9 ml. of distilled water giving a concentration of 1:10,000 acridine orange. One ml. of the 1:10,000 acridine orange is added slowly to 9 ml. of 20% KOH solution giving a final dilution of 1:100,000 acridine orange. This alternative method may be used to advantage with thick specimens in which some background fluorescence is troublesome.

The material to be studied is placed on a well polished slide and 2–3 drops of the prepared stain is then added. The preparation is carefully mixed with an applicator stick for proper staining and covered with a well polished cover slip. The preparation is then gently heated briefly over an open flame and examined under the microscope. All preparations were examined under both blue-violet and white light illumination.

RESULTS

Scrapings from lesions of untreated culturally proven cases of dermatomycosis could be rapidly and easily scanned for the presence of fungi. The hyphae fluoresce brightly against a darkened background under blue-violet light. Under white light illumination, the hyphae could be found with slightly more difficulty, and occasional artifacts resembling hyphae sometimes proved a problem in identification. Switching to blue-violet light illumination readily revealed artifacts as such in that they did not fluoresce, or if they did fluoresce, as rarely occurred, their structure was different from that of hyphae.

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Similar findings were experienced with hair clippings.

DISCUSSION

The KOH-acridine orange technic has been shown to be superior to the simple KOH preparation. It retains the simplicity of the latter and in addition provides a means of confirming KOH evaluations, ruling out false positive results from KOH evaluations, and finally, and possibly most important, allowing organisms previously missed by KOH preps to be picked up with the addition of the acridine orange stain.

The KOH acts to partially clear the cells of properties which otherwise would accept the acridine orange. The latter, however, is still accepted by the fungi and with the proper filters fluoresces against a darker fluorescent background. The procedure is quite versatile in that the viewer readily may switch back and forth from white light, under which conditions the specimen is a simple KOH preparation, to filtered blue-violet light and the fluorescent technic. This switching back and forth provides a means of valid discrimination between hyphae and artifact as the latter does not fluoresce, usually, or if fluorescence occurs, structural differences from hyphae are easily seen. In addition, the fluorescent technic will pick up organisms which are obscured by the classical KOH technic.

As to the practicality of this procedure, ordinary white light compound microscopes may be converted easily to blue light by means of the proper filters. A blue-violet activator filter is placed in front of the light source and yellow barrier filters in the eye-pieces.[3] This combination of matched filters is available for approximately $30, a not excessive outlay for routine and office laboratories.

In addition to its use as a tool to demonstrate fungi in skin scrapings and hair clippings, the KOH-acridine orange stain may prove of value in the demonstration of fungi in sputum, spinal fluid, exudates, and other clinical materials. With this technic we have been able to demonstrate *B. dermatitidis* in sputum with ease.

SUMMARY

The combination of a fluorescent stain for fungi, acridine orange, with 10–20% potassium hydroxide provides a simple method for the demonstration of fungi in skin scrapings and hair clippings. By means of inexpensive filters it is possible to switch back and forth from white light and the traditional KOH examination to blue-violet light and fluorescent examination. With this method it is possible to scan materials rapidly and accurately and to distinguish between artifacts and fungi.

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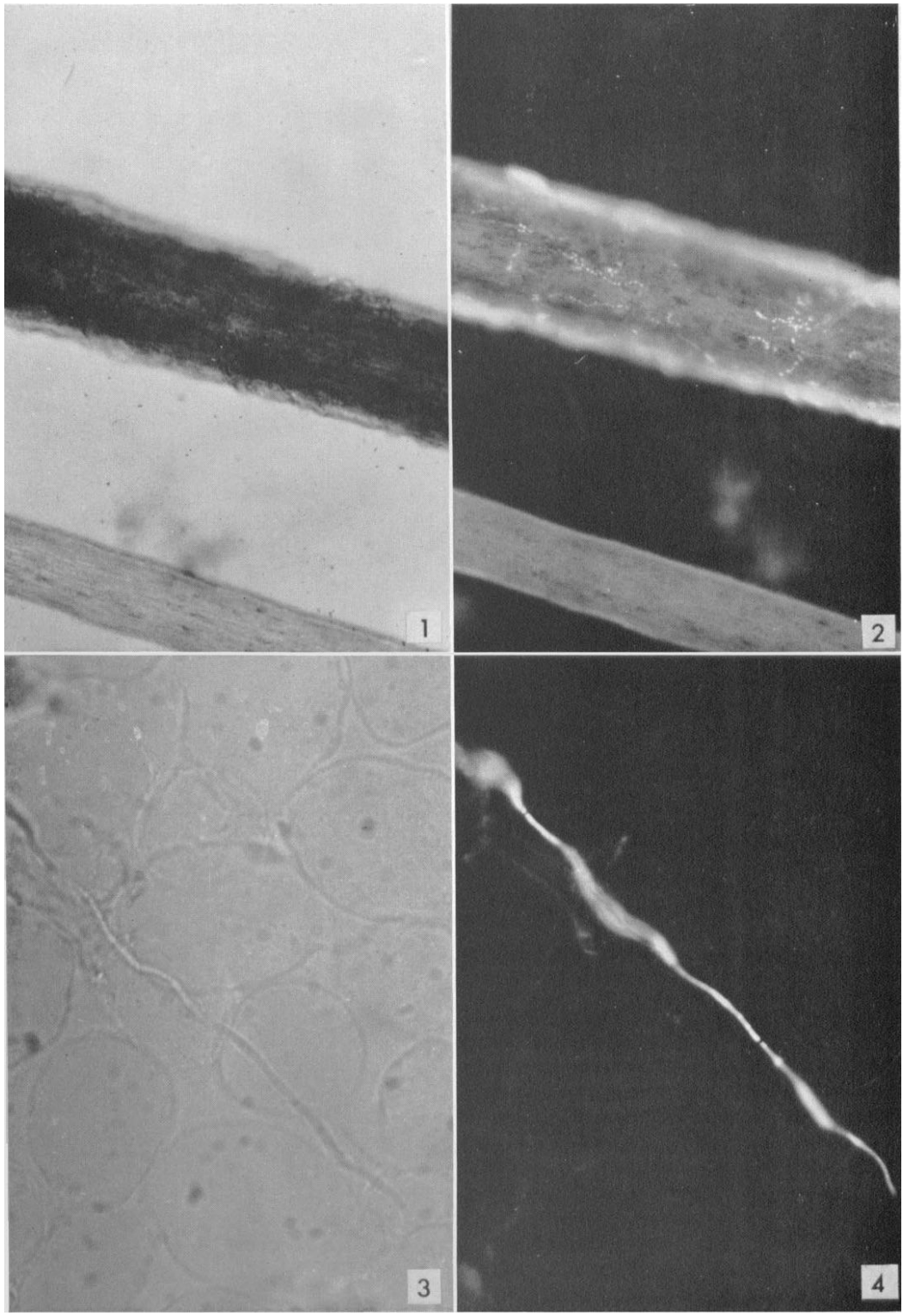
[3] A Leitz or Zeiss blue light fluorescent filter (BG 12, 4 mm. thick) is used at the light source which should be adjusted for Koehler illumination. Corning sharp-cut yellow filters (No. 3486, 3-69, 3 mm. thick, cut to fit the inside diameter of the eyepiece) are used as barrier filters.

Fig. 1. Hair shaft as visualized by white light. The fungus can be faintly visualized in roughly parallel growth. This preparation corresponds to the usual KOH preparation. (KOH-acridine orange stain.)

Fig. 2. Hair shaft as visualized by blue-violet light. The fungus fluoresces brightly producing easy visualization. An uninfected hair is shown for comparison. (KOH-acridine orange stain.)

Fig. 3. Skin scrapings as visualized by white light. The long hypha has the characteristic appearance seen in usual KOH preparations. (KOH-acridine orange stain.)

Fig. 4. The same field as in Fig. 3 as visualized by blue-violet light. The fungus fluoresces brightly against a darkened background; thus it is easy to scan the field rapidly and accurately for fungi. In the photograph there is some diffusion of the fluorescence due to different focal planes of the hyphae. Under the microscope this is overcome by focusing continuously along the length of the hyphae. (KOH-acridine orange stain.)



FIGS. 1–4