LXXXVII. ON THE SOLUBILITY OF SOME PICRATES AND THE DETERMINATION OF GUANIDINES IN URINE.

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In a paper published about a year ago, Sharpe [1925] refers to certain criticisms by the present writer [Greenwald, 1924] of the methods employed by Burns and Sharpe [1917], Findlay and Sharpe [1920] and Natrass and Sharpe [1921] for the detection and determination of guanidines in urine. Essentially, these were that creatine might be oxidised to methylguanidine by the mercuric salts employed by Burns and Sharpe and that the method employed by Findlay and Sharpe and by Natrass and Sharpe made no adequate provision for the separation of creatinine and of ammonium salts. In order to meet this criticism, Sharpe proposed a slight modification of the method previously employed. The urine (250 cc.) is treated, in succession, with tannic acid, $\text{Ba}(\text{OH})_2$, H_2SO_4 and BaCO_3 , filtering off each precipitate as produced. The final filtrate is evaporated to a syrup and extracted with absolute alcohol. The extract is evaporated and the residue then obtained is extracted with 20 cc. alcohol. The alcohol is then evaporated and the residue is dissolved in 10 cc. water. After adding 10 cc. of a saturated aqueous solution of picric acid, the mixture is set aside for crystallisation. Guanidines, if present, should crystallise out as the picrates, whereas creatinine should remain in solution.

Although proposed as a method for the determination of *guanidines*, the experimental data presented refer only to the isolation of *guanidine*. It seems to be assumed that the same method will be applicable to the determination of methylguanidine and of dimethylguanidine. That the picrates of these substances might be more soluble than is guanidine picrate seems not to have been considered.

Even guanidine picrate, as was shown by Medes [1925], is not sufficiently less soluble than creatinine picrate to permit of a satisfactory separation of the former from an excess of the latter. If the quantity of guanidine picrate is less than one-third of the amount of creatinine picrate, such crystals as separate must contain creatinine picrate. Only if at least 20 mg. of guanidine are present for each 113 mg. of creatinine is it at all possible for pure guanidine picrate to separate from a solution containing the two picrates.

With methylguanidine and dimethylguanidine, the separation is hopeless. As is indicated in the table, the solubilities of the picrates of creatinine, methylguanidine and dimethylguanidine, at both 10° and 20° , are almost

identical. Consequently, no pure crystals of methylguanidine or dimethylguanidine picrate can separate from a solution that also contains creatinine picrate, unless the amount of the latter is much smaller than the amount of the methylguanidine, or dimethylguanidine, picrate.

Sharpe's method calls for the concentration of the extract from 250 cc. of urine to a final volume of 20 cc. This quantity of urine would, ordinarily, contain approximately 250 mg. of creatinine. This might form 755 mg. of creatinine picrate. Sharpe adds only 10 cc. of a saturated solution of picric acid, so that not more than about 150 mg. of creatinine picrate could be formed. But even this amount is sufficient to saturate, not 20 cc. of water, but 80 cc. It is quite evident that, in Sharpe's experiments, practically all of the creatinine must have been converted into creatine, or other substances, during the manipulation. But the extent of such conversion must vary greatly from experiment to experiment.

Sharpe suggests that creatinine might be removed by treatment with blood charcoal but no experimental data are presented. Folin and Denis [1916], whom Sharpe quotes, used blood charcoal to remove most of the creatinine from urine, not from a solution of creatinine picrate. Whether or not guanidines are also removed by the blood charcoal was not determined either by Folin and Denis or by Sharpe.

There is still another source of error. Ammonium salts are supposed to be almost completely separated by their insolubility in alcohol. With many urines, the relation between Na, K, NH_4 , Cl, SO_4 , etc., in the concentrated filtrates may be such as to give an alcoholic extract not containing appreciable quantities of ammonium salts, but, with some urines, it seems quite likely that the 20 cc. of final alcoholic extract should contain ammonium chloride. According to Lobry de Bruyn [1892, 1893], 100 g. of a saturated solution of ammonium chloride in absolute alcohol contain, at 17–19°, from 0.62–0.67 g. of NH_4Cl . It follows that 100 cc. would contain 0.5 g. and 20 cc. 0.1 g. This amount would yield 0.44 g. of ammonium picrate, enough to saturate not only 20 cc. of water but more than twice this volume.

It may be, of course, that the solubility relationships in the concentrated solution of urea and other urinary constituents obtained by Sharpe are not those prevailing in simple aqueous solutions. But, in the absence of any evidence that this is the case, Sharpe's claim for a method for the determination of guanidines cannot be accepted.

Solubility of some picrates at 10° and 20°.

Picrate of	Milligrams per 100 cc.	
	10°	20°
Creatinine*	130	184
Guanidine†	43	64
Methylguanidine	132	178
Dimethylguanidine	117	162
Ammonium	697	1020

* Calculated from the results of Medes, who gives 122 at 7.5° and 182 at 21°.

† " " " " 34.5 at 7.5° and 61 at 21°.

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