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An Unknown Reducing Urinary Substance in Myasthenia Gravis.

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In the course of the determination of urinary inorganic phosphate by the method of Fiske and Subbarow[1] in a case of myasthenia gravis it was observed that the phosphomolybdic acid became reduced and an intense blue color developed before the reducing agent, aminonaphtholsulfonic acid, was added. The same immediate reduction of the phosphomolybdic acid did not take place after the acid digestion in the course of the total phosphorus determination. It was also observed that occasionally in this particular urine the inorganic phosphate figures slightly exceeded the values for total phosphorus. Attempts to elucidate this phenomenon have led to our present conception that we deal with a hitherto unknown reducing urinary substance.

We have obtained the same color reaction from the urines of two other cases of myasthenia gravis, but have failed to demonstrate the same in one case of muscular dystrophy and in one case of muscular atrophy, diseases in which as in myasthenia gravis a creatinuria is or may be present. Of our second and third cases of myasthenia gravis, which at the present show a milder clinical picture than our first case and from which only random samples of urine were obtained, none developed as deep a color as our first case. In one we designate the color as weak but definite, in the other as of moderate intensity. In normal urines we have never observed this reaction, which also must be in accordance with the experiences of Fiske and Subbarow. The only other condition in which we have observed a similar behavior is alkaptonuria.

The new compound reduces alkaline copper solution more slowly than does glucose, thus in the Folin and Berglund method for the sugar of normal urine considerable color development takes place on boiling beyond 8 minutes. With this method and 8 minutes boiling the 24 hours urine of our patient shows a reducing value corresponding to 2.5-3 gm. of glucose. On starvation as well as on high carbohydrate feeding these values vary only as much as may be explained from the corresponding known variations in the so called sugar values of normal urines. High meat diet seems to cause a slight increase.

The compound present does not ferment, does not increase its reducing value of acid hydrolysis as used in the Folin and Berglund method, does not give the phloroglucin or orcin tests for pentoses. An osazone could not be obtained. The compound does not reduce alkaline picrate at room temperature nor interfere with the matching of the color in the creatinine determination. During picric acid hydrolysis as used in Folin's creatine determination an alteration is produced which somewhat throws off the color developed in the subsequent creatinine determination.

Attempts to isolate the compound have revealed the following facts: it is not precipitated by phosphotungstic acid, by tannic acid, by mercuric chloride nor by neutral lead acetate. With basic lead acetate it is partly carried down with the lead precipitate, from which it may again be removed by washing with water. It is nonvolatile and cannot be distilled from water by steam distillation. It is somewhat soluble in ethyl alcohol, ether, chloroform, acetone, toluol, xylol and petroleum ether. It may be extracted by alcohol from the urine after saturation with ammonium sulphate. It becomes oxidized in the course of weeks on exposure to air. It is oxidized by hydrogen peroxide, bromine, ferric chloride or by heating with nitric or sulfuric acid. It is destroyed by mercuric nitrate. The compound reduces phosphomolybdic acid in both acid and alkaline solution, apparently more rapidly in acid solution. It reduces phosphotungstic acid in alkaline solution.

For further differentiation of this compound against homogentisic acid it may be stated that this urine does not darken on the addition of ammonia and that our compound does not reduce ammoniacal silver solution at room temperature. Homogentisic acid reduces phosphomolybdic acid more rapidly, almost instantly, while the complete reaction with our compound requires several minutes. Both give a weak Millon's reaction. So far no precipitates have been obtained with saturated sodium bisulphite nor with semicarbazide. Schiff's aldehyde reaction was positive to a considerably greater extent than with any normal urines tested. The color obtained on our urine corresponded approximately to the color given by formaldehyde at a dilution of 1 :50,000. It might here be recalled that formaldehyde does not reduce phosphomolybdic acid.

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[1] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, lxvi, 375.