

by nearly all of them. Thorium is precipitated by aniline, *o*-toluidine, xyloidine, diethyl aniline, dimethyl aniline, pyridine, piperidine and quinoline. Aniline *o*-toluidine, diethyl aniline, benzylamine, pyridine and piperidine are the only precipitants of cerium, while with lanthanum, neodymium and praseodymium, the precipitants are benzylamine and piperidine.

Quinoline will separate thorium from neodymium and zirconium from the latter, while aniline separates thorium and also zirconium from lanthanum. The latter and thorium are also separated by quinoline. With *o*-toluidine cerium may be separated from praseodymium, and the latter and thorium may be separated by aniline. Finally, xyloidine will separate zirconium from lanthanum, as well as from praseodymium.

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A NEW METHOD FOR THE APPROXIMATE DETERMINATION OF URIC ACID IN URINE.

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SEVERAL of the most important methods for the quantitative determination of uric acid in urine, depend on the practically complete precipitation of the uric acid, as a magnesia silver urate, by the addition of an ammoniacal silver solution and a magnesia solution to the urine.

This method of precipitation is open to several objections:

First, the ammoniacal magnesia solution causes a voluminous precipitate of triple phosphate to separate from the urine. This precipitate, if not previously removed, contaminates the silver urate precipitate, rendering filtration and washing difficult owing to its large bulk. In Ludwig's method¹ the phosphates are thrown down together with the uric acid. Salkowski² recommends the removal of the phosphates by means of an ammoniacal magnesia solution and filtering the urine, previous to the addition of the ammoniacal silver solution. Folin and Shaffer found³ that if this

¹ *Ztschr. anal. Chem.*, **21**, 148 (1882).

² *Ztschr. physiol. Chem.*, **14**, 50 (1890).

³ *Ibid.*, **32**, 553 (1901).

method be followed, a loss of about 0.002 gram of uric acid for 100 cc. of urine results.

Second, the ammoniacal silver solution not only precipitates the uric acid but also the xanthine bases contained in the urine.

Third, the silver precipitate obtained by this method from urine, is not entirely insoluble in dilute solutions of ammonia.

Fourth, the precipitate is generally dark colored, or gradually blackens owing to a reduction of the ammonia silver solution. Salkowski¹ also noticed this. Haycraft² and Herman³ found that the addition of sodium bicarbonate hinders but does not entirely prevent this reduction.

In an attempt to do away with the use of ammonia to which at least three of the above-mentioned difficulties could be traced, a sulphite-silver solution was tried.

The solubility of silver chloride in an aqueous solution of sodium sulphite is not generally known, as no mention of it can be found in Comey's "Dictionary of Chemical Solubilities."

It was found by us that at ordinary room temperature, 100 cc. of a saturated aqueous solution of sodium sulphite dissolves about 1 gram of silver chloride. In the experimental work a twentieth normal solution, containing 7.175 grams of silver chloride in 1 liter was employed.

By adding the sulphite-silver solution, to a solution of uric acid, strongly alkaline with sodium carbonate, it was found that all the uric acid was thrown down as an almost white, flocculent precipitate which settles after several minutes and which can be readily filtered out and washed. This precipitate dissolves to a considerable extent in cold water, but is practically insoluble in dilute solutions of sodium carbonate.

From the analyses of the precipitate, the ratio of silver to nitrogen was found to agree with that calculated for 1 atom of silver to 1 molecule of uric acid. From the fact that the uric acid is completely precipitated in the absence of magnesia salts, and also that uric acid cannot form neutral salts with alkalis in the presence of carbon dioxide or soluble carbonates as claimed by Allen and

¹ *Ztschr. physiol. Chem.*, **14**, 40 (1890).

² *Ztschr. anal. Chem.*, **25**, 169 (1886).

³ *Ztschr. physiol. Chem.*, **12**, 497.

Beusch¹, further, from the conditions under which the precipitation was made, it is probable that the precipitate has the following composition: $\text{AgC}_5\text{H}_3\text{N}_4\text{O}_3$.

When the sulphite-silver solution is added to urine, made strongly alkaline with sodium carbonate, a yellowish white flocculent precipitate forms, which settles after a few minutes and can be readily filtered out and washed. The precipitate does not darken during washing, or on exposure to the air. By this method the voluminous precipitate of the triple phosphates is avoided, as the addition of a considerable excess of a saturated sodium carbonate solution, to the urine, throws down only a slight precipitate. This need not be removed previous to the addition of the sulphite-silver solution. By adding from 10 to 15 grams of Rochelle salt to 100 cc. of urine it will remain clear even after the addition of an excess of a sodium carbonate solution.

A series of parallel determinations upon a number of urines were made, using both the silver-ammonia and the sulphite-silver methods of precipitation. The silver or nitrogen or both of these constituents of the precipitates were determined. By calculating all the results to uric acid, it was found that those obtained by the silver-ammonia method were from 0.001 to 0.006 gram (uric acid) higher for 100 cc. of urine, than the results obtained by the sulphite silver method.

On examining the precipitates obtained by both methods, according to the Salkowski² method for the determination of xanthenes, it was found, that the difference in the results obtained by the two methods could be partly, if not entirely accounted for, by the fact that the sulphite-silver solution does not precipitate the xanthine bases as completely as the ammonia-silver solution. Which of the 10 xanthine bases that have been discovered in urine are, and which are not, precipitated by the sulphite-silver solution has not as yet been determined.

EXPERIMENTAL PART.

A. Uric Acid.

1. 0.150 gram uric acid was dissolved in a hot solution of sodium

¹ *Ann. Chem. Pharm.*, **65**, 181 (1848).

² *Pflüger's Archiv.*, **69**, 280 (1898).

carbonate and diluted to 300 cc. with water. An excess of a magnesium nitrate solution and 30 cc. of twentieth-normal sulphite-silver solution were added. After standing one-half hour, the precipitate was filtered off and washed free of the excess of silver and of chlorine with water. The precipitate and filter were digested in a Kjeldahl flask with concentrated sulphuric acid and potassium sulphate, and heated until almost colorless. On cooling, the mixture was diluted with water, the silver was separated as silver sulphide, by means of a sodium sulphide solution, and after being filtered out and washed was determined by the Volhard method. The filtrate was made alkaline with sodium hydroxide and the ammonia was distilled into a standard sulphuric acid solution.

The silver required 7 cc. of tenth-normal ammonium thiocyanate.

$$7 \times 0.0168 = 0.1176 \text{ gram uric acid.}$$

The ammonia neutralized 28 cc. of tenth-normal sulphuric acid.

$$28 \times 0.0014 \times 3 = 0.1176 \text{ gram uric acid.}$$

The ratio of the silver to nitrogen corresponds to 1 atom of silver to the molecule of uric acid. It was afterwards found that the loss of uric acid was due to the solubility of the precipitate in water.

2. 0.250 gram of uric acid was dissolved in a dilute potassium hydroxide solution (free from chlorine) and made up to 500 cc. with water.

100 cc. of this solution was titrated according to Hopkins' method. Required 13.1 cc. of twentieth-normal potassium permanganate.

$$13.1 \times 3.75 = 0.0491 \text{ gram uric acid.}$$

To 250 cc. of this solution, an excess of magnesium nitrate solution was added, 25 cc. of a saturated aqueous sodium carbonate solution, and 25 cc. of twentieth-normal sulphite-silver solution. The precipitate was filtered off and washed with a dilute solution of sodium carbonate. To the first wash solution a little sodium sulphite was also added. The precipitate and filter were placed in a beaker and boiled with concentrated nitric acid (free of chlorine). Then it was diluted with water and boiled to expel the

oxides of nitrogen. When cold the solution was titrated with a thiocyanate solution, using ferric alum as an indicator.

Required 36.6 cc. fiftieth-normal ammonium thiocyanate.

$$\frac{36.6 \times 2 \times 0.00168}{2.5} = 0.0492 \text{ gram uric acid for 100 cc. of the solution.}$$

3. A solution of 0.250 gram of uric acid in 500 cc. was made as in (2).

I. 100 cc. by Hopkins' method required 13.3 cc. twentieth-normal potassium permanganate.

II. 100 cc. by Hopkins' method required 13.3 cc. twentieth-normal potassium permanganate.

$13.3 \times 3.75 = 0.0499 \text{ gram uric acid in 100 cc of the solution.}$

To 100 cc. of the solution, 15 cc. of a saturated solution of sodium carbonate, and 10 cc. of twentieth-normal sulphite-silver solution were added. The precipitate was treated as in (2). Required 14.7 cc. of fiftieth-normal ammonium thiocyanate = 0.0494 gram uric acid.

Another 100 cc. was treated in exactly the same way. Required 14.8 cc. of fiftieth-normal ammonium thiocyanate = 0.0497 gram uric acid.

Therefore the uric acid was completely precipitated by the sulphite-silver solution in the absence of magnesium salts.

B. Experiments with Urine.

The following methods of precipitation were employed:

1. *The Sulphite-Silver Method.*—A measured amount of urine was taken. After neutralizing with a saturated aqueous solution of sodium carbonate, 15 cc. excess for each 100 cc. of urine was added, and then 10 cc. of twentieth-normal sulphite-silver solution was added. In many of the determinations an excess of magnesium nitrate solution was also introduced, but this was found to be unnecessary. After standing one hour, the precipitate was filtered off and washed free of the excess of silver and of chlorine with a dilute solution of sodium carbonate.

2. *The Ammonia-Silver Method.*—A measured amount of urine was taken. For each 100 cc. was added 10 cc. of the Ludwig mag-

nesia mixture. The urine was then diluted to a known bulk and filtered through a dry filter. An aliquot portion of the filtrate was taken and for each 100 cc. urine represented by the filtrate 5 cc. of a tenth-normal ammoniacal silver solution was added. After standing one hour, the precipitate was filtered off and washed free of the excess of silver and of chlorine with a very dilute ammonia solution. If the nitrogen of the precipitate was to be determined, it was washed with water to remove the ammonia.

To determine the silver and nitrogen contained in these precipitates the filter and precipitate were boiled with water containing a little magnesium oxide to remove the last traces of ammonia, and then the method as described under *A 1* was followed. When only the silver contained in these precipitates was to be determined, the method described under *A 2* was used. The results are given in the following table:

Urine.	Method of precipitation.	Silver per 100 cc. of urine.	Silver calculated to uric acid.	Nitrogen per 100 cc. of urine.	Nitrogen calculated to uric acid.
1	¹ Sulphite-silver	0.0404	0.063
	Ammonia-silver ...	0.0414	0.0645
2	¹ Sulphite-silver	0.0329	0.0507
	Ammonia-silver ...	0.0331	0.0517
3	¹ Sulphite-silver	0.0348	0.0542	0.0154	0.0462
	Sulphite-silver	0.0359	0.0558
	Ammonia-silver ...	0.035	0.0546	0.0164	0.0492
4	¹ Sulphite-silver	0.0301	0.0468
	Sulphite-silver	0.0301	0.0468
	Ammonia-silver ...	0.0339	0.0528	0.0167	0.0501
5	¹ Sulphite-silver	0.0324	0.0504
	Sulphite-silver	0.0324	0.0504
	Ammonia-silver ...	0.0339	0.0528
6	² Sulphite-silver	0.0274	0.0426
	Ammonia-silver ...	0.0291	0.0454
7	² Sulphite-silver	0.0346	0.0541
	Ammonia-silver ...	0.0363	0.0561
8	² Sulphite-silver	0.0229	0.036	0.0115	0.0346
	Ammonia-silver ...	0.0286	0.044	0.0141	0.0423

From the invariably lower results obtained by the sulphite-silver method, it was suspected that the xanthine bases of the urine were

¹ Magnesium solution also added.

² Mixed urine containing sugar.

not precipitated by the sulphite-silver solution. In order to determine this fact the following experiments were tried:

1. By means of the Salkowski method¹ the xanthine bases from 900 cc. of urine were obtained in a dilute sulphuric acid solution. This solution was diluted with water to 100 cc.

Fifty cc. of this solution were made alkaline with ammonia and the xanthine bases precipitated as silver salts by the addition of an ammoniacal silver solution. The silver contained in the precipitate was determined by the Volhard method. Required 18.4 cc. of fiftieth-normal ammonium thiocyanate.

To 50 cc. of the same solution an excess of sodium carbonate was added and then the sulphite-silver solution. The silver, in the xanthine-silver precipitate, was titrated by the Volhard method. Required 15.7 cc. of fiftieth-normal ammonium thiocyanate.

The difference in the silver content of the xanthine precipitates obtained by the two methods was therefore:

$18.4 - 15.7 = 2.7$ cc. fiftieth-normal ammonium thiocyanate = 0.0058 gram silver for 450 cc., or 0.0013 gram silver for 100 cc. of urine.

Calculated to uric acid this difference amounts to 0.002 gram per 100 cc. of urine.

2. The silver, in the precipitate from 100 cc. of a urine obtained by the sulphite-silver method, required 16.1 cc. fiftieth-normal ammonium thiocyanate.

The xanthine-silver, obtained by applying the Salkowski method to the precipitate from 300 cc. of urine thrown down by the sulphite-silver method, required 5.7 cc. of fiftieth-normal ammonium thiocyanate.

The uric acid in 100 cc. of this urine was

$$\left(16.1 - \frac{5.7}{3}\right) \times 2 \times 0.00168 = 0.0477 \text{ gram.}$$

Similarly the silver in the precipitate from 100 cc. of urine obtained by the ammonia-silver method, required 16.8 cc. of fiftieth-normal thiocyanate; and the xanthine-silver from 300 cc. of this urine obtained by the Salkowski method required 7.8 cc. of fiftieth-normal ammonium thiocyanate.

¹ Neubaum Vogel's "Analyse des Harns," p. 829, 10th ed.

The uric acid in 100 cc. of the urine was

$$\left(16.8 - \frac{7.8}{3}\right) \times 2 \times 0.00168 = 0.0477 \text{ gram.}$$

3. Another urine was treated in the same way.

The silver in the precipitate obtained according to the sulphite-silver method from 100 cc. of urine, required 14.8 cc of fiftieth-normal ammonium thiocyanate.

The xanthine silver, from 300 cc. of urine by sulphite-silver method, required 7.8 cc. of fiftieth-normal ammonium thiocyanate.

The uric acid in 100 cc. of the urine is

$$\left(14.8 - \frac{7.8}{3}\right) \times 2 \times 0.00168 = 0.041 \text{ gram.}$$

The silver from 100 cc. of the urine by the silver-ammonia method, required 15.5 cc. of fiftieth-normal ammonium thiocyanate.

The xanthine silver obtained by the Salkowski method from 300 cc. of the urine, required 9 cc. of fiftieth-normal ammonium thiocyanate.

The uric acid in 100 cc. of the urine is

$$\left(15.5 - \frac{9}{3}\right) \times 2 \times 0.00168 = 0.042 \text{ gram.}$$

The difference in the results found by the two methods, can be partly accounted for by the fact that the sulphite-silver solution does not precipitate the xanthine bases completely.

The use of a sulphite-silver solution instead of an ammoniacal silver solution for the precipitation of uric acid in urine, has the advantage that by its use the precipitation of the bulky earthy phosphates is avoided, the precipitate does not darken, as no reduction of the silver solution takes place and finally the precipitate can be readily filtered off and washed. The work will be continued.
