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Colorimetric Estimation of Penicillin in Aqueous Solutions.*†

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It was recently shown that acid hydrolyzates of penicillin gave a blue-violet coloration with the ninhydrin reagent.[1] Moreover, the same workers reported that the color intensity of this reaction ran parallel to the antibacterial activities of their preparations.

Colorimetric analysis of amino acids by the ninhydrin reagents has been reported by other investigators.[2,3]

The writer has modified the ninhydrin reagents so that small amounts of crystalline penicillin can be estimated with fairly consistent results, provided that temperature, duration of heating, and pH are rigorously controlled. These experiments have been performed on aqueous solutions of crystalline penicillin (Merck), with potency of 1650 units/mg.

The procedure is as follows:

To graduated 10 cc tubes are added 3 to 9 μg of freshly diluted solution of penicillin, 0.5 cc of 0.2 N H_2SO_4, 4 mg of ninhydrin (Eastman) treated with norite (Coleman and Bell). The tubes are then covered with glass vials and heated in boiling water bath for 20 minutes in subdued light. The tubes are then removed, cooled and treated with 0.2 cc of freshly prepared 10% pyridine (Mallinckrodt), 20 mg of norited $(NH_4)_2SO_4$ (Baker)† the volumes equalized with distilled water and then reheated for 15 minutes. The tubes are removed, cooled and made up to 6 cc with water.

The pH of these solutions should be from 4.7 to 5.0. They are then read in the Coleman spectrophotometer against reagent blanks at 550 μ. Blanks and penicillin solutions are prepared in triplicate.

The actual values obtained are not absolute but vary somewhat with the reagents and experimental conditions. However, straight line calibrations (semi-log)) may be obtained for any one set of reagents and conditions.

An illustrative set of values is as follows:

Concentration penicillin, gamma	Light transmission, %
3	88.5
6	79
9	71.5

Summary. A colorimetric technic for analysis of 9 μg or less of crystalline penicillin is described.

* The writer is indebted to Merck & Co., Rahway, N.J., for their generous gift of penicillin.

† Research paper No. 579, Journal Series, University of Arkansas.

1 Abraham, E. P., Chain, E., Baker, W., and Robinson, R., *Nature,* 1943, **151**, 107.

2 Harding, V. J., and Maclean, R. M., *J. Biol. Chem.*, 1915, **20**, 217.

3 Virtanen, A. I., and Laine, T., *Skand. Arch. f. Physiol.*, 1938, **80**, 392.

† 25 cc of 20% $(NH_4)_2SO_4$ is shaken with 250 mg norite and filtered.

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Parasympathomimetic Effect of Aqueous Humor in Human Eyes with and Without Chronic Simple Glaucoma.

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Velhagen[1] demonstrated that the aqueous humor of certain animals produced a parasympathomimetic effect on the isolated frog heart. Engelhart[2] one year later instilled eserine in the eyes of rabbits to prevent the destruction

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2 Engelhart, E., *Arch. f. d. ges. Physiol.*, 1931, **227**, 220.