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Evaluation of the Rupp-Schied-Thiel Method as a Test for Thiocyanate in the Urine.*

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While applying the Rupp-Schied-Thiel^{1, 2} thiocyanate procedure to pathological urines, cancerous, and non-cancerous and to normals, as given in the preceding paper, the question arose as to the degree of specificity of the reaction when applied to a number of substances known to occur in urine or possibly there. Among the substances possibly in urine found to behave like thiocyanate in the Rupp-Schied-Thiel procedure are ergothioneine, urinary proteose, and oxyproteic acids, and to some degree uric acid. Four normal urines were studied for total apparent thiocyanate by the Rupp-Schied-Thiel procedure; for ergothioneine-like material colorimetrically against blood ergothioneine by the Behre and Benedict³ procedure; urinary proteose by the method of Oriel and Barber⁴; and for oxyproteic acids as barium salts according to Pregl.⁵ In each case the thiocyanate equivalent of the fractions, (1) ergothioneine-like material, (2) proteose, and (3) barium oxyproteic acids was determined in the Rupp-Schied-Thiel procedure. The thiocyanate equivalents of fractions listed subtracted from the total apparent thiocyanate shows much less thiocyanate than given by the Rupp-Schied-Thiel method. The conclusion is drawn that the Rupp-Schied-Thiel method is not a measure of thiocyanate if applied directly to the urine. The same objection holds for the Munk⁶ gravimetric method which depends on the precipitation of thiocyanate by silver nitrate and melting the precipitate with sodium carbonate and potassium nitrate to oxidize the sulfur to sulfate, which is then precipitated as barium sulfate. Other urinary sulfur compounds than thiocyanate are included in this method.

The interfering material, however, can be eliminated. Thus,

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¹ Rupp, E., and Schied, A., *Ber. Chem. Ges.*, 1902, **35**, 2191.

² Thiel, A., *Ber. Chem. Ges.*, 1902, **35**, 2766.

³ Behre, J. A., and Benedict, S. R., *J. Biol. Chem.*, 1929, **82**, 11.

⁴ Oriel, G. H., and Barber, H. W., *Lancet*, 1930, II, 231.

⁵ Pregl, F., *Arch. Physiol.*, 1899, **75**, 87.

⁶ Munk, I., *Virchows Arch.*, 1877, **69**, 350.

based on the findings that strong alcohol does not dissolve certain interfering substances such as ergothioneine, proteose, the barium salt of oxyproteic acid, or barium urate but does dissolve barium thiocyanate a somewhat improved procedure has been employed as follows: An aliquot of urine generally 50 cc. is treated with a saturated aqueous solution of barium hydroxide as long as a precipitate forms. The mixture is filtered. The filtrate is brought to dryness on the water bath and is extracted with warm absolute alcohol and filtered. The procedure of extraction with alcohol and filtering is repeated. Then the alcohol is evaporated, the residue dissolved in 25 cc. of water and precipitated with silver nitrate. Then the Rupp-Schied-Thiel iodine procedure is applied.

Such a procedure has given a return of 97.2% for potassium thiocyanate in pure solution, a return of 95% for thiocyanate added to the urine and shows in urine amounts of thiocyanate very much smaller than the original Rupp-Schied-Thiel procedure applied to urine directly. Four normal urines tested for thiocyanate by this procedure gave values of 14.2 mg., 5.31 mg., 17.6 mg., and 25.5 mg. of HCNS in 24-hour samples as compared respectively with 101.3 mg., 66.2 mg., 116.1 mg., and 140.6 mg. HCNS in the original Rupp-Schied-Thiel procedure.

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Dissociation of *Pneumococcus*: A New Colony Variant.

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I. *A New Colony Variant of *Pneumococcus*.* R colonies of pneumococcus, suitably spaced on blood agar, are allowed to age for 6 to 7 days at 37°C. Under these conditions many of the colonies give rise to a variety of secondary growths including daughter colonies, peripheral excrescences and a peculiar form of localized, marginal outgrowth with a rough, fungoid appearance. These various forms of secondary growth have been observed in some detail and the rough, localized, marginal outgrowth represents an unusual phase of development. When transfers are made from such areas to fresh blood agar plates a new type of colony is produced. Its ap-