SPORE IDENTIFICATION IN SCRAPINGS *

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The purpose of this work was to find a simple method by which mold spores contained in scrapings could be identified. Many stains have been used by various authors, but the technic employed either requires too much time or is so complicated that it is of little service to the practitioner.

Under ordinary circumstances, the immersion of the scrapings containing spores in 10 per cent. potassium hydroxid solution, in many instances, is quite sufficient to make a diagnosis. There are, however, cases in which only a few spores are present, and on examination the question arises whether the globules seen are those of degenerated keratin, fatty or protein substances, or the spores themselves. Unfortunately, this method goes no farther in making a differential diagnosis.

In this series of experiments it was originally intended, relying on the density of spores, to precipitate some material in them (by mordant methods or otherwise), which would not be washed out by subsequent solvents or differentiating agents. The methods suggesting themselves for this purpose were: (1) Exposure to heat, and (2) the use of various mordants and dyes.

EFFECTS OF EXPOSURE TO HEAT

Assuming that mold spores were more resistant to heat than the scrapings in which they were contained, it seemed possible by exposing them to heat long enough, to scorch scrapings to a greater degree than the spores and thereby produce a differential picture. The heat effects were first tried on pure cultures of laboratory saprophytes.

Material and Method.—Coverslips were cleansed in alcohol to render them fat free. They were then placed on a flat surface. One small drop of water was placed on the center of the coverslip; to this a loopful of spores was added and then spread over its surface. It was grasped between the thumb and forefinger and gently warmed over a Bunsen burner until dry. After all moisture had evaporated the coverslip was grasped with a Stewart's forceps and passed through the flame, right side up until the spores were scorched to a light brown color. The best results were obtained by passing the coverslips

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through an arc of about 12 cm., the center of which cut the Bunsen burner flame, 11 cm. in height, about 3 cm. below its tip, each pass requiring one second for its completion. At the end of thirty seconds (or the same number of passes), the spores were mounted in balsam and examined microscopically.

By this method the spores and mycelia were all scorched to a brown and were clearly discernible. The spores contained a reddish refractile granule which made them quite characteristic.

Scrapings containing spores were next examined. It was necessary to tease the scrapings to smaller pieces with dissecting needles in order to have a more uniform exposure of the spores to the heat. By this method the spores appeared as small, dark brown globules both outside and inside the mycelia, and those contained in the mycelia were much darker in contrast to the refractile mycelia which stood out conspicuously when examined under high power.

This method being so simple and requiring so little time for its completion, deserves to be considered when a hurried diagnosis is to be made.

USE OF MORDANTS AND DYES

This problem was first attacked from the standpoint that a mordant could be used to penetrate the spores, and with subsequent washings the solution used could be washed from the scrapings and not from the spores.

Material and Method.—In these experiments were used: (1) pure cultures of laboratory saprophytes; (2) spores and normal scrapings together, and (3) scrapings containing spores.

Pure cultures of spores were placed in a drop of water on a coverslip, then scattered over its surface and slowly dried by heat, as in the method previously described.

Mordants Used.—1. A 10 per cent. solution of silver nitrate was used from one to ten minutes to stain spores, after which they were washed in water and dried. Other spores were immersed in pyrogallic acid from one to five minutes after the silver nitrate was washed off, but showed little improvement. The spores stained a yellow, brown or black depending on the length of time they were exposed to the silver solution. No differentiation was possible with the spores in the scrapings.

2. Ferric chlorid solutions were used with potassium ferrocyanid solution for staining periods lasting from one to ten minutes. The pure culture of spores took a slight blue color; otherwise little was accomplished.

3. Mercuric chlorid followed by potassium iodid in solutions proved absolutely valueless.

4. Zinc iodin stain, used by botanists, was worthless.

The mordant methods in general will color the spores and scrapings to about the same extent in the short time allowed for their action. It is quite likely when a permanent color is desired and the time required for staining becomes a secondary matter that a differentiation could be obtained.

Dyes.—(1). Methyl violet alone stains spores deep purple or violet and in this way is satisfactory, but it also stains scrapings (keratin), etc., and is not per se differential.

(2). Carbolfuchsin stains spores deep red. Mixtures of both solutions produced so slight a differentiation (the blue predominated in the spores and the red in the scrapings) that they were useless for our purposes.

(3). Loeffler's methylene blue and (4) Leishman's stain were substituted for the methyl violet, (5) eosin for the fuchsin, with no better success. (6) Besson's stain was tried. This appeared to have some selective action for the spores in that they were stained a *dark* blue and the tissue a *pale* blue. It was fairly satisfactory.

The method employed:

1. Besson's, two minutes, excess water poured off.
2. Washed in water from one fourth to one half minute.
3. Decolorized in 95 per cent. alcohol, one half minute.
4. Washed in water, one fourth minute, to remove excess alcohol.
5. Dried by heat.
6. Mounted in balsam.

(7). Mixture of Besson's and carbolfuchsin was tried, but the differentiation was not sharp.

As gentian violet (in the Besson's combination) seemed to have a selective action for the spores and mycelia, it was tried in various percentages. Its use on (1) spores only, (2) spores and scrapings, and (3) ringworm fungus contained in scrapings, demonstrated it to be a satisfactory spore stain.

The next problem was to find a satisfactory stain for the scraping substrate. The stain which eventually proved to be the best in this case was orange G. Satisfactory results were obtained with: Solution No. 1, saturated alcoholic solution of gentian violet, 9; distilled water, 91. Solution No. 2, orange G, 2; alcohol, 95 per cent., 20; distilled water, 80.

1. Stain with No. 1 one half minute; pour off excess.
2. Immerse in 95 per cent. alcohol, one half minute.
3. Immerse in distilled water one fourth minute to remove excess alcohol.

4. Counterstain with No. 2, one fourth minute, pour off excess.
5. Immerse in 95 per cent. alcohol, from one fourth to one half minute.
6. Immerse in distilled water from one fourth to one half minute.
7. Dry over flame.
8. Mount in balsam.

This brings out the spores as a deep blue and the scrapings as a yellow or deep orange. If the scrapings containing the spores be teased sufficiently, this sharp differentiation occurs, but when the spores are embedded too deeply in the scraping the time allowed for this method is hardly sufficient for them to take the stain at all.

As these stains proved quite satisfactory the next step was to combine them and simplify the method. It was hoped that a combined stain could be made which was strong enough in gentian violet to overstain the specimen and then by immersing in alcohol, the excess gentian violet would be removed from the tissue and leave only the blue spores in a field of orange. Various percentage combinations were tried, but with all, very little orange G remained after the preparation was decolorized in 95 per cent. alcohol long enough to remove all of the gentian violet from the scrapings. The specimen was decolorized in different percentage strengths of alcohol, but with no better success. It was then found that by making the solution alkaline all of the orange G was removed, and by making the stain distinctly acid with acetic acid, the orange G was retained.

The formula that gave the best results is saturated alcoholic solution of gentian violet, 2.5; distilled water, 17.5; orange G solution (No. 2), 9; chemically pure acetic acid, 1; alcohol, 95 per cent., 5.

The method used was:

1. Place scrapings in small drop of water on cover slip.
2. Tease thoroughly with dissecting needles.
3. Dry over flame, being careful not to scorch.
4. Stain two minutes, pour off excess.
5. Immerse in 95 per cent. alcohol, from one fourth to one half minute.
6. Immerse in distilled water, from one fourth to one half minute, pour off excess.
7. Dry by heat.
8. Mount in balsam.

The spores and mycelia take the blue, and the scrapings the yellow. Normal scrapings were used repeatedly and stained by this method, but no gentian violet remained in the specimen; nothing simulated mold spores.

This stain brings out the spores very clearly when free in the tissue, but when they are within the mycelia, as in tinea cruris and circinata, the gentian violet will penetrate very little or not at all. The mycelia, even in these cases, will appear as pale yellow refractile strands traversing the field, and are easily recognized.

In examining hairs, it is necessary to tease the scrapings from the hair to a certain extent, in order that the stain can penetrate more easily. Tinea capitis may appear violet rather than the deep blue of the versicolor spores.

CONCLUSIONS

1. Heat may be used to identify the presence of spores in scrapings.

2. An acidulated solution of gentian violet and orange G makes a differential spore stain which may be manipulated with the simplest technic, requiring only five or six minutes for the finished preparation.