intensity and shade of the color produced. Within our experience, however, no thiamin-free substance has yielded a positive reation. Mixtures of unknown composition are readily de-vitaminized by shaking with Decalso at p_H 4.0–4.5 (2), (3).

For good results, it is important to plan the quantities of reagents used so that the coupling reaction takes place in a mixture containing 20–25% ethyl (or methyl) alcohol.

The specificity of the Prebluda-McCollum color test depends largely on the fact that the colored thiamin derivative is insoluble in both acids and alkalis. We always extract the color from an acid medium, to avoid the annoyance of the emulsions which tend to form when shaking alkaline solutions. We then wash the colored toluol solution with sodium hydroxide solution, to remove foreign colored substances containing acidic groups such as carboxyl or phenol hydroxyl.

SUMMARY

A simple and rapid method for the quantitative estimation of thiamin chloride has been described. The method is based upon the Prebluda-McCollum reaction. It has successfully been applied to the analysis of mixtures of pharmaceutical interest, containing as little as twenty micrograms of thiamin chloride per Gm. of sample.

REFERENCES

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ADDENDUM

Colorimetric Determination of Thiamin Chloride in the Presence of Ascorbic Acid.— In the foregoing paper, several examples were given of a colorimetric determination of thiamin chloride in pharmaceutical products. It was stated that the presence of vitamins A, B_2 and D causes no difficulty with the method, but that the presence of vitamin C necessitates a special pre-treatment. Both ascorbic acid and its oxidation products prevent the development of the colored thiamin derivative upon which the determination depends. It was discovered that the addition of calcium ion (magnesium and barium are partially effective) to the mixture of thiamin chloride and oxidized ascorbic acid adequately prevented interfering reactions. The mechanism by which this is accomplished is not yet clarified.

The example given below presents details of the modified method. The product chosen for description consists of a suspension of vitamins A, B_1, B_2, C and D in a fat.

Method: Transfer about one Gm. of the thoroughly mixed fatty mass to a tared 50-cc. Erlenmeyer flask. Add 5 cc. monochlorobenzene and then, from a burette, 10 cc. of $N/10$ HCl for each mg. of thiamin chloride estimated to be in the sample taken. Stopper and shake the flask thoroughly for five minutes, then centrifuge the contents until the aqueous layer is perfectly clear. The fatty material and vitamins A and D are retained by the monochlorobenzene. Thiamin chloride, riboflavin and ascorbic acid are all quantitatively extracted by the acid water.

Take one cc. of the aqueous extract in a clean test-tube, add 1 cc. 50% ethanol, then, drop by drop, strong fresh bromine water until the color is permanently a deeper yellow than the original solution. Add one drop of 4% sodium salicylate solution to take up excess bromine, then 2 cc. of 50% ethanol and finally 1 cc. of approximately $N/1$ $CaCl_2$. Warm in a water-bath at 60° C. and add 2 cc. of freshly prepared diazo solution.

(The diazo solution is prepared by mixing 10 cc. of 0.03% *p*-amino acetophenone in $N/5$ HCl with 2-cc. 0.1% $NaNO_2$. Place in an ice-bath for at least 3–4 minutes, then add 3 cc. of 10% NaOH just before using.)

Allow the tube to stand at 60° C. for 10–15 minutes, cool, acidify with a few drops of $4N$ HCl, add 8 cc. toluol, shake well and transfer to a small separatory funnel. Discard the aqueous layer, then wash the colored toluol with (1) about 15 cc. water; (2) about 15 cc. 4% NaOH; (3) about 15 cc. water. Transfer the colored toluol to a small centri-

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

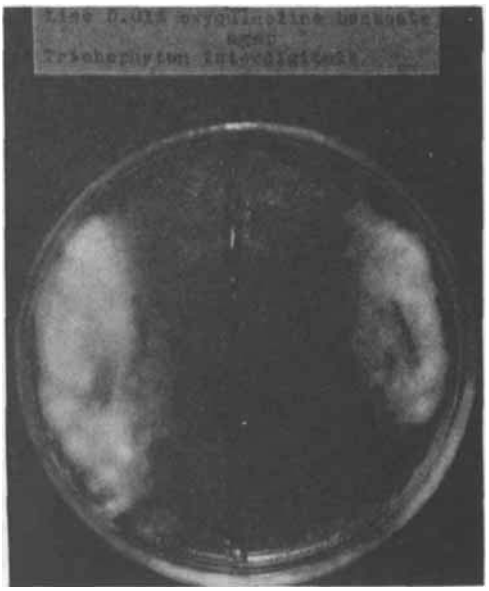

Fig. 1.

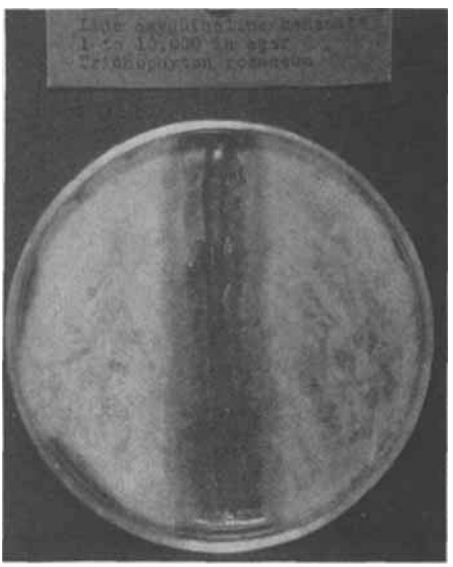

Fig. 2.

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