cc. of normal saline to which one-half cc. of N/1 HCL was added. This suspension was boiled for 15 minutes and centrifuged. The supernatant fluid was drawn off, neutralized and recentrifuged. This clear extract was used in the precipitin tests.

Anti-sera were prepared in rabbits. For direct precipitin reactions undiluted serum was run against the extract diluted from 1:10-1:8000. For absorption test undiluted serum was mixed with a heavy suspension of organisms. Enough were added to absorb completely the precipitin in the serum for the absorbing organisms. A grouping of the moniliae can be made by this test:

Group I—*M. albicans, M. psilosis,* and *M. candida* showed almost identical reactions. They showed cross precipitation in very high dilution, indicating that these strains contained similar antigens.

Group II—*M. parapsilosis* and M. type 1 reacted alike, showing their similarity. Cross precipitations between the first group of moniliae and the second were noticed only in very low dilutions.

Group III—*M. krusei* did not seem to contain any precipitinogen similar to the other types.

Absorption tests showed a higher degree of specificity for each of the 3 groups. These 2 methods described, one biochemical and one serological, seem to show a definite correlation and give a simpler and more tangible means of differentiation of the species of moniliae.

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Further Contributions to Methods of Barbital Research.

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In addition to procedures previously described,[1] we have adopted the principles of Myers' and Wardell's[2] blood cholesterol method for extraction of barbiturates.

A convenient amount of acidulated urine, blood (1 to 2 cc.), or well-ground, liquified tissues is pipetted into a small mortar containing sufficient Plaster of Paris to form a dry mixture, which is then

[1] Koppanyi, Murphy and Krop, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1405.

[2] Myers and Wardell, *J. Biol. Chem.*, 1918, **36**, 147.

pulverized and transferred to a paper extraction shell. This shell is inserted into a perforated glass tube connected to a reflux condenser. Ten volumes of chloroform (10 to 20 cc.) are poured into the extraction flask, which in turn is heated to boiling on a water-bath for half an hour. The chloroform extract is then filtered and tested. If the test is negative the extract may be concentrated over a water-bath.

We have increased the sensitivity of our test previously described[3] by using cobalt acetate and, instead of the methyl alcohol solution of barium hydroxide, a 0.5% solution of lithium hydroxide in absolute methyl alcohol. Two or 3 drops of the 1% cobalt acetate and 0.5% lithium hydroxide reagents will produce a definite blue color in chloroform extracts of concentrations as low as 0.0025%. However, chloroform extracts can be concentrated 20 times, thus making the test sensitive to 1.25 parts per million.

We have described our test as colorimetric determination, but without the actual use of a colorimeter, because the colors produced by barium or other metallic hydroxides soon precipitate or fade if transferred to colorimeter cups. We have now succeeded in adopting the test for colorimetric readings with standard colorimeters. The procedure is as follows:

One cc. of the unknown chloroform extract is treated first with 0.05 cc. of a 1% cobalt acetate solution in absolute methyl alcohol and 0.3 cc. of a 5% (by volume) solution of iso-propyl amine (Research Laboratories, Eastman Kodak Co.) in absolute methyl alcohol. If barbiturates are present in the extract, a reddish-violet color develops, which is then compared in micro-cups with the color produced under the same conditions by barbital solutions of known strength in chloroform. We found that a 0.025% solution of barbital in chloroform usually suffices as a standard, against which unknown solutions over a wide range may be read. A simple formula, which may be used with any standard colorimeter, giving the number of milligrams of barbiturate per cc. of chloroform extract using the 0.025% solution as the standard is:

$$\frac{\text{Reading of standard} \times 0.25}{\text{Reading of Unknown}} = \text{Concentration of Unknown}$$

[3] Koppanyi, Murphy, and Krop, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 542.