COVER-GLASS CULTURES AND THEIR POSSIBILITIES IN STUDYING EPIDERMIC FUNGI.*

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The popular methods for studying the macroscopic and microscopic appearances of the epidermic fungi have been limited to the tube and plate cultures and their modifications on various culture media, and the hanging drop of bouillon ably demonstrates the structural formation of the growth.

By the plate culture Sabouraud, Colcott, Fox and others have suggested a classification of the *tinea* by their clinical appearances and their behavior on solid media. Although they have distinguished certain of the parasitic growths, yet their differentiation from some of the common molds which form cultures not unlike the growth of the trichophyton, can only be corroborated by the microscope.

Smears from cultures and cover-glass impressions only suggest stages of a growth and forms of fructification, without a complete history of vegetation.

The method which I have entitled cover-glass cul-

Fig. 1.—Moist chamber for cover-glass cultures.

tures consists in culturing the parasite on a cover-glass, using any nutrient media desired, placed in a moist chamber and incubated at the room temperature.

The advantage of this method over the hanging drop consists in the facility for observing the development long after the possibilities afforded by the latter, and by providing a method of registering various stages by staining and mounting.

These specimens exhibit the beautiful arrangement of the formation of mycelia from spores, the growth of the colony above and below the media and the character of the fruit-bearing bodies or conidia, without disturbing the picture as it occurs in life.

The scheme consists in placing a drop or two of culture medium on a cover glass which has been passed through a flame. If solid medium is used, it must be liquefied by gently heating and then dropped on the cover glass with a platinum loop. The hair or scale is then embedded while the medium is soft. I generally prepare from four to eight cover glasses for each subject. These are cultured in specially prepared moist chambers, which consist of a shallow glass dish furnished with a circular barrier of glass, one-half inch in height, separating it into two distinct compartments and afford-

ing a shoulder over which is fitted a thin glass dish three inches in height. In the inner compartment are placed Petri dishes holding the prepared cover glasses (Fig. 1). Moisture is produced by keeping lime water in the bottom vessel outside the hooded barrier. This excludes the air and prevents evaporation of the medium. The dishes must be replenished with water frequently.

Fig. 2.—Hair from small-spored fungus, demonstrating the process of budding.

Lime water is preferred, as it precludes the possibility of contamination. This is further prevented by packing a rope of cotton in the water around the cover, a precaution invaluable if the water should become vaporized. This has not infrequently happened in my experience.

Fig. 3.—Same hair as Fig. 2, but at later stage.

I have sometimes found it necessary to prevent drying of the medium by placing the cover glasses on moist circles of blotting paper in each Petri dish.

Room temperature is sufficient for the growth of the epidermic fungi and less favorable for contaminations that flourish above that point. The average period of

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incubation is three days, but the microscopic study may commence after the first twenty-four hours.

A cover glass is held to a slide by a drop of water and the commencement of the growth observed. If sufficient progress of the culture is evident, it may be photographed before staining and mounting. Daily inspection should thus be made, and if further development occurs, a record of the same may thus be preserved.

This process permits the observer to study the growth

gelatin and peptone agar being preferred. This he placed in a glass plate kept cool with ice. When solid, the cover glass was removed and half the jelly taken away by means of a platinum wire, forming a half moon. The slide was then placed in a perpendicular position, the free border thus formed then inoculated, permit-

Fig. 4.—Hair from small-spored fungus, five days' growth, under high power.

Fig. 6.—A hair of class 2, after five days' growth.

of the living culture through all stages, from the formation of mycelium in and above the medium to the full development of a perfect colony, thus exhibiting the form and character of the fruit bearers and the manner of reproduction.

The removal of the culture by the platinum loop, known as the "smear," will not convey to the mind of

ting the aerial portion to grow upward in the empty half of the hole. About twenty of these slides were thus prepared and held upright in a glass vessel with grooved corks; the bottom of the vessel was covered with blotting paper saturated with a solution of sublimate. The method of Unna has the advantage that the growth can be viewed from the side, but it can not be preserved by staining and mounting, nor can the first stages of the development of a colony be studied.

The value of cover-glass cultures will be more fully

Fig. 5.—A cultured hair from the small-spored fungus.

Fig. 7.—Cultured hair; multiple kerion class.

the observer the process that he has wantonly destroyed. This difficulty Dr. Unna endeavored to overcome by a process which enabled him to study the growth of the living fungi, and which he published in 1888 in a paper entitled "Cultivation of Epidermic Fungi."

The plot consists in sealing a perforation in a slide with a cover glass smeared with vaselin. This cell is filled with some nutrient medium, a mixture of peptone,

appreciated if we study the progress of living cultures, prepared slides and microphotographs. My work in this field has been purely experimental as yet, and I have made no attempts to classify the epidermic fungi; but I hope by the early publication of this paper to interest others and by their assistance to eventually establish a class for these parasites and discover the relation they bear to the common molds.

These results can be accomplished by combining the clinical picture and the microscopic findings with the cover-glass and plate cultures. In studying the trichophytosis the three classes, according to the doctrine of Sabouraud, should be observed.

The microphotographs represent a few of the variations in the growth of the microsporon and the megalosporon, and the two forms of the common molds which

hair, also from the small-spored variety, after forty-eight hours' growth, with straight mycelium. Figure 6 shows a hair after five days' growth and corresponding to class 2.

Figures 7, 8 and 9 belong to class 3, and illustrate an

Fig. 8.—Hair illustrating an unusual form of kerion.

not infrequently contaminate our cultures.

Figures 2 and 3 illustrate the development of the fungus from a hair which clinically and microscopically belonged to the first of the three classes of Sabouraud. Figure 2 represents a budding occurring on the shaft and the root after the first twenty-four hours' growth.

Fig. 9.—Another hair, illustrating kerion.

Figure 3 exhibits a sprouting of the bud later, showing a number of sprouts tapering from the hair to the margin of the culture, there expanding into numerous filaments; also a dense network of straight mycelial threads are seen radiating from the hair to the margin.

Figure 4 shows a hair after five days' incubation, taken from class 1. Figure 5 represents a cultured

Fig. 10.—Ribbons of conidia after culture.

unusual form of kerion, the lesions consisting of numerous small abscesses invading the greater portion of the scalp. In some instances they were not unlike favus, and a few were clinically diagnosed as such. The patches are conspicuous by the complete loss of hair and an apparent depressed scar. All cases have recovered in less than a year, with a complete restoration of the hair.

Fig. 11.—Ribbons of conidia in an uncultured hair.

Out of seven cases observed, five were girls; the eldest were 16 and 17 years of age. The history and description of these cases will be the subject of a paper which will be published later.

Figures 10 and 11 illustrate ribbons of conidia or chlamydospores, frequently seen in cover-glass culture. Figure 9 is a microphotograph of a culture, and is the

same process found in an uncultured hair after digestion in a solution of caroid and stained.

Figures 12 and 13 represent two molds. Figure 11 is a living unstained culture of *penicillium* photographed from above, and Figure 12 a variety of *aspergillus*. All specimens are from stained preparations, except Figure 12.

Fig. 12.—Unstained cover-glass culture of *aspergillus*.

DISCUSSION.

DR. JAY F. SCHAMBERG, Philadelphia—I have seen some of the cover-glass preparations made by Dr. Wallis in this ingenious manner; the method appears to facilitate the study of the various pathogenic fungi, particularly those that develop in hairs. The photomicrographs illustrate the manner in

Fig. 13.—Stained specimen of *aspergillus*.

which the mycelial threads grow out through the hair shafts into the surrounding culture medium.

DR. W. S. GOTTHEIL, New York City—I had occasion to study contagious fungoid scalp disease when I was consultant to a large orphan asylum in New York some time ago. The trouble had been prevalent for some time before my attention was called to it; and 460 cases were found among the 900 inmates. Some idea of the importance of an outbreak of this

usually trivial disease under these conditions is shown by the fact that it nearly led to the permanent closure of the institution; that it took eighteen months to eradicate; and that it cost \$30,000 for the hire of and fitting up of buildings, physicians, nurses, etc. We did a good deal of culture work, and finally came to the conclusion that parasites indistinguishable from the trichophyton could be obtained from the scalps of all the children, even those who were apparently unaffected. Whether the fungus in these latter cases was attenuated or not I do not know, but we finally abandoned the culture test as entirely unreliable.

DR. L. DUNCAN BULKLEY, New York City—It is interesting to see, after many years, the same plan gone back to which many of us used before culture methods were known. Many years ago, twenty or thirty, I used the only way we knew of then, growing the culture on a plate and regulating the temperature of the room by the heater, and I have watched the growth of the parasite exactly as in the pictures presented by Dr. Wallis. I think this brings up a very practical matter and is a desirable statement. When a man doing scientific work shows the general practitioner who is not up on bacteriology in general how he may begin in a certain way with an ordinary slide and cover-glass and learn about vegetable parasites, this Section is doing what it is intended it should do, namely, helping the general practitioner to understand these things.

DR. J. F. WALLIS—I am interested in hearing that others have been before me in this work, especially Dr. Bulkley. I thought I was entirely original in this line of work until I read Dr. Unna's paper, published in 1888, and I still believe my process differs from others, as it enables one to preserve the various stages of the growth as well as to study the living colony. Dr. Gottheil's experience in finding cultures from normal hairs proves that growths from other methods are not reliable. It was this difficulty that suggested cover-glass cultures which enables one to observe the spores within the diseased hair develop into mycelial threads and this, I think, without doubt demonstrates the cause of the disease. To make the experiment more perfect I think it prudent to cleanse the diseased area of the scalp with cotton and alcohol, and this is an excellent scheme to collect the hair stubs, as they can be readily found on the cotton when they can not be seen on the scalp or epilated with forceps. This I also depend on as a test, and in my experience it is diagnostic. I depend on this test as a means of studying the progress of treatment.

NEOPLASM (EPITHELIOMA) OF THE PULP.*

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The great importance of this subject from a clinical as well as a purely pathologic point of view must be my excuse for presenting this rather incomplete study. It is with diffidence that I put the case on record, for in looking through the literature at my command I have so far been unable to locate any other like condition. If we exclude the hypertrophies which have been described under the heading of "Polypus of the Pulp or Tooth," then I am led to the supposition that this may be the first case recorded of an exceedingly rare condition. In reviewing the literature, Ziegler¹ speaks of "tumors of dental tissue formed in later life, and described by dental pathologists as odontinoids."² Accordingly as they consist of enamel, dentin, cement, or a combination of

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1. Ziegler: Special Pathological Anatomy, section ix, p. 593, 1897.

2. Ullrich: Ueber feste Neubildungen in der Zahnhöhle. 1852.