

I am working. The incompleteness of this report is to be accounted for by the fact that this piece of work was done during such spare time as I could find while teaching three classes with laboratory superintendence every afternoon.

### THE RAPID ESTIMATION OF URIC ACID IN URINE.<sup>1</sup>

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THE estimation of uric acid presents well recognized difficulties, and the results obtained by any of the known methods are always open to criticism on the side of accuracy. While we admit this fact, it must also be admitted that the object for which the estimations of uric acid in urine are usually undertaken, does not require extreme accuracy. For diagnostic purposes, slight errors may usually be disregarded. Another factor often regarded by both the person afflicted and the physician, as of great importance, is the time and trouble and hence the expense to be incurred in making a correct diagnosis. The methods now in vogue for the estimation of uric acid are so time-consuming that only the chemist with a well-appointed laboratory can undertake them. This fact has prevented the mass of physicians from having such estimations made, and has deprived many unfortunate sick from the benefits to be derived from a scientific diagnosis of their ailments.

A very considerable number of methods have been proposed for the estimation of uric acid. One of the oldest methods depends upon the fact that the acid is almost insoluble in water acidulated with hydrochloric acid. The acid is set free by hydrochloric acid, and after forty-eight hours it is collected on a filter, dried at 100° C., and weighed. This method has been shown to be very unreliable, as a very considerable amount of uric acid may remain in solution. Fokker, Salkowski, and Hopkins precipitate the uric acid with ammonium chloride as acid ammonium urate, filter, wash, decompose with hydrochloric acid, let stand two hours, filter, wash, dry, and weigh on the filter. Or, the precipitated ammonium urate may be titrated with potassium permanganate, or with twentieth-normal acid. It has also been proposed to treat this precipitate with Fehling's or Pavey's copper solution, both of which are reduced by uric

<sup>1</sup> Read at the meeting of the New York Section, May 7, 1897.

acid, and thus arrive at the amount of the acid present by the amount of copper reduced. Either of these methods necessitates a rather troublesome filtration of the gelatinous ammonium urate.

Bayrac evaporates the urine to dryness, treats the residue with hydrochloric acid, washes the residue with alcohol, transfers to a special apparatus, and heats nearly to boiling with a solution of sodium hypobromite, and collects and measures the nitrogen, as in an urea determination. Haycraft precipitates the uric acid with silver nitrate in excess, in presence of ammonia, filters with the aid of a filter pump, dissolves the precipitate in nitric acid, and estimates the silver in the solution by Volhard's method, from which he calculates the uric acid.

This method has been much used, in spite of the numerous observers who have shown that the results are too high, on account of the fact that xanthin, hypoxanthin, and possibly other substances are thrown down by silver nitrate. Ludwig precipitates with an ammoniacal silver nitrate solution, in presence of an excess of magnesium mixture, filters, washes, decomposes the silver magnesium urate with sodium hydrogen sulphide, filters off the silver sulphide, evaporates the filtrate to a small bulk, acidifies with hydrochloric acid, lets the mixture stand to crystallize, filters through glass wool, washes, dissolves out any sulphur with carbon disulphide, dries at  $100^{\circ}$  C., and weighs.

This method is a modification of that used by Salkowski, but the latter<sup>1</sup> claims the priority for Ludwig's modification. Moreover, he states that hydrochloric acid does not precipitate all the uric acid from its solutions, and quotes the results of Maly and Hoffman,<sup>2</sup> who have shown the same thing. They found that silver nitrate will always precipitate some uric acid in the filtrate from the hydrochloric acid. Salkowski, in the same article, after a somewhat critical review of a number of the methods that have been proposed, declares that the methods of Haycraft, Hermann, and Czapek are all unreliable.

Salkowski claims to have been the first to propose the precipitation of uric acid by silver nitrate in an ammoniacal solution.<sup>3</sup>

<sup>1</sup> *Ztschr. phys. Chem.*, 14, 54.

<sup>2</sup> *Pflüger's Archiv.*, 6, 201.

<sup>3</sup> *Virchow's Archiv.*, 52, 61.

He then proposed to add an excess of silver nitrate, filter, acidify with nitric acid, filter, and estimate the excess of silver as chloride. Since then, he has thrown some doubt upon the constancy of composition of the silver compound with uric acid, upon which the method is based. Other authors have not all accepted this conclusion, and several have based processes upon the supposition that the composition of the double urate of silver and magnesium is a definite compound. It is admitted that the silver urate undergoes a partial decomposition during the rather tedious process of filtration and washing. Haycraft and Hermann both claim that this is not the case when the urine is treated with sodium bicarbonate and ammonia, before adding the silver solution, and hastening the filtration by means of the filter pump.

Cammerer<sup>1</sup> proposes a method in which he removes the phosphates with magnesia mixture, and then precipitates the uric acid with silver nitrate, filters, dries the residue, and estimates the nitrogen by the Kjeldahl method, and calculates the uric acid from the nitrogen found. This method avoids the following source of error in Ludwig's process. In decomposing the precipitated urate of silver with hot solution of alkaline hydrogen sulphate, a part of the uric acid is decomposed, as pointed out by Hopkins and E. Groves.<sup>2</sup> It has been proposed by several authors to estimate this acid by precipitation with a mixture of copper sulphate and sodium bisulphite, and estimation of the nitrogen in the precipitate. Arthaud and Butte have proposed a method by the use of a standard solution of cuprous thiosulphate. Martin Krüger<sup>3</sup> proposes a process based upon this principle, which he claims gives good results. Balke states that this process is not reliable when applied to the urine.

From this hasty and incomplete review of the methods that have been proposed for the estimation of uric acid in the urine, it will be seen that the processes are complicated as a rule, and the results are not in all cases reliable. As remarked above, none of these methods are rapid enough to be considered as clinical methods.

Czapek<sup>4</sup> has proposed a process intended to shorten the time

<sup>1</sup> *J. Chem. Soc.*, 56, 1040.

<sup>2</sup> *Chem. News*, 66, 107.

<sup>3</sup> *Ztschr. phys. Chem.*, 21, 317.

<sup>4</sup> *Ztschr. phys. Chem.*, 12, 502.

required. He adds to 150 cc. urine, eighteen cc. of a tenth-normal solution of silver nitrate, thirty cc. of a twenty per cent. ammonia, and fifteen cc. of magnesia mixture. The volume is now brought to 300 cc. and filtered through a large filter. Fifty cc. of this filtrate are taken for the titration, to determine the amount of silver in the solution. This is done in a flask by a solution of sodium hydrogen sulphide or potassium hydrogen sulphide. The flask is stoppered with a cork containing a small glass tube, to serve as an outlet for steam when the contents of the flask are boiled. The sodium hydrogen sulphide is added until the steam from the flask browns a strip of lead paper held before the outlet tube. The sodium hydrogen sulphide is then titrated against the silver solution in a similar manner. The amount of silver found in the filtrate, deducted from that added to the urine, gives the quantity precipitated with the uric acid. The results obtained by Czapek were good when pure uric acid was operated upon, but far from satisfactory when applied to the urine. The sources of error in this process are :

1. Too large a factor in uric acid for one cc. of the silver solution.
2. The precipitation of the xanthin bases by silver nitrate, and their calculation as uric acid, as in the Haycraft process.
3. The reduction of a part of the silver nitrate to metallic silver, while boiling the filtrate during the titration. This loss of silver is reckoned as having been precipitated with the uric acid.
4. In making up the solution to 300 cc., after precipitating the uric acid and magnesium ammonium phosphate, no account is made of the volume of this precipitate. In one trial he found the precipitate to measure seven and a half cc.

Errors 2 and 3 will be very considerable in fever urines, and in all concentrated urines, as his results show.

The author shows that the error, as compared with Ludwig's process, is from 0.002 to 0.004 gram in 100 cc. of normal urines, while in fever urines it reached in one case as much as 0.023 gram.

I have devised a process of direct titration, which I believe corrects most of these errors. The method is based upon the

complete precipitation of uric acid from the urine by silver nitrate, in the presence of an excess of magnesia mixture and ammonia, and the detection of the end reaction by potassium, sodium, or ammonium hydrogen sulphide. The titration is performed in a hot solution, to prevent the precipitation of the xanthin bases by silver nitrate.

The process is conducted as follows : To fifty cc., or 100 cc., of the clear urine, add five cc. of the magnesia mixture and about ten cc. of ammonium hydroxide (sp. gr. 0.960), or enough to give a decided excess. Warm the solution on a water-bath, and add from a burette a fiftieth-normal solution of silver nitrate. From time to time a drop is removed from the solution, by means of a dropper pipette, with a bit of absorbent cotton wound tightly over the end, so as to make an efficient filter, and after removing the cotton filter, bring it in contact with a drop of the weak sodium hydrogen sulphide solution on a white surface. Experiments with pure water, showed that it required one-half cc. of the silver solution in fifty cc., or one cc. in 100 cc., to give a marked reaction. This amount must therefore be deducted from the reading. The titration is continued until a dark ring or cloud is seen at the line of contact of the two drops, showing the presence of silver in the solution. Each cc. of silver solution corresponds to 0.00336 gram of uric acid, and the number of cc. used (less one-half cc. for each fifty cc. of urine) multiplied by this factor, gives the number of milligrams of uric acid in the urine taken. From this we may easily calculate the amount excreted in twenty-four hours, the only figure of clinical value.

As soon as the process is complete the precipitate rapidly settles, and it is best to draw off a drop or two from this clear supernatant liquid and test it carefully again. We may also check our work by running in another drop of the silver solution, to see if it produces a cloud, or to see if the precipitation be complete. As there is no excess of silver in the hot liquid at any time, there can be no reduction of silver.

If, after the titration is complete, the solution be cooled, it will usually be found that it will require from one to three cc. of the silver solution to again produce the end reaction, because of the

precipitation of the xanthin bases by the silver, in a cold solution.

This amount is usually greater in concentrated and fever urines than in normal urines, and it is this fact that invalidates Salkowski's older process, Haycraft's, Czapek's, and Denige's methods, in such urines, as they all depend upon the estimation of the excess of silver in the filtrate from the precipitated uric acid. I have found that the xanthin bases are but slightly, if at all, precipitated by silver nitrate from hot urine. It is shown in the following way :

Baginski, of Berlin, has shown that Hofmeister's method of precipitation with hydrochloric and phosphotungstic acids completely removes xanthin bodies from urine. This is confirmed by Alexander von Pohl, who, in a communication to the Paris Academy of Sciences, October 10th, 1892, uses this reaction for the quantitative estimation of leucomaines in urine. Baginski<sup>1</sup> makes use of this precipitation of these bodies for their quantitative estimation. He found normal urine to contain about 0.0027 to 0.003 gram of xanthin bodies in 100 cc. of urine.

I found, by a series of trials, that the titration of a hot urine gave the same result as the same urine after the removal of the precipitate produced by hydrochloric and phosphotungstic acids, and filtered while hot. In the cold solution a part of the uric acid is also thrown down, but this is soluble in hot water, as I have also proved, by trials with solutions of pure uric acid.

I have found the difference between the hot and cold titration, in normal urines, to be usually about one cc. in fifty cc., but in abnormal urines it has been as high as three cc. If we accept the formula of the silver xanthin precipitate, as  $\text{Ag}_2\text{O} \cdot \text{C}_8\text{H}_4\text{N}_4\text{O}_2$ , the factor for the fiftieth-normal silver nitrate will be 0.0015 gram. This would give for 100 cc.  $2 \times 0.0015 = 0.003$  gram for the average amount of xanthin bodies, calculated as xanthin. It will be seen that this agrees with the results found by Baginski. In febrile urines, and in concentrated urines the amount of these bodies is greater.

On one occasion, a specimen of morning urine passed by a healthy man, after an unusually hearty meal accompanied by some champagne, and followed by a night without sleep and

<sup>1</sup> *Ztschr. phys. Chem.*, 8, 399.

with hard work, gave a difference between the hot and cold titration of three cc. in fifty cc. of urine, instead of one cc., as on the preceding day. We have here, then, a method that gives important information outside of the quantity of uric acid. I have not had time to investigate how completely we can separate uric acid from the xanthin bases, by this process, but the results seem to point to it as a valuable clinical method, for their quantitative estimation. It has the advantage over all other methods with which I am acquainted, in that it avoids the necessity of any filtrations, and is a direct titration instead of an indirect one. It is simple, and gives good results when applied to solutions of pure uric acid. A solution of pure dry uric acid was made, with the assistance of sodium phosphate and just enough sodium hydroxide to make the solution clear, and containing one gram to the liter. Fifty cc. of this solution, containing 0.050 gram uric acid required 15 to 15.5 as the result of a number of titrations. As it took one-half cc. of the silver solution to give the reaction in fifty cc. of water, we have, after deducting this amount, 14.5 and 15 as the limits of test. This gives 0.04872 to 0.0504 as the variations in the results obtained. These results were repeated on three separate solutions. The process was then tried as follows: Fifty cc. of urine was measured out and titrated, cold, to the appearance of the end-reaction. Fifty cc. of the above solution of uric acid was then added, and the titration continued. It required sixteen cc. of the silver solution. Deducting one cc. from the result, we have fifteen cc. as the corrected reading, which agrees with the reading with the pure uric acid solution. It seems, therefore, that it is possible to estimate by this method with reasonable accuracy, uric acid in watery solutions, and to obtain good results in estimating the acid in urine to which it has been added. I regret that I have not had time to compare the result obtained by this process with those obtained with other methods. It has become general to compare new methods with Ludwig's, which gives lower results than most others. It seems to me likely that the results obtained by such a complicated process are likely to vary. The author admits that the results are about two per cent. low, and this has been confirmed by others. The

following is the result of three estimations made by my method as compared with Ludwig's :

	Ludwig. Gram.	Direct titration. Gram.
No. 1. Uric acid in 100 cc ...	0.0822	0.08064
No. 2. " " " 100 " ...	0.0506	0.05370
No. 3. " " " 100 " ...	0.0684	0.07064

The relation of uric acid to urea is generally regarded as an important guide in the determination of a normal or abnormal excretion of uric acid, and it is believed by many to be more important than a statement of the actual amount of uric acid eliminated. This ratio is variously stated as from one to thirty-three, to one to sixty. The ratio varies with the method used for the estimation of uric acid. In the above three urines the ratio was as follows :

	Ludwig.	Direct titration.
No. 1 .....	1 to 33.7	1 to 34.4
No. 2 .....	1 to 37.0	1 to 34.8
No. 3 .....	1 to 50.0	1 to 48.5

Although the number of comparative tests is too small to prove the entire reliability of the process, as compared with Ludwig's, these results indicate that it is a valuable clinical method. It is also valuable as a rapid and approximately accurate method of estimating the unoxidized xanthin bases, which have an important clinical bearing, when present in the urine in excess.

In conclusion, I would call attention to the fact, that the results by this process are more apt to be higher than the truth, than lower, on account of the difficulty of seeing the first appearance of the dark cloud, in the test. It will, therefore, be best in individual cases, for the observer to make a test of the process with a solution containing a known amount of uric acid, as a guide to the appearance of the end-reaction. With a little practice, the end-reaction can be determined with accuracy.