males), in addition to the lack of fertility without pipette transfer of sperm, suggests that no spermatozoa had been collected during the fall mating season and that the few present to fertilize the eggs were those retained from the laboratory procedure.

Fertile eggs were usually attached to the water plants; those which dropped to the bottom of the aquaria had been forced out after too short a stay in the cloaca for fertilization to occur. There was marked individual variation not only in the number and fertility of the eggs laid (33 fertile of 48 laid, 57 of 120, 32 of 160, 20 of 37), but also in the size and degree of their maturity, in the duration of the egg-laying period (6, 17, 6, and 4 days), and in the relation of this period to the total number of implants received (9, 10, 7, and 4, respectively). The third female produced, during the last 2 days of her egg-laying, very small immature eggs, unpigmented and pressed end to end in a long trailing albuminous strand.

Isolated female Newts from the same source given implants in February, 1933, laid only non-fertile eggs. Non-treated females laid no eggs. Further work was prevented by deaths of both sexes, probably due to the 2 species of tapeworms found on autopsy.

The embryos developing from the induced eggs of January, 1932, showed a high degree of asymmetry, appearing for the most part before the closure of the neural folds. Duplications of balancers or of gills, supernumary or fused digits were frequent. Except for these latter minor variations, 94 animals were selected as apparently normal upon arrival at the eating stage and were reared for 6 weeks on ostracods, 2 species of daphnids, and enchytreids. Synthetic diets, used for the remaining period of larval growth, led to a range in length from 32 to 47 mm. at the time of metamorphosis.

7133 P

Precipitin and Fermentation Reactions of the Moniliae.

JOHN HENDERSON LAMB AND MARGARET LAIN LAMB. (Introduced by J. Howard Brown.)

From the Departments of Pathology and Bacteriology and of Dermatology, Johns Hopkins University.

In the course of the study of smears from cases of bronchial asthma and routine cultures taken from cases of ringworm infection of the feet, numerous yeast-like organisms were encountered, especially

those of the monilia group. Since fermentation reactions and serological tests proved to form a very important basis for the identification of the moniliae, as well as other yeasts, further studies have been made of these 2 characteristics.

Previous investigators, in the study of fermentation reactions, have made their observations on extract broth plus 1% fermentable sugars with Andrade's solution as an indicator, and Durham tubes for determination of gas. Because of the differences in the results of these authors, some method seemed necessary to investigate this test further. Pork infusion fermented broth (the carbohydrates in the meat infusion were removed by fermentation with *B. coli* before the peptone was added) was used with addition of the various carbohydrates. The medium was inoculated with monilia and incubated at 37°C. The pH of the inoculated media was followed every 2 or 3 days for 20 to 40 days by the colorimetric method described by Brown.[1] Eight or 10 drops of the uninoculated media gave distinctly positive reactions for sugar with Benedict's solution. Those cultures found to give a negative chemical test for sugar after a period of incubation were considered to have fermented the carbohydrates.

The hydrogen-ion concentration of the cultures of moniliae in 2% sugar broth over a period of 40 days showed a wide divergence in the amount of acidity produced in the fermenting process. Several strains, although showing little if any acidity, fermented the sugar as shown by its disappearance indicated by the negative Benedict's test. Taking the disappearance of the sugars as a basis for division, the moniliae fall into 3 groups:

A first group, *M. albicans, M. candida* and *M. psilosis,* utilizes completely sucrose, dextrose, maltose, xylose, levulose and galactose, but does not attack inulin. A second group including *M. parapsilosis* and M. type 1 (Stovall),[2] utilizes 4 of the above sugars not fermenting maltose or inulin and rarely xylose. A third group includes *M. krusei* which does not utilize xylose, sucrose, maltose, galactose or inulin.

As a further step in identifying these organisms precipitin reactions were investigated. Previous descriptions of methods used for the making of extracts of these fungi for the test were time-consuming and complicated. A simpler method was devised. The organisms were cultivated on Sabouraud's agar in Petri dishes. After 48 hours the heavy surface growth was scraped off, emulsified in 10

[1] Brown, J. H., *J. Lab. and Clin. Med.,* 1924, **9**, 23.
[2] Stovall, W. D., *J. Infect. Dis.,* 1932, **50**, 73.

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.

7134

Further Contributions to Methods of Barbital Research.

THEODORE KOPPANYI, WILLIAM S. MURPHY AND STEPHEN KROP.

From the Department of Pharmacology and Materia Medica, Georgetown University School of Medicine.

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.