

work, however, the effects of the hormones are quite different and anterior lobe cannot replace the thyroid hormone.

To test our claim 6 larvae of the Utah axolotl were most carefully thyroidectomized. As complete thyroidectomy of salamander larvae is very difficult and regeneration of remnants not visible under the dissecting microscope takes place frequently, the injections of an anterior lobe extract prepared in our laboratory, as described in Article I of this series, were started in 4 of the thyroidectomized larvae immediately (one day) after the operation. Two normal control larvae of the same stage and approximately same size were injected at the same time with the same sample of anterior lobe powder as the experimentals. Both controls metamorphosed after the sixth injection, 11 days after the first injection. None of the experimentals showed even a sign of metamorphosis after the ninth injection, fifteen days after the first injection. The injections were stopped after the ninth injection: 51 days after the first injection, the experimentals are still completely larval.

Anterior lobe substance has absolutely no effect on metamorphosis if the thyroid is absent. It is possible that Spaul's axolotls had not been completely thyroidectomized. Anterior lobe is a specific thyroid stimulator, but it cannot replace the thyroid hormone.

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Action of Several Disinfectants on Yeasts.

FRED W. TANNER AND GEORGE D. BOLLAS.

From the Department of Bacteriology, University of Illinois, Urbana.

Ten strains of yeasts were subjected to the action of gentian violet, mercurochrome-220, iodine and acriflavine. The technic involved the use of a plate very much like that used by Churchman. The plate which we used, however, had 2 control areas instead of one. The agar containing the dye was added to the middle compartment, leaving one compartment on each side without the disinfectant. Gentian violet was studied in 3 ways: (1) application of the dye directly to the cells; (2) behavior of the organisms on dye-agar; and (3) attempts to raise the tolerance of the organisms for the dye. All of the cultures except one were susceptible to the presence of gentian violet. This organism, when stained according to Henrici's¹ technic for applying the Gram stain to yeasts, was Gram

¹ Henrici, *J. Med. Res.*, 1914, xxx, 409.

negative. The observation that 9 of the yeasts were Gram positive explains their inability to endure the presence of gentian violet. It was found that when the dye was employed at a dilution of 1 to 80,000 no growth appeared on the dye media even after several days' incubation. On the other hand, a dilution of 1 to 140,000 allowed very slow growth after from 3 to 4 days incubation. Raising the temperature greatly increased the action of the dye. Attempts to raise the tolerance of yeasts to gentian violet were successful although the extent varied for each strain. These results indicate that yeasts, like bacteria, can be divided into two groups depending on their reaction to the Gram stain. Others who worked on this subject are E. B. Clark² and Faber and Clark.³ Our results confirm their conclusions.

Mercurochrome 220 was studied in the same manner. It was less active on yeasts and required a much longer time to kill the fungi. A concentration of 0.4% repressed growth when the disinfectant was added to the medium. Mercurochrome-220 diffused quite rapidly from the dye agar into the plain agar so that no growth was secured near the line demarking the 2 kinds. No increased tolerance could be attained.

Tincture of iodine has had extensive use as a skin disinfectant. Consequently, a study of its toxic action on yeast cells was included in this investigation. Tincture of iodine was prepared by dissolving 5 gm. of potassium iodide in 5 cc. of distilled water. To this solution, 7 gm. of iodine were added and finally sufficient 95% ethyl alcohol to make 100 cc. This element was found to have a powerful action on yeasts. When tincture of iodine was incorporated in media in a concentration 1-100, the yeasts were completely destroyed. Attempts to increase the tolerance of yeasts to this element were entirely negative. Windisch⁴ stated that the behavior of iodine with yeast could be classified as a special case of the Arndt-Schultz law. In very high dilutions the cell is stimulated by the iodine but in low dilutions the cells are killed.

The action of acriflavine on the strains used was quite variable. Five of the yeasts used were distinctly resistant. When incorporated in the medium, a dilution of 1 to 500 prevented growth.

Summary and Conclusions: The results may be briefly summarized as follows: Gram positive yeast, like bacteria, can be classified into gentian violet positive, while gram negative are likewise gentian violet negative. Yeasts which are gentian violet positive are

² Clark, E. B., *J. Inf. Dis.*, 1926, xl, 423.

³ Faber, H. K., and Clark, E. B., *Am. J. Dis. Child.*, 1927, xxxiv, 408.

⁴ Windisch, W., *Woch. Brau.*, 1927, xlv, 516.

destroyed when stained for one hour with 5 drops of an aqueous saturated solution of this dye. Yeasts which are gentian violet negative are unaffected by this treatment. When the dye is incorporated into media at a dilution 1:80,000 it will completely destroy the fungi, while at a dilution of 1:140,000 growth is inhibited. As the temperature increases the time required to destroy the stained organisms decreases, and increases as the temperature decreases. Tolerance towards gentian violet could be produced with most of the yeasts studied.

Mercurochrome-220 soluble when incorporated into media at a concentration of 0.4% destroys the yeasts planted on it. Yeasts treated with 5 drops of a 2% solution of mercurochrome are destroyed after 8 to 11 hours in perfect contact with the dye. No tolerance towards mercurochrome could be produced by the organisms studied.

Tincture of iodine, prepared according to U. S. Pharmacopoeia, will destroy yeasts instantaneously when one drop of this solution is applied to one cc. of a heavy suspension. At a dilution of 1:50, yeasts are destroyed in less than 5 minutes when treated with only 2 drops of this dilution. When 5 drops are used it kills the organisms instantaneously. Tincture of iodine when incorporated into media in a dilution of 1:100 will completely destroy the yeasts. No tolerance towards tincture of iodine could be produced with the organisms under study.

Neutral acriflavine has a marked bactericidal inhibitory action on some yeasts but in others its effect is practically insignificant.