XXIII. THE DETERMINATION OF GUANIDINES IN URINE AS PICRATES

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BURNS and SHARPE [1917] described a marked increase in the amount of guanidine and methyl-guanidine in the blood and urine of dogs in tetania parathyreopriva and in the urine in idiopathic tetany in children.

The method then used was based upon that of Koch and involved the use of mercuric chloride. It has been criticised by Greenwald [1924] on the ground that by the use of the mercuric salt creatine may be split into guanidine. Further, the precipitation with auric chloride is unsatisfactory as the salt is easily decomposed even by light; and, as the gold salt of guanidine is not very insoluble, there is a considerable loss. The objection that creatinine in the urine may in our investigations have been estimated along with the guanidine seems to be negatived by the finding of Burns [1917] that after administration of guanidine there is little if any increase of creatinine.

Later Findlay and Sharpe [1920], and Nattrass and Sharpe [1921] recorded the presence of methyl- or dimethyl-guanidine in the urine in two cases of adult tetany. The method used depends upon precipitation by tannic acid and subsequent precipitation of the bases with picric acid. By this method the objections to the process previously used are eliminated. The danger that creatinine, potassium and ammonium picrates may be precipitated along with guanidine picrate has to be considered, but the difference in solubility and character of the crystals obviates this danger.

This method has also been criticised by Greenwald on the ground that the precipitate may have in part consisted of creatinine and ammonium picrate [1924, p. 332].

Since the publication of the method in 1920, improvement in some particulars has been the object of study, and the modification now described has been devised.

METHOD.

250 or 500 cc. of urine were treated with a 10 % solution of tannic acid. This was added in small quantities at a time and shaken after each addition. The precipitant was added until the supernatant liquid appeared green in colour. The precipitate was allowed to settle and filtered off by suction. To the filtrate $\text{Ba}(\text{OH})_2$ was added in excess, the precipitate of barium tannate

allowed to settle and filtered. To remove the excess of hydroxide a few cc. of a 1 % H_2SO_4 solution were added in slight excess; this excess was then neutralised by adding a cream of BaCO_3 , testing with litmus. The filtrate from this was evaporated to a syrup which usually crystallised on cooling. When cool the mass was extracted thoroughly with absolute alcohol and filtered: about 60 cc. of alcohol were necessary for 250 cc. of the original urine. The alcohol was evaporated off and the mass again extracted with about 20 cc. of alcohol. All the guanidine bases, creatinine, traces of potash, soda and ammonia are in the extract. The alcohol was again evaporated off and the residue dissolved in 10 cc. water. A saturated aqueous solution of picric acid was now added until the total volume was about 20 cc. This was set aside to crystallise.

The following tests show the applicability of the method:

1. *Recrystallisation of mixed picrates.* 50 mg. creatinine hydrochloride and 50 mg. guanidine hydrochloride were dissolved in a few cc. of water and saturated picric acid was added to precipitate the bases. The precipitate was filtered and dissolved in about 20 cc. of water at about 80° and set aside to crystallise.

Recovery: 47.8 mg. (95.6 %) guanidine hydrochloride.

The creatinine picrate separated on further evaporation. Its presence, along with the guanidine or methyl-guanidine picrates may be recognised by Jaffe's test and its amount estimated by Folin's method.

2. *Recovery of guanidine from mixtures of urinary chlorides.* 50 mg. creatinine hydrochloride, 50 mg. guanidine hydrochloride, 100 mg. potassium chloride and 100 mg. sodium chloride were dissolved in a little water and the mixture was evaporated to dryness, as in the final stage of the process of extraction of bases. The crystalline residue was treated in the usual way with 15-20 cc. absolute alcohol, filtered, and the alcohol evaporated off. Guanidine and creatinine were retained in the filtrate along with a little potassium.

After evaporation 10 cc. of water were added, then 10 cc. of saturated picric acid solution. The guanidine was precipitated immediately. After the guanidine picrate had been separated by filtration further evaporation was allowed to occur and creatinine picrate was obtained. On still further evaporation to 10 cc. characteristic hexagonal needles of potassium picrate were obtained, but this was not estimated. A little more creatinine was obtained in this fraction.

Recovery: 43.8 mg. (87 %) guanidine hydrochloride and 27 mg. (43.4 %) creatinine hydrochloride.

Sodium picrate is so very soluble that it would be impossible to precipitate it in this way.

3. *Addition of guanidine and creatinine to normal urine.* 50 mg. creatinine hydrochloride and 50 mg. guanidine hydrochloride were dissolved in 250 cc. normal urine and the full process of extraction carried out. The creatinine was not estimated.

Recovery (in two similar experiments): 42.9 and 44.8 mg. (85.5 and 89.6 %) guanidine hydrochloride.

Should creatinine picrate appear along with the guanidine picrate it was found that a safe and reliable method of eliminating it was fractional crystallisation. The mixed picrates were heated with just sufficient water at about 80° to dissolve the less soluble guanidine salt; this was set aside and the guanidine picrate separated free from creatinine and potassium.

As an alternative to this method of crystallisation, the creatinine can be removed by adding pure blood charcoal in slightly acid solution. Folín and Denis [1916] claim that upwards of 90 % of the creatinine is thus eliminated.

Ammonium picrate is more soluble than the potassium salt and it comes down only in concentrated solutions. It is very easily identified by the deep colour of the crystals and by their very constant crystalline form.

SUMMARY.

1. A method for the estimation of guanidine and methyl-guanidine is described. The process is simple and rapid and the proportion of guanidine recovered is nearly 90 %, whilst the separation from creatinine and potassium is complete. The photographs (Plate I) should assist in the identification of the picrates found in the various fractions of the final stage.

2. As mercuric chloride is not used the possibility of conversion of other bases (creatine) to guanidine is eliminated.

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Guanidine picrate. $\times 150$.

Methyl-guanidine picrate. $\times 150$.

Dimethyl-guanidine picrate. $\times 150$.

Creatinine picrate. $\times 150$.