

gr. 1.125) should be added to the solution resulting from the action of extract of malt on the material under examination. After heating for one hour in a flask immersed in a boiling water-bath, making allowance for the time required for the solution to attain the temperature of the bath, the solution is cooled, enough sodium hydroxide is added to neutralize 90 per cent. of the hydrochloric acid used, the solution made up to a definite volume, filtered on a dry filter, if necessary, and the reducing power determined by Fehling's solution. 100 parts of glucose found in this manner represent 93 parts of starch in the original material. *The chemist should determine for himself, with pure glucose, the ratio between glucose and copper oxide or copper for the solutions and method which he uses.*

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## THE BEHAVIOR OF URIC ACID IN THE URINE AND THE EFFECT OF ALKALIES ON THE SOLUBILITY OF URIC ACID IN THE URINE.<sup>1</sup>

BY FRANCIS H. MCCRUDDEN.

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WHEN urine cools, a precipitate of uric acid, or sodium urate, or a mixture of the two, very frequently occurs. But not all the uric acid precipitates. The cause for this precipitation, and the factors which determine how much uric acid shall remain in solution after equilibrium is reached, have always been points of controversy. The subject is one of very great importance in medicine. Theories of gout and the uric acid diathesis and methods of treatment in these diseases have often been evolved from a study of the behavior of the uric acid in the urine.

At first the precipitation was thought to be due to a simple decrease in solubility on cooling, and that the greater the quantity of uric acid present the larger the precipitate. Later it was supposed that an increase in the acidity of the urine takes place on cooling, changing the sodium urate to insoluble uric acid. Again, the alkaline sodium phosphate was supposed to play the rôle of a uric acid solvent. The quantity of alkaline sodium phosphate present, the ratio between the alkaline and the acid sodium phosphate, the quantity of salts present, the amount of coloring-mat-

<sup>1</sup> Read at the St. Louis Meeting of the American Chemical Society.

ter, and other factors have all been supposed by different authors to have some bearing on the matter. None of the theories have explained the behavior of uric acid in the urine. The effect of alkalis on the solubility of uric acid in the urine has also been the subject of a great deal of controversy, and different experimenters have come to entirely different conclusions.

The view most generally held is that in the warm urine, uric acid is present as sodium acid urate. As the urine cools, the alkaline sodium phosphate changes to acid sodium phosphate, and this latter changes the sodium urate to uric acid. The uric acid is insoluble and therefore precipitates. The fact that when cold urine has stood for several hours the amount of uric acid in solution is very much greater than the solubility of uric acid would lead us to expect, has been attributed to the tendency of uric acid to become colloidal.

This explanation cannot be accepted. His<sup>1</sup> has shown that when a colloidal solution of uric acid is shaken with solid uric acid, the colloid precipitates. When a cold urine is shaken with solid uric acid until equilibrium is reached, there is still, according to Klemperer,<sup>2</sup> more uric acid in solution than the solubility of uric acid warrants.

An explanation of the behavior of uric acid in the urine is offered if we turn to physical chemistry for assistance. The affinity constant,  $k$  in the equation

$$\frac{C_H \times C_U}{C_{HU}} = k \text{ is } 0.0000151$$

according to His and Paul.<sup>3</sup> ( $C_H$  stands for the concentration of the hydrogen ions,  $U$  for the concentration of the negative uric acid ions and  $C_{HU}$  for the undissociated uric acid.)  $C_{HU}$  is a constant, equal, at 18°, to 0.0001363.<sup>1</sup> Then

$$\frac{C_H \times C_U}{0.0001363} = 0.0000151,$$

or the solubility product<sup>1</sup>

$$C_H \times C_U = 0.0000151 \times 0.0001363 = 206 \times 10^{-12}.$$

<sup>1</sup> W. His, jun.: "Die Harnsäureablagerungen des Körpers und die Mittel zu ihrer Lösung." Vortrag. auf der Versammlung Deutscher Naturforscher und Aerzte in Hamburg. *Therapie der Gegenwart*, Neue Folge, 3, 434 (1901).

<sup>2</sup> G. Klemperer: "Untersuchungen über die Lösungsverhältnisse der Harnsäure im Urin." Verhandlungen des XX Kongresses für innere Medizin zu Wiesbaden (1902).

W. His and T. Paul: "Physikalische-chemische Untersuchungen über das Verhalten der Harnsäure und ihrer Salze in Lösungen 1 Abhandlungen." *Ztschr. physiol. Chem.*, 31, 1 (1900-1901).

In the 1500 cc. of urine daily excreted, there is on the average 0.75 gram uric acid excreted in the form of sodium acid urate. This is 0.5 gram per liter, or, dividing by the molecular weight, about 0.003 gram-molecule per liter. A salt in such dilute solution is almost completely dissociated, so that in an aqueous solution of sodium acid urate of the concentration found in the urine,  $C_U$  in equation (1) becomes 0.003. Then

$$C_H \times 0.003 = 206 \times 10^{-12}$$

or

$$C_H = \frac{206 \times 10^{-12}}{0.003} = 6.7 \times 10^{-8}.$$

If 2 grams uric acid per day were excreted, a rather high limit,  $C_H$ , would be about  $2 \times 10^{-8}$ . If only 0.1 gram uric acid were excreted per day with the ordinary amount of urine, or if in a diseased condition the amount of urine should increase to 4 liters and only 0.4 gram uric acid were excreted,  $C_H$  would increase to  $34 \times 10^{-8}$ . In other words, in an aqueous solution of sodium urate of the concentration of average urine we can have hydrogen ions present in a concentration of only about  $7 \times 10^{-8}$ . With the ordinary variations in the amount of urate present in urine, this figure would vary from about  $4 \times 10^{-8}$  to  $15 \times 10^{-8}$ . In solutions of sodium urate of the concentration of urine in extreme pathological cases, it might vary from  $2 \times 10^{-8}$  to  $35 \times 10^{-8}$ . If the concentration of the hydrogen ions increases beyond the limit set by the equation

$$C_H \times C_U = 206 \times 10^{-12},$$

then undissociated uric acid will form, and precipitation of uric acid will occur.

The average acidity of urine expressed by the concentration of the hydrogen ions is  $300 \times 10^{-8}$  according to Rohrer,<sup>1</sup> and  $150 \times 10^{-8}$  according to Höber and Jankowsky.<sup>2</sup> Rohrer found the value to vary in different urines from  $40 \times 10^{-8}$  to  $510 \times 10^{-8}$ . Höber and Jankowsky obtained results varying from  $100 \times 10^{-8}$  to  $1000 \times 10^{-8}$ . In a very few pathological cases the value was slightly outside these limits. We can see then why uric acid should precipitate from cold urine. The concentration of the hydrogen ions is too great to permit the presence of the large

<sup>1</sup> L. Rohrer: "Die Bestimmung der Harnacidität auf elektrometrischem Wege." *Pflüger's Arch.*, 86, 586 (1901).

<sup>2</sup> Höber and Jankowsky: "Die Acidität des Harns vom Standpunkt der Ionenlehre." *Beiträge zur chemischen Physiologie und Pathologie*, 3, 525 (1903).

quantity of negative uric acid ions present, so precipitation of uric acid occurs. The fact that uric acid does not always precipitate immediately from the cold urine is due, as His<sup>1</sup> has shown, to the fact that the insoluble uric acid goes into the colloidal condition and does not precipitate for some time. Even when hydrochloric acid is added to the solution, some of this insoluble uric acid is not precipitated for weeks. Pfeiffer,<sup>2</sup> His,<sup>1</sup> and Klemperer<sup>3</sup> have shown that this colloidal uric acid can be precipitated by shaking the solution thoroughly with solid uric acid.

Klemperer showed that when urine is thoroughly shaken with solid uric acid a variable part, but not the whole of the uric acid present, is precipitated from solution. An explanation of this fact is easily given. The acidity of urine as determined by titration, that is, the "potential acidity," is about 10,000 times as great as the real acidity. As soon as the "actual" hydrogen ions present are used up by titration with alkali, more of the "potential" hydrogen ions present are set free to preserve the equilibrium indicated by the equation

$$\frac{C_H \times C_A}{C_{HA}} = k,$$

where  $C_{HA}$  is used to indicate the acid or acid bodies to which urine owes its acidity,—probably chiefly  $\text{NaH}_2\text{PO}_4$ . The same thing occurs when the "actual" H ions are carried out of solution by precipitation as uric acid. If merely enough uric acid disappears from solution to use up the "actual" hydrogen ions present, the "actual" acidity of the solution remains practically unaffected, for immediately more H ions are set free from the undissociated HA until equilibrium is reached.

In the equation

$$\frac{C_H \times C_{\text{HPO}_4}}{C_{\text{H}_2\text{PO}_4}} = k,$$

we know that  $C_{\text{H}_2\text{PO}_4}$  is large in comparison with  $C_H$  and  $C_{\text{HPO}_4}$ . Then for small changes in  $C_{\text{H}_2\text{PO}_4}$  due to setting free of H and  $\text{HPO}_4$  to establish equilibrium after disappearance of H on account of precipitation of uric acid, we can consider  $C_{\text{H}_2\text{PO}_4}$  prac-

<sup>1</sup> W. His: "Die Harnsäureablagerung des Körpers und die Mittel zu ihrer Lösung." *Therapie des Gegenwart*, Neue Folge, 3, 434 (1901).

<sup>2</sup> E. Pfeiffer: "Zur Aetiologie und Therapie der Harnsäuren Steine." *Verh. des 5 Kongresses für innere Medizin*, Wiesbaden, 1886, p. 444.

<sup>3</sup> G. Klemperer: "Untersuchungen über die Lösungsverhältnisse der Harnsäure in Urine." *Verhandlungen des 10 Kongresses für innere Medizin*, Wiesbaden, 1903, p. 219.

tically constant. We know that on account of the presence of  $\text{Na}_2\text{HPO}_4$  largely dissociated to  $\text{Na}_2$  and  $\text{HPO}_4$ ,  $C_{\text{HPO}_4}$  is large in comparison with  $C_{\text{H}}$  so that for small changes in  $C_{\text{HPO}_4}$  due to the further dissociation of  $\text{H}_2\text{PO}_4$  to establish equilibrium after disappearance of the "actual" H ions,  $C_{\text{HPO}_4}$  may be considered practically constant. If we consider

$$\frac{C'_{\text{H}} \times C'_{\text{HPO}_4}}{C'_{\text{H}_2\text{PO}_4}} = K$$

the initial equation, then after precipitation of a very little uric acid, we have the new equation

$$\frac{C''_{\text{H}} \times C''_{\text{HPO}_4}}{C'_{\text{H}_2\text{PO}_4}} = K,$$

where  $C''_{\text{HPO}_4} = C'_{\text{HPO}_4}$ , approximately and  $C''_{\text{H}_2\text{PO}_4} = C'_{\text{H}_2\text{PO}_4}$ , approximately. Therefore  $C_{\text{H}''} = C_{\text{H}'}$  approximately. In other words, after a small amount of uric acid has precipitated, carrying away all of the "actual" H ions, the concentration of the H ions is almost the same as before, so that the solubility constant for uric acid is still exceeded, and more uric acid precipitates. After considerable quantities of uric acid have precipitated, the loss of H ions from the  $\text{H}_2\text{PO}_4$  will decrease the value for  $C_{\text{H}_2\text{PO}_4}$ , and the  $\text{HPO}_4$  ions formed at the same time will increase the value for  $C_{\text{HPO}_4}$ . Then  $C_{\text{H}}$  must decrease in order to maintain equilibrium. Obviously a time will come when this decrease in concentration of the H ions and the decrease in concentration of the negative U ions brought about through loss of uric acid by precipitation will be so great that  $C_{\text{H}} \times C_{\text{U}}$  will be less than  $206 \times 10^{-12}$ . Then no more uric acid will precipitate.

Let us take the value for normal urine  $C_{\text{H}} = 150 \times 10^{-8}$ , and the amount of uric acid excreted as 0.75 gram in 1000 cc. urine. Then  $C_{\text{U}}$  (gram-molecules of urate per liter) = 0.003. Let us suppose that by spontaneous precipitation of uric acid or by addition of alkali,  $C_{\text{H}}$  has decreased to  $15 \times 10^{-8}$  or to 0.1, its initial value. Then  $C_{\text{H}} \times C_{\text{U}} = 206 \times 10^{-12}$  because  $15 \times 10^{-8} \times C_{\text{U}} = 206 \times 10^{-12}$  and

$$C_{\text{U}} = \frac{206 \times 10^{-12}}{15 \times 10^{-8}} = 15 \times 10^{-4} = 0.0014.$$

In this case equilibrium is reached when the acidity of the urine has been decreased to 0.1 of its initial value and 0.5 of the uric acid has precipitated.

Ritter,<sup>1</sup> it will be remembered, found that the equilibrium is reached more quickly, that is, less uric acid is precipitated, when large quantities of  $\text{Na}_2\text{HPO}_4$  are present than when small quantities of  $\text{Na}_2\text{HPO}_4$  are present. This is easy to understand. The addition of  $\text{Na}_2\text{HPO}_4$  means the addition of  $\text{HPO}_4$  ions. The formation of  $\text{H}_2\text{PO}_4$  by the union of even a large part of the H ions present with some of this extra  $\text{HPO}_4$  would scarcely affect the total value for  $\text{H}_2\text{PO}_4$  since  $\text{H}_2\text{PO}_4$  is so large in comparison with H. Therefore  $C_H \times C_{\text{HPO}_4} = K \times C_{\text{H}_2\text{PO}_4} = \text{a constant}$  in this case. Then addition of  $\text{HPO}_4$  will decrease  $C_H$  in the same ratio as  $C_{\text{HPO}_4}$  increases. Further we know that  $\text{Na}_2\text{HPO}_4$  is alkaline. That is, a solution of  $\text{Na}_2\text{HPO}_4$  contains OH ions. (This is due to the fact that the reaction  $\text{Na}_2^{++} + \text{HPO}_4^{--} + \text{H}^+ + \text{OH}^- = \text{Na}_2^{++} + \text{H}_2\text{PO}_4^- + \text{OH}^-$  takes place to some extent.) On addition of a solution of  $\text{Na}_2\text{HPO}_4$  to one of  $\text{NaH}_2\text{PO}_4$ , the reaction  $\text{H}^+ + \text{OH}^- = \text{HOH}$  takes place, thus still further decreasing the concentration of the H ions. Therefore, the value of  $C_H$  in the equation

$$C_H \times C_U = 206 \times 10^{-12}$$

will be reached sooner when  $\text{Na}_2\text{HPO}_4$  is added to the solution, and will necessitate the precipitation of a smaller quantity of uric acid before equilibrium is reached.

The effect of the addition of a small quantity of alkali to a cold urine would be the same as the effect of a slight precipitation of the uric acid. The hydroxyl ions from the alkali would combine with the "actual" H ions in order that the value

$$C_H \times C_{\text{OH}} = 0.64 \times 10^{-14}$$

should not be exceeded. But then, just as the precipitation of a little uric acid by carrying away the actual hydrogen ions affects the values in the equation

$$\frac{C_H \times C_{\text{HPO}_4}}{C_{\text{H}_2\text{PO}_4}} = k$$

very little, so the value of  $C_H$  would not be much affected by the addition of a little alkali. Therefore, since  $C_H \times C_U = \text{a constant}$ , and the addition of a little alkali does not change H, U is not changed, so that the addition of a little alkali does not change the solubility of uric acid in cold urine. It has, in fact, been

<sup>1</sup> A. Ritter: "Über die Bedingungen für die Entstehung harnsäurer Sedimente, ein Beitrag zur Theorie der Gicht." *Ztschr. f. Biol.*, 35, 155 (1897).

shown by experiment, as we shall see later, that partial neutralization of the acidity of a cold urine by alkali does not give it the power of dissolving uric acid crystals. When, however, enough alkali is added to very decidedly decrease  $C_{H_2PO_4}$ , and correspondingly increase  $C_{HPO_4}$ ,  $C_H$  is decreased to maintain equilibrium, and when  $C_H$  is decreased enough to approach the concentration allowed by the equation

$$C_H \times C_U = 206 \times 10^{-12},$$

then the solubility of uric acid begins to be increased.

With increase in temperature,  $k$  increases.  $C_{HU}$ , the value for the solubility of the undissociated uric acid, also increases. Therefore,

$$C_H \times C_U = k \times C_{HU}$$

is very largely increased, but since  $C_U$  is constant for any given urine, the value

$$C_H = \frac{k \times C_{HU}}{C_U}$$

is greatly increased. In other words, the concentration of the H ions, which can exist in solution with a definite amount of negative urate ions, is increased with the temperature. With increase in temperature there is also an increase in the dissociation of the other weak bases and acids in the urine. But the OH ions from the bases neutralize the H ions from the acids according to the equation  $H + OH = HOH$ . If the increase in the supply of the OH ions is equal to the increase in the supply of H ions, the actual number of the H ions will remain the same with increase in temperature. In the equation

$$C_H \times C_U = k \times C_{HU}$$

or

$$C_U = \frac{k \times C_{HU}}{C_H},$$

we have, in such a case,  $k$  increasing,  $C_{HU}$  increasing, and  $C_H$  constant. Therefore,  $C_U$  must increase; that is, the amount of negative uric acid ions or sodium urate that can remain in solution is greater. Or, to put it more generally, if the increase in concentration of the OH ions is rapid enough to prevent the actual concentration of the  $C_H$  ions in any urine from increasing as rapidly as the value for  $C_H$  in the equation

$$C_H = \frac{k \times C_{HU}}{C_U},$$

then the solubility of the uric acid will increase with increase in temperature. If the concentration of the OH ions increases faster than the concentration of the H ions, the actual value for the concentration of the H ions will decrease with increase in temperature. In this case, which, as we shall see, is probably the condition in normal urine, the solubility of the uric acid will increase with rise in temperature faster than it will increase in pure water. As the value  $C_{H'}$  for the actual concentration of H ions decreases, and the value  $C_{H''}$  for the concentration of H ions permitted by the equation

$$C_H = \frac{k \times C_{HU}}{C_U}$$

increases, a point will be reached where  $C_{H'} = C_{H''}$ . As we pass this point uric acid becomes the strongest acid in the urine, and, in fact, we have proof that, at 37°, uric acid is the strongest acid in the urine.

Tunicliffe and Rosenheim<sup>1</sup> took a set of flasks containing 100 cc. of urine each and containing an excess of undissolved uric acid, added to each 0.2 gram of different alkalis and allowed the solution to remain at a temperature of about 37° C. until saturated. The alkalis used were piperidine, lysidine, and piperazine. These authors found that the amount of uric acid dissolved by each was proportional to the solubility of their respective urates. If there were a stronger acid than uric acid present, we should have the condition described on pages 7 and 8, that is, these alkalis would not effect the solubility of the uric acid. On addition of a little alkali the H ions would disappear to form HOH. Immediately new H ions would be supplied by dissociation of the stronger acid, HA. In the case of warm urine, however, as the H ions disappear, more are supplied by further dissociation of HU. But this decreases the concentration of the undissociated HU. Therefore, more uric acid goes into solution to supply the deficiency.

The precipitation of sodium acid urate in urine is due to the fact that the product

$$C_{Na} \times C_U = k \times C_{NaU}$$

is exceeded. Either a high concentration of sodium salts or a large amount of uric acid might bring about a precipitation of

<sup>1</sup> F. Tunicliffe and O. Rosenheim: "Piperidine as a Uric Acid Solvent, a Comparative Study." *Lancet*, July 23, 1908.



sodium urate. We can see a reason, therefore, for a precipitation of the urate in alkaline urine, a condition which sometimes occurs. A precipitation of both uric acid and sodium acid urate such as we often have in urine will occur when the concentration of Na ions, H ions, and negative U ions is such that the values for the solubility products of both the uric acid and the sodium urate are exceeded.

What now will be the effect of alkali on a urine from which uric acid precipitates while still warm? Obviously, in this case, the solubility product,

$$C_H \times C_U = k,$$

is exceeded even in the warm urine. Uric acid is not the strongest acid in solution. We have, in this case, the condition usually found in cold urine. The addition of a little alkali will not, therefore, affect the solubility of uric acid. Addition of large amounts of alkali will have some effect on the solubility of the uric acid.

By the addition, then, of alkali to a normal urine we should increase the solubility of uric acid in it at the body temperature, and by the addition of considerable quantities of alkali to a urine from which uric acid precipitates while still warm, we should expect to increase its power of dissolving uric acid at the body temperature. As it is the latter case with which we have to deal in the practical therapeutics of uric acid calculi and gravel, we should, for the best results, give as much alkali as it is possible to give without making the urine alkaline. We can see the reason for the apparently contradictory results of different experimenters on the effect of alkali on the solubility of uric acid in urine. The result depends on the temperature, the quantity of alkali, and on the conditions in the urine.

We hope to determine the dissociation constants

$$k = \frac{C_H \times C_{HPO_4}}{C_{H_2PO_4}} \text{ (at } 18^\circ\text{),}$$

$$k_2 = \frac{C_H \times C_{HPO_4}}{C_{H_2PO_4}} \text{ (at } 37^\circ\text{),}$$

$$k_3 = \frac{C_H \times C_U}{C_{HU}} \text{ (at } 37^\circ\text{),}$$

and to study the "actual" and "potential" acidity of different

urines at 18° and at 37°, their content of alkaline and acid sodium phosphate and uric acid, and the amount of uric acid precipitated on cooling, in order that the condition in urine be made still clearer, and that we may be able to predict quantitatively from the chemical composition and acidity of the urine, the effect of a certain amount of alkali on a urine and the amount of uric acid which will precipitate spontaneously on cooling. We can then, perhaps, do something for those suffering from uric acid calculi and gravel. Later we hope to study the blood and tissue fluids by physico-chemical methods. This rich and unexplored field must certainly offer a good harvest to the physiological chemist. The results will, perhaps, throw light not only on gout but on many other of those diseases which are classed as disorders of metabolism.

CHEMICAL LABORATORY,  
MASSACHUSETTS GENERAL HOSPITAL,  
BOSTON, MASS.

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## THE GERMINATION OF BARLEY.

BY ARVID NILSON.

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ACCORDING to the prevalent theory, the germination of the barley is caused largely by the action of enzymes in the presence of oxygen, moisture, and a suitable temperature.

Of the enzymes contained in the barley, the diastase and the peptase have been most thoroughly investigated. The diastase changes the insoluble starch into soluble sugar, and the peptase is supposed to change the insoluble albumen into soluble, coagulable albumen, albumoses, peptones, and amido-bodies.

Late researches, however, have made the existence of enzymes in the barley rather doubtful, and more than one investigator has failed to detect enzymes in appreciable quantities, even after the steeping of the barley. It is only after the growth is well on the way that the enzymes make their appearance in quantity, and they, therefore, seem to be rather the consequence than the cause of the growth.

I do not thereby deny that the enzymes are indispensable to the germination, but claim that they are not the primary cause of the growth, and that some other agent must be looked for