fuge tube, spin until perfectly clear and compare in the colorimeter with a standard solution.

The standard is prepared by mixing 1 cc. of $N/10$ HCl with 1 cc. of 50% ethanol containing 100γ B_1. To parallel conditions in the actual analysis, we add to this mixture one drop of bromine water, one drop of 4% sodium salicylate, then proceed exactly as with the sample.

A Modification of the Agar Cup Method Suitable for the Estimation of the Fungistatic Action of Powders and Ointments*

By Arthur E. Meyer

In experiments of estimating the penetrating and growth inhibiting action of compounded products on *Trichophyton*, we found the agar cup test not quite satisfactory, for which reason a modification was worked out, which we would call the "l ne test."

EXPERIMENTAL

The substances investigated were oxyquinoline benzoate and malachite green, as such and incorporated in powder and ointment respectively. The powder contained 0.3% oxyquinoline benzoate and 3% boric acid in pure talcum. The first ointment contained 0.3% oxyquinoline benzoate and 3% boric acid in a 20% lanolin-water basis, the second 0.2% oxyquinoline benzoate with 0.01% malachite green with 3% boric acid and the same basis.

The fungicide properties of both active substances had previously been tested by the method of Burlingame and Reddish, showing that the 0.1% solution of oxyquinoline benzoate destroys *Epidermophyton interdigitale* after 15 minutes, 0.1% malachite green after 5 minutes, whereas *Trichophyton rosaceum* was not destroyed by either substance at the concentration used within 30 minutes.

The line test was performed as follows. A moderately thick plate of Sabouraud's medium was poured in Petri dishes, and with a sterile knife a 2 mm. wide strip was cut out across the plate through the center. The strip is easily lifted out with a sterile spatula. A sterile glass rod laid across the dish may serve as a guide to the knife. It is advisable to incubate the plate for a few days to make sure that no contamination has taken place. During that time the dish should be sealed with parafilm to prevent drying out.

Three days cultures of *Trichophyton rosaceum* and 5 days cultures of *E. interdigitale* were used. For that purpose a few cc. of sterile broth was poured on the fungus surface and the latter thoroughly suspended by rubbing with a sterile cotton swab. The same swab was used immediately to wipe the suspension over the whole surface of the divided plates. The wiping will occasionally cause slight scratches on the smooth surface of the agar, which are an inconvenience if photographs are to be taken, but do not influence the value of the test. In testing the

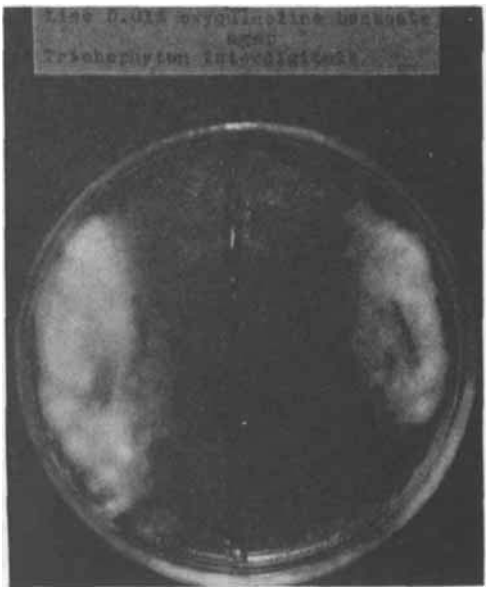

Fig. 1.

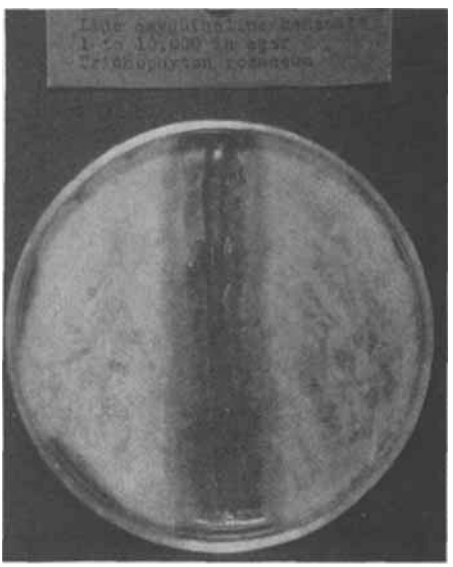

Fig. 2.

* From the Research Laboratories of the Maltine Company, Brooklyn, N. Y.

fungicides themselves, they were poured into the strip as a 0.01% solution in liquefied agar, at a temperature slightly above congealing point. The powder was mixed with water to a thick paste and applied, similarly to the ointments, by squeezing a strip out of a narrow hole, *i. e.*, using at in tube. The strip was placed in the canal in the agar and gently pressed down with a sterile spatula.

The fungi grow within due time in parallel lines to the center strip and give an illustrating picture of the efficacy and diffusion of the fungicide. There is no difficulty in inverting the dishes for incubation; even the powder stays perfectly within the strip.

From the experiments reported it may be concluded that *E. interdigitale* is too sensitive to be used at the concentration of the drugs as employed.

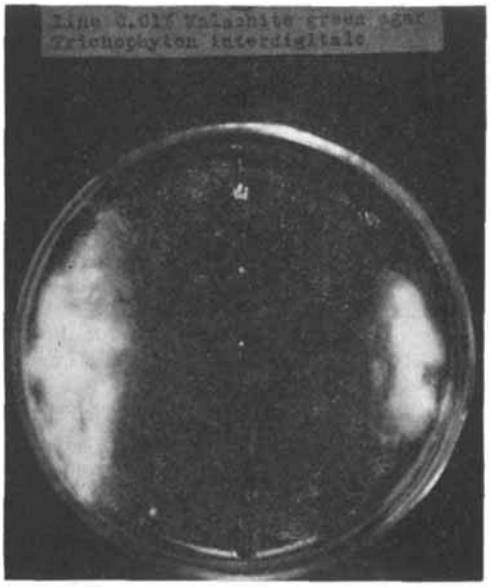

Fig. 3.

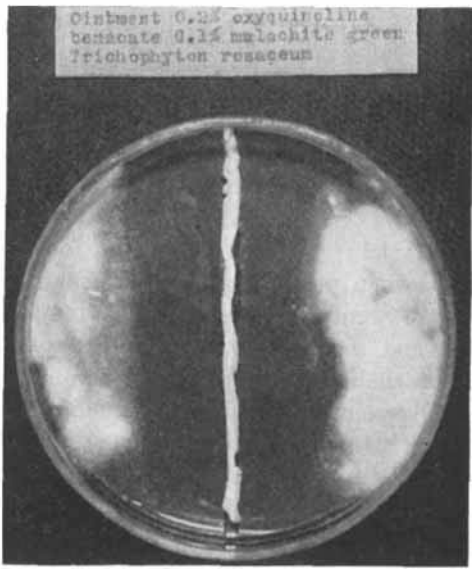

Fig. 4.

Table I.—Readings on Line Tests

No. of Plates	Material Used	Type Organism	Readings
1	0.01% Malachite green agar	*Trichophyton rosaceum*	After 2 days' growth at $1^1/_2$-cm. distance on both sides, after 3 days at 1-cm. distance. No further narrowing of sterile zone
2	0.01% Malachite green agar	*Trichophyton rosaceum*	After 4 days' growth at $1^1/_4$-cm. distance on both sides
3–4	0.01% Malachite green agar	*E. interdigitale*	After 5 days' parallel growth at $2^1/_2$-cm. distance on both
5-6	0.01% Oxyquinoline benzoate agar	*Trichophyton rosaceum*	After 2 days' growth to 1-cm. distance, after 3 days 0.7-cm. distance, after 4 days 0.2-cm. distance; no further change
7–10	0.01% Oxyquinoline benzoate agar	*E. interdigitale*	After 6 days varying from 1 to $2^1/_2$-cm. distance
11–12	Powder 0.3% Oxyquinoline benzoate	*Trichophyton rosaceum*	After 2 days' growth at $2^1/_2$–3-cm. distance, after 3 days 1–2-cm. distance
13–14	Powder 0.3% Oxyquinoline benzoate	*E. interdigitale*	No growth
15	Powder 0.3% Oxyquinoline benzoate	*E. interdigitale*	After 7 days two small cultures at 3-cm. distance
16	Ointment 0.3% Oxyquinoline benzoate	*Trichophyton rosaceum*	After 4 days' growth at 2-cm. distance, no further change
17	Ointment 0.3% Oxyquinoline benzoate	*Trichophyton rosaceum*	After 7 days' growth on one side only at 3-cm. distance
18–19	Ointment 0.3% Oxyquinoline benzoate	*E. interdigitale*	No growth
20–22	Ointment 0.2% Oxyquinoline benzoate 0.1% Malachite gr.	*Trichophyton rosaceum*	After 5 days' maximum growth at 2–$2^1/_2$-cm. distance
23	Ointment 0.2% Oxyquinoline benzoate 0.1% Malachite gr.	*E. interdigitale*	No growth
24	Ointment 0.2% Oxyquinoline benzoate 0.1% Malachite gr.	*E. interdigitale*	After 5 days very small colony at edge of dish on one side only

The method described has the advantage that the surface of contact between disinfectant and medium is considerably increased and shows better the evenness of action than is the case with the agar cup method. It also imitates the use of thin layers as applied for practical purposes.

Rapid Staining Methods in Plant Histology

By Robert S. McLean and Edward J. Ireland†*

The routine methods for the differential staining of plant tissue for histological study in use to-day require from three to twenty-four hours or more. McClung (1929) and Chamberlain (1932) recommend for routine work a primary stain of Safranin for three to twenty-four hours at room temperature, and a counterstain of hæmatoxylin for one-half to six hours. Chamberlain (1932) recommends six hours or more for a primary stain of Malachite Green or Methylene Blue, and one-half to one minute for counterstaining with Eosin or Erythrosin. These procedures are typical with respect to the time required to obtain an effective differential stain.

Obersteiner (1878) used a solution of carmine over a bath of boiling water for staining nerve cells. Bacteriologists use heat for staining bacterial spores and such organisms as *Mycobacterium tuberculosis* which "take" a stain slowly. By the use of heat in the "acid-fast" technique, the tuberculosis organism is stained in four or five minutes where twelve to twenty-four hours at room temperature are required.

Since heat is applicable in bacteriological staining, it seemed logical to assume that it would also be effective in botanical staining. Therefore the use of heat was investigated and successfully used in the following methods.

EXPERIMENTAL

To avoid the numerous transfers through increasing and decreasing concentrations of the alcohol, 95 per cent ethyl alcohol was used as the solvent for the stains in all cases except for one of the counterstains, Methyl Blue. For this stain, 80 per cent ethyl alcohol was used since the dye was very slightly soluble in the 95 per cent alcohol. The few stains which have a standardized formula were prepared with the directed solvent.

Paraffin sections of ten to twenty microns thickness fixed to slides with Mayer's albumin fixative were used throughout the series of experiments.

METHOD I

The first method which gave consistently good differentiation and showed considerable saving of time was conducted as follows:

1. Two to four drops of the primary stain (or enough to cover the sections) were placed on the slide, the solvent ignited and allowed to burn off completely.
2. The slide was washed briefly but thoroughly in 95 per cent ethyl alcohol. This was best accomplished by agitating the slide in the alcohol contained in a deep beaker or Coplin jar. The washing removed the excess stain from the slide and partially removed the primary stain from the cellulose tissue.
3. Four to six drops (or enough to flood the sections) of the counterstain were placed on the slide and allowed to act for ten to thirty seconds as required (see Table I). For the slower acting counterstains, Methyl Blue and Orange G especially, the solvent was burned off as in (1). With woody sections even the faster acting counterstains gave best results when flamed.
4. The slide was again washed in 95 per cent ethyl alcohol as in (2), cleared and examined.

Table I.—Relative Approximate Time Required for the Various Counterstains for the Above Process

Group I 5–10 Seconds	Group II 10–20 Seconds	Group III 20–30 Seconds
Erythrosin	Eosin Y	Orange G
Eosin B	Picric Acid	Methyl Blue
Orange I	Acriflavine	Methyl Red
Acid Fuchsin	Congo Corinth	
	Bismarck Brown	
	Hæmatin	

The chief difficulty encountered in this technique was the manipulation of the counterstains. The time required to obtain the proper degree of differentiation varied with the counterstain used and also with the nature of the sections, but this variation was only a matter of a few seconds. For example, Acid Fuchsin used on leaves or on herbaceous stems required only about 5 to 10 seconds, Eosin Y required 10 to 15 seconds and Methyl Blue about 20–30 seconds. On more woody sections these time ranges increased about 5 to 10 seconds. The lengths of time as classified in Table I were found to be only approximate. Since the time during which the counterstain was allowed to act was, in most cases, so very short, accurate timing was impossible.

Perhaps the best method of handling the counterstain is to flood the sections with the counterstain, allow it to act for five to ten seconds, wash briefly in

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