tissues of an adult dog which had been subjected to two fasts of 117 days and 104 days in duration respectively, possessed catalytic powers which were much more comparable with the catalytic powers of normal tissues than with the catalytic powers of the tissues of another adult dog which had been subjected to but a single fast 48 days in length. Arguments against drawing any important conclusions from these facts may of course be adduced from the standpoint of individuality. However, in view of other related data already mentioned as obtained from repeated fasters[1] we are willing to stand upon our interpretation. That our data upon catalase values may properly be interpreted as indicating the efficacy of "repeated fasting" is brought out still more clearly in connection with the work of Battelli and Stern[2] in which they determined that the catalytic power of the tissues was an index of functional activity. On the basis of this finding, therefore, our observation of higher catalase values for the tissues of adult "repeated fasters" as compared with adult "initial fasters" may be taken as indicating the more efficient functional activity of the repeated faster.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, INDIANA UNIVERSITY.]

THE DECOMPOSITION OF URIC ACID BY ORGANIC ALKALINE SOLVENTS.

BY HANNAH STEVENS AND CLARENCE E. MAY.

Received December 11, 1910.

From time to time, there have appeared in the literature statements regarding the decompositions uric acid underwent when exposed to the action of alkaline inorganic solvents. Various chemists, Austin, Schittenhelm, Folin and others have noted a loss of uric acid while using an alkali, such as sodium hydroxide, as solvent. Austin has quite conclusively shown the effects of inorganic alkalis in splitting uric acid. He digested solutions of uric acid in Na_2CO_3, Li_2CO_3, Na_2HPO_4 and NaOH respectively for different periods of time and at different temperatures. He proved beyond a doubt that uric acid was destroyed by alkalies, even when acting in the cold, and that this destruction was greatly increased when the temperature was increased to that above room temperature. Sodium hydroxide was found to be by far the most active, for although a solution of only 0.024 gram NaOH per 400 cc. of water was allowed to act on some of the acid, he was able to recover only about 50 per cent. of his acid. Sodium carbonate proved to be least destructive. Schittenhelm also, in using sodium hydroxide as a solvent lost about one-sixth of his uric acid. Further, Folin found that uric acid was decom-

[1] Howe and Hawk, *loc. cit.*

[2] Battelli and Stern, *loc. cit.*

posed fairly quickly by the action of ammonium hydroxide and that the presence of ammonium salts prevented this destruction. He made use of this deportment in determining uric acid as ammonium urate by precipitation with ammonium hydroxide in the presence of another ammonium salt, preferably the sulfate.

It has been the purpose of this series of experiments to determin the stability of uric acid in some few organic alkaline solvents and where there was decomposition, to ascertain whether it reached a maximum at which all further action ceased, or whether it continued to increase from day to day; also to determin whether an increase in the concentration of the solvent affected in any marked way the stability of uric acid in that particular solvent. No attempt has been made to bring out the effect of heat on this destructive property, as all experiments were carried on at room temperature. There has also been no effort made to study the cleavage products formed in the decomposition and only such facts are known about them as were incidentally brought to notice during this investigation.

The uric acid used was some that had been purified by dissolving in dilute sodium hydroxide and reprecipitating by the passage of CO_2 through the solution. This residue was thoroughly washed and dried and was found to be ash-free and quite pure, as was indicated by ignition of some of the material and by a Kjeldahl nitrogen determination. This uric acid sample was used in the first two piperazine solutions, that used in the other experiments was a preparation of Merck's.

The Folin-Schaffer method of uric acid determination was taken as the standard method throughout this work. This method depended on taking a 100 cc. portion of the uric-acid-bearing material, and treating it with 25 cc. of the Folin-Schaffer reagent (a solution composed of 500 grams ammonium sulfate and 5 grams uranium acetate together with 60 cc. of 10 per cent. acetic acid in 650 cc. of water). On the addition of this reagent the mixture was filtered and the filtrate was allowed to stand twenty-four hours. The uric acid that separated was filtered and washed with 10 per cent. ammonium sulfate, then dissolved with hot water, filtering into the original beaker. After the addition of 15 cc. of conc. sulfuric acid, the mixture was titrated against a 0.05 N $KMnO_4$ solution. Each cubic centimeter of the permanganate corresponded to 0.00375 gram uric acid. When there was any deviation from this method of procedure, it will be noted.

The solvents used were piperazine, urotropin and lycetol. Of these, piperazine was by far the best solvent and there was no difficulty in making a solution of any strength desired. The solubility of piperazine urate is given in the literature as 1 : 50 in water at 17°. Urotropin was

found to be only a fair solvent. The maximum amount of uric acid that we found would dissolve in a liter of water containing 15 grams urotropin, at room temperature, was 0.35 gram. Larger amounts refused to dissolve completely. At room temperature, lycetol was a very poor solvent of uric acid since no appreciable quantity of the acid could be made to dissolve. In a hot solution a small quantity dissolved yet it was immediately precipitated again on cooling.

Each stock piperazine solution was made up by dissolving the given weight of uric acid in cold water in a graduated flask into which a weighed amount of piperazine had been placed. This solution together with washings was filtered into a graduated flask and diluted immediately to the mark. Samples of 100 cc. each were taken from day to day and the uric acid content was determined by the Folin-Schaffer method. Washings of 100 cc. 10 per cent. $(NH_4)_2SO_4$ were used and the final residue was taken up in about 150 cc. boiling water; after the addition of 15 cc. concentrated sulfuric acid, the mixture was titrated with standard permanganate. When the uric acid residue was dissolved in hot water prior to titration, there was always a yellow residue left on the paper. No attempt was made to determin the nature of this residue. No account was taken of the loss of uric acid through washing, as the error made in this way was uniform in each case and the estimated amount of uric acid destroyed was independent of this small loss.

TABLE I.—TWO GRAMS URIC ACID DISSOLVED IN 2000 CC. WATER CONTAINING SIX GRAMS PIPERAZINE.

	I. 2 days.	II. 6 days.	III. 8 days.	IV. 12 days.	V. 15 days.	VI. 21 days.	VII. 29 days.	VIII. 36 days.	IX. 43 days.
Cc. $KMnO_4$ used in titration of	2.0	19.2	15.8	7.0	1.0	8.5	5.5	6.3	5.5
duplicates	2.0	19.2	15.8	...	0.8	8.4	6.1	6.3	...
Grams uric acid recovered in	0.0557	0.0534	0.0443	0.0185	0.0026	0.0224	0.014	0.023	0.0204
duplicates	0.0557	0.0534	0.0443	....	0.0021	0.0223	0.016	0.023	....
Gms. uric acid the solution should	0.08	0.0856	0.0856	0.08	0.0856	0.08	0.08	0.08	....
have contained (in duplicate)	0.08	0.0856	0.0856	....	0.0856	0.08	0.08	0.08	....

DUPLICATE OF TABLE I.

	I. 2 days.	II. 6 days.	III. 8 days.	IV. 13 days.	V. 15 days.
Cc. $KMnO_4$ consumed (duplicates)	18.1	15.0	14.1	10.3	9.8
	18.1	15.0	14.2	10.5	9.8
Grams uric acid recovered (in duplicate)	0.0662	0.055	0.051	0.0377	0.0359
	0.0662	0.055	0.052	0.0398	0.0359
Grams uric acid the solution should have contained (in duplicate)	0.08	0.08	0.08	0.08	0.08
	0.08	0.08	0.08	0.08	0.08

TABLE II.—TWO GRAMS URIC ACID DISSOLVED IN 2000 CC. WATER CONTAINING FOUR GRAMS PIPERAZINE.

	I. 1 day.	II. 4 days.	III. 7 days.	IV. 13 days.	V. 20 days.	VI. 27 days.	VII. 34 days.
Cc. $KMnO_4$ consumed (duplicates)	18.2	14.1	3.4	13.6	10.8	9.2	6.2
	10.6	18.7	3.7	13.6	11.0	10.9	7.0
Grams uric acid recovered (duplicates)	0.0507	0.0352	0.0089	0.0359	0.0285	0.0342	0.0231
	0.0293	0.0494	0.0095	0.0359	0.0290	0.0400	0.0260
Grams uric acid the solution should have contained (duplicates)	0.0856	0.08	0.0856	0.08	0.08	0.08	0.08
	0.0856	0.08	0.0856	0.08	0.08	0.08	0.08

TABLE III.—TWO GRAMS URIC ACID IN 500 CC. WATER CONTAINING SIX GRAMS PIPERAZINE.

	I. 3 days.	II. 9 days.	III. 13 days.	IV. 17 days.	V. 22 days.
Cc. $KMnO_4$ consumed (duplicates)	24.0	22.1	19.9	18.6	15.3
	23.9	22.0	20.0	18.4	15.4
Grams uric acid recovered (duplicates)	0.088	0.081	0.0733	0.068	0.059
	0.087	0.080	0.0729	0.068	0.0579
Theory	0.1	0.1	0.1	0.1	0.1
	0.1	0.1	0.1	0.1	0.1

In Series I, II and III in Table I, the permanganate solution was 0.74375 $N/20$. It was found that the uric acid had a tendency to separate from solution after the addition of the Folin-Schaffer reagent, so dilution with 50 cc. of water was tried at this stage and an aliquot portion of the resulting mixture (100 cc.) was used. In II, III and V, this dilution was made but as it did not seem to affect the crystallization, it was abandoned without further trial. In IV, V, VI and VII, 0.73155 $N/20$ $KMnO_4$ was used. In IV, one sample was lost entirely and freshly prepared Folin-Schaffer reagent was used in which all the ammonium sulfate had not dissolved. This lack of ammonium sulfate might possibly account for the results obtained. In VIII and IX, 0.99416 $N/20$ $KMnO_4$ was used. In IX, also only one sample was taken, hence the blank in the table. In the duplication of this work (Table I) the amount of uric acid found uniformly ran higher. This might be accounted for by the fact that a different preparation of uric acid was used. There was also no tendency of the acid to crystallize out as in the first work. The permanganate used was 0.978 $N/20$.

In Table II, the permanganate used was 0.743575 $N/20$ in I, 0.73155 $N/20$ in II, III, IV, and V, 0.99416 $N/20$ in VI and VII. In I and II, dilution was made to prevent the crystallization of the uric acid but nothing was gained; the uric acid continued to separate from solution.

In Table III, since a quite concentrated solution was being used, it was necessary to follow a slightly different treatment. Only 25 cc. portions were pipetted out and these were diluted to 225 cc. to prevent the precipitation of the uric acid on the addition of the Folin-Schaffer

reagent. The amount of this reagent was 30 cc. instead of 25 cc. as in the former work. It was found that with solutions of this concentration, unless this precaution of dilution was taken the uric acid was completely thrown out of solution on the addition of the reagent. Sufficient dilution prevented any precipitation whatever and it was not necessary to filter in this case.

The amount of ammonium hydroxide was increased from 5–10 cc. in order to keep a constant ammonium hydroxide concentration. The residue was taken up in 200 cc. boiling water and acidified with 30 cc. concentrated sulfuric acid before titration.

On examination of these tables, it is evident that uric acid was attacked in a piperazine solution. In all three solutions of different strengths there were losses of uric acid that can be accounted for in no way except through the action of piperazine. Conditions were uniform in every case and any error in the method would remain constant throughout. Even with only two days' action, there was quite an appreciable loss of uric acid. These experiments extended over periods of from one to forty-two days and in the main the decomposition seemed to gradually continue throughout the period of the investigation. We found on maximum beyond which all action ceased.

A comparison of the concentrations of the piperazine solutions will show that the solution used in Table III was just twice as great as that of the solution used in Table I. The solution used in Table II was weaker than either of the former. The amount of uric acid involved was the same in each case. Corresponding to the difference in concentration of the solvent, no change in the amount of uric acid recovered was noted. This amount grew uniformly less in all three cases, according to the time through which action was allowed to continue. There was no marked increase in the decomposition induced by the increased piperazine concentration, but it continued at about the same rate in all cases. The destruction seemed to continue independent of the concentration of the solvent.

The second solvent whose destructive action on uric acid was studied was urotropin. As stated previously this did not prove to be a very good solvent and even in small quantities, with a reasonably concentrated solution of urotropin, considerable agitation was necessary to effect solution. In the first solution the attempt was made to dissolve 1 gram of uric acid in one liter of water containing 10 grams urotropin. Only a small fraction of the acid dissolved and it was necessary to filter into a flask and take samples immediately in order to determin how much uric acid had gone into solution.

The same method was followed in this work as in the work with piperazine except that it was necessary to filter on the addition of the Folin-

Schaffer reagent to some of the piperazine urate solutions while in the case of the urotropin solutions, it was never necessary to filter the solution at this stage. A yellow residue insoluble in hot water was obtained on the final precipitation of uric acid as ammonium urate. Solutions of only two different concentrations were studied but the results obtained were sufficient to indicate the action. The following tables show the results obtained.

TABLE IV.— LESS THAN ONE GRAM URIC ACID DISSOLVED IN 1000 CC. CONTAINING 10 GRAMS UROTROPIN.

	I. 1 day.	II. 7 days.	III. 14 days.
Cc. $KMnO_4$ consumed (triplicates)	3.8	3.8	3.8
	3.8	3.8	3.8
	3.5	3.8	3.8
Grams uric acid recovered	0.0137	0.0137	0.0137
	0.0137	0.0137	0.0137
	0.0128	0.0137	0.0137

TABLE V.—0.1 GRAM URIC ACID IN 400 CC. WATER CONTAINING 6 GRAMS UROTROPIN.

	I. 7 days.	II. 14 days.
Cc. $KMnO_4$ consumed (duplicates)	4.4	4.6
	4.6	4.6
Grams uric acid recovered	0.0161	0.0168
	0.0168	0.0168
Theory	0.02	p.02
	0.02	0.02

From the results it seemed that urotropin had no effect on uric acid under the conditions employed. In the first case where the exact acid content was not known, samples were taken the same day. Those taken a week and even two weeks later showed exactly the same amount of uric acid present as there was in the first place. There could have been no destructive action exerted by the urotropin. In the second solution, no sample was taken the first day, as the exact uric acid content was known; but after seven and fourteen days respectively there was still the same amount of acid present. The titration does not check with the actual amount of uric acid known to have been present, but if the correction is made for the solubility of uric acid in washings, etc., part of this loss may be accounted for. There was a 100 cc. filtrate on the precipitation of uric acid with ammonium hydroxide and 100 cc. 10 per cent. ammonium sulfate solution were used in washing.

The strength of the urotropin solution did not seem to affect the destruction of uric acid to any marked extent. An increase of 5 grams solid urotropin per 1000 cc. of solution brought the same results as far as destructive action was concerned. Basing conclusions on these results, it would seem that in cold solutions urotropin did not have the power of

splitting uric acid. Further, the concentration of the solvent in no way changed its action on uric acid to bring about decomposition under such conditions as were used.

Decompositions by Means of Ammonia.—After studying the extent of action of a few alkaline solvents on uric acid, it seemed desirable to study the action of ammonia with regard to the decomposition it may bring about with uric acid, both in the presence and absence of ammonium salts. That ammonia alone has the power to split uric acid is quite well known. A few experiments were made in this laboratory to gain some idea of the extent of its decomposition at room temperature. Dilute ammonia (1 per cent.) was used in one instance in an attempt to dissolve 1 gram of the acid. The ammonium hydroxide was added to small portions of the acid at a time so that the disappearance of the acid would show that some small amount had dissolved. The uric acid was only slightly soluble in ammonium hydroxide. The solution was filtered and 100 cc. samples were taken the same day. It was found that on the addition of the Folin-Schaffer reagent a heavy white precipitate separated and no further precipitate could be obtained after filtering, on the addition of concentrated ammonia. To prevent the separation of the acid on the addition of the reagent, the solution was barely neutralized with acetic acid. After this treatment no precipitate at all was formed. The entire solution was then treated with 5 cc. of concentrated ammonium hydroxide. At this stage there resulted a heavy yellowish precipitate that for the most part was soluble in hot water, a condition quite contrary to that obtained when piperazine and urotropin were used as solvents. This residue was practically completely dissolved in 100 cc. of boiling water and on titrating with permanganate only three drops sufficed to give a very distinct color to the solution, thus showing that practically all the uric acid had disappeared prior to the titration. The heavy precipitate previously mentioned must have been a decomposition product of uric acid; at any rate, the substance did not consume permanganate to any extent.

A second solution of 1 per cent. ammonium hydroxide was made to completely dissolve 0.2 gram uric acid. This was best accomplished by agitation and by the addition of ammonium hydroxide in small quantities and decanting off from time to time to a graduated flask. Samples of this solution were run the next day with exactly the same result as before. The entire amount of uric acid as such had disappeared, since three drops of the standard permanganate solution produced a permanent color.

The next phase of the work had to do with the determination of uric acid in silver magnesium urate by the common methods and another method involving the use of ammonia. Having noted the use of sodium

sulfide in place of hydrogen sulfide in the decomposition of silver urate (the Salkowski modification of the Ludwig H_2S-silver urate method for the determination of uric acid) it seemed desirable to prepare some silver magnesium urate and allow weighed quantities of this urate to be acted on respectively by H_2S, Na_2S and $(NH_4)_2S$.

In the preparation of this silver urate about 10 grams of Merck's uric acid were dissolved in the least quantity of cold dilute sodium hydroxide necessary for complete solution. The resulting solution, about one liter in volume, was filtered into a tall gas generator cylinder and was treated with equal volumes of magnesia mixture and ammoniacal silver nitrate as long as a heavy white precipitate settled out. The mixture was allowed to stand until the precipitate of silver magnesium urate settled. The supernatant liquid was siphoned off, replaced by dilute ammonia, stirred, allowed to settle and the second supernatant liquid was siphoned off. This was the best way we found for washing the precipitate free from impurities so this washing procedure was repeated several times. The white precipitate was filtered by means of suction, using a porous plate, and dried. The dry urate was pulverized in a mortar and run through a 100-mesh sieve. To insure like conditions up as far as the treatment with the respective sulfides, the urate thus obtained was used as starting material. This was granular, finely divided and a pure white color.

The magnesia mixture contained 100 grams magnesium chloride dissolved in dilute ammonium hydroxide and treated with solid ammonium chloride sufficient to dissolve the precipitate formed. The resulting mixture was diluted to one liter.

The ammoniacal silver nitrate was made as follows: 26 grams of silver nitrate were dissolved in water and enough ammonium hydroxide was added to completely dissolve the precipitate formed. The solution was then diluted to one liter.

The sodium sulfide used in the Salkowski modification of the Ludwig H_2S method was made by dissolving 10 grams of sodium hydroxide in a liter of water. One-half of this solution was saturated with hydrogen sulfide and then mixed with the other half of the solution.

After the preparation of several grams of the silver magnesium urate the three reagents H_2S, Na_2S and $(NH_4)_2S$ corresponding to the Ludwig, Salkowski and the authors' methods respectively were allowed to react to precipitate silver as the sulfide and liberate the uric acid in a separable form.

The Ludwig Method.—In this method 0.2 gram of our urate was accurately weighed and placed in a beaker containing 100 cc. water. The mixture was heated to near boiling and allowed to react while still hot with a current of hydrogen sulfide. The heating and passage of the gas

was continued for several minutes. During this time the white gritty precipitate was replaced by the usual black silver sulfide precipitate. However it was only after repeated heatings and saturations with hydrogen sulfide that the complete conversion of silver urate to silver sulfide was brought about. The resulting solution was filtered and the residue was washed with hot hydrogen sulfide water. On evaporation to about 100 cc. volume and acidification with a few drops of concentrated hydrochloric acid, a heavy white precipitate of uric acid separated. On standing over night the solution yielded more uric acid that was filtered and washed slightly with 100 cc. cold water. By means of hot water the uric acid precipitate was allowed to dissolve and drain back from the filter to the original precipitation beaker. After adding 30 cc. concentrated sulfuric acid the resulting slightly warm solution was titrated immediately with a standard permanganate solution.

By the Salkowski-Ludwig method, weighed quantities of the urate (0.2 gram) were suspended in 100 cc. portions of water in respective beakers. On being heated to near boiling the mixtures were treated with the sodium sulfide solution until an excess was present. This was indicated by the odor. The mixture was maintained at near boiling on a water bath for ten minutes. Meanwhile, by gradual stirring with a rod, the granules were broken up and more thoroughly converted to sulfide. The black solution was then filtered and given the same treatment as in the case of the use of the Ludwig method.

By the general deportment of ammonia and uric acid, it was suspected that ammonium sulfide used to replace sodium or hydrogen sulfide might have a tendency to cause the loss of some uric acid in any determination employing that reagent. Hydrogen sulfide seemed to us to not act vigorously enough in the conversion of silver urate to silver sulfide. The sodium sulfide method, while getting around the problem of complete conversion of sulfide, introduced an error due to the subsequent action of the alkali on the uric acid. Thinking the use of ammonium sulfide would get around this alkaline decomposition at least to an extent worthy of a method, the authors developed and used the following method with more or less success.

Equal portions of the urate were suspended in about 100 cc. of water and treated with ammonium sulfide until the mixture smelled strongly of ammonia. (The sulfide was the ordinary yellow sulfide; the amount of silver urate used in all cases was 0.2 gram.) The solution was then heated on a water bath for about 10 minutes with constant stirring until the silver was completely precipitated as silver sulfide. This was shown by the thorough blackening of the precipitate and the change from a granular to a flocculent form. The solution was allowed to cool slightly and then filtered. The residue was washed with boiling water until the total

volume of the filtrate was about 300 cc. A few drops of concentrated hydrochloric acid were added to the filtrate and this was concentrated to about 75 cc. when 15–20 drops more of hydrochloric acid were added and the solution was allowed to stand 18 hours when it was filtered. It was at this point that the difficulty in the process arose. On account of the excess of sulfur that had separated from solution, it was found impossible to dissolve all the uric acid in hot water. The sulfur seemed to prevent the contact between uric acid and water and it also prevented sharp end points in the subsequent titrations. Hence a scheme was devised to eliminate the sulfur by a mixed solvent in which uric acid was insoluble. Some work was done using carbon disulfide alone as a solvent but it was found that a mixture of equal parts carbon disulfide and chloroform seemed better as a sulfur solvent under the conditions. (By actual test under similar titration conditions sulfur did not consume permanganate nor have any influence on the sharpness of the end point. Uric acid mixed with sulfur gave a very uncertain end point with permanganate.)

The uric acid sulfur residues were washed with hot water and the washings were allowed to reach a maximum volume of 100 cc. Then the precipitation beaker still containing some sulfur and uric acid and the filter containing the main portion of the precipitate were dried thoroughly. The sulfur was then dissolved by means of 75–100 cc. of CS_2–$CHCl_3$ mixture that had been heated almost to its boiling point. The washings were discarded and the beaker and filter were again dried thoroughly. After this treatment the uric acid could easily be taken up in boiling water and titrated. Sharp end points were almost always obtained. Just before the addition of any permanganate the uric acid was dissolved in dilute potassium hydroxide. This was necessary for a sharp end point in the titration.

After working out the details for the determination of uric acid by each of the three methods, equal weighed quantities of the magnesium silver urate were taken and in these samples the uric acid was determined four times by each method, besides two determinations by the authors' method using carbon disulfide alone as a sulfur solvent. The uric acid residues were titrated with the same permanganate solution but the results were not calculated. There was no account taken of loss through washings and dilution, as exactly the same conditions prevailed in all three methods and any errors from that source were equal.

From a comparison of the results of these methods it will be noted that in our hands the hydrogen sulfide treatment was far superior to either of the others. The destructive action of the alkali was distinctly noticeable in both the sodium sulfide and ammonium sulfide treatments. These two checked very closely and the ammonia method gave slightly

higher results than the sodium method but perhaps the higher results would not justify the extra difficulty involved.

TABLE VI.—0.2 GRAM SILVER MAGNESIUM URATE INVOLVED.

	H_2S method. Cc.	Na_2S method. Cc.	$(NH_4)_2S$ method. Cc.
Cc. Standard $KMnO_4$ consumed	26.9	22.6	22.5
	27.0	22.7	22.8
	27.0	22.6	22.8
	27.0	22.6	22.7
	..	..	22.7[1]
	..	..	19.0[1]

The Influence of the Presence of Neutral Salts.—The Folin-Schaffer method for the determination of uric acid is based upon the fact that it is possible to precipitate uric acid as ammonium urate in the presence of ammonium sulfate without any loss of acid by the alkaline decomposition. The ammonium sulfate in the Folin-Schaffer reagent not only has a salting-out effect but it also prevents the destruction of uric acid.

Some time was given by the authors to an investigation of the action of piperazine on uric acid while various salts were present. The piperazine solutions were of the same strength as those used in the former experiments and consisted of 1 gram uric acid dissolved in a liter of water containing 3 grams piperazine. The piperazine was dissolved in about 400 cc. water and the weighed amount of uric acid was washed into this solution. At once the acid dissolved; the solutions were diluted to about 900 cc. in graduated liter flasks. The inorganic salts used were ammonium sulfate, disodium hydrogen phosphate and sodium chloride. Ten grams each of the above salts were carefully weighed and added to different piperazine urate solutions, only one neutral salt being allowed to be present in any one urate solution at a time. The resulting solutions were each diluted to the marks on the graduated flasks. On the addition of the ammonium sulfate the uric acid was largely thrown out of solution and on standing 24 hours still more uric acid had separated. The sodium monohydrogen phosphate did not completely dissolve and on standing 24 hours part of the material already in solution had separated from the solution. On the addition of the sodium chloride, solution was complete but after standing 24 hours an appreciable wart-like sediment had formed. Only one organic salt was used; this was lycetol, which is the tartrate of piperazine. The piperazine solution was prepared in the same way as the former ones and 5 grams lycetol were added. The latter dissolved practically completely. After 24 hours' standing, there was a slight separation of warty nodules.

Each solution of piperazine urate containing a neutral salt was allowed to stand 24 hours and then was filtered, the filtrate being preserved for

[1] The last two results were obtained with the use of CS_2 alone.

future use. In order to determine how much uric acid did not go into solution, the residue and original flasks were washed with four small portions (25 cc. each) of cold water and then dissolved in about 500 cc. of boiling water. It was impossible to dissolve in 500 cc. hot water all the uric acid that had separated from the ammonium sulfate solution since the amount of uric acid involved was large. This residue however was washed into a beaker and dissolved with about 90 cc. concentrated sulfuric acid and this solution was added to the contents of the original flask. It was titrated with standard permanganate. The other three uric acid residues dissolved completely on the filter in hot water and these solutions were titrated at once after the addition of 75 cc. of concentrated sulfuric acid to each solution.

After two days' standing, samples of 100 cc. each were taken from each original filtrate that had been set aside previously. It was found that no further separation of solids had taken place except in the case of the sodium chloride solution, where it was necessary to filter again. To each of the samples taken, 15 grams solid ammonium sulfate and 5 cc. concentrated ammonium hydroxide were added. A heavy white precipitate was formed in each solution except in those samples taken from the ammonium sulfate solution. The various ammoniacal (NH_4OH) solutions were allowed to stand 24 hours when they were filtered and the residues were each washed with 100 cc. of 10 per cent. ammonium sulfate solution. The residues were then dissolved in about 200 cc. of hot water; after the addition of 30 cc. concentrated sulfuric acid to each solution, titrations were made with standard permanganate.

The same filtrate that furnished the two-day samples was also used to furnish samples after five more days' standing. These samples were taken and their uric acid content examined according to the directions just described. Still more uric acid had separated from the sodium chloride solution, so much that it was impossible to tell the exact uric acid content of the portion of solution involved.

TABLE VII.

	$(NH_4)_2SO_4$.	Na_2HPO_4.	NaCl	Lycetol.
Grams uric acid undissolved in 1000 cc.	1.0823	0.0164	0.0452	0.0148
Grams uric acid found per 100 cc. after two days	none	0.09248	0.09404	0.10028
(duplicates)	none	0.09248	0.09248	0.10068
Grams uric acid found after seven days. Per 100	none	0.0717	...	0.0846
cc. (duplicates)	none	0.0717	...	0.0839

At the outset 1 gram of uric acid had been placed in each of the piperazine solutions containing the respective neutral salts.

The results of this part of the work were not as satisfactory as we would have liked. It was very difficult to carry the work out quantitatively and the process itself depended on quantitative work to show

the qualitative nature of the results. From the actual figures it would seem that we recovered in several cases more uric acid than we started with. We had no reason to doubt the factor on the permanganate solution used and it was not impossible that we have incorporated from the filtrations certain impurities that used up permanganate thus making our results, in terms of uric acid, run high. In any event, uric acid tended to separate from solution. Fairly concordant results were obtained for the uric acid content after two days. After seven days' standing, the results of the determinations showed a loss of uric acid and this information in itself was very desirable. Any error in the factor of the permanganate solution would have been uniform, for every effort was made to see that the respective uric acid solutions and residues were given the same treatment throughout the work, except where indicated. The presence of a salt did not entirely prevent the destruction of some uric acid in piperazine solutions. Ammonium sulfate seemed to completely salt out the uric acid; when once out of solution the piperazine seemed unable to act on the uric acid. Sodium chloride seemed to work in the same direction. Na_2HPO_4, NaCl and lycetol prevented the initial destruction of uric acid but with continued action, destruction of uric acid resulted to a quite marked extent.

Summary.

From the results of this work, it is quite evident that piperazine had a marked ability to dissolve uric acid in the cold. Urotropin, lycetol and ammonium hydroxide, although acting as bases, were poor uric acid solvents.

Piperazine, ammonium sulfide and ammonium hydroxide had marked destructive ability uricolytically, in the absence of neutral salts. Urotropin seemed to have but little effect on uric acid under the conditions employed. In the presence of certain neutral salts, decompositions of uric acid dissolved in piperazine were not so vigorous as in the absence of the salts. However, with continued action, there was a destruction of some of the uric acid.

In the boiling aqueous ammonium sulfide solution there seemed to be no reaction with uric acid to form thiouranil. Ammonium sulfide decomposed uric acid however to an extent nearly equal to that produced by sodium sulfide.

The work involved in this paper was done as partial fulfilment of the requirements in chemistry for the degree Master of Arts at Indiana University and this paper embodies the results of the investigation as presented in the thesis. The authors were particularly interested in any decompositions uric acid might suffer while using piperazine as a solvent, since piperazine is used so largely in medicine as a good uric acid solvent.

The neutral salts selected were salts that would naturally be present in the living tissues.

Bibliography.

1. Ueber die quantitative Bestimmung der Harnsäure in Harn: Folin and Schaffer, *Z. physiol. Chem.*, **32**, 552.

2. The Uricolytic Enzyme: Austin, *J. Med. Research*, **16**, (N. S. 11) 71.

3. Ueber das Uricolytische Ferment: Schittenhelm, *Z. physiol. Chem.*, **45**, 161.

4. Determinations of Uric Acid: Hoppe Seyler-Thierfelder, *Handbuch der Chem. Analyse*, (eighth edition) page 587.

5. Solubility of Piperazine Urate: (*Ber.*, **23**, 3718); also Richter, *Organische Chemie*, eleventh edition, 1909, Bd. I, p. 641.

6. A Note on the Behavior of Uric Acid toward Animal Extracts and Alkalies: (Protection of Uric Acid from Alkali by Presence of Protein), by Mitchell, *J. Biol. Chem.*, **3**, 145.

7. The Ludwig-Salkowski Determination of Uric Acid: Hammarsten, *Textbook of Physiological Chemistry*, first English of sixth German edition, pages 576–7.

BLOOMINGTON, IND.

[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY, U. S. DEPT. OF AGRICULTURE.]

AN ELECTRICALLY HEATED VACUUM FRACTIONATION APPARATUS.

BY H. S. BAILEY.

Received December 23, 1910.

In developing methods for the analysis of essential oils, it was found desirable to improve, if possible, upon the ordinary apparatus for fractional distillation *in vacuo* and, as a result of a year or more experimentation with various styles of apparatus, the following has finally been adopted.

The suggestion made by Richards,[1] that fractional distillations can be carried on much more expeditiously by the use of a heating coil within the distillation flask itself than when the heat is applied externally, led us to use a flask of the style shown in the accompanying sketch, A.

For working with 50 cc. portions of oil, this should have a capacity of approximately 150 cc. The heater B is a small coil of German silver or nichrome wire attached to platinum leads which in turn are sealed into the bottom of the flask. As it was desirable to distil off all but the last 10 per cent. (5 cc.) from the sample of oil and to leave as near as possible exactly this amount in the flask, it was necessary to have the bottom of the container as small as practicable. For this reason, it was made long and narrow, the 5 cc. mark being a little above the top of the heater. A fractionating head, C, of the Ladenberg type, is attached to the flask by a ground joint, the upper end of this head being extended some distance above the outlet tube so that the stopper through which

[1] THIS JOURNAL, 30, 1282; 31, 1200.