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A Colorimetric Test for Guanidine.

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In studies pertaining to muscular dystrophies, attention was given to the possibility that simple guanidine derivatives might play a part in the etiology of these abnormalities. Guanidine is a protoplasmic poison as early shown by Gergens and Baumann[1] for animals and Kawahita[2] for plants. Just what part it might play in the phenomena of health and disease has been problematical since satisfactory tests for guanidine in body fluids, tissues, and excretions have been lacking. Various color tests have been recommended for guanidine by Tieg,[3] Marston,[4] and Weber.[5] These tests have not been found satisfactory since they give color more or less with methyl guanidine which may be formed from creatine or creatinine by oxidation as early shown by Dessaignes,[6] and corroborated by Ewins,[7] Baumann and Ingvaldsen,[8] and by Greenwald.[9]

Accordingly, a new test for guanidine $NH{:}C(NH_2)_2$ with a high degree of specificity was devised. This procedure runs as follows:

Color reaction for guanidine. To 1 cc. of an aqueous or decinormal hydrochloric acid solution of guanidine carbonate or hydrochloride, containing 0.5 mg. of guanidine base, add 1 cc. of a freshly made 1% solution of 1.2 naphthoquinone-4-sodium sulphonate followed by 0.3-0.5 cc. of N NaOH. Let stand 15 minutes at 20°C. or, better, 1-2 minutes in a boiling water bath. Cool under running tap water, add 0.3 cc. of a 25% urea solution and acidify with 1 cc. of concentrated HCl, mix, and add 1 cc. of concentrated HNO_3 (density 1.42). The guanidine solution becomes a bright red while other compounds tested, with a few exceptions later detailed, go to yellow. The guanidine reaction is definitely red with solutions containing 0.05 mg. guanidine per cc. With 0.5-1 mg. of guanidine

1 Gergens, E., and Baumann, E., *Pflügers Arch. f. Physiol.*, 1876, **12**, 265.
2 Kawahita, I., *Bull. Coll. Agr. Tokyo*, 1908, **6**, 161.
3 Tieg, I. W., *Australian J. Exp. Biol. Med. Sci.*, 1924, **1**, 93.
4 Marston, H. R., *Australian J. Exp. Biol. Med. Sci.*, 1924, **1**, 99.
5 Weber, C. J., *J. Biol. Chem.*, 1928, **78**, 465.
6 Dessaignes, M., *Compt. rend.*, 1854, **38**, 839.
7 Ewins, A. J., *Biochem. J.*, 1916, **10**, 103.
8 Baumann, L., and Ingvaldsen, T., *J. Biol. Chem.*, 1918, **35**, 277.
9 Greenwald, I., *J. Am. Chem. Soc.*, 1919, **41**, 1109.

per cc. a brown red precipitate is formed, soluble in alcohol. In such cases add a few cc. of alcohol to standard and unknown.

In estimating guanidine in mixtures of amino acids more naphthoquinone must be added. Two cc. of the 1% aqueous solution of 1.2 naphthoquinone-4-sodium sulphonate have been found ample in all cases detailed in this paper.

The urea is added to lessen fading of the red color. If methyl guanidine is present in appreciable amounts, and only nitric acid is used, the final solution starts yellow but gradually develops a brown color. If concentrated hydrochloric acid is added before the nitric acid, methyl guanidine gives no red color. If no methyl guanidine is present the addition of hydrochloric acid before the nitric can be dispensed with.

In this procedure the color is red with guanidine, guanidine glyoxylic acid, and guanidine oxalic acid (which readily split off the glyoxylic acid and oxalic acid), yellow with methyl guanidine, amino guanidine (which starts red but fades quickly to yellow), glycocyamine, glycocyamidine, creatine, creatinine, hydantoic acid, arginine, biuret, xanthine, methyl xanthine, adenine, guanine, uric acid, allantoin, alloxan, glycine, alanine, phenylalanine, aspartic acid, valine, cysteine, cystine, glutamic acid, leucine, proline, tryptophane, histidine, histamine, lysine, thymine, uracil, urea, thiourea, acetone, pyruvic acid, catechol, adrenaline, dioxyphenylalanine, hydrazine, dextrose, parabanic acid, urethane, etc., each 1 mg. per cc. in solution or suspended. The only positive interferers are large amounts of ammonia or methylamine, indol, and benzidine.

Benzidine can be easily eliminated by precipitation as a sulphate and filtering. Indol is readily volatilized by heating in a slightly acid solution. Thus 1 cc. each of 2 indol solutions, one containing 1 mg. of indol in 95% alcohol and the other a saturated aqueous solution, treated with 0.2 cc. of N HCl and heated on the water bath for 10 minutes, before making the guanidine test, were negative.

In biological media, methyl amine and ammonia will rarely if ever be met with in amounts sufficient to interfere with the guanidine test. If such amounts are present, the interference can be eliminated by precipitating the guanidine with picric acid as done in the reaction dealing with urine. Guanidine readily forms an insoluble picrate which gives the guanidine reaction, while the picrates of methyl amine and ammonia are readily soluble.

In a mixture containing 10 mg. each of tyrosine, phenylalanine, dioxyphenylalanine, cystine, leucine, alanine, glycine, valine, proline, aspartic acid, glutamic acid, arginine (as carbonate), lysine,

histidine, tryptophane, creatinine, creatine and 100 mg. of guanidine (as guanidine hydrochloride, 161.7 mg. in 200 cc. of 0.1 N HCl), guanidine can be matched quantitatively against the same quantity of guanidine in 0.1 N HCl.

In this case, however, more of the naphthoquinone must be used because of the large content of amino acids. The following procedure gave practically quantitative results: (A) To 1 cc. of the guanidine amino acid mixture add 2 cc. of 1% aqueous solution of 1.2 naphthoquinone-4-sodium sulphonate, mix and add 0.5 cc. of N NaOH, heat in water bath for 1 minute, cool, and add successively with mixing 0.5 cc. of 25% aqueous urea solution, then 0.5 cc. of concentrated HCl and 1 cc. of concentrated HNO_3 and finally 5 cc. of 95% alcohol to put precipitate into solution. (B) One cc. of guanidine (0.5 mg. per cc.) in 0.1 N HCl similarly treated. (B) set at 20, (A) reads 20.7.

Guanidine added to urine. The quantitative determination of guanidine added to urine offers difficulties. In some urines the detectable guanidine speedily disappears, due apparently to tying with urinary ingredients. In a number of cases guanidine added to urine has been estimated with 90-100% accuracy shortly (within an hour) after the addition to the urine. Even in these cases the urine requires special treatment before applying the color reaction, the details of which are somewhat outside the scope of the present paper. Aside from the addition of guanidine to urine no unsubstituted guanidine has been found in any of the normal or pathological urines examined. As will be shown, however, by Sullivan, Hess, and Irreverre, in progressive muscular dystrophy and especially in pseudohypertrophic muscular dystrophy, there occur in the urine simple guanidine derivatives which by mild oxidation yield guanidine. Such guanidine derivatives have not been found in normal urine in any appreciable amounts nor in any other pathological urine so far investigated.