FREE ENERGY AND OTHER DATA FOR EIGHT COMPOUNDS OF PHYSIOLOGICAL INTEREST

Thesis by

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In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena California

1939

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INTRODUCTION

Purines and their related compounds are widely distributed in nature. Among the substances and biological materials which contain purines as their components are chromosomes, viruses, bacteriophages, sperm, cell nuclei, leaf growth factors, coenzymes, tissue extracts and body fluids. In vivo the purines are readily synthesized even by the simplest organisms. The study of the energetic relationship of these compounds to one another is of interest in indicating whether their synthesis may proceed spontaneously or whether other energy yielding reactions must also take part. The mechanism of synthesis of purines in turn is of interest in its relation to the much wider field of biology indicated by their distribution.

The standard free energies of formation of solid crystalline adenine, hypoxanthine, guanine, xanthine, uric acid, allantoin and alloxan have been determined by Stiehler and Huffman (1),(2). For the consideration of reactions taking place in an aqueous medium it is necessary to know the standard free energies of formation of the various species involved. In order to be able to calculate the free energies in solution other data are required, the determination of which constitutes the problem of this thesis.

These include the determination of solubilities, dissociation constants, activities and vapor pressures. In addition some data on leucine have been included because the older values are inaccurate due to the contamination of leucine with methionine (3). A complete redetermination of the above described physico-chemical constants has been undertaken.

Utilizing the above described data calculations have been made of the standard free energy of formation of each of the compounds in its aqueous solution. The equilibrium relations of purines and their degradation products have also been computed.

EXPERIMENTAL METHODS

The experimental methods adopted will be considered first. Since these differ only in detail from one compound to the next a general description will be given here. The more particular details will be described in connection with the discussion of the individual compounds. A brief consideration will also be given to the fundamental concepts essential for a clear understanding of the meaning of the various physico-chemical constants determined.

SOLUBILITIES

Solubility is essentially the measure of an equilibrium constant in a heterogeneous system in which the solid phase is in equilibrium with the dissolved phase. A general reaction may be written as,

$$\text{Solvent} + X_{\text{solid}} \rightleftarrows X_{\text{dissolved}} + \text{Solvent}_{(\text{sat.sol.})}$$

the temperature of the reaction being defined. The equilibrium constant is therefore,

$$K = \frac{(X_{\text{dissolved}})\ (\text{Solvent}_{(\text{sat.sol.})})}{(X_{\text{solid}})\ (\text{Solvent}_{(\text{liq.})})}$$

But since $X_{(\text{solid})}$ is in its standard state its activity

is unity and in a dilute solution the activity of the solvent in the solution is nearly equal to that of the pure solvent, we may write

$$K_c = X_{\text{dissolved}}$$

where the subscript on K_c indicates that this equilibrium constant has the dimensions of concentration. The equilibrium constant K_c is usually called solubility in dilute solutions.

It is to be noticed that the solubility equilibrium does not imply that the solid phase is anhydrous. It specifically refers to that phase which is in equilibrium with the dissolved phase. This is of importance where the solid forms a hydrate and will be referred to in that connection.

The apparatus which was used for the determination of solubilities included solubility tubes, thermostatically controlled water-bath and a rocking device for shaking the solubility tubes, together with weighing bottles, a drying oven etc.

The solubility tubes are merely ⊥ shaped tubes such as may be seen in the diagram in Figure 1. When such solubility tube is rocked back and forth a thorough agitation of its contents is obtained. These tubes were

generally about three quarters full or less to give maximum agitation. Various sizes of solubility tubes ranging from 15 to 250 ml. were used depending on the solubility of the compound and the amount of saturated solution required for the solubility determination.

A constant temperature water bath served to maintain the solubility tubes at 25 ± .03°C and 50 ± .05°C (the two temperatures at which the solubility determinations were made). This water bath was equipped with a motor driven rocking device which rocked back and forth the solubility tubes. The solubility tubes were clamped to a horizontal bar connected with the rocker. The arrangement of the apparatus was such that the lower portion of the solubility tube was immersed in water.

The solubilities were determined in the following manner. An excess of a given solid was introduced into a solubility tube which was then about three quarters filled with redistilled water. The tube was then stoppered to prevent contamination with carbon dioxide, ammonia etc. This was merely a precaution in order to minimize the sources of error in the determinations. The solubility tube was then placed in the thermostat and agitated. Sufficient time was allowed for the solubility equilibrium to be established.

In order to ascertain whether equilibrium had been reached solubility determinations were made on several successive days. Constant values were obtained in most cases in one or two days. From supersaturated solutions the equilibrium was in some cases more slowly attained than from the undersaturated solutions. Further confirmation of the attainment of equilibrium was obtained by approaching it from both the undersaturated and the supersaturated side. To approach the equilibrium from the undersaturated side the solid was simply agitated with water in a solubility tube as already described. To approach the equilibrium from the supersaturated side the solid in presence of water was first equilibrated for one day as described above, the solubility tube was removed from the thermostat and heated in a water bath for 15 to 20 minutes at $35^{\circ}C$ for the solubility determinations at $25^{\circ}C$, and at $65^{\circ}C$ for the solubility at $50^{\circ}C$. The solubility tube was then replaced in the thermostat and equilibrated for another one or two days before samples were removed for determination.

For the solubility determinations portions of the saturated solution were transferred directly into a weighing bottle by means of a siphon and suction as

shown in the illustration in Figure 1. A cotton plug was inserted into the end of the siphon to serve as a filter. In some cases it was necessary to use good grade Gooch crucible washed asbestos in order to obtain a clear filtrate. The asbestos was held in place by a small cotton plug on either side.

During the transfer of the saturated solution from the solubility tube to the weighing bottle in no case was there any tendency observed of the solute to crystallize out due to small temperature differences between the solution and the siphon. During the manipulations the weighing bottles were kept stoppered in order to prevent loss of water by evaporation.

The weighing bottles used were carefully cleaned in a chromic acid bath washed and dried in an oven. Under this treatment the Jena glass bottles changed in weight very little from day to day. Quite often their mass remained constant to within .1 mg although over a longer period of time the change in mass was larger than this. All the weighings were done on an analytical balance to .1 mg. A slightly larger margin was allowed in determining the mass of the solution.

For the gravimetric procedures the mass of the weighing bottles was determined beforehand then samples

REMOVING

SOLUBILITY SAMPLES

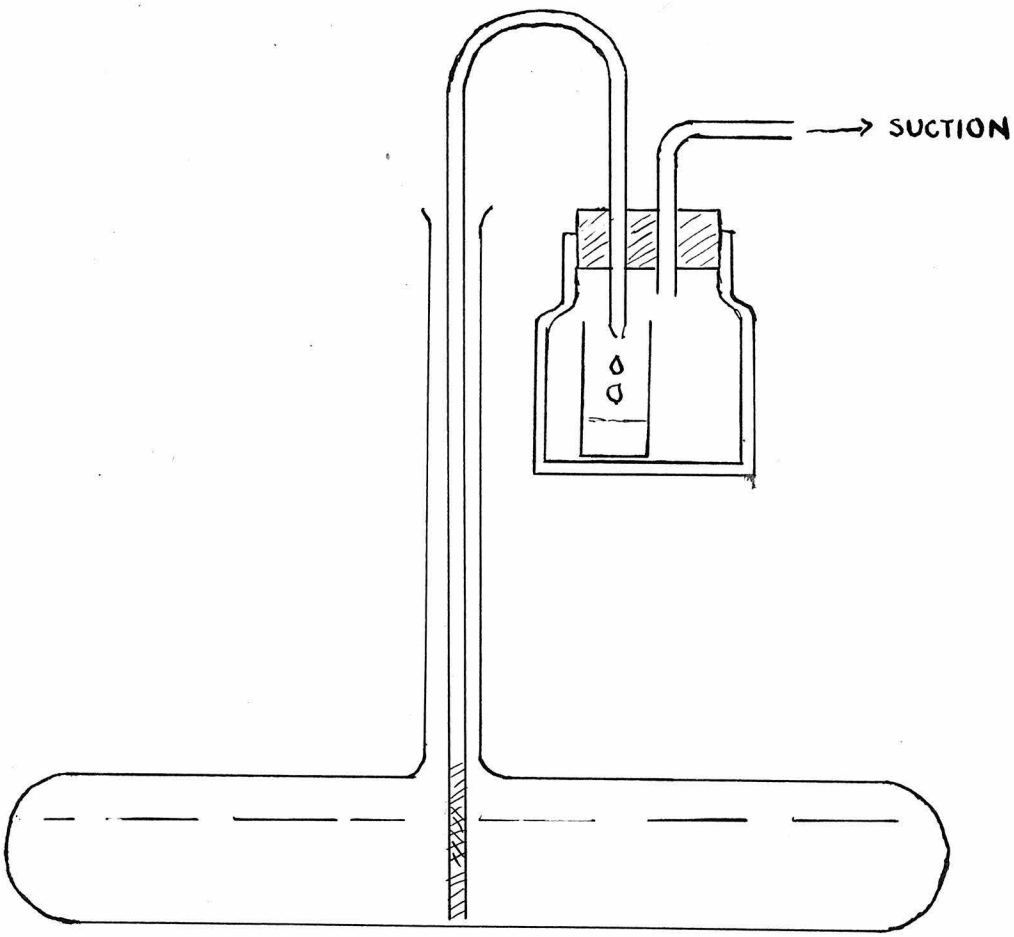


FIGURE 1

of the saturated solution were taken and the bottles with the solution were weighed. These were placed in a drying oven at 100°C and evaporated t o dryness. During the evaporation the lids were so kept on the weighing bottles as to protect the solutions from contamination with dust particles. At all times the oven was used only for quantitative work. Under these conditions the evaporation proceeded smoothly without bumping and consequently with no loss of solution and with no contamination. The residue was dried to constant weight also at 100°C. Several preliminary experiments drying the residue at 110°C and at 135°C showed that there was no noticeable change in the weight of the residue at the higher temperature. Drying the residue at 100°C was also used by Dalton and Schmidt in their solubility determinations of the amino acids (4).

The mass of the residue of the solute was obtained as the difference between the mass of the weighing bottle plus the residue and its mass empty. Similarly the mass of the solvent is the difference between the weighing bottle plus the residue.

Due to the fact that most of the compounds were only slightly soluble the mass of the residue was small,

of the order of magnitude of 10 mg. Since this was determined as the difference between two large numbers a high accuracy was required in the weighings. Thus .1 mg difference in the mass represents a 1% error. Such factors as the absorption of a thin film of moisture on the relatively large surface of the weighing bottle are significant in this type of work.

In order to minimize the errors due to the change in the mass of the weighing bottles themselves parallel experiments using distilled water were done with each determination. The second weighing bottle was then used as a counterpoise or tare in weighing the weighing bottle plus the residue. The masses of the empty weighing bottles were also determined by the counterpoise method.

For this purpose the weighing bottles were paired in such a way that their masses were within .1 gm. of each other. In this way the weighings could be done using only the chain of the chainomatic balance and were therefore independent of the brass weights. Furthermore since each bottle was nearly of the same dimensions and mass and had undergone the same treatment in drying any changes in their masses would be nearly identical and would thus tend to cancel out in the counterpoise weighing.

For the least soluble members of the purine group namely uric acid, xanthine and guanine the gravimetric method was not sufficiently accurate so that colorimetric methods were adopted.

The amounts of xanthine and guanine in solution were determined using the Koessler and Hanke diazo method (5). The diazotized sulfanilic acid in the reagent couples with xanthine and guanine in an alkaline medium to yield azo dyes. The color that is developed can be measured quantitatively in a colorimeter.

The application of this method to a quantitative determination of purines was made by Hunter (6). Since solutions of pure compounds were used the usual objections of the non specificity of the reaction were absent. Furthermore suitable standards as will be later described treated in the same way as the test solutions were used for comparison. Care was taken to make the two solutions to be compared of nearly the same concentrations. In this way the several errors of the method were minimized.

All the solubilities were done on at least two different preparations of each compound where sufficient material was available. The temperatures used were

25 ± .03°C and 50 ± .05°C. The solubilities were expressed as grams of solute per kilogram of water.

The results of the solubility determinations for any one compound were conveniently tabulated, the arrangement in the table being as follows. One table was made to include the solubilities obtained when the solubility equilibrium was approached from the undersaturated side. A second table included the results when the equilibrium was approached from the supersaturated side. In the first column of the table the determinations are numbered consecutively. The second column gives the length of time during which the solubility tube was equilibrated before the determination was made. The solubilities are given in the third column. The fourth column represents the deviations of the individual results from the group mean. The group mean was obtained by averaging the results of the solubility determinations for any one preparation of the compound under consideration and is given at the end of the group in the third column. The final column gives the deviations from the general mean. The general mean was obtained by averaging all the results in the table. An analysis of the results in this manner brings out more clearly

the variations in the different preparations of the same compound and also their relation to the final result.

The precision errors were calculated according to the method of Rossini (7) using the formula $\pm 2\sqrt{\Sigma\Delta^2/n(n-1)}$ where $\Sigma\Delta^2$ is the sum of the squares of the deviations from the mean and n is the number of determinations. In order to obtain the final values of the solubilities the results approached from the undersaturated and the supersaturated side of the equilibrium were averaged. The precision errors were combined using the formula $\pm\sqrt{a^2 + b^2}$, where a and b are the respective precision errors of the two individual results.

DISSOCIATION CONSTANTS

In all cases except one the dissociation constants were determined by electrometric titration using a glass electrode and a Beckman pH meter to follow the H^+ concentration.

The pH meter was first adjusted using a phthalate buffer of known pH of 3.97. Then a given solution of known volume and concentration was titrated with a standard acid or base. The concentration of the solutions titrated was determined by the solubility of the compound

in question as for example a solution of xanthine which was .0001 molar was used on account of the sparing solubility of xanthine in water. The concentration of the standard acid or alkali used in the titrations was in turn determined by the concentration of the solution titrated. The use of a micro burette of 1 ml. capacity which could be easily read to .005 ml. made it possible to use the standard reagent of higher concentrations thus avoiding dilution of an already dilute solution. In this manner maximum accuracy was obtained from the titrations.

The titrations were done by adding known amounts of the standard acid or base to a given volume of the solution being titrated the pH of the solution being recorded after each addition of the reagent. Care was taken to have the solutions at 25°C during the titration. The accuracy of the pH determinations under the usual working conditions was about ±.05 pH units. In the higher alkaline range the glass electrode becomes inaccurate. A correction was applied from a graph supplied with the instrument for this purpose.

All the titrations were done in duplicate and the results were averaged. The volume of the reagent added was plotted against the pH of the solution to give a

titration curve. Since the dissociation constant varies with the dilution a correction is desirable. A titration of the water blank was therefore done with each titration using the same volume of the solution (water) and of the reagent. The volume of the reagent used for the water blank was subtracted from the amount used in titrating the aqueous acid or base for each pH thus giving a corrected titration curve. The corrected titration curve thus represents a graphic method of obtaining a dissociation curve.

Such a corrected titration curve is sometimes used to obtain the dissociation constant directly from the graph (8). It is preferable, however, to calculate the dissociation constants from the titration data by means of appropriate equations.

The dissociation constants were calculated from the equations

$$pK_a = pH + \log \frac{C_A - C_{Na^+} - C_{H^+}}{C_{Na^+} + C_{H^+}}$$

and

$$pK_b = pK_w - pH + \log \frac{C_A - C_{Cl^-} + C_{H^+}}{C_{Cl^-} - C_{H^+}}$$

where

C_A is the concentration in moles per liter of all forms of the substance titrated.

C_{Na^+} is the total concentration of Na^+ in moles per liter

C_{Cl^-} is the total concentration of Cl^- in moles per liter.

C_{H^+} is the concentration of H^+ in moles per liter

pK_W is 14.00 at 25°C (9).

Guanine was found to be too insoluble to be titrated in the above manner hence the solubility method of Hitchcock (10) was used. Xanthine was done by both the titrimetric and the solubility methods.

The relation between solubility and dissociation is given by

$$S = S_o + \frac{S_o K_b C_{H^+}}{K_W}$$

and

$$S = S_o + \frac{S_o K_a}{C_{H^+}}$$

where,

S is the solubility of all forms of the substance in grams per liter at a given pH.

S_o is the solubility of the undissociated substance

which is usually taken as the solubility in water. It is also expressed in grams per liter

C_{H^+}, K_w , K_b , K_a have the same significance as before.

The results obtained from the titrations were averaged to give a final value. Sometimes the first and last values were not included in the mean, as the accuracy of the dissociation constants is least when the ratio of the free acid to the salt is far from unity. The solubility method however can only be applied when the ratio of the free acid or base to its salt is quite small. It has been found to be fairly accurate under these conditions.

ACTIVITIES

At infinite dilution dissolved substances behave as perfect solutes. At higher concentrations they deviate from the laws of perfect solutions and a correction factor is required to make them conform to these laws. This factor is known as the activity coefficient and the concentrations corrected in this manner are known as activities. The relation between concentration and activity may be expressed by the equation

$$M\gamma = a$$

where M is the concentration in molality, γ is the activity coefficient and a is the activity.

In practice the activity coefficients are determined only for the more soluble substances. As already stated above in dilute solutions the laws of perfect solutions are more nearly obeyed hence it is common practice to assume as a pretty good approximation that the activities in dilute solutions are equal to concentrations. The line of demarcation is usually made at .1 molal concentration where the activity correction is already small and where the experimental methods begin to be inaccurate.

The molal solubilities of the purines were found to be less than .01 and that for allantoin .0373. It is therefore safe to assume their activities equal to their concentrations. The molal solubility of leucine was found to be .167 just on the borderline where the activity measurements become inaccurate. Alloxan alone was found to be sufficiently soluble for an experimental determination of its activity coefficients.

VAPOR PRESSURES OF HYDRATE SYSTEMS

Just as in the case of solubilities the hydration of substances can best be considered from the point of view of heterogenous equilibria. Let us consider a substance X which forms a hydrate $X.H_2O$. This hydrate will decompose to form water and the anhydrous substance X . If such a system is put in a closed vessel an equilibrium

will finally be established

$$X.H_2O_{(s)} \rightleftarrows H_2O_{(g,p)} + X_{anhydrous(s)}$$

where p is the equilibrium pressure of the hydrate-anhydrous transformation.

The equilibrium constant for this reaction is given by

$$K = \frac{H_2O_{(g,p)} \times X_{anhydrous(s)}}{X.H_2O_{(s)}}$$

Now since X anhydrous and X,H_2O are in their standard states their activities are unity. The activity or fugacity of water is equal to its vapor pressure, hence the equilibrium constant becomes

$$K_p = P$$

where p is again the equilibrium pressure of the hydrate-anhydrous transformation. The subscript of K_p indicates that the equilibrium constant has the dimensions of pressure.

Let us suppose a case where X forms a trihydrate but no intermediate hydrates. The equilibrium involved is then given by

$$X.3H_2O_{(s)} \rightleftarrows 3H_2O_{(g,p)} + X \text{ anhydrous}(s)$$

The equilibrium constant is therefore

$$K = \frac{P^3 \times X_{anhydrous\ (s)}}{X.3H_2O\ (s)}$$

and

$$K_p = p^3$$

It will be seen that it is the equilibrium constant that is of interest for the purpose of free energy considerations and not the vapor pressure although in the case of a monohydrate these are identical.

It should also be pointed out that the vapor pressure of the hydrate does not refer to the hydrate alone but to the equilibrium system.

Among the compounds studied alloxan, adenine and xanthine are known to form hydrates. It was necessary to determine whether these hydrates were formed at 25°C and if stable at this temperature it was also necessary to determine the vapor pressure of the hydrate-anhydrous system. The other compounds studied did not form hydrates.

The general method employed will now be considered. Where this was feasible the solid phase was completely dissolved in water at elevated temperatures. It was then allowed to come to equilibrium by agitating it for several days in a solubility tube placed at 25°C in a thermostat. A solubility determination was made in order to ascertain whether the equilibrium had been attained. The solid phase was then removed by filtration on a Hirsch funnel and superficially dried between filter papers. This solid

was then placed on a tared watch glass and its weight determined. It was then placed in a small vacuum chamber together with about 5 ml. of sulfuric acid solution contained in a small glass vessel. The system was evacuated on the water pump for one or two minutes and finally set away in a 25°C incubator. After several days the sample was removed and the loss in weight determined.

The rationale of the method is the following. The sulfuric acid solution acts as a desiccant to remove any water of wetting that still adheres to the crystals after superficially drying the solid. If the vapor pressure of the hydrate system is less than that of the sulfuric acid solution no further desiccation will take place after the water of wetting has been removed. If the solid is then dried in an aven at 100°C the water present will be removed. The water removed in this manner must then have been bound as water of crystallization. On the other hand if the vapor pressure of the hydrate system is greater than that of the sulfuric acid solution all of the water will be lost to the desiccant.

By using a series of sulfuric acid solutions of varying concentrations the vapor pressure of the hydrate system can be determined from the vapor pressure of that sulfuric acid solution to which the hydrate neither loses nor gains any water. Experimentally this is obtained as the average of two

concentrations near one another, to one of which the hydrate system loses water vapor and not to the other.

The vapor pressures of the sulfuric acid solutions of different concentrations were obtained from the International Critical Tables ([11]). The concentrations were checked by specific gravity determinations. These were done by weighing 5ml. of the solution on the balance.

MATERIALS

A brief account of the source and the method of preparation and purification of the compounds used in this investigation is given below. A number of these were the preparations described by Stiehler and Huffman (1) and they will be designated as combustion samples.

Adenine I - combustion sample, adenine a.

Adenine II - This was prepared by the hydrolysis of yeast nucleic acid by the method of Hunter and Hlynka ([12]). The adenine hydrochloride obtained was crystallized three times from dilute hydrochloric acid. It was then converted into the free base by dissolving the hydrochloride in water and neutralizing the solution with sodium carbonate. The free adenine which precipitated out was crystallized four times from water. Microscopic bipyramid crystals were obtained. Attempts to grow larger crystals were unsuccessful as adenine

deposited as a crusty precipitate on the walls of the container. The needle crystal form as in Adenine I above could not be obtained under any of the conditions tried which included seeding adenine solutions with these crystals and crystallizing at different temperatures and varying dilutions.

Guanine I and Guanine II were both combustion samples.

Hypoxanthine I, II, and III were also combustion smaples.

Xanthine I was a combustion sample.

Xanthine II and III were Hoffmann La Roche preparations decolorized with charcoal and crystallized four times from water.

Xanthine IV was a Hoffmann La Roche preparation treated the same way as II and III above. A special effort was made to obtain large crystals by first heating Xanthine in presence of a large amount of its saturated solution to 60^{o}C and then cooling it at 8^{o}C over night in a refrigerator. This procedure was repeated for a period of one week.

Uric Acid I was a combustion sample.

Uric Acid II was Pfanstiehl C.P. uric acid crystallized three times from water. It crystallized readily to

yield well defined crystals.

Allantoin I was a Hoffmann La Roche preparation.

Allantoin II was a combustion sample.

Alloxan was a Hoffmann La Roche preparation crystallized once from water and dried in vacuo over sulfuric acid.

d- and l- Leucine were methionine free samples prepared by Fox (13).

ADENINE

THE SOLUBILITY OF ADENINE

The solubility of adenine was determined by the gravimetric method. Approximately 10 gram portions of the saturated solution were used for each determination and the weight of the residue obtained on evaporation was determined as already described.

The results of the solubility determinations of adenine at 25°C are given in tables I and II. Taking the mean of the general means we obtain for the solubility of adenine at this temperature 1.13 ± .04 grams per 1000 grams of water.

The variation of the individual determinations constitutes the largest error. The variation of the results on the two different preparations is not significant. Also there is little difference between the values obtained approaching the equilibrium from the undersaturated or the supersaturated side. It also appears that saturation is obtained in a relatively short time from the undersaturated side but that there is a tendency for the solution to remain supersaturated when the equilibrium is approached from the supersaturated side.

The results of solubility determinations at 50°C are given in tables III and IV. The results obtained give for the solubility of adenine at this temperature 3.4 ± .2 grams per 1000 grams of water. It is at once apparent that there is some disagreement between the individual results on the two different preparations of adenine. Adenine I and Adenine II as will be recalled differ in crystalline form and it is likely that their solubilities differ, this difference becoming more apparent at higher temperatures. Unfortunately an insufficient amount of Adenine I was available. As has been mentioned above no success was had in attempting to prepare the needle crystalline form of adenine. The discrepancy between the individual determinations is not large and the agreement between the values obtained when the equilibrium was approached from the undersaturated and the supersaturated side is also quite satisfactory.

According to Koessel (14) .92 grams of adenine dissolve in 1000 grams of water at room temperature. Tafel and Ach (15) found its solubility in boiling water to be 25 grams per 1000 grams of water. These data are of limited quantitative value only, but indicate the same order of magnitude.

It is convenient to have the solubilities expressed in terms of molalities. At 25°C the molality of a saturated solution of adenine from our results was found to be .00836 and at 50°C .0252

For the purpose of calculating the percentage dissociation the pH of a saturated solution is required. It was found to be 6.6 at 25°C.

TABLE I

SOLUBILITY OF ADENINE at 25°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg. Water	Dev.from Gr. Mean	Dev.from Gen Mean
ADENINE I				
1	1	1.17	.03	.04
2 w	1	1.21	.07	.08
3 w	½	1.12	-.02	-.01
4	½	1.10	-.04	-.03
5	1	1.16	.02	.03
6	2	1.06	-.08	-.07
7	3	1.13	-.01	.00
8	4	1.13	-.01	.00
9	1	1.15	.01	.02
10	2	1.16	.02	.03
11	3	1.22	.08	.09
12	4	1.21	.07	.08
13	2	1.07	-.07	-.06
14	3	1.09	-.05	-.04
15	4	1.14	.00	.01
16	3	1.11	-.03	-.02
17	4	1.15	.01	.02
18	5	1.21	.07	.08
		1.14	±.02	

TABLE I (con)

Ex.No.	Days Equilibrated	Sol'y Gm/Kg. Water	Dev.from Gr. Mean	Dev.from Gen.Mean
ADENINE II				
19	2	1.11	.04	-.02
20	3	1.10	.03	-.03
21	4	1.05	-.02	-.08
22	2	1.04	-.03	-.09
23	4	1.05	-.02	-.08
24	5	$\frac{1.09}{1.07}$	$\frac{.02}{\pm .02}$	-.04
	Mean of group means	1.10	$\pm$.03	
	General mean	1.13	$\pm$.02	

W after the experiment number in the first column indicates that water was added to the solute of the previous determination.

TABLE II

SOLUBILITY of ADENINE at 25°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg. Water	Dev.from Gr.Mean	Dev.from Gen.Mean
ADENINE I				
1	2	1.33	.19	.20
2 w	2	1.18	.04	.05
3	5	1.17	.03	.04
4 w	5	1.14	.00	.01
5	6	1.10	-.04	-.03
6	7	1.10	-.04	-.03
7 w	7	1.08	-.06	-.05
8	8	1.04	-.10	-.09
9	9	1.08	-.06	-.05
10	10	1.11	-.03	-.02
11 w	6	1.17 / 1.14	-.03 ± .05	.04
ADENINE II				
12	3	1.14	.01	.01
13	3	1.18	.05	.05
14	6	1.12	-.01	-.01
15	6	1.14	.01	.01
16	8	1.14	.01	.01

TABLE II (con)

Ex/No.	Days Equi-librated	Sol'y Gm/Kg. Water	Dev.from Gr. Mean	Dev. From Gen. Mean
17	8	1.11	-.02	-.02
18	18	1.11	-.02	-.02
19	10	1.10	-.03	-.03
		1.13	± .02	
Mean of group means		1.13	± .05	
General mean		1.13	± .03	

TABLE III

SOLUBILITY of ADENINE at 50°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
ADENINE I				
1	1	3.18	.00	-.16
2	2	3.22	.04	-.12
3	4	3.14	-.04	-.20
4	5	3.17 / 3.18	-.01 / ±.03	-.17
ADENINE II				
5	1	3.45	.03	.11
6	1	3.47	.05	.13
7	2	3.41	-.01	.07
8	2	3.44	.02	.10
9	3	3.36	-.06	.02
10	3	3.42	.00	.08
11	4	3.38	-.04	.04
12	4	3.44 / 3.42	.02 / ±.03	.10
Mean of group means		3.30	± .04	
General mean		3.34	± .08	

TABLE IV

SOLUBILITY of ADENINE at 50°C

Equilibrium Approached from the Supersaturated Side

	Ex.No.	Days Equilibrated	Sol'y Gm/Kg. Water	Dev.from Gr. Mean	Dev.from Gen.Mean
ADENINE I					
	1	2	3.46	.22	.04
	2	3	3.34	.10	-.08
	3	4	2.91	-.33	-.51
			3.24	±.33	
ADENINE II					
	4	1	3.68	.19	.26
	5	1	3.77	.28	.35
	6	2	3.39	-.10	-.03
	7	2	3.39	-.10	-.03
	8	3	3.41	-.08	-.01
	9	3	3.44	-.05	.02
	10	4	3.43	-.06	.01
	11	4	3.40	-.09	-.02
			3.49	±.09	
	Mean of group means		3.36	±.54	
	General mean		3.42	±.15	

HYDRATION OF ADENINE AT 25°C

Adenine is known to form both a monohydrate and a trihydrate. In order to determine whether the solid phase in equilibrium with a saturated solution of adenine at 25°C is hydrated or not the following experiments were done. Adenine was completely dissolved in hot water and equilibrated in the same way as in the solubility experiments. After four days equilibrium was attained as judged by a solubility determination. The solid phase was then removed and superficially dried between filter papers.

It should be possible to remove the water of wetting by drying the samples of adenine, prepared as above, in presence of a desiccant the vapor pressure of which is higher than that of the hydrate system but lower than that of the saturated solution of adenine. Experiments of this type were done using sulfuric acid solutions of known vapor pressures. In table V below, the vapor pressures in mm. of Hg. of the sulfuric acid solution used is given in the second column. In the third, fourth and fifth columns are given the weights of the superficially dried sample, of the same sample dried for four days in presence of a sulfuric acid solution and the weight of the sample dried in an oven at 100°C. The last column

indicates the number of molecules of water remaining as water of hydration. All experiments were done at 25°C.

This series of experiments indicates that adenine does not form a hydrate under these conditions. The last experiment in the series shows that water of wetting is not removed when the superficially dried sample of adenine is placed in presence of water alone. Although some water appears to have been lost it was shown to be due to a preliminary loss during the evacuation of the vacuum chamber. Even if a hydrate were formed having a vapor pressure of this order of magnitude it would contribute little to the free energy change and could be neglected.

TABLE V

HYDRATION OF ADENINE AT 25°C

No	Vap.Press of H_2SO_4 Soln.	Wt of Superfic. Dried Adenine	Wt of Desicc. Dried Adenine	Wt of Oven Dried Aden.	Molec of aq.
1	19.40	.229	.186	.186	0
2	21.40	.091	.067	.067	0
3	22.54	.095	.075	.075	0
4	23.25	.098	.073	.073	0
5	Water	.148	.128	.103	1.8

DISSOCIATION CONSTANTS OF ADENINE AT 25°C

The dissociation constants of adenine were determined by electrometric titration. Fifty ml. of .00222 molar adenine solution was titrated with 1.2 ml. of .0989 N HCL and Na OH respectively. Each titration was done in duplicate and the pH readings were averaged. The dissociation constants were calculated as described above. A titration of a water blank was also done. The results are given in tables VI and VII. Figure II shows the titration curves of the adenine solutions and the corresponding water blanks. A corrected titration curve is also given.

An average of the pK values included between asterisks was taken.

The value obtained for pK_a is 9.90 which gives for the acid dissociation constant K_a a value of 1.26×10^{-10}.

The value of pK_b was found to be 9.86 which gives for the basic dissociation constant K_b a value of 1.38×10^{-10}.

The limiting factor in the accuracy of the above titrations is the high dilutions of the solutions which it was necessary to employ. The constancy of the dissociation constant in the middle range of the titration

curve is an indication that the values obtained are quite satisfactory.

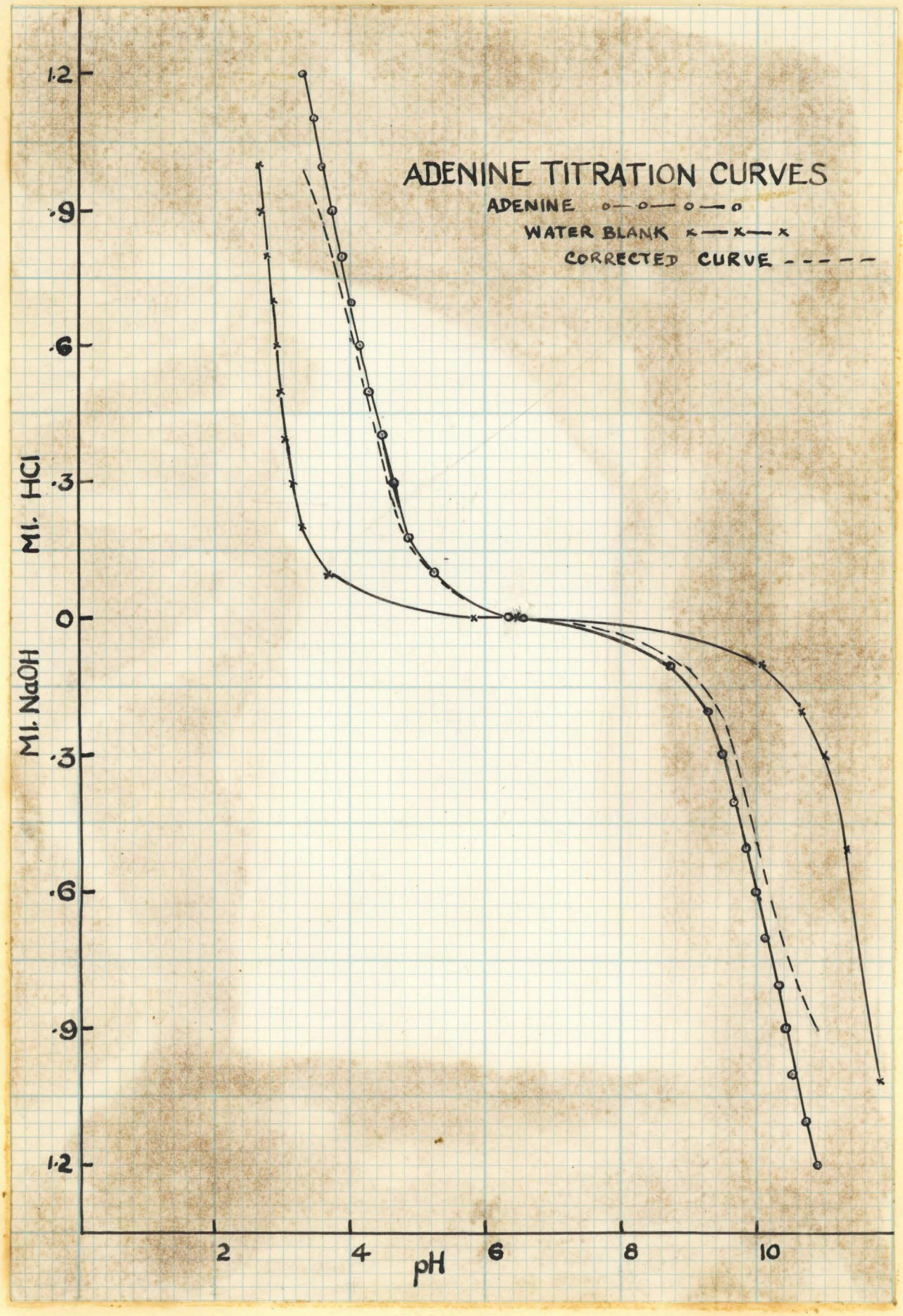

ADENINE TITRATION CURVES
ADENINE o—o—o—o
WATER BLANK x—x—x
CORRECTED CURVE - - - - -
Ml. HCl
Ml. NaOH
1.2
.9
.6
.3
0
.3
.6
.9
1.2
2
4
pH
6
8
10

FIGURE 2

TABLE VI

ACID DISSOCIATION CONSTANT OF ADENINE AT 25°C

NaOH ml	pH of Adenine Solution	pH of Water Blank	pK_a
0.0	6.57	6.50	....
.1	8.76	10.08	9.77
.2	9.25	10.68	9.89*
.3	9.48	11.00	9.92
.4	9.67	.....	9.92
.5	9.83	11.37	9.93
.6	9.99	.....	9.93
.7	10.14	.....	9.93
.8	10.28	.....	9.88
.9	10.41	.....	9.80*
1.0	10.55	11.85	8.62
1.1	10.71	.....	8.98
1.2	10.87	.....	....

Average pK_a 9.90

K_a 1.26×10^{-10}

TABLE VII

BASIC DISSOCIATION CONSTANT OF ADENINE AT 25°C

HCL ml.	pH of Adenine Solution	pH of Water Blank	pK_b
0.0	6.31	5.83	....
.1	5.22	3.68	9.81
.2	4.85	3.35	9.83*
.3	4.62	3.18	9.84
.4	4.44	3.04	9.84
.5	4.29	2.96	9.85
.6	4.14	2.86	9.86
.7	4.00	2.82	9.86
.8	3.86	2.74	9.85
.9	3.72	2.69	9.88
1.0	3.58	2.64	9.89*
1.1	3.43	....	9.94
1.2	3.30	....	....

Average pK_b 9.86

K_b 1.38×10^{-10}

HYPOXANTHINE

THE SOLUBILITY OF HYPOXANTHINE

Hypoxanthine solubility determinations were done by the gravimetric method. Portions of about 20ml. of the saturated solution were required to give a residue of about 14 mg. Since the capacity of the weighing bottles used was about 10 ml. two successive samples were evaporated to dryness and the total residue was determined. The results are given in tables VIII to XI.

At 25°C the solubility was found to be .73 ± .01 grams per 1000 grams of water. Although all the preparations were carefully purified combustion samples there appears to be some variation in their solubilities. It is more likely that this variation is due to the low precision of the method. In addition it also appears that approaching the equilibrium from the supersaturated side the solutions tend to remain supersaturated for several days.

At 50°C the solubility was found to be 2.01 ± .06 grams per 1000 grams of water. At higher temperatures hypoxanthine is more soluble and the gravimetric method gives results of greater precision. The agreement among the individual determinations as well as among the

different preparations is much better. The agreement between the results obtained from the undersaturated solution and those from the supersaturated solution is also better at the higher temperature.

The solubility of hypoxanthine in water has been determined by several investigators. A summary of their results is given for comparison.

Temperature	Solubility in Gm/Kg Water	Investigator
Room	1.03	Bruhns (16)
"	.63	"
"	.53	"
"	.92	Scherer (17)
17°C	1.50	Stutzer (18)
19°C	.71	Fischer (19)
23°C	.73	"
100°C	14.30	"

The results obtained by Fischer are in substantial agreement with ours.

For convenience our results are given here in terms of molalities. The solubility of hypoxanthine at 25°C was found to be .00536 moles per 1000 grams of water and at 50°C .0148 moles per liter.

The pH of a saturated solution was found to be 6.4 at 25°C.

TABLE VIII

SOLUBILITY OF HYPOXANTHINE AT 25°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg. Water	Dev. from Gr. Mean	Dev.from Gen.Mean
HYPO-				
XANTHINE I				
1	2	.684	-.031	-.054
2	3	.681	-.034	-.037
3	4	.716	.001	-.002
4	5	.787	.072	.069
5 w	2	.666	-.049	-.052
6	4	.740	.025	.022
7	5	.710	-.005	-.008
8	6	.758	.043	.040
9	7	.776	.061	.058
10 w	3	.702	-.013	-.016
11	4	.694	-.021	-.024
12	5	.710	-.005	-.008
13	6	.769	.054	.051
14	7	.704	-.011	-.014
15	4	.695	-.020	-.023
16	5	.693	-.022	-.025
17	6	.697	-.018	-.021
18	7	.691	-.024	-.027
1 9	8	.706 / .715	-.009 ± .016	-.012

TABLE VIII (con)

Ex.No.	Days Equilibrated	Sol'y Gm/Kg. Water	Dev. from Gr. Mean	Dev.from Gen.Mean
HYPO-				
XANTHINE II				
20	2	.682	-.025	-.036
21	3	.705	-.002	-.013
22	4	.729	.022	.011
23	5	.703	-.004	-.015
24	6	.701	-.006	-.017
25 w	3	.701	-.006	-.017
26	5	.721	.014	.005
27	6	.743	.036	.025
28 w	3	.681	-.026	-.037
		.707	± .014	
HYPO-				
XANTHINE III				
29	2	.735	-.001	.017
30	3	.748	.012	.030
31	4	.727	-.009	.009
32	5	.740	.004	.022
33	6	.751	.015	.033
34 w	2	.722	-.014	.004
35	3	.733	-.003	.015
36	4	.731	-.005	.013
		.736	± .007	
Mean of group means		.719	±.022	
General mean		.718	±.010	

TABLE IX

SOLUBILITY OF HYPOXANTHINE AT 25°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
HYPO-				
XANTHINE I				
1	2	.750	.018	.000
2	3	.739	.007	-.011
3	4	.725	-.007	-.025
4	5	.741	.009	-.009
5	6	.705	-.027	-.045
6	7	.718	-.014	-.032
7	8	.720	-.012	-.030
8	9	.736	.004	-.014
9	10	.711	-.021	-.039
10	11	.777 / .732	.045 ± .014	.027
HYPO-				
XANTHINE II				
11	3	.748	- .002	-.002
12	4	.740	-.010	-.010
13	5	.759	.009	.009
14	8	.745	-.005	-.005
15	9	.749	-.001	-.001
16	10	.760 / .750	.010 ± .006	.010

TABLE IX (con)

	Ex.No.	Days Equilibrated	Sol'y Gm/Kg. Water	Dev.from Gr. Mean	Dev.from Gen.Mean
HYPO-					
XANTHINE III					
	17	4	.768	-.001	.018
	18	5	.774	.005	.024
	19	6	.772	.003	.022
	20	7	.783	.014	.033
	21	8	.808	.039	.058
	22 w	3	.751	-.018	.001
	23	4	.739	-.030	-.011
	24	5	.763	-.006	.013
	25	6	.761	-.008	.011
	26	7	.768	-.001	.018
			.769	± .012	
	Mean of group means		.750	± .019	
	General Mean		.750	± .009	

TABLE X

SOLUBILITY OF HYPOXANTHINE AT 50°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg. Water	Dev.from Gr. Mean	Dev.from Gen.Mean
HYPO-				
XANTHINE I				
1	1	2.02	.02	.01
2	2	1.96	-.04	-.05
3	3	2.00	.00	-.01
4	4	1.97	-.03	-.04
5	5	2.05	.05	.04
		2.00	±.03	
HYPO-				
XANTHINE II				
6	6	1.94	-.02	-.07
7	1	2.05	.09	.04
8	2	1.97	.01	-.04
9	3	2.00	.04	-.01
10	4	1.82	-.14	-.19
11	5	1.98	.02	-.03
12	6	1.97	.01	-.04
		1.96	±.05	

TABLE X (con)

	Ex. No.	Days Equilibrated	Sol'y Gm/Kg. Water	Dev.from Gr. Mean	Dev.from Gen.Mean
HYPO-					
XANTHINE III					
	13	1	2.17	.11	.16
	14	2	2.18	.12	.17
	151	3	2.01	-.05	.00
	16	4	2.02	-.04	.01
	17	5	2.01	-.05	.00
	18	6	1.98	-.08	-.03
			2.06	±.07	
	Mean of group means		2.01	±.09	
	General mean		2.01	±.04	

TABLE XI

SOLUBILITY OF HYPOXANTHINE AT 50°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg. Water	Dev.from Gr. Mean	Dev.from Gen.Mean
HYPO-				
XANTHINE I				
1	2	1.91	-.06	-.13
2	3	2.01	.04	-.03
3	4	1.96	-.01	-.08
4	5	1.99	.02	-.05
5	6	1.96	-.01	-.08
6	7	2.03 / 1.97	.06 / ± .03	-.01
HYPO-				
XANTHINE II				
7	2	1.98	-.04	-.06
8	3	2.14	.12	.10
9	4	2.04	.02	.00
10	5	1.99	-.03	-.05
11	6	1.99	-.03	-.05
12	7	2.01 / 2.02	-.01 / ± .05	-.03

TABLE XI (con)

Ex.No.	Days Equilibrated	Sol'y Gm/Kg. Water	Dev.from Gr. Mean	Dev.from Gen.Mean
HYPO-				
XANTHINE III				
13	2	2.13	.00	.09
14	3	2.15	.02	.11
15	4	2.13	.00	.09
16	5	2.10	-.03	.06
17	6	2.14	.01	.10
18	7	2.15	.02	.11
		2.13	± .02	
Mean of group means		2.04	± .06	
General mean		2.04	± .04	

THE DISSOCIATION CONSTANTS OF HYPOXANTHINE AT 25°C

Both the acid and basic dissociation constants were determined by means of an electrometric titration. Fifty ml. of .0022 molar hypoxanthine was titrated with 1.2 ml. of .0989 N. HCL. The same quantity of this solution was also titrated with 1.2 ml. of .0989 N. NaOH. The results obtained are given in tables XII and XIII. These are represented graphically in Figure 3.

The average value for pK_a was found to be 8.97 which gives for the acid dissociation constant of hypoxanthine a value of 1.07×10^{-9}. The variation of pK_a values is small except for the last few.

The basic dissociation constant of hypoxanthine is quite small. Due to the fact that it was necessary to use very dilute solutions of hypoxanthine for the titrations and also because the dissociation constant is small accurate results could not be obtained. However taking an average of the pK_b values in the middle portion of the titration curve some idea is obtained as to the order of magnitude of the basic dissociation. The pK_b value obtained is 12.7 and hence the dissociation constant comes out to be in the neighborhood of 2.0×10^{-13}. Filitti (20) using the solubility method found the acid dissociation constant of hypoxanthine to be 2.12×10^{-12}. In a subsequent paper she reported K_a 2.13×10^{-11} and K_b 8.07×10^{-13} at 40°C.

TABLE XII

THE ACID DISSOCIATION CONSTANT OF HYPOXANTHINE AT 25°C

NaOH ml	pH of Hypox. Sol'u	pH of Water Blank	pK_a
.0	6.50	6.50	....
.1	7.99	10.08	9.01
.2	0.35	10.68	9.01
.3	8.59	11.00	9.02
.4	8.77	.....	9.02
.5	8.93	11.31	9.02
.6	9.08	.....	9.01
.7	9.23	.....	9.00
.8	9.38	.....	8.97
.9	9.54	.....	8.91
1.0	9.15	11.85	8.81
1.1	9.99	.....	8.91
1.2	10.30	.....	....

Average pK_a 8.97

Ka 1.07×10^{-9}

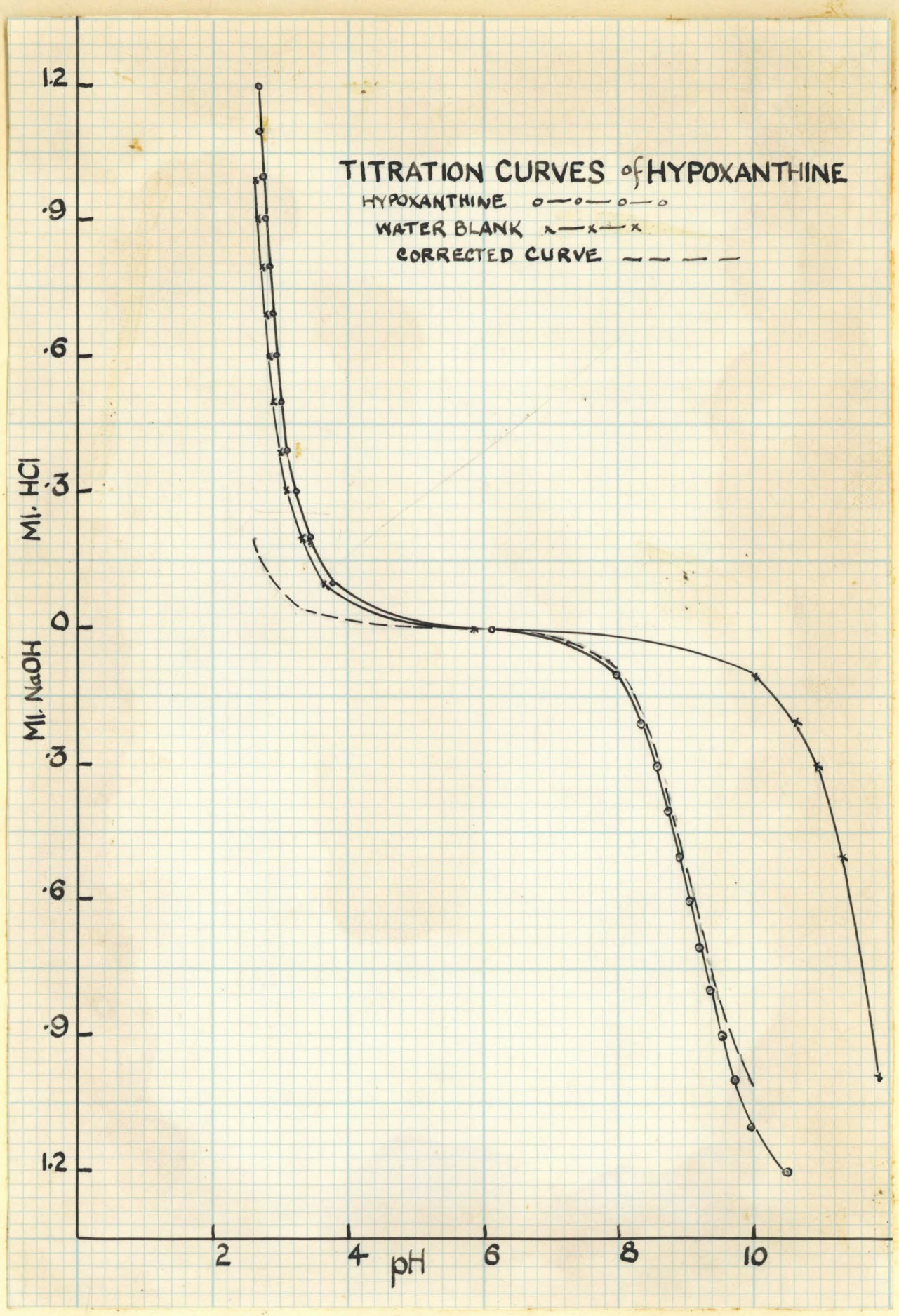

TITRATION CURVES of HYPOXANTHINE
HYPOXANTHINE
WATER BLANK
CORRECTED CURVE
Ml. HCl
Ml. NaOH
1.2
.9
.6
.3
0
.3
.6
.9
1.2
2
4
pH
6
8
10

FIGURE 3

TABLE XIII

THE BASIC DISSOCIATION CONSTANT OF HYPOXANTHINE AT 25°C

HCL	pH of Hypox. Sol'u	pH of Water Blank	pK_b
.0	6.12	5.82	.....
.1	3.78	3.68	12.06
.2	3.45	3.35	12.29
.3	3.26	3.18	12.56*
.4	3.12	3.04	12.73
.5	3.02	2.96	12.93
.6	2.95	2.86	12.66
.7	2.89	2.82	12.45
.8	2.82	2.74	12.87
.9	2.78	2.69	12.58*
1.0	2.72	2.64	13.07
1.1	2.68	....	13.04
1.2	2.62	....	.....

Average pK_b 12.7

K_b 2.0×10^{-13}

*Average taken of values between asterisks.

GUANINE

SOLUBILITY OF GUANINE

For the quantitative determination of guanine in saturated solution a colorimetric method was employed using the Koessler and Hanke diazo reagent (5). The procedure was as follows. A standard solution of guanine was prepared by dissolving 3 mg. of guanine in a liter of redistilled water. The rate of solution is slow and can be increased by pulverizing the solid with a blunt stirring rod and heating on a water bath.

Having the standard, a suitable portion of about 5 ml. of a saturated guanine solution was then withdrawn from a solubility tube. The determination of the amount of guanine in the saturated solution was then made by making the following solutions and comparing them in a colorimeter.

a) 5ml. standard solution + .1gm.Na_2CO_3 + 5ml. diazo reagent.

b) 5ml. saturated guanine solution + .1gm.Na_2CO_3 + 5ml. diazo reagent.

A brownish color develops first and persists for about 15 minutes. The solutions finally become clear yellow and are ready for comparison in about 25 minutes. The initial brownish color is probably due to the slow deamination of guanine. In making a colorimetric

comparison care should be taken to have the same shade of color in both the standard and test solution. If each solution is treated in the same way and sufficient time is allowed for the color to develop this difficulty can in large measure be avoided. An average of 10 readings on the colorimeter was taken.

For the solubility at 50°C the proportion of the reagents was altered as follows. The standard was made by dissolving 5 mg. of guanine in a liter of 1.1% Na_2CO_3 solution. The solutions compared in the colorimeter were,

a) 5ml. standard guanine solution + 5ml. diazo reagent.

b) 2ml. saturated guanine solution + 3ml. of 1.1% Na_2CO_3 solution + 5ml. diazo reagent.

Other details are the same as before.

The results obtained for the solubility of guanine at 25°C are given in tables XIV and XV. The difference between the results on two different preparations as well as between those obtained from the undersaturated and the supersaturated side of the equilibrium appears to be within the precision of the method. Combining the results as previously explained we obtain as the solubility of guanine at 25°C .0034 ± .0001 grams per 1000 grams of water. This corresponds to a .0000225 molal solution at saturation.

The solubility of guanine at 50°C was found to be .0131 ± .0004 grams per 1000 grams of water. The molality of the saturated solution comes out to be .0000867 at this temperature. Here, as above, the limiting factor in the precision of the result is the precision of the method.

Wood ([21]) determined the solubility of guanine at 40.1°C using the gravimetric method and found it to be .039 grams per 1000 grams of water. Several obvious reasons for the large discrepancy between the results of Wood and our results may be offered. Since guanine is not readily crystallized it has to be purified by successive reprecipitations. The purity of the two preparations might have been different. With compounds having a small solubility widely different results might be obtained if a small amount of impurity were present. A second and also a likely explanation might be the difference in the methods. The sources of error in a gravimetric method in the determination of the solubilities of slightly soluble compounds are considerable. It was for this reason that a colorimetric method was adopted in our determinations.

Further reasons for the discrepancy between the results obtained by Wood and our results will be suggested in connection with the determinationation of the dissociation constants.

TABLE XIV

SOLUBILITY OF GUANINE AT 25°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Days Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
GUANINE I				
1	2	3.8	.4	.4
2	2	3.4	.0	.0
3	3	3.4	.0	.0
4	3	3.3	-.1	-.1
5	4	3.3	-.1	-.1
6	4	3.4	.0	.0
7	5	3.5	.1	.1
8	5	3.3	-.1	.1
		3.4	± .1	
GUANINE II				
9	2	3.7	.2	.3
10	2	3.5	.0	.1
11	3	3.3	-.2	-.1
12	3	3.4	-.1	.0
13	4	3.5	.0	.1
14	4	3.6	.1	.2
15	5	3.2	-.3	-.2
16	5	3.4	-.1	.0
		3.5	± .1	
Mean of group means		3.4	± .1	
General mean		3.4	± .1	

TABLE XV

SOLUBILITY OF GUANINE AT 25°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Days Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
GUANINE I				
1	2	3.2	-.3	-.2
2	2	3.3	-.2	-.1
3	3	3.3	-.2	-.1
4	3	3.3	-.2	-.1
5	4	3.6	.1	.2
6	4	3.6	.1	.2
7	8	3.6	.1	.2
8	8	3.8	.3	.4
		3.5	± .2	
GUANINE II				
9	2	3.3	-.1	-.1
10	2	3.5	.1	.1
11	3	3.2	-.2	-.2
12	3	3.3	-.1	-.1
13	4	3.3	-.1	-.1
14	4	3.3	-.1	-.1
15	8	3.6	.2	.2
16	8	3.7	.3	.3
		3.4	± .1	
Mean of group means		3.4	± .2	
General mean		3.4	± .1	

TABLE XVI

SOLUBILITY OF GUANINE AT 50°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Days Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
GUANINE I				
1	7	11.8	-1.2	-1.5
2	7	12.0	-1.0	-1.3
3	8	12.4	-.6	-.9
4	8	12.8	-.2	-.5
5	9	13.0	.0	-.3
6	9	12.5	-.5	-.8
7	10	12.3	-.7	-1.0
8	1	14.4	1.4	1.1
9	1	13.7	.7	.4
10	1	13.6	.6	.3
11	1	13.6	.6	.3
12	1	13.2	.2	-.1
13	2	13.3	.3	0
14	2	13.1	.1	-.2
15	2	13.0	.0	-.3
16	2	13.1	.1	-.2
17	2	13.3 / 13.0	.3 / ±.3	0

TABLE XVI (con)

Ex.No.	Days Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
GUANINE II				
18	1	12.3	-1.3	-1.0
19	1	13.6	.0	.3
20	1	13.7	.1	.4
21	1	13.4	-.2	.1
22	1	13.2	-.4	-.1
23	2	14.5	.9	1.2
24	2	14.0	.4	.7
25	2	13.4	-.2	.1
26	2	13.0	-.6	-.3
27	2	13.4	-.2	.1
28	3	12.5	-1.1	-.8
29	3	14.0	.4	.7
30	3	14.1	.5	.8
31	3	14.2	.6	.9
32	3	14.2	.6	.9
		13.6	± .3	
Mean of group means		13.3	± .4	
General mean		13.3	± .3	

TABLE XVII

SOLUBILITY OF GUANINE AT 50°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Days Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
GUANINE I				
1	1	13.5	.6	.7
2	2	13.0	.1	.2
3	3	13.1	.2	.3
4	1	13.1	.2	.3
5	1	13.4	.5	.6
6	1	13.0	.1	.2
7	1	13.0	.1	.2
8	1	13.2	.3	.4
9	2	13.5	.6	.7
10	2	12.0	-.9	-.8
11	2	12.9	.0	.1
12	2	12.5	-.4	-.3
13	2	12.5	-.4	-.3
14	2	12.4	-.5	-.4
15	3	13.0	.1	.2
16	3	12.9	.0	.1
17	3	12.8	-.1	0
18	3	12.8	-.1	0
19	3	12.8	-.1	0
20	3	12.8	-.1	0
		12.9	±.2	

TABLE XVII (con)

Ex.No.	Days Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
GUANINE II				
21	1	14.0	1.4	1.2
22	1	12.5	-.1	-.3
23	1	12.7	.1	-.1
24	1	12.3	-.3	-.5
25	1	12.7	.1	-.1
26	2	13.7	1.1	.9
27	2	12.4	-.2	.4
28	2	12.4	-.2	.4
29	2	12.6	.0	-.2
30	2	13.0	.4	.2
31	4	12.8	.2	0
32	4	12.0	-.6	-.8
33	4	11.5	-1.1	-1.3
34	4	11.6	-1.0	-1.2
35	4	13.0	.1	.2
		12.6	± .9	
Mean of group means		12.8	± .4	
General mean		12.8	± .2	

THE BASIC DISSOCIATION CONSTANT AT 25°C

As has been already pointed out the dissociation constant of guanine was done by the solubility method. Small amounts of guanine were put into a series of solubility tubes and equilibrated with successively increasing concentrations of hydrochloric acid for a period of two days. Portions of the saturated solution were removed and the solubility determined as previously described. The standard solution of guanine used for comparison was made up in hydrochloric acid solution of the same concentration as the solution being tested in order to promote solution and to have the same conditions for the development of color with the diazo reagent. Simultaneously with the solubility determinations of pH were made. The dissociation constants were then calculated in the manner described above. The value obtained for the solubility of guanine in water was used as the minimum solubility.

The results obtained for the dissociation constant of guanine at 25°C are given in table XVIII. The error in the pH determinations is estimated as .05 pH units. Since a difference between the minimum solubility and the solubility in the hydrochloric acid is involved in the calculation a large error is introduced where this

difference is small, ie., in dilute hydrochloric acid solutions. Furthermore, the dissociation constant is small and is therefore in a region where its determination is difficult. The results obtained therefore, show wide variation.

The average value of pK_b taken between the figures marked with asterisks is 10.90. This gives as the dissociation constant $K_b = 1.3 \times 10^{-11}$. This value of the dissociation constant is taken to represent the order of magnitude rather than an accurate result.

The variation of solubility of guanine in hydrochloric acid solutions and consequently its dissociation is represented graphically in Figure 4.

Wood (21) using his solubility values in tenth normal and twentieth normal hydrochloric acid found the basic dissociation constant of guanine at 40.2°C to be $.807 \times 10^{-11}$. The dissociation constant appears to be smaller even at a higher temperature than that obtained in our experiments. It is suggestive of some contamination.

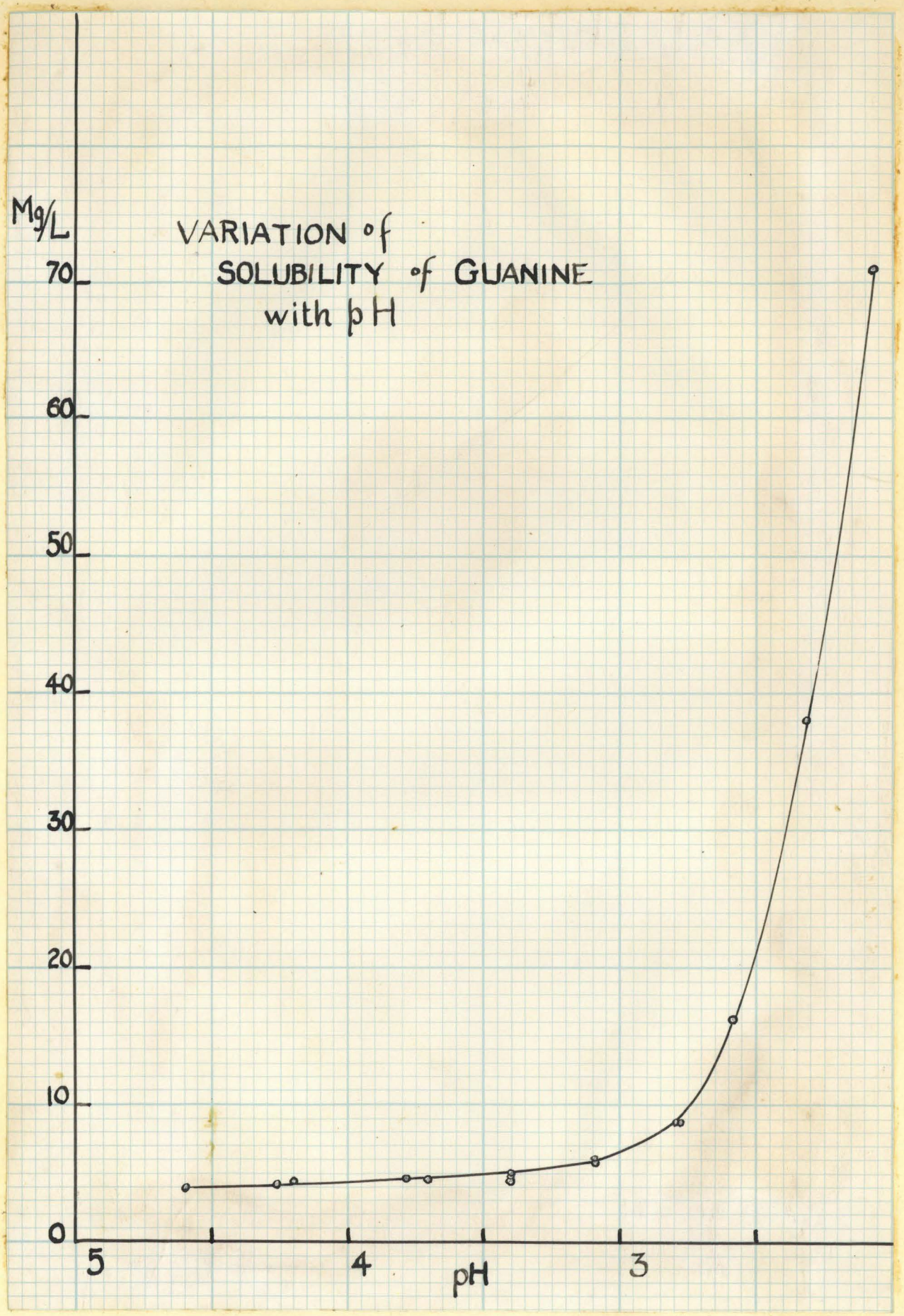

Mg/L
VARIATION of
SOLUBILITY of GUANINE
with pH
70
60
50
40
30
20
10
0
5
4
3
pH

FIGURE 4

TABLE XVIII

DISSOCIATION CONSTANT OF GUANINE AT 25°C

Ex.No.	Solubility Mg/Kg of Water	pH of Guanine Sol'n in HCl	pK_b
1	3.96	4.57	10.22
2	4.03	4.57	10.08
3	4.42	4.19	10.27
4	4.65	3.78	10.25
5	4.87	3.69	10.88*
6	4.20	3.39	10.93
7	4.85	3.39	10.99
8	5.00	3.39	10.94
9	6.05	3.08	11.03
10	5.75	3.08	11.08
11	8.73	2.79	11.01
12	8.70	2.76	11.04
13	16.70	2.58	10.82
14	38.00	2.31	10.68
15	71.00	2.06	10.71
16	129.00	1.79	10.64*

pK_b 10.90

K_b 1.3×10^{-11}

XANTHINE

THE SOLUBILITY OF XANTHINE

The solubility of xanthine was determined by the colorimetric method of Koessler and Hanke (5). The method was as follows. A standard solution was prepared by dissolving 3 mg. of finely pulverized xanthine in 200 ml. of water thus giving a concentration of 15 mg. per kilogram of water. Under ordinary conditions the rate of solution is slow but with the aid of the blunt end of a stirring rod and sometimes heat solution is finally effected.

A suitable portion of the saturated solution of xanthine was transferred from a solubility tube into a weighing bottle. The tests were performed by adding to each of the test tubes 2 ml. of the Koessler and Hanke diazo reagent, followed by 5 ml. of 1.1% Na_2CO_3 solution to one and an equal quantity of a saturated xanthine solution to the other. About 20 minutes was allowed for the yellow color to develop. Comparison was made in a colorimeter in the usual way.

The quantities of the reagents for the 50°C solubility were modified so as to obtain a suitable intensity of color in each of the solutions. The standard was made by dissolving 3 mg. of xanthine in 50 ml. of 1.1% Na_2CO_3

solution thus giving a concentration of 60 mg. per 1000 grams of water. The test was done by using,

a) 2ml. of diazo reagent + 5 ml. of 1.1% Na_2CO_3 solution + 1 ml. of saturated xanthine solution.

b) 2 ml. of diazo reagent + 4 ml. of 1.1% Na_2CO_3 solution + 1 ml. standard solution + 1 ml. water.

The solutions were compared after 20 minutes.

The solubility of xanthine at 25°C was found to be .0164 ± .0007 grams per 1000 grams of water. A saturated solution is therefore taken as .000108 molal. From tables XIX and XX it may be seen that the agreement of the results on the two preparations of xanthine used is satisfactory. There also appears to be little difference whether the solubility equilbrium is approached from the undersaturated or the supersaturated side. The largest variation is among the individual determinations. It therefore appears that the precision of the result is determined by the precision of the method.

The results of the solubility determinations at 50°C are shown in tables XXI and XXII. The solubility of xanthine at this temperature was found to be .067 ± .004 grams per 1000 grams of water. The saturated solution is therefore .000440 molal at this temperature. Although there is a considerable variation among the individual

determinations the largest discrepancy is due to the fact that xanthine I has a lower solubility than the other two preparations.

In this connection it is instructive to examine the graph in Figure 6. If it is recalled that the solubility of xanthine in water was found to be 16.4 mg. per 1000 grams and that it increases very rapidly with a slight change in pH, it will be seen that the solubility of xanthine will be considerably affected by small amounts of contaminants. In this light it is possible to understand the large variation in the results obtained by various investigators. A summary of their results is given below.

Temperature	Solubility Gm/Kg Water	Investigator
10°C	.470	Strecker (22)
16	.615	"
16	.0675	Sundwick (23)
16	.069	Almen (24)
17	2.600	Stutzer (18)
40	.075	Staedeler (25)
40	.182	Wood (21)
100	1.380	Strecker (22)
100	.725	"
100	.885	Staedeler (25)
100	.770	Almen (24)

The conclusion that has been drawn from results such as above is that the solubility of xanthine depends on the method of preparation. It is more logical to conclude that the solubility depends on the purity of the preparations, and the above results may then be explained by the varying amounts of impurities in the xanthine preparations. The effect of purification is to decrease the solubility of xanthine. We believe that our results are more accurate than any of those reported in the literature. Our results on the dissociation constant of xanthine also support this conclusion.

TABLE XIX

SOLUBILITY OF XANTHINE AT 25°C

Equilibrium approached from the Undersaturated Side

	Ex.No.	Days Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
XANTHINE I					
	1	1	16.0	-.2	-.3
	2	2	15.9	-.3	-.4
	3	11	15.1	-1.1	-1.2
	4	12	17.8	1.6	1.5
	5	14	15.8	-.4	-.5
	6	16	15.7	-.5	-.6
	7	18	17.4	1.2	1.1
			16.2	±.7	
XANTHINE IV					
	8	2	17.5	1.1	1.2
	9	3	15.9	-.5	-.4
	10	5	15.5	-.9	-.8
	11	6	16.5	.1	.2
	12	7	16.8	.4	.5
			16.4	±.7	
	Mean of group means		16.3	±1.0	
	General mean		16.3	±.5	

TABLE XX

SOLUBILITY OF XANTHINE AT 25°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Days Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
XANTHINE I				
1	3	17.1	.3	.6
2	4	18.5	1.7	2.0
3	5	16.5	-.3	.0
4	6	16.3	-.5	-.2
5	15	17.5	.7	1.0
6	16	16.1	-.7	-.4
7	17	15.9	-.9	-.6
		16.8	±.7	
XANTHINE IV				
8	2	15.9	-.1	-.6
9	3	15.9	-.1	-.6
10	5	15.9	-.1	-.6
11	6	16.5	.5	.0
12	7	15.7	-.3	-.8
		16.0	±.3	
Mean of group means		16.4	±.8	
General mean		16.5	±.5	

TABLE XXI

SOLUBILITY OF XANTHINE AT 50°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Days Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
XANTHINE II				
1	1	70.5	1.4	3.9
2	2	73.5	4.4	6.9
3	3	78.0	8.9	11.4
4	4	70.2	1.1	3.6
5	5	60.2	-8.9	-6.4
6	7	66.2	-2.9	-.4
7	8	66.6	-2.5	.0
8	9	68.2	-0.9	1.6
9	10	68.2	-0.9	1.6
		69.1	±3.0	
XANTHINE I				
10	1	68.7	8.9	2.1
11	2	62.1	2.3	-4.5
12	3	58.4	-1.4	8.2
13	4	55.6	-4.2	-11.0
14	6	59.0	-.8	-7.6
15	7	58.4	-1.4	-8.2
16	8	56.6	-3.2	-10.0
		59.8	±3.0	

TABLE XXI (con)

Ex.No.	Days Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
XANTHINE III				
17	1	70.3	1.1	3.7
18	1	71.4	2.2	4.8
19	2	68.7	-.5	2.1
20	2	71.4	2.2	4.8
21	3	69.2	.0	2.6
22	3	67.7	-1.5	1.1
23	4	69.2	.0	2.6
24	4	66.7	-2.5	.1
25	5	68.7	-.5	2.1
26	5	68.7	-.5	2.1
		69.2	± .9	
Mean of group means		66.0	± 5.0	
General mean		66.6	± 2.0	

TABLE XXII

SOLUBILITY OF XANTHINE AT 50°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Days Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
XANTHINE II				
1	2	69.7	.6	2.9
2	3	71.4	2.3	4.6
3	4	68.7	-.4	1.9
4	6	68.2	-.9	1.4
5	7	66.7	-2.4	-.1
6	8	69.7	.6	2.9
		69.1	± 1.3	
XANTHINE I				
7	1	66.7	3.8	.1
8	2	60.0	-2.9	-6.8
9	3	60.4	-2.5	-6.4
10	4	67.2	4.3	.4
11	5	60.8	-2.1	-6.0
12	6	62.1	-.8	-4.7
		62.9	± 2.7	

TABLE XXII (con)

EX.NO.	Days Equi-librated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
XANTHINE III				
13	1	67.7	-.4	.9
14	1	70.3	2.2	3.5
15	2	67.7	-.4	.9
16	2	62.1	-6.0	-4.7
17	3	70.6	2.5	3.8
18	3	71.0	2.9	4.2
19	4	68.7	.6	1.9
20	4	66.7	-1.4	-.1
		68.1	± 2.0	
Mean of group means		66.7	± 4.0	
General mean		66.8	± 3.0	

HYDRATION OF XANTHINE AT 25°C

It has been shown that xanthine crystallizes out of water with one molecule of water of crystallization. (26) The following experiments were done to demonstrate whether a hydrate is formed at 25°C.

Solid xanthine was equilibrated with its aqueous solution for three or four days under the conditions of the solubility experiments. The solid phase was removed by filtration and superficially dried between filter papers. These samples were then submitted to drying in presence of sulfuric acid solutions of known vapor pressures as in the case of adenine. After four days the samples were removed, weighed, and then further dried in an oven at 100°C. The results are given in table XXIII.

TABLE XXIII

HYDRATION OF XANTHINE

	Vap.Press of H_2SO_4 soln	Wt of Superfic. Dried Xanth.	Wt of Desicc. Dried Xanth.	Wt of Oven Dried Xanth.	Water of Hydr.
	MM of Hg	Grams	Grams	Grams	
1.	20.80	.087	.076	.0750	0
2.	21.40	.065	.049	.049	0
3.	22.54	.087	.066	.066	0
4.	23.25	.200	.175	.175	0
5.	Water	.089	.071	.066	.5

It is seen from the above experiments that water is completely lost from the xanthine samples to sulfuric acid solutions having vapor pressures of 23.25 mm of mercury or less. In Experiment 5 the preliminary loss was found to be due to evacuation of the system for one minute. It also indicates that if a hydrate were formed it would have retained more than .5 molecules of water as is indicated. From the above experiments it therefore appears that xanthine does not form a hydrate at 25°C.

THE DISSOCIATION CONSTANT OF XANTHINE AT 25°C

The dissociation constant of xanthine was done both by the titrimetric and the solubility methods.

For the titration of xanthine 100 ml. of .0001 molar solution was titrated with 1 mn. of .01 N. NaOH. The results are shown in table XXIV. There is an apparent drift in the values of pK_a probably due to the absorption of CO_2 from the air during the titration. It was also necessary to work with extremely dilute solutions which made the titration subject to error on this account. The average value of pK_a obtained is 7.06.

Because the results of the titration were not entirely satisfactory the dissociation constant of xanthine was also done by the solubility method. Small amounts of solid xanthine were equilibrated under the conditions of the solubility experiments with varying concentrations of naOH solution. After three days the solubility of the saturated solutions was determined. pH determinations were also made on each solution by means of a glass electrode and a Beckman pH meter. The results are given in table XXV. The limiting factor in the precision of these experiments was the pH determination. In the first place the pH values tended to drift

somewhat during the determination and in the second place a small change in the pH caused a considerable change in the pK_a value. The average value for pK_a of xanthine by the solubility method was found to be 7.10. Combining the results obtained by the two methods we get pK_a equal to 7.1 and the dissociation constant equal to 7.9×10^{-8}. It is to be noticed that the results obtained by either method are substantially the same.

The value for the dissociation constant of xanthine is given by Wood (21) as 1.10×10^{-10}. Such a small value would be expected if the xanthine preparation were insufficiently pure. This same indication of insufficient purification has already been referred to in connection with the solubility of xanthine. Furthermore judging from the ease with which xanthine dissolves in NaOH solutions as indicated by the steepness of the curve in Figure 6 and by the close structural relationship of xanthine to uric acid one would expect the dissociation constant to be higher than that given by Wood. It is interesting to point out the contrast between the dissociation constant and the pH solubility curve of guanine and xanthine. Guanine has a small dissociation constant hence its solubility changes very little until

the concentration of hydrochloric acid is fairly large. Xanthine on the other hand immediately increases in solubility even when only a small amount of NaOH has been added.

From all the evidence presented it appears that our value for the dissociation constant is more accurate than that given by Wood.

TABLE XXIV

ACID DISSOCIATION CONSTANT OF XANTHINE AT 25°C

NaOH ml	pH of Xanthine Solution	pH of Water Blank	pK_a
.0	5.95	5.90	....
.1	6.30	6.20	7.20
.2	6.54	6.54	7.24
.3	6.77	7.00	7.12
.4	6.94	7.75	7.12
.5	7.05	8.10	7.05
.6	7.19	8.50	7.01
.7	7.30	8.70	6.93
.8	7.43	8.90	6.83 *
.9	7.54	9.00	6.59
1.0	7.66	9.10	....

Average pK_a 7.06

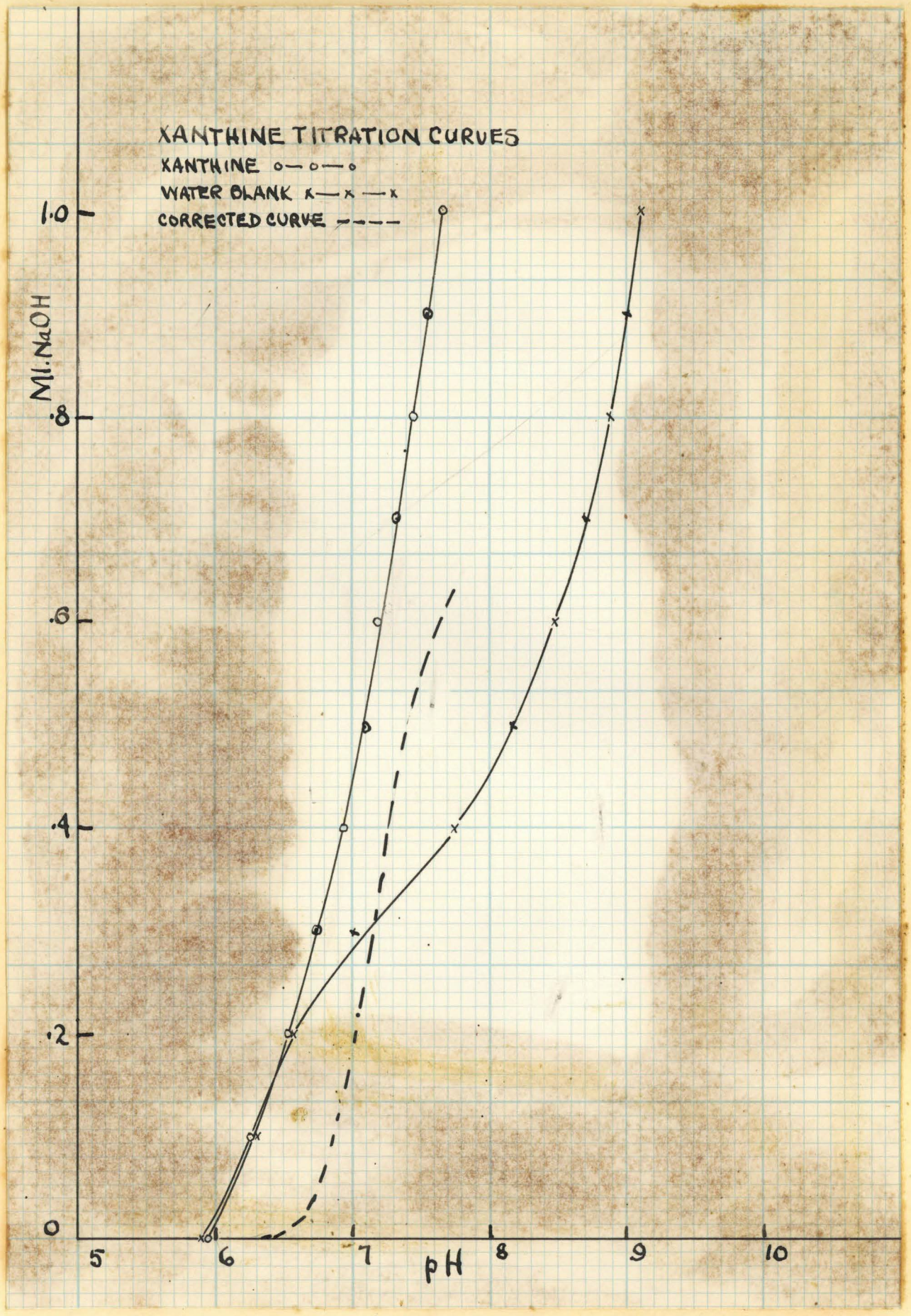

XANTHINE TITRATION CURVES
XANTHINE o—o—o
WATER BLANK x—x—x
CORRECTED CURVE - - -
Ml. NaOH
1.0
.8
.6
.4
.2
0
5
6
7
pH
8
9
10

FIGURE 5

TABLE XXV

DISSOCIATION CONSTANT OF XANTHINE AT 25°C FROM SOLUBILITY

EX. No.	pH of Xanthine Solution	Solubility Mg/Kg Water	pK_a
1	7.15	35.6	7.09
2	7.65	62.7	7.20
3	7.76	107.0	7.02
4	8.46	277.0	7.10

Average pK_a 7.10

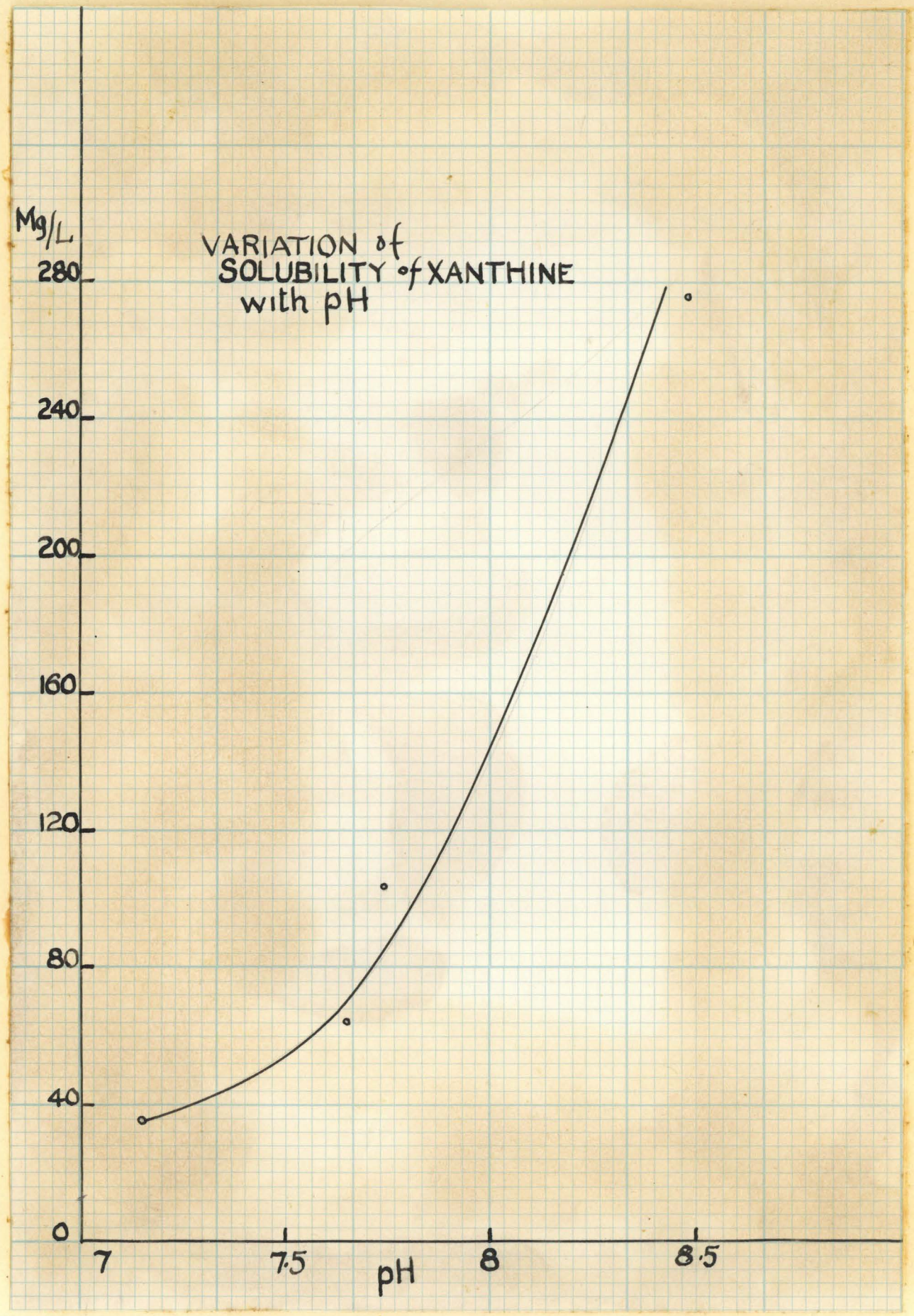

Mg/L
VARIATION of
SOLUBILITY of XANTHINE
with pH
280
240
200
160
120
80
40
0
7
7.5
pH
8
8.5

FIGURE 6

URIC ACID

THE SOLUBILITY OF URIC ACID

For the determination of the amount of uric acid in solution the colorimetric method of Benedict and Franke (27) was adopted. This method depends ona blue color which is developed when to a uric acid solution is added arseno-phosphotungstic acid reagent followed by a solution of sodium cyanide. The color is measured quantitatively in a colorimeter.

It was established by His and Paul (28) that the chief difficulty in the determination of the solubility of uric acid is its instability in aqueous solutions. In our experiments, therefore, uric acid was equilibrated with its aqueous solution only for a limited period of time. That equilibrium is established in a relatively short time was also shown by His and Paul in their work.

The details of our procedure are as follows. A portion of the saturated solution of uric acid was transferred from the solubility tube into a weighing bottle. It was then treated in the same way as the standard solution of uric acid. The standard solution was prepared by dissolving 5 mg. of uric acid in 250 ml. of water. This was readily effected by shaking. A new standard was prepared each day.

The solutions for colorimetric comparison were made up in 50 ml. flasks by adding,

a) 10 ml. of standard uric acid solution + 5 ml. NaCN solution + 1 ml. of arsenophosphotungstic acid reagent.

b) 5 ml. of saturated uric acid solution + 5 ml. water + 5 ml. NaCN solution + 1 ml. arsenophosphotungstic acid reagent.

After 5 minutes these were diluted to 50 ml. with water and compared in a colorimeter.

For the solubility at 50°C 2 ml. of the saturated solution was diluted to 10 ml. with water and then treated in the same way as before.

The results for the solubility of uric acid at 25°C are given in tables XXVI and XXVII. It will be seen that the values obtained when the solubility equilibrium was approached from the supersaturated side are somewhat higher than those obtained when the equilibrium was approached from the undersaturated side. This may be due to supersaturation or probably to decomposition. In the attempt to avoid decomposition supersaturation could not readily be avoided and conversely. The solubility of uric acid at 25°C is taken to be .041 ± .001 grams per 1000 grams of water. The molality of the saturated

solution of uric acid at this temperature is taken as .000244.

The results of the determinations of the solubility of uric acid at 50°C are given in tables XXVIII and XXIX. The solubility is taken to be .117 ±.004 grams per 100 grams of water. A saturated solution is therefore taken as being .000696 molal. There appears to be no systematic variation in the determinations.

Our results are in good agreement with those of His and Paul (28) and Gudzent (29) if a proper interpolation is made to account for the variation of solubility with temperature.

A summary of the earlier determinations of the solubility of uric acid is given by His and Paul. Although it is interesting historically the failure of the early investigators to recognize the instability of uric acid solutions makes their results of little value.

TABLE XXVI

SOLUBILITY OF URIC ACID AT 25°C

Equilibrium Approached from the Undersaturated Side

Ex. No.	Hours Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
URIC ACID I				
1	8	42.7	1.9	2.0
2	22	41.6	.8	.9
3	12	39.7	-1.1	-1.0
4	48	38.4	-2.4	-2.3
5	24	41.1	.3	.4
6	12	40.2	-.6	-.5
7	36	41.6	.8	.9
		40.8	±1.1	
URIC ACID II				
8	24	40.5	0.0	-.2
9	24	40.5	0.0	-.2
		40.5	± 0.0	
Mean of group means		40.7	± 1.1	
General mean		40.7	± .8	

TABLE XXVII

SOLUBILITY OF URIC ACID AT 25°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Hours Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
URIC ACID I				
1	12	39.5	-.6	-1.9
2	12	40.0	-.1	-1.4
3	12	41.0	.9	-.4
4	12	40.7	.6	-.7
5	24	39.1	-1.0	-2.3
6	24	39.5	-.6	-1.9
7	24	40.5	.4	-.9
8	24	40.5	.4	-.9
		40.1	±.5	
URIC ACID II				
9	12	42.2	-.5	.8
10	12	42.2	-.5	.8
11	12	42.5	-.2	1.1
12	12	44.5	1.8	3.1
13	24	43.5	.8	2.1
14	24	41.6	-1.1	.2
15	24	41.6	-1.1	.2
16	24	43.7	1.0	1.3
		42.7	± .7	
Mean of group means		41.4	± .9	
General mean		41.4	± .8	

TABLE XXVIII

SOLUBILITY OF URIC ACID AT 50°C

Equilibrium Approached from the Undersaturated Side

	Ex.No.	Hours Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
URIC ACID I					
	1	7	112	-3	-5
	2	9	96	-19	-21
	3	24	100	-15	-17
	4	8	120	5	3
	5	24	121	6	4
	6	12	120	5	3
	7	24	106	-9	-11
	8	10	116	1	-1
	9	24	122	7	5
	10	13	126	11	19
	11	24	129	14	12
	12	12	128	13	11
	13	24	105	-10	12
	14	10	108	-7	9
	15	12	121	6	4
	16	12	115	-0	-<
	17	12	118	3	1
	18	12	117	2	0
	19	12	117	2	0
	20	8	112	-3	-5

TABLE XXVIII (con)

	Ex.No.	Hours Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
	21	8	114	-1	-3
	22	8	112	-3	-5
	23	8	112	-3	-5
	24	8	113	-2	-4
			115.	± 4.	
URIC ACID II					
	25	8	127	7	10
	26	8	127	7	10
	27	8	124	4	7
	28	8	123	3	6
	29	8	124	4	7
	30	12	119	-1	2
	31	12	124	4	7
	32	12	124	4	7
	33	12	128	8	11
	34	12	128	8	11
	35	8	107	-13	-10
	36	8	106	-14	-11
	37	8	111	-9	- 6
	38	8	111	-9	-6
	39	8	110	-10	-7
			120	± 4	
	Mean of group means		117	± 6	
	General mean		117	± 3	

TABLE XXIX

SOLUBILITY OF URIC ACID AT 50°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Hours Equilibrated	Sol'y Mg/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
URIC ACID I				
1	6	120	3	2
2	6	118	1	0
3	6	122	5	4
4	6	125	8	7
5	24	109	-8	-9
6	24	113	-4	-5
7	24	115	-2	-3
8	24	115	-2	-3
		117	± 4	
URIC ACID II				
9	6	116	-3	-2
10	6	114	-5	-4
11	6	114	-5	-4
12	6	115	-4	-3
13	24	123	4	5
14	24	110	-9	-8
15	24	130	11	12
16	24	128	9	10
		119	± 5	
Mean of group means		118	± 7	
General mean		118	± 3	

THE DISSOCIATION CONSTANT OF URIC ACID AT 25°C

The dissociation constant of uric acid was determined by electrometric titration. 100 ml. of .000238 molar uric acid solution was titrated with 1.7 ml. of .0143 N. NaOH. The results of the titration are shown in table XXX. It will be seen that in the middle portion of the titration the pK_a values are in good agreement with one another. Uric acid is a fairly strong acid and can therefore be titrated satisfactorily in spite of its slight solubility.

The average value of pK_a obtained from the titration is 5.76. The dissociation constant is therefore taken as 1.74×10^{-6}. From careful conductivity measurements at 18°C. His and Paul (28) found the dissociation constant to be 1.51×10^{-6}, a result which is in substantial agreement with our value. Young and Musgrave (30) give 8×10^{-6} for the dissociation constant of uric acid. Their value was derived graphically from titrinetric data.

TABLE XXX

DISSOCIATION CONSTANT OF URIC ACID AT 25°C

NaOH	pH of Uric Acid Solution	pH of Water Blank	pK_a
.0	4.96	6.80	....
.1	5.06	7.51	6.02
.2	5.18	8.40	5.94
.3	5.28	8.77	5.87
.4	5.38	9.00	5.84#
.5	5.46	9.12	5.77
.6	5.56	9.22	5.79
.7	5.64	9.29	5.86
.8	5.72	9.37	5.75
.9	5.82	9.44	5.74
1.0	5.94	9.48	5.76
1.1	6.01		5.74
1.2	6.12		5.69
1.3	6.28		5.72
1.4	6.41		5.71*
1.5	6.56		5.50
1.6	6.81		5.38
1.7	7.35		....
		Average pK_a	5.76

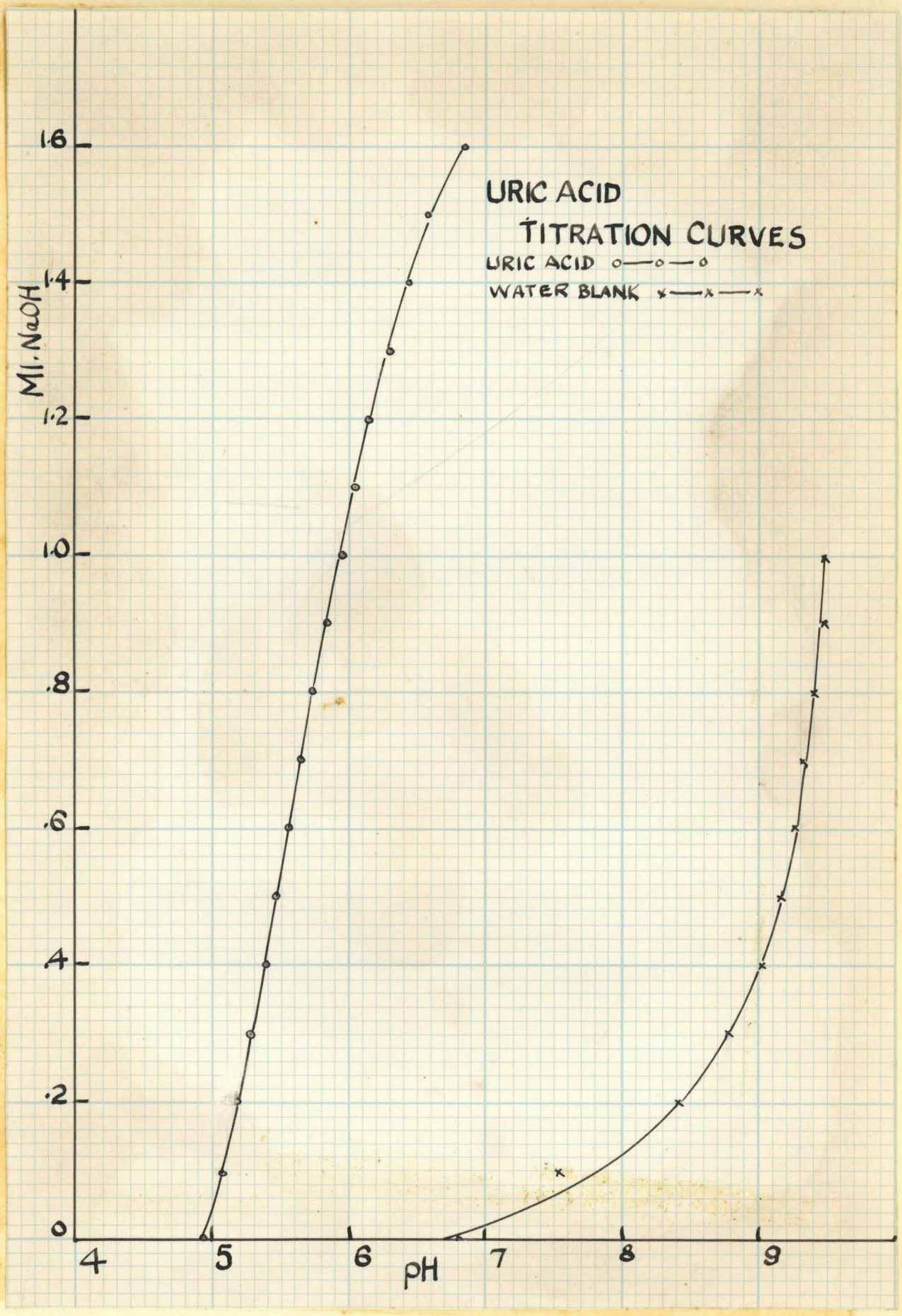

URIC ACID
TITRATION CURVES
URIC ACID o—o—o
WATER BLANK x—x—x
Ml. NaOH
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0
4
5
6
pH
7
8
9

FIGURE 7

ALLANTOIN

THE SOLUBILITY OF ALLANTOIN

The solubility of allantoin was determined by the gravimetric method. The result of the solubility determinations at 25°C are given in tables XXXI and XXXII and at 50°C in tables XXXIII and XXXIV. It appears that Allantoin II has a smaller solubility. The tendency of allantoin to supersaturate is also noticeable. While there are some large discrepancies among the individual results the solubility of allantoin is sufficiently large to make these deviations relatively small.

The solubility of allantoin at 25°C is taken to be 5.92 ± .1 . The molality of the saturated solution is .037$_3$. The solubility of allantoin at 50°C was found to be 18.1±.3 grams per 1000 grams of water. The molality of the saturated solution was taken to be .114 .

According to Martignon (31) the solubility of allantoin in water is 7.7 grams per 1000 grams of water at ordinary temperature. Schulze and Barbieri (32) give 5.4 grams per 1000 grams of water at 22°C a result which is on good agreement with ours. Allantoin is also reported as easily soluble in hot water.

TABLE XXXI

SOLUBILITY OF ALLANTOIN AT 25°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
ALLANTOIN I				
1	2	5.99	-.01	.13
2	3	6.00	.00	.14
3	4	6.09	.09	.23
4	2	5.89	-.11	.03
5	4	5.98	-.02	.12
6	5	5.94	-.06	.08
7	6	6.08	.08	.22
		6.00	± .05	
ALLANTOIN II				
8	2	5.73	.01	-.13
9	3	5.72	.00	-.14
10	4	5.75	.03	-.11
11	2	5.67	-.07	-.19
12	5	5.70	-.02	-.16
13	6	5.68	-.04	-.18
14	7	5.76	.04	-.10
		5.72	± .03	
Mean of group means		5.86	± .06	
General mean		5.86	± .08	

TABLE XXXII

SOLUBILITY OF ALLANTOIN AT 25°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Days Equi-Librated	Sol'y Gm/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
ALLANTOIN I				
1	3	6.17	.18	.19
2	4	6.05	.06	.07
3	5	5.89	-.10	-.09
4	6	6.00	.01	.02
5	6	5.90	-.09	-.08
6	7	5.93	-.06	-.05
7	8	5.97	-.02	-.01
8	9	6.03	.04	.05
9	11	5.98	-.01	.00
		5.99	± .06	
ALLANTOIN II				
10	3	6.34	.37	.36
11	4	6.09	.12	.11
12	5	5.96	-.01	-.02
13	6	5.92	-.05	-.06
14	7	6.16	.19	.18
15	8	5.95	-.02	-.03
16	9	5.84	-.13	-.14
17	10	5.77	-.20	-.21
18	12	5.71	-.26	-.27
		5.97	± .13	
Mean of group means		5.98	± .14	
General mean		5.98	± .07	

TABLE XXXIII

SOLUBILITY OF ALLANTOIN AT 50°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg Water	Dev.from Gr. Mean	Dev.from Gen. Mean
ALLANTOIN I				
1	1	17.81	-.04	-.07
2	2	17.71	-.14	-.17
3	3	17.88	.03	.00
4	4	17.92	.07	.04
5	5	17.93	.08	.05
6	6	17.85	.00	-.03
7	7	17.86	.01	-.02
		17.85	± .06	
ALLANTOIN II				
8	2	17.74	-.15	-.14
9	3	17.76	-.13	-.12
10	4	18.08	.19	.20
11	5	18.22	.33	.32
12	6	17.67	-.22	-.21
		17.89	± .26	
Mean of group means		17.88	± .27	
General mean		17.88	± .10	

TABLE XXXIV

SOLUBILITY OF ALLANTOIN AT 50°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
ALLANTOIN I				
1	1	17.73	-.26	-.49
2	2	18.12	.13	-.10
3	3	18.09	.10	-.13
4	4	18.11	.12	-.11
5	5	17.99	.00	-.23
6	6	17.90	-.09	-.32
		17.99	±.12	
ALLANTOIN II				
7	1	18.85	.39	.62
8	2	17.68	-.58	-.54
9	3	18.51	.05	.29
10	4	18.53	.07	.31
11	5	18.47	.01	.25
12	6	18.70	.24	.48
		18.46	± .30	
Mean of group means		18.23	± .32	
General mean		18.22	± .23	

THE DISSOCIATION CONSTANT OF ALLANTOIN AT 25°C

To determine the dissociation constant of allantoin 10 ml. of .038 molar solution was titrated with an equal volume of .038 N. NaOH. The results of the titration are given in table XXXV. The titration curve is shown in Figure 8.

It will be seen that the pK_a values are quite consistent so that an average value should give a good dissociation constant. The average pK_a value was found to be 8.67 which gives for the dissociation constant of allantoin at 25°C $K_a = 2.14 \times 10^{-9}$.

Wood (21) determined the dissociation constant of allantoin by following the rate of catalysis of the saponification of methyl acetate by allantoin hydrochloride. His value is $K_a = 1.17 \times 10^{-9}$. The titrimetric method is to be considered to be more reliable. Wood's work was done at a time when the measurement of H had not yet been developed to a high degree. His determination gives a result of the same order of magnitude as ours.

TABLE XXXV

DISSOCIATION CONSTANT OF ALLANTOIN AT 25°C

NaOH ml	pH of Allantoin Solution	pH of Water Blank	pK_a
.0	6.21	5.16	....
.25	7.14	10.66	8.73
.50	7.46	10.99	8.62
.75	7.63	11.14	8.73
1.00	7.73	11.24	8.67
1.50	7.93	11.39	8.68
2.00	8.06	11.46	8.66
2.50	8.16	.....	8.64
3.00	8.28	11.56	8.65
4.00	8.62	11.64	8.80
5.00	8.62	11.66	8.62
6.00	8.81	11.72	8.64
7.00	9.00	11.73	8.63
8.00	9.21	11.75	8.62
9.00	9.52	11.76	....
10.00	10.00	11.78	....

pK_a 8.67

K_a 2.14×10^{-9}

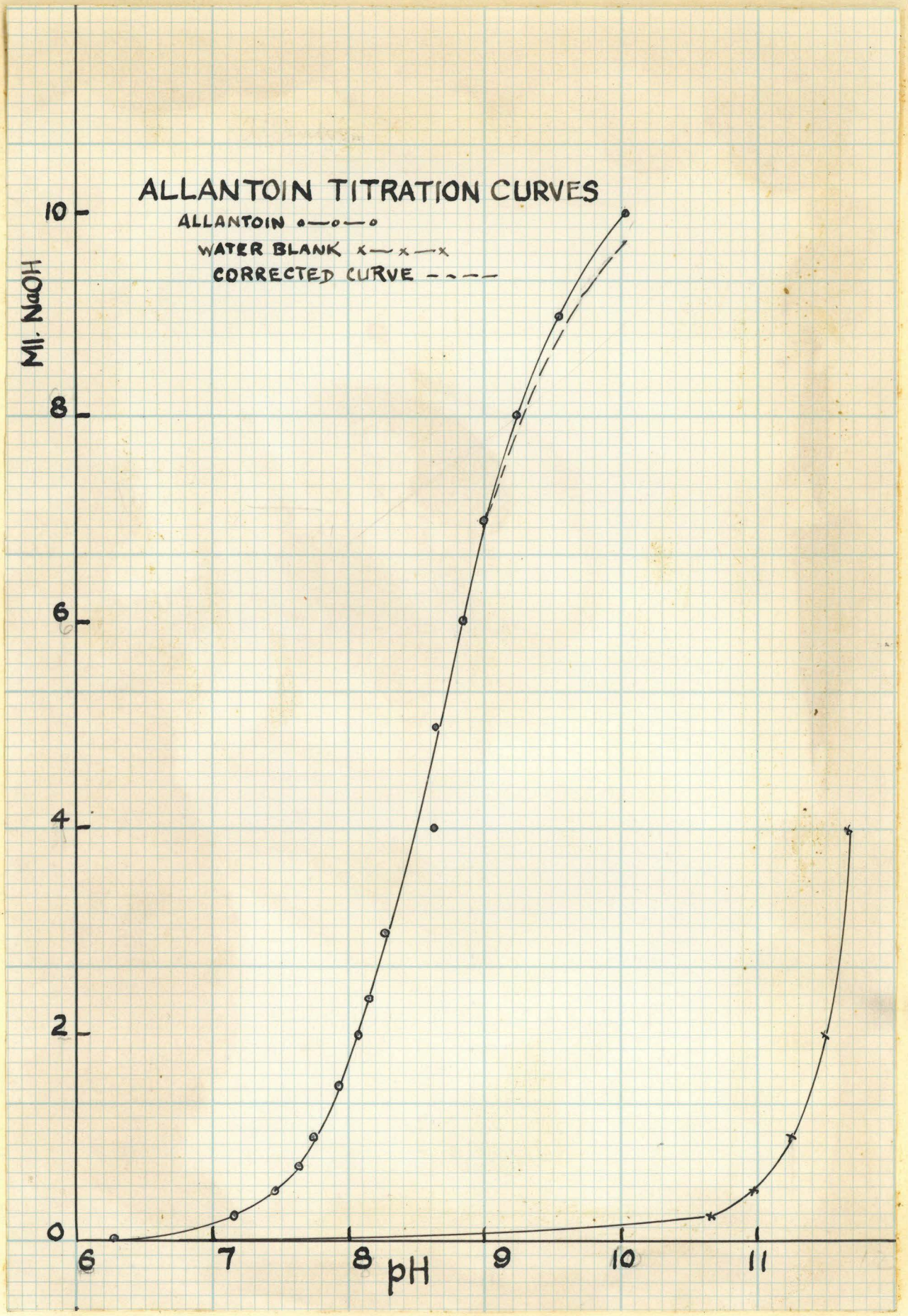

ALLANTOIN TITRATION CURVES
ALLANTOIN o—o—o
WATER BLANK x—x—x
CORRECTED CURVE - - - -
Ml. NaOH
10
8
6
4
2
0
6
7
8
9
10
11
pH

FIGURE 8

ALLOXAN

THE SOLUBILITY OF ALLOXAN

There are no quantitative data in the literature on the solubility of alloxan.

The solubility of alloxan was determined gravimetrically. There are several points of departure from the general method which should be noted. Since alloxan is very soluble only small portions of the saturated solution were required for a single determination. About .5 grams of the solution was used. If the samples were larger some difficulty was encountered in the drying.

Drying was done in vacuo over sulfuric acid at room temperature in order to prevent decomposition. It required about a week for the samples to dry to constant weight. Under these conditions alloxan contains one molecule of water of constitution which is sometimes referred to as water of crystallization.

In order to obtain reliable and consistent results one must be aware of the difficulties encountered due to the instability of alloxan. It decomposes to urea, oxalic acid, alloxantin and carbon dioxide even when dry as was shown by Gortner (33). When in a closed system several explosions have been reported (34), (35), (36). In aqueous solutions this decomposition is accelerated

considerably which makes the determination of any physical constants of alloxan somewhat difficult. Freshly crystallized alloxan was used in all the experiments and the time of contact with water was also decreased to a minimum.

The results of the solubility determinations of alloxan are given in tables XXXVI, XXXVII, XXXVIII and XXXIX. At 25°C the solubility was found to be 416± 2 grams per 1000 grams of water, corresponding to 2.60 molal solution at saturation.

At 50°C the solubility was found to be 719± 2 grams per 1000 grams of water, corresponding to 4.49 molal solution at saturation.

Because of the high solubility it is rather easy to obtain results with considerable precision. Some uncertainty is introduced on account of the instability of alloxan although this has been reduced by working under favorable conditions.

Note- the molecular weight of the stable modification of alloxan at 25°C is taken as 160.087. This corresponds to a formula $C_4H_4O_5N_2$.

TABLE XXXVI

SOLUBILITY OF ALLOXAN AT 25°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Hours Equilibrated	Solubility Gm/Kg Water	Dev. from the Mean
1	24	417.6	1.0
2	"	416.0	-.6
3	"	416.8	.2
4	"	416.2	-.4
5	"	416.5	-.1
	Mean	416.6 ±.6	

TABLE XXXVII

SOLUBILITY OF ALLOXAN AT 25°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Hours Equilibrated	Solubility Gm/Kg Water	Dev. from the Mean
1	24	418.5	2.4
2	"	415.5	-.6
3	"	416.6	.5
4	"	415.3	-.8
5	"	414.6	-1.5
	Mean	416.1 ±1.4	

TABLE XXXVIII

SOLUBILITY OF ALLOXAN AT 50°C

Equilibrium Approached from the Undersaturated Side

Ex. No.	Hours Equilibrated	Solubility Gm/Kg Water	Dev. from the Mean
1	3	717.1	-2.0
2	"	719.5	.4
3	"	720.7	1.6
4	"	719.4	.3
5	"	718.9	-.2
	Mean	719.1 ± 1.2	

TABLE XXXIX

SOLUBILITY OF ALLOXAN AT 50°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Hours Equilibrated	Solubility Gm/Kg Water	Dev. from the Mean
1	3	718.5	-.3
2	"	718.4	-.4
3	"	717.9	-.9
4	"	718.9	.1
5	"	720.3	1.5
	Mean	718.8 ± .8	

THE DISSOCIATION CONSTANTS OF ALLOXAN AT 25°C

The dissociation constants of alloxan were determined by electrometric titration. 10 ml. of .0937 molar alloxan solution was titrated with 10 ml. of .0989 N. NaOH for the first dissociation constant and an additional 10 ml. of NaOH for the second dissociation constant.

During the first part of the titration the pH values were observed to drift especially if a sufficient length of time were allowed. In order to obviate this difficulty the initial pH readings were taken. During the second part of the titration of the first dissociation constant steady potentials were obtained. Good pH readings were also obtained throughout the titration of the second constant.

A considerable uncertainty has therefore been introduced into the value of the first dissociation constant due to the instability of alloxan. An average of the values obtained in the middle portion of the titration was taken. pK_a was found to be 6.70 giving for the dissociation constant 2.09×10^{-7}. The results are shown in table XL.

The second dissociation constant is much more satisfactory as may be seen from the data in table XLI. An average of the pK_a values gives 8.66. The dissociation constant comes out to be 2.19×10^{-9}.

The dissociation constant of alloxan was determined by Wood (~~21~~ 21a) and by Trubsbach (37) who give 2.32×10^{-7} and 4.11×10^{-5}. Both investigators used the conductivity method and both found that the conductivity changed during the determination. The value given by Wood is of the same order of magnitude as our first dissociation constant. By the conductivity method the second dissociation constant was not detected.

The titration curve of alloxan is given in Figure 9.

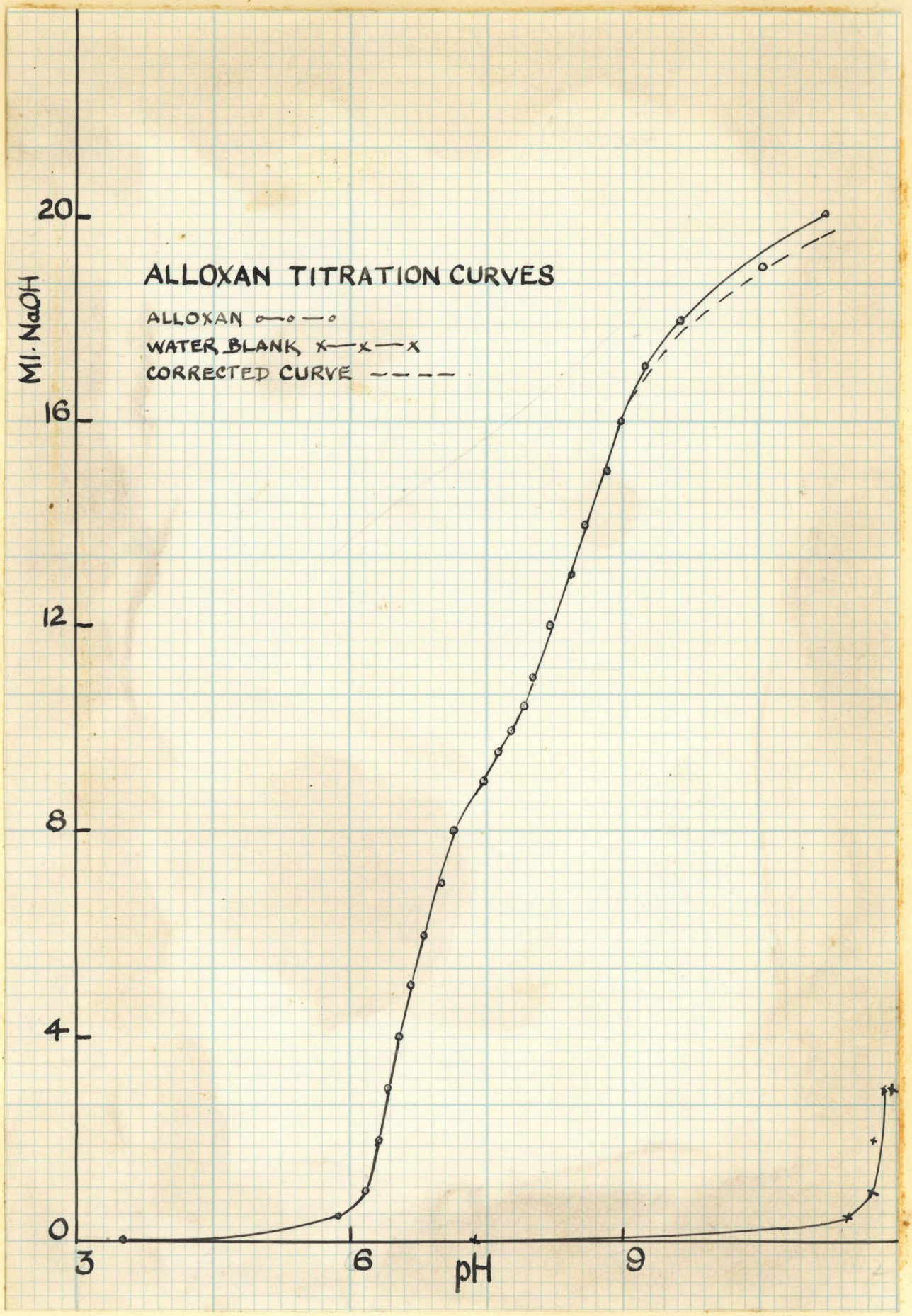

ALLOXAN TITRATION CURVES
ALLOXAN o—o—o
WATER BLANK x—x—x
CORRECTED CURVE – – – –
Ml. NaOH
20
16
12
8
4
0
3
6
9
pH

FIGURE 9

TABLE XL

THE FIRST DISSOCIATION CONSTANT OF ALLOXAN AT 25°C

NaOH ml	pH of Alloxan Solution	pH of Water Blank	pK_1
0.0	3.50	7.36	....
.5	5.90	11.50	7.16
1.0	6.17	11.70	7.10
1.5	6.25	.....	6.98
2.0	6.32	11.85	6.87*
2.5	6.40	.....	6.86
3.0	6.41	11.90	6.75
3.5	6.48	.....	6.71
4.0	6.57	...	6.71
4.5	6.64	.	6.68
5.0	6.68		6.63
5.5	6.74		6.59
6.0	6.83		6.59
6.5	6.93		6.58*
7.0	6.99		6.54
7.5	7.08		6.50
8.0	7.17		6.44
8.5	7.31		6.37
9.0	7.45		6.20
9.5	7.60		....
10.0	7.75		....
		pK_1	6.70

TABLE XLI

THE SECOND DISSOCIATION CONSTANT OF ALLOXAN AT 25°C

NaOH ml	pH of Alloxan Solution	pK_2
10.0	7.75	9.21
10.5	7.90	8.82
11.0	8.01	8.73 *
11.5	8.13	8.70
12.0	8.23	8.68
12.5	8.32	8.65
13.0	8.42	8.63
13.5	8.51	8.65
14.0	8.60	8.65
14.5	8.69	8.64
15.0	8.79	8.65
15.5	8.89	8.65
16.0	8.99	8.65
16.5	9.10	8.65
17.0	9.23	8.64
17.5	9.41	8.68
18.0	9.63	8.64
18.5	9.83	....
19.0	10.51	....
	pK_2	8.66

VAPOR PRESSURE OF ALLOXAN-ALLOXAN TRIHYDRATE SYSTEM AT 25°C

What is usually referred to as alloxan monohydrate is the stable modification of alloxan at 25°C and the water of crystallization is actually water of constitution. It will be referred to simply as alloxan in conformity with the terminology of Stiehler and Huffman (1), (2). On this basis the tetrahydrate becomes a trihydrate.

The vapor pressure of alloxan-alloxan trihydrate has been determined by the method previously described. Large alloxan trihydrate crystals are easily prepared by crystallizing alloxan from water. Such crystals were removed from the mother liquor and superficially dried between filter papers. Because the crystals are large the water of wetting can be almost completely removed in this manner. Crystals of alloxan trihydrate were submitted to desiccation in presence of solutions of sulfuric acid of known vapor pressure. The loss in weight of the alloxan trihydrate crystal was determined after four days. The results are given in table XLII.

The dehydration is slow so that the loss of water was not complete in all cases. The loss, however, is sufficient to indicate whether the vapor pressure of the hydrate system is greater or less than that of the

sulfuric acid solution. The vapor pressure of alloxan-alloxan trihydrate system is taken as the average of experiments 4 and 5, i.e., 21.1 ± .3 mm of mercury.

Alloxan was also shown to hydrate readily when the anhydrous form was placed in presence of water vapor at 25°C.

TABLE XLII

VAPOR PRESSURE OF ALLOXAN HYDRATE SYSTEM AT 25°C

No.	Vap.Press. H_2SO_4 Sol.	Wt of Allox. Hydr.Xtal	Wt After Desiccat.	Mol. of Water Remaining
	mm	gms.	gms.	
1	10.9	.682	.439	.9
2	17.8	.372	.274	.0
3	19.4	.186	.149	.7
4	20.8	.075	.061	.8
5	21.4	.075	.075	3.0
6	22.4	.208	.210	3.0

ACTIVITY COEFFICIENTS OF ALLOXAN AT 25°C

Because alloxan is unstable in aqueous solutions a suitable method was developed for determining the activities under such conditions. The method depends on the principle of isothermal distillation. The procedure was as follows.

The apparatus consisted of a small desiccator with water at the bottom so that the atmosphere inside was saturated with water vapor. Into this desiccator were placed small glass cups of approximately the same dimensions and ½ ml. capacity. They were placed on a suitable copper block with their places numbered. Into two such vessels were placed .25 ml. of standard NaCl solutions, the concentrations of which differed by about 10%. Into two other cups were placed .25 ml. of alloxan solution of such a concentration that its vapor pressure was between those of the two standards. It was possible to select this concentration by assuming the behavior of alloxan to be the same as that of equimolar urea for which the vapor pressures are known (38). Sometimes preliminary runs had to be made in order to determine the proper concentrations to be used in the actual experiment.

The glass cups were weighed with their respective solutions. Care was taken to prevent evaporation during the manipulations by covering the vessels with a glass cover. The vessels were weighed uncovered. The vessels were then placed on the copper block and put in the desiccator. The desiccator was placed in a 25°C incubator. The length of time required for an appreciable amount of distillation to take place was about 12 to 15 hours. For the more concentrated solutions the time could be shortened to about 8 or 10 hours. The temperature in the incubator remained constant to within .1° or less.

After a suitable period of time the vessels on the copper block were removed from the desiccator, covered with a glass cover and finally weighed after about 5 minutes. This time was required for the water film on the outside of the vessels to evaporate in order to increase the accuracy of the weighings. An example will illustrate the details more clearly.

TABLE XLIII

VAPOR PRESSURE DETERMINATION

Solution	Molality	Initial Wt. grams	Final wt. grams	Gain mg.
NaCl	1.130	.7921	.7964	4.3
NaCl	1.300	.7448	.7516	6.8
Alloxan	2.500	.7377	.7432	5.5
Alloxan	2.500	.7980	.8032	5.2

The initial and final weights refer to the total weight of the vessel plus the solution. Since it is the gain in weight that is required the absolute amount of the solution need not be known. The volumes of the solutions were measured with a pipette and were .25 ml. in all cases.

The average gain of the alloxan solution from the above data is 5.35 mg. The agreement between the two duplicates was usually better than in this example. It will be seen that 2.500 molal alloxan solution is isotonic or isopiestic with a NaCl solution which is between 1.130 and 1.300 molal and by interpolation this comes out to be 1.194 molal. On the average the results obtained agreed within 1%.

In this manner the isotonic solutions corresponding to four different concentrations of alloxan were determined. Each result was obtained as the average of several determinations. Table XLIV contains the data obtained.

TABLE XLIV

ISOTONIC NaCl AND ALLOXAN SOLUTIONS

Molality of Alloxan Soln.	Molality of Isotonic NaCl Soln.	Value of 100R	Vapor Press.
.700	.3803	3.27	23.460
1.250	.6277	3.27	23.269
1.8575 (1.875)	.9294	3.29	23.034
2.500	1.1960 (1.2010)	3.32	22.827 (22.807)

The calculation of vapor pressures was made by the use of the formula

$$100R = \frac{100(p_o - p)}{Mp_o}$$

given in the I.C.T. where P_o is the vapor pressure of pure water, p is the vapor pressure of NaCl solution and M is the molality. (g. NaCl / 1000 g. H_2O) R is defined by the equation and is the fractional vapor pressure lowering per mole of solute. The values of R for NaCl were obtained from I.C.T.

A graph of vapor pressure against molality was made for alloxan using the values from the table above. This is shown in Figure 10. Activity coefficients were calculated for several convenient rounded off molalities according to the method of Lewis and Randall (39) using the h/m function. In table XLV the results of these calculations are shown.

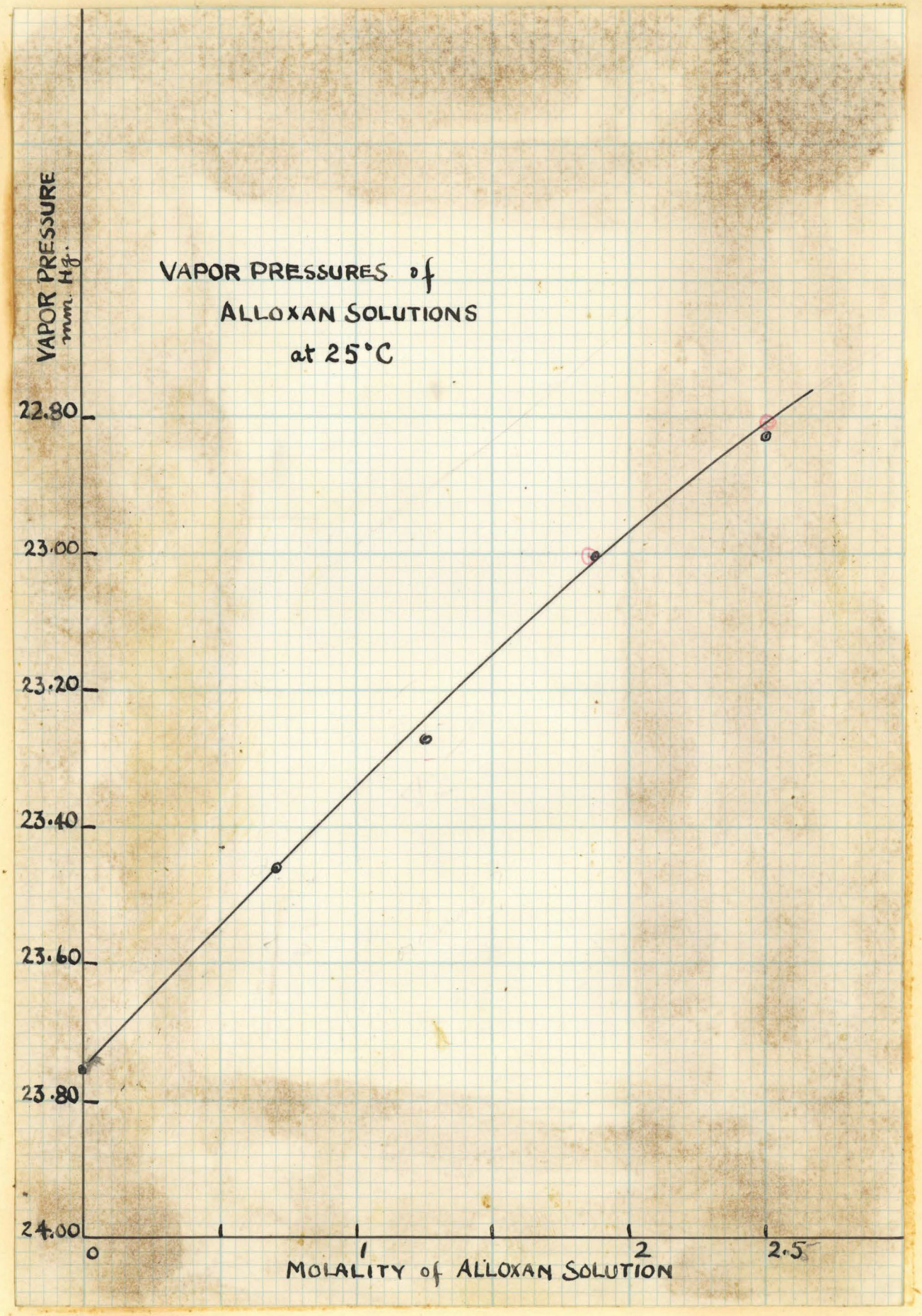

VAPOR PRESSURE mm. Hg.
VAPOR PRESSURES of ALLOXAN SOLUTIONS at 25°C
22.80
23.00
23.20
23.40
23.60
23.80
24.00
0
1
2
2.5
MOLALITY of ALLOXAN SOLUTION

FIGURE 10

Recalc. by Dobbl. gives extinction coefficient = .81 ± .02 at 2.59 m. (= satl'd solin.) see his notes in possession of Dr. Bowdoch.

TABLE XLV

ACTIVITY COEFFICIENTS OF ALLOXAN

Molality of Alloxan Soln.	Vapor Press. Mm of Hg	h/m	Activity Coefficient
.50	23.545	.0092	.9984
1.00	23.340	.0138	.9953
1.50	23.140	.0274	.9880
2.00	22.967	.0315	.9809
2.50	22.810	.0393	.9705
2.60	22.780	.0403	.9682

In order that the method of calculation be made clear all steps of the calculation of the activity coefficient for saturated alloxan solution are given. The vapor pressure of 2.60 molal alloxan solution is obtained directly from the graph in Figure 10. This value is 22.780 mm. Hg. The vapor pressure lowering is given by the formula $\frac{p_o-p}{p_o}$ which amounts to .04108.

The vapor pressure of water is a measure of the fugacity or activity of the solvent a_1. The value of $\ln a_1$ is next obtained by means of the formula

$$\ln a_1 = -\frac{p_o-p}{p_o} - \frac{1}{2}\left(\frac{p_o-p}{p_o}\right)^2 - \cdots$$

by substituting .04108 for the fractional vapor pressure lowering. $\ln a_1$ is thus -.04193.

The h function is related to the activity of the solvent by

$$h = \frac{55.51 \ln a_1}{m} + 1$$

where m is the molality and is 2.60 in this case. This gives a value of .1049 for h and .0403 for h/m.

The integral of h/m from 0 to m is obtained by graphic integration as shown in Figure 11. Such graphic integration gives -.05793. This value together with the value for h is substituted in the equation

$$\ln a_2/m = -h - \int_0^m h/m \, dm$$

to give the activity coefficient of alloxan of .9682 when evaluated.

It is to be noted that the h/m function is very sensitive so that some care is necessary in drawing the vapor pressure curve in Figure 10 in order to avoid tremendous distortion in the h/m values.

Other values were calculated in exactly the same manner.

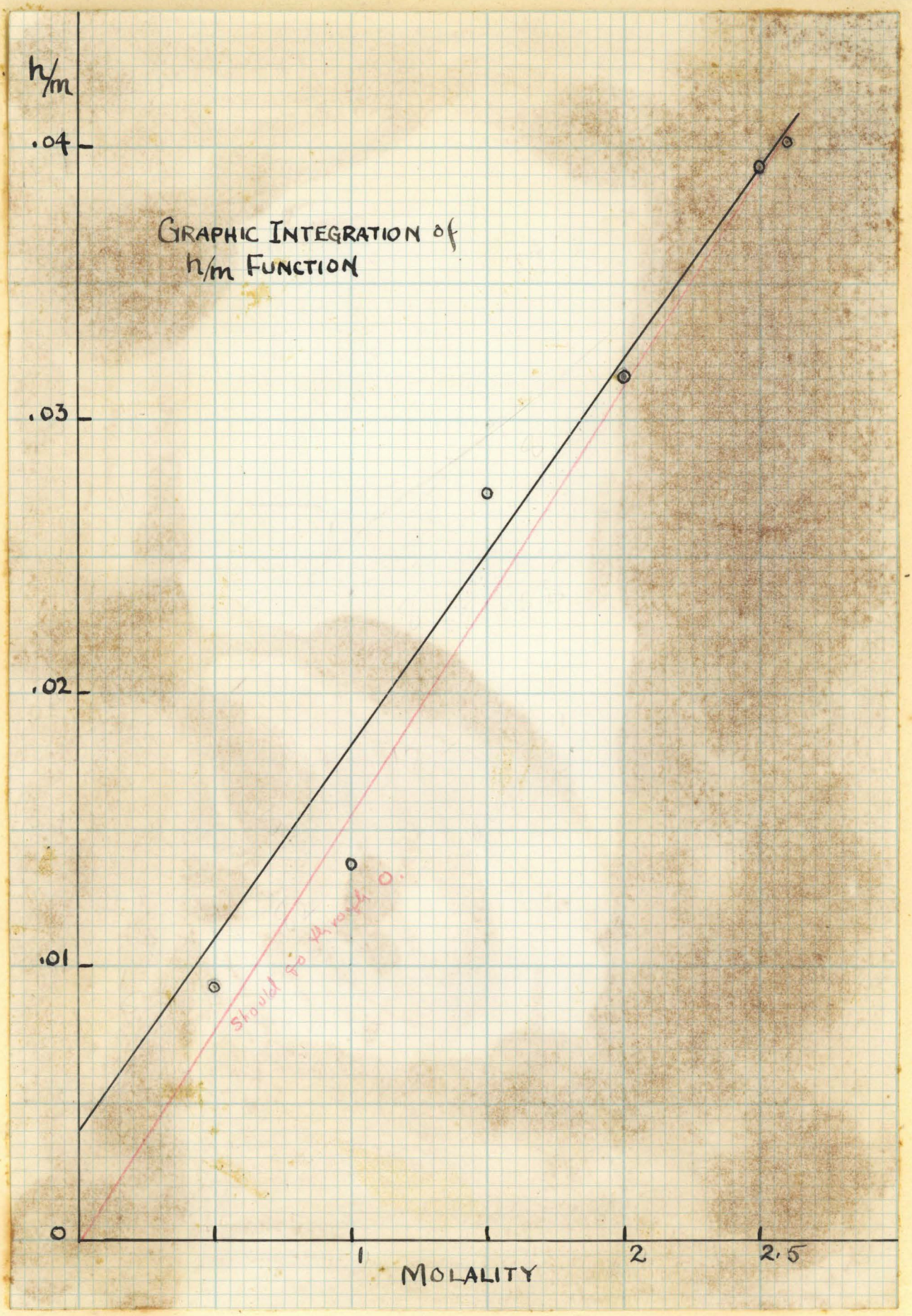

h/m
.04
.03
.02
.01
0
GRAPHIC INTEGRATION of
h/m FUNCTION
should go through 0.
1
2
2.5
MOLALITY

FIGURE 11

LEUCINE

THE SOLUBILITY OF LEUCINE

It has been shown by Muller (3) that leucine prepared from natural sources is invariably contaminated with methionine. Fox (13) has been able to prepare methionine free leucine. Our determinations on the solubility of leucine were done on methionine free preparations. Both the optical isomers were used.

The solubility of d- and l- leucine at 25°C was found to be 22.0 ±.2 grams per 1000 grams of water. The results are given in tables XLVI and XLVII. The precision of the results is quite satisfactory. There appear to be no systematic variations. The molality of saturated leucine solution at this temperature is .167.

The solubility of d- and l- leucine at 50°C was found to be 26.6 ± .3 grams per 1000 grams of water the molality of the saturated solution at this temperature being .202. The results obtained by approaching the equilibrium from the supersaturated are somewhat higher but the discrepancy is not large.

The results given by Dalton and Schmidt (4) on the solubility of l- leucine are about 10% higher than our values. One would expect a higher value for the

solubility if the preparations were contaminated with methionine. The work of other investigators is subject to the same criticism.

The pH of a saturated solution of leucine at 25°C was found to be 6.5.

TABLE XLVI

SOLUBILITY OF LEUCINE AT 25°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
d-Leucine				
1	2	21.86	-.17	-.17
2	3	22.14	.11	.11
3	5	22.02	-.01	-.01
4	7	22.06	.03	.03
5	8	22.06	.03	.03
		22.03	± .09	
l-Leucine				
6	2	21.88	-.14	-.15
7	4	22.12	.10	.09
8	5	22.06	.04	.03
9	8	22.02	.00	-.01
10	9	22.03	.01	.00
		22.02	± .08	
Mean of group means		22.00	± .11	
General mean		22.03	± .06	

TABLE XLVII

SOLUBILITY OF LEUCINE AT 25°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
d-Leucine				
1	2	22.17	.25	.13
2	19	21.88	-.04	-.16
3	21	21.73	-.19	-.31
4	22	21.69	-.23	-.35
5	23	22.14	.22	.10
		21.92	±.20	
l-Leucine				
6	2	22.17	.01	.13
7	19	22.21	.05	.17
8	21	21.98	-.18	-.06
9	22	22.10	-.06	.06
10	23	22.35	.19	.31
		22.16	±.12	
Mean of group mean		22.04	±.20	
General mean		22.04	±.14	

TABLE XLVIII

SOLUBILITY OF LEUCINE AT 50°C

Equilibrium Approached from the Undersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg Water	Dev.from Gro Mean	Dev.from Gen.Mean
d-Leucine				
1	1	26.61	-.17	.02
2	2	26.64	-.14	.05
3	3	27.14	.36	.55
4	4	26.04	-.74	-.55
5	5	27.11	.33	.52
6	6	27.14	.36	.55
		26.78	±.34	
l-Leucine				
7	1	26.48	.08	-.11
8	2	26.39	-.01	-.20
9	3	26.19	-.21	-.40
10	4	26.42	.02	-.17
11	5	26.33	-.07	-.26
12	6	26.58	.18	-.01
		26.40	±.11	
Mean of group means		26.59	±.36	
General mean		26.59	±.22	

TABLE XLIX

SOLUBILITY OF LEUCINE AT 50°C

Equilibrium Approached from the Supersaturated Side

Ex.No.	Days Equilibrated	Sol'y Gm/Kg Water	Dev.from Gr. Mean	Dev.from Gen.Mean
d-Leucine				
1	2	26.40	-.33	-.27
2	3	26.64	-.09	-.03
3	4	26.76	.03	.09
4	5	26.88	.15	.21
5	6	26.82	.09	.15
6	7	26.88	.15	.21
		26.73	± .15	
l-Leucine				
7	2	26.59	-.02	-.08
8	3	26.68	.07	-.01
9	4	26.77	.16	.10
10	5	26.63	.02	-.04
11	6	26.79	.18	.12
12	7	26.22	-.39	-.45
		26.61	± .17	
Mean of group means		26.72	± .23	
General mean		26.67	± .12	

THE DISSOCIATION CONSTANTS OF l-LEUCINE AT 25°C

The dissociation constants were determined on methionine free l-leucine. 10 ml. portions pf .0972 molar solution were titrated with 10 ml. of .0989 N. NaOH and HCl respectively. The results have been calculated on the basis of the classical theory and are given in tables L and LI. A titration curve is shown in Figure 12.

The average pK_a value was found to be 9.70, the dissociation constant therefore being 2.0×10^{-10}. This value agrees fairly closely with the values found by Sano (40), Harris (41), and Winkleblech (42). Wood (43) gives a value much smaller than this.

The average value of pK_b obtained is 12.4 giving for the dissociation constant K_b 3.98×10^{-13}. This result is much smaller than that given by the above mentioned investigators. The determination of dissociations constants in this range is subject to several practical difficulties. However the precision of the results in our determinations appears to be good and the discrepancy may be due to the methionine contamination. Methionine has a basic dissociation constant in this range and would be a likely source of error.

The zwitter ion constants for optically active leucine from the above results are $K_a = 2.51 \times 10^{-2}$ and $K_b = 5.01 \times 10^{-5}$.

TABLE I

BASIC DISSOCIATION CONSTANT OF l-LEUCINE AT 25°C

HCl	pH of Leucine Solution	pH of Water Blank	pK_b
.0	5.54	6.80	....
.5	3.04	2.53	12.3
1.0	2.68	2.15	12.4
1.5	2.55	2.00	12.3
2.0	2.38	1.87	12.4
2.5	2.27	1.80	12.4
3.0	2.18	1.72	12.4
3.5	2.10	1.65	12.4
4.0	2.05	1.60	12.4
4.5	2.00	1.58	12.3
5.0	1.93	1.58	12.4
5.5	1.88	....	12.4
6.0	1.83	1.58	12.4
6.5	1.78	....	12.4
7.0	1.73	1.54	12.5
7.5	1.68	....	12.5
8.0	1.63	1.45	12.6
8.5	1.60	....	12.6
9.0	1.57	1.43	12.6
9.5	1.54	....	12.6
10.0	1.52	1.38	....

pK_b 12.4

TABLE LI

ACID DISSOCIATION CONSTANT OF l-LEUCINE AT 25°C

NaOH	pH of Leucine Solution	pH of Water Blank	pK_a
.0	6.32	7.50	....
.5	6.46	11.50	9.72*
1.0	8.77	11.70	9.72
1.5	8.96	.....	9.71
2.0	9.12	11.85	9.71
2.5	9.23	.....	9.70
3.0	9.35	11.90	9.71
3.5	9.46		9.72
4.0	9.53		9.72
4.5	9.65		9.71
5.0	9.70		9.68
5.5	9.77		9.66
6.0	9.88		9.68
6.5	9.97		9.66
7.0	10.07		9.67
7.5	10.19		9.75*
8.0	10.27		9.63
8.5	10.42		9.62
9.0	10.58		9.55
9.5	10.78		9.30
10.5	11.03		....

pK_a 9.70

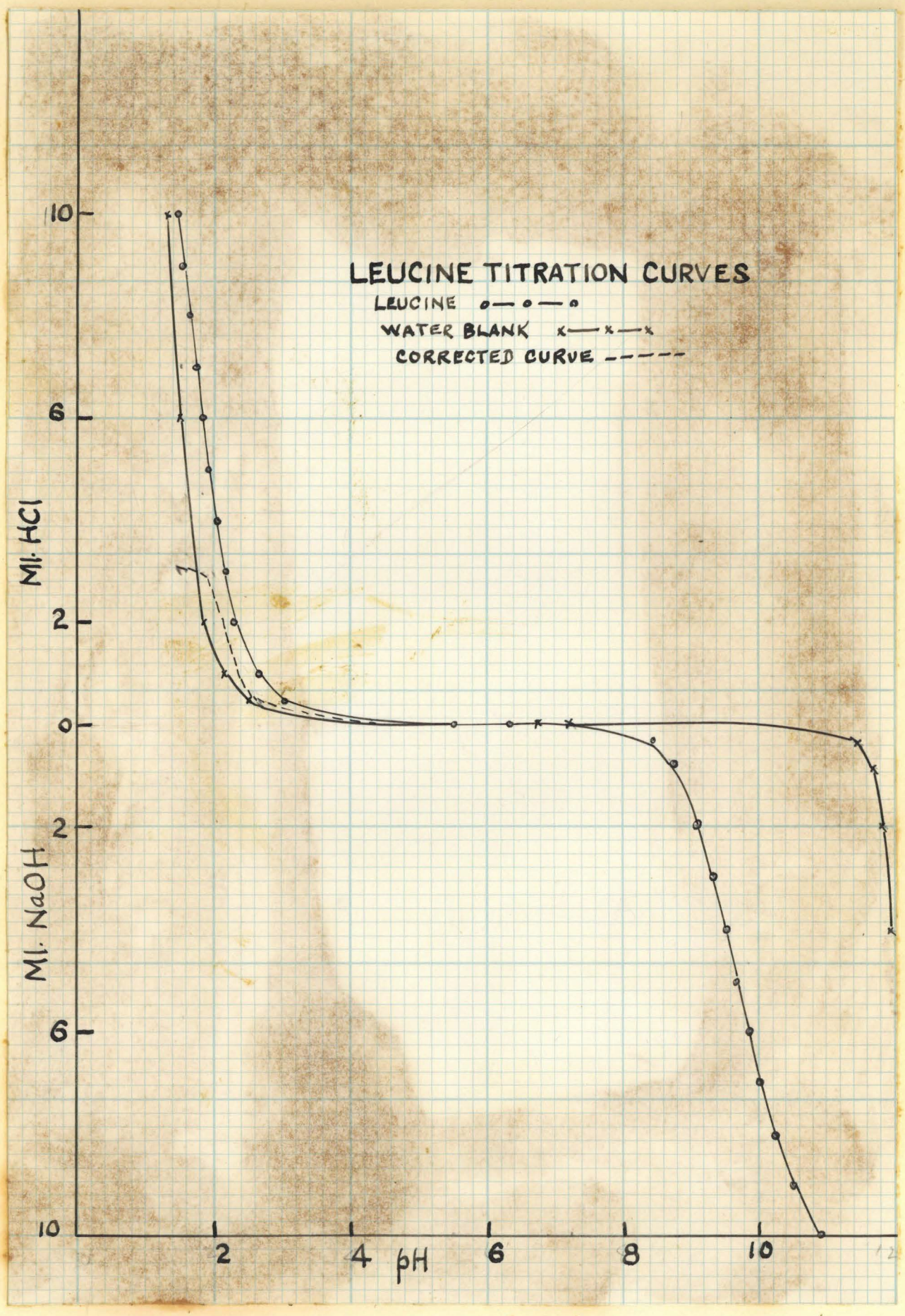

LEUCINE TITRATION CURVES
LEUCINE o—o—o
WATER BLANK x—x—x
CORRECTED CURVE - - - - -
Ml. HCl
Ml. NaOH
10
6
2
0
2
6
10
2
4
pH
6
8
10

FIGURE 12

TABLE LII

SUMMARY OF SOLUBILITY DATA

Compound	Solubility at 25°C Gm/Kg Water	Solubility at 50°C Gm/Kg Water
Adenine	1.13 ± .04	3.4 ± .2
Hypoxanthine	.73 ± .01	2.01 ± .06
Guanine	.0034 ± .0001	.0131 ± .0004
Xanthine	.0164 ± .0007	.067 ± .004
Uric Acid	.041 ± .001	.117 ± .004
Allantoin	5.9 ± .1	18.1 ± .2
Alloxan	416. ± 2.	719. ± 2.
d- and l-Leucine	22.0 ± .2	26.6 ± .3

TABLE LIII

SUMMARY OF DISSOCIATION CONSTANTS AT 25°

Compound	K_a	K_b
Adenine	1.26×10^{-10}	1.38×10^{-10}
Hypoxanthine	1.07×10^{-9}	2.0×10^{-13}
Guanine	.....	1.3×10^{-11}
Xanthine	7.9×10^{-8}	.....
Uric Acid	1.74×10^{-6}	.....
Allantoin	2.14×10^{-9}	.....
Alloxan	2.0×10^{-7}	.....
	2.19×10^{-9}	
l-Leucine	2.00×10^{-10}	3.98×10^{-13}

TABLE LIV

SUMMARY OF OTHER DATA

pH of saturated solution of adenine at 25°C 6.6

pH of saturated solution of hypoxanthine at 25°C 6.4

pH of saturated solution of d- and l- leucine at 25°C 6.5

Vapor pressure of alloxan - alloxan trihydrate system at 25°C 21.1 ± .3 mm. of mercury

Vapor pressure of saturated solution alloxan at 25°C 22.78 mm. of mercury

The activity coefficient of alloxan in saturated solution at 25°C .9682

DERIVED DATA

The following general considerations of the methods used in the calculation of the free energies of formation of the ionic and the non ionic species and other pertinent data are given here for reference. One example of each calculation is given in detail in order to illustrate the application of the methods. The heats of solution are calculated for the temperature range 25 - 50°C. All the other data are calculated for the temperature 298.1K.

THE HEAT OF SOLUTION

If the solubilities at two temperatures are known the heats of solution may be readily derived by the application of the van't Hoff equation,

$$\frac{d\ln K}{dT} = \frac{\Delta H}{RT^2}$$

where T is the absolute temperature, R is the gas constant, ΔH is the heat of solution and K is the equilibrium constant. Since, as has been explained, the solubility is the measure of the equilibrium constant we may replace K by M where M is the solubility in moles per 1000 grams of water. The value of ΔH will then be in calories per mole of solute.

When the compound under consideration forms a hydrate K represents the equilibrium constant of the reaction

$$X.H_2O \text{ (solid)} \longrightarrow X\text{(aq.)} + H_2O \text{ (sat. sol.)}$$

$$K = \frac{(X \text{ (aq.)}) \ (H_2O \text{ (sat. sol.)})}{(X.H_2O \text{ (solid)})}$$

Since the activity of the water in the saturated solution is not very different from unity in most cases and since the logarithm of K is used in the calculations the error introduced by using the solubility value instead of K is small. In the calculation of the heat of solution of alloxan this approximation will be made.

Over a short temperature interval ΔH may be assumed to remain constant. Substituting M for K in the van't Hoff equation and integrating between temperatures T_1 and T_2 we obtain

$$\log M_2/M_1 = \frac{\Delta H}{2.303R}\left(\frac{T_2 - T_1}{T_2T_1}\right)$$

where M_1 is the solubility at the temperature T_1 and M_2 the solubility at T_2. Since these are known the value of the heat of solution ΔH can be readily calculated.

As an illustration we shall calculate the heat of solution of adenine. The solubility of adenine at 25°C is .00836 moles per 1000 grams of water or $M_1 = .00836$ and $T_1 = 298.1$. Similarly $M_2 = .0252$ and $T_2 = 323.1$. The value for R is taken as 1.987. Substituting these values in the integrated form of the van't Hoff equation

$$\log .0252/.00836 = \frac{\Delta H}{2.303 \times 1.987}\left(\frac{323.1 - 298.1}{323.1 \times 298.1}\right)$$

and solving for ΔH the heat of solution of adenine comes out to be 8,450 calories per mole.

The heats of solution of the other compounds were calculated in the same way. The results are summarized in table LV.

The heats of solution are useful in calculating the value of the free energy change or the equilibrium constant at any desired temperature if this value is known at a given temperature. This holds within the temperature range for which the heats of solution have been calculated. For this purpose the van't Hoff equation is employed in the same way as already described.

TABLE LV

THE HEATS OF SOLUTION

FOR TEMPERATURE RANGE 25-50°C

Compound	ΔH Heat of Solution Calories per Mole
Adenine	8,450
Hypoxanthine	7,780
Guanine	10,330
Xanthine	10.760
Uric Acid	8,030
Allantoin	8,550
Alloxan	4,180
Leucine	1.460

THE EXTENT OF DISSOCIATION OF SOLUTES IN THEIR SATURATED SOLUTIONS AT 25°C

The extent of dissociation of the various compounds may be calculated if their dissociation constants are known. The method of calculation will depend on the nature of the compound under consideration and upon the approximations that can be made.

The fraction of the dissociated molecules of a weak acid or base may be calculated from Ostwald's dilution law $\alpha = -\frac{vK}{2} + \sqrt{vK + \frac{v^2K^2}{4}}$

where α is the degree of dissociation, v the volume in liters containing one mol of the electrolyte and K is the dissociation constant.

The calculation of the extent of dissociation of uric acid is of this type and is as follows. From its solubility v the volume in liters required to dissolve one mol of uric acid is 4098. The dissociation constant is 1.74×10^{-6}. Substituting in the above equation

$$\alpha \quad -\frac{4098 \times 1.74 \times 10^{-6}}{2} + \sqrt{vK + \ldots\ldots} \quad = \quad .081$$

the last term under the square root sign being negligible. The fraction of uric acid that is dissociated in its saturated solution at 25°C is thus .081. Hence the undissociated fraction is .919.

Using the same expression the extent of dissociation of guanine, xanthine, allantoin and alloxan have been calculated. Although alloxan has two acid dissociation constants, the dissociation is so slight that the second dissociation has been neglected.

If both acid and basic groups are present in the same molecule then the amount of the undissociated species may be calculated from Sorensen's formula (44)

$$\rho = \frac{1}{1 + \frac{K_a}{H^+} + \frac{K_b H^+}{K_w}}$$

where ρ is the fraction of the undissociated ampholyte. The H^+ concentration of the saturated solution must be known. These have been determined.

The fraction of undissociated adenine will now be calculated. The H^+ of its saturated solution is 2.5×10^{-7}, K_a is 1.26×10^{-10} and K_b is 1.38×10^{-10}. Substituting these values in the equation together with K_w 1×10^{-14}

$$\rho = \frac{1}{1 + \frac{1.26 \times 10^{-10}}{2.5 \times 10^{-7}} + \frac{1.38 \times 10^{-10} \times 2.5 \times 10^{-7}}{1 \times 10^{-14}}}$$

$$= .996$$

The calculation of the undissociated fraction of hypoxanthine was done in the same way.

When dealing with zwitter ions Sorensen's formula is modified to

$$\alpha = \frac{1}{1 + \frac{H^+}{K_A} + \frac{K_w}{K_B H^+}}$$

The fraction of undissociated leucine was calculated by use of this formula. The value of K_A is 2.5×10^{-2} of K_B 5.0×10^{-5} and of H^+ 3.16×10^{-7}. The fraction of leucine in the zwitter ion form is therefore

$$\alpha = \frac{1}{1 + \frac{3.16 \times 10^{-7}}{2.5 \times 10^{-2}} + \frac{1 \times 10^{-14}}{5.0 \times 10^{-5} \times 3.16 \times 10^{-7}}}$$

$$= .9992$$

The fractions of the undissociated species of all of the compounds are summarized in table LVI.

TABLE LVI

THE FRACTION OF THE UNDISSOCIATED SPECIES IN THEIR SATURATED SOLUTION AT 25°C

Compound	pK_a	pK_b	pH of Sat.Sol	Fraction Undiss.
Adenine	9.90	9.86	6.6	.996
Hypoxanthine	8.97	12.7	6.4	.997
Guanine	....	10.90	...	.999
Xanthine	7.1	.....	...	.973
Uric Acid	5.76	.....	...	.919
Allantoin	8.67	.....	...	1.000
Alloxan	6.70;8.66	.....	...	1.000
Leucine*	1.6	4.3	6.5	.999

*The dissociation constants of leucine are the zwitter ion constants.

ACTIVITIES

The activities of adenine, hypoxanthine, guanine, xanthine, uric acid and allantoin were assumed to be equal to their concentrations. Since these compounds are only slightly soluble in water it is assumed that they do not deviate much from behavior of perfect solutes.

The activity of alloxan in its saturated solution was determined to be .9682 (see above). The activity coefficient of l-leucine has been estimated by Dalton and Schmidt (4) to be 1.07.

The activity correction may then be simply applied by the use of the equation,

$$a = M\gamma$$

where a is the activity of the solute in the saturated solution, M is the solubility in moles per liter and γ is the activity coefficient.

THE FREE ENERGY OF FORMATION OF UNDISSOCIATED SOLUTE IN THE STANDARD SOLUTION AT 25°C

The free energy changes involved in going from the standard free energy of formation of the anhydrous solid compound to a solution in which the undissociated solute is at one molal activity will next be considered.

Suppose first that the solute does not form a hydrate. Then the free energy of the solute in its saturated solution is equal to the free energy of the solid with which it is in equilibrium,

$$X \text{ solid} \longrightarrow X \text{ aq. sat.}; \quad \Delta F = 0. \quad (1)$$

The free energy change in this process is zero.

If the solute forms a hydrate, of the general formula $X.3H_2O$, the changes taking place may be represented as below,

$$X_S + 3H_2O_{(g\ p)} \longrightarrow X.3H_2O_{(s)} \ ; \Delta F_1 = 0$$

$$3H_2O_{(g,\ P_o)} \longrightarrow 3H_2O_{(g,p_1)} \ ; \Delta F_2 = 3RT \ln P_1/P_o$$

$$X.3H_2O_{(s)} \longrightarrow X_{(aq.sat.)} + 3H_2O_{(sat.sol)}; \Delta F_3 = 0$$

$$3H_2O \text{ (sat.sol.)} \longrightarrow 3H_2O,(l) \ ; \Delta F_4 = 3RT \ln P_{(l)}/P_s$$

$$3H_2O \text{ (l)} \longrightarrow 3H_2O_{(g,p_o)} \ ; \Delta F_5 = 3RT \ln P_o/P_{(l)}$$

where P_o represents the vapor pressure of pure water in the standard gaseous state, P_i the vapor pressure of the hydrate-anhydrous system, P_l the vapor pressure of pure water in the liquid state and P_s the vapor pressure of the saturated solution. All of the reactions are considerdd to take place at 25°C.

By summung up all the above reactions we obtain

$$X_{(s)} \longrightarrow X(\text{sat.aq.}) \quad ;\Delta F = 3RT\ln P_i/P_{sat} \quad (2)$$

The free energy change in the transformation of one mol of anhydrous X, to a saturated solution in which the hydrate $X.3H_2O$ is in equilibrium with the aqueous phase may be obtained from equation (2) if the vapor pressure of the hydrate - anhydrous transformation is known, together with the vapor pressure of the saturated solution.

As an illustration, the free energy change in the transfer of one mol of alloxan to a large amount of its saturated solution will be considered. It has been shown above that alloxan in its saturated solution is in equilibrium with its trihydrate as the solid phase. The vapor pressure of the anhydrous - trihydrate transformation was found to be 21.1 mm of Hg, and the vapor pressure of the saturated solution was found to be 22.78 mm. of Hg at 25°C. The free energy change in the process

$$\text{Alloxan(solid anhydrous)} \longrightarrow \text{Alloxan(sat.sol.)}$$

is given by equation (2)

$$\Delta F = \frac{3RT\ln 21.1}{22.78} = -136 \text{ Calories.}$$

Alloxan was the only compound found to form a hydrate.

We shall next proceed to evaluate the free energy change per mol in the transfer from a saturated solution to one in which the undissociated solute ia at one molal activity at 298.1°K. This is given by the equation,

$$X(\text{sat.sol.}) \longrightarrow X_{(a=1)} \text{ undiss} \; ; \; \Delta F = -RT\ln M\gamma\rho \quad (3)$$

where γ is the activity coefficient, ρ is the fraction of undissociated solute in the saturated solution, and M is the molality of the saturated solution.

As an example the free energy change in the transfer of one mol of l-leucine from its saturated solution to one in which the neutral form is at one molal activity, will be calculated. The molality of leucine in its saturated solution was found to be .167. The activity coefficient in its saturated solution is given by Dalton and Schmidt as 1.07 (4). The fraction of undissociated leucine was found to be .999. Substituting these values in the equation (3), we obtain,

$$\Delta F = -RT\ln M\gamma\rho$$

$$= -1364 \log (.167 \times .999 \times 1.07)$$

$$= 1{,}020 \text{ Calories}$$

Having the standard free energies of formation, and having calculated the free energies involved in going from the solid anhydrous compounds to their solutions in which the undissociated species is at one molal activity, the free energies of formation of the undissociated compounds in their standard solutions have been calculated. The data are given in table LVII. In the second column are given the standard free energies of formation of each compound at 298.1°K. The values of Stiehler and Huffman (2) corrected by Huffman (45) were used.

The third column gives the free energy changes at 298.1°K, involved in the transfer of one mole of anhydrous solute to a saturated solution. In the next three columns are given the molalities of the saturated solution, the activity coefficients used, and the fraction of undissociated (or neutral) molecules in the saturated solutions, all at 298.1°K. The seventh column gives the free energy changes involved in the transfer of one mole of solute from the saturated solution to a solution in which the undissociated solute is at one molal activity at 298.1°K.

The final column gives the standard free energy of formation of the undissociated solute in aqueous

solution at one molal activity at 298.1°K. This was obtained by the summation of columns 2, 3, and 7.

TABLE LVII

THE FREE ENERGIES OF FORMATION IN CALORIES PER MOLE OF THE UNDISSOCIATED COMPOUNDS IN THEIR STANDARD SOLUTIONS AT 25°C

Substance	$\Delta F^{\circ}_{f\,(Solid)}$	$X_{(s)} \rightarrow X_{(aq.sat.)}$	M.sat.sol.	γ	ρ	$X_{(aq.sat.)} \rightarrow X_{(a=1,undiss)}$ $\Delta F = -RT\ln M\rho\gamma$	$\Delta F^{\circ}_{f\,(a=1)}$ undiss.
Adenine	71,960	.....	.00836	1.	.996	2,840	74,800
Hypoxanthine	18,700	.....	.00536	1.	.997	3,100	21,800
Guanine	11,710	.....	.0000225	1.	.999	6,340	18,050
Xanthine	-39,310	.....	.000108	1.	.973	5,430	-33,880
Uric Acid	-90,130	.....	.000244	1.	.919	4,980	-85,150
Allantoin	-106,430	.....	.0373	1.	1.000	1,950	-104,480
Alloxan	-181,910	- 140..	2.60	.968	1.000	-550	-182,600
Leucine d- and l-	-82,480	.....	.167	1.07	.999	1,020	-81,460

THE FREE ENERGY OF FORMATION OF IONS IN THEIR STANDARD SOLUTION AT 25°C

Sometimes it is preferable to consider a reaction as taking place between ions rather than between neutral molecules. It is then necessary to know the free energy of formation of ions in their standard solutions.

Let us consider an acid of the general formula XH, which ionizes in aqueous solution according to the equation

$$XH_{(a=1,aq.)} \longrightarrow X_{(a=1,aq.)} + H^{+}_{(a=1)}$$

The free energy of ionization is given by the equation

$$\Delta F = -RT\ln K \qquad (4)$$

where K is the ionization constant of the reaction.

For a general case we may write the free energy of ionization as

$$\Delta F = -RT\ln K, K_2 -- Kn$$

where K_1, K_2--Kn represent the dissociation constants. This equation may be applied to the free energy of ionization of the cation, or the anion alone, or the bivalent ion etc.

It is to be noticed that the basic dissociation constants are ordinarily calculated in such a manner that they include the dissociation of water. In order

to be applied directly in the above equation they must therefore be expressed in terms of the dissociation of the hydrogen ion.

The following equations will illustrate this relationship. Suppose that a substance X ionizes according to the following scheme, all the reactants and products being at one molal activity

$$X + H_2O \rightarrow X \cdot HOH \rightarrow XH^+ + OH^-$$

Water ionizes according to

$$HOH \rightarrow H^+ + OH^-$$

Subtracting, we obtain

$$X + H^+ \rightarrow XH^+$$

The ionization of bases therefore consists of the gain of protons or hydrogen, whereas the ionization of acids involves the loss of protons or hydrogen ions. If the dissociation constants are written out for each reaction, it will be seen that they are related through the ionization constant of water, i.e.,

$$K_w/K_b = K_{H^+}$$

where K_w is the ionization constant of water, K_b the ordinary dissociation constant of the base, and K_{H^+} the basic dissociation constant in terms of H^+.

For convenience, K_W/K_b is used rather than K_H. On this basis the free energy of ionization of a base is given by

$$\Delta F = RT\ln K_W/K_b \qquad (5)$$

The free energies of formation of ions in aqueous solution at one molal activity at 298.1°K may be derived as follows.

Consider the ionization reaction of the compound HX to give

$$XH_{(a=1 \text{ undiss.aq.})} \longrightarrow X^-_{(a=1 aq.)} + H^+_{(a=1)}$$

We know the free energy of ionization from equations (4) and (5) above. We also know the free energy of formation of $XH_{(a=1 undiss.aq.)}$ The free energy of formation of $H^+_{(a=1)}$ is taken as zero. Now, the free energy of the reaction is equal to the sum of the standard free energies of the reactants less the standard free energy of the products, hence,

$$\Delta F^{\circ}_{f(a=1,\ ion.aq)} = F^{\circ}_{f(a=1 \text{ undiss.aq})} - RT\ln K$$

In table LVIII the free energies of formation of ions in their standard solutions at 298.1°K are given. In the second column are given the free energies of formation of the undissociated ions in their standard solutions.

These are taken from table LVII. The free energies of ionization are given in the fourth column. In the fifth column are given the free energies of formation of the anions at one molal activity in aqueous solution at 298.1°K. These are obtained as the sum of columns 2 and 4.

Similarly in the remaining columns the free energies of formation of the cations in the standard solution are given.

TABLE LVIII

THE FREE ENERGIES OF FORMATION OF IONS IN THEIR STANDARD SOLUTIONS AT 298.1°K

Compound	$\Delta F_{f\,(a=1,\text{undiss.})}^{\circ}$ Calories	pK_a	$-RT\ln K_a$ Calories	$\Delta F_{f\,(a=1\ \text{anion})}^{\circ}$ Calories	pK_w-pK_b	$RT\ln K_w/K_b$ Calories	$\Delta F_{f\,(a=1\ \text{cation})}^{\circ}$ Calories
Adenine	74,800	9.90	13,500	88,300	4.14	-5.650	69,150
Hypoxanthine	21,800	8.97	12,240	34,040	1.3	-1.770	20,030
Guanine	18,050	....	....	.....	3.10	-4,230	13,820
Xanthine	-33,880	7.10	9,680	-24,200	....	....	....
Uric Acid	-85,150	5.76	7,860	-77,290	....	....	....
Allantoin	-104,480	8.67	11,830	-92,650	....	....	....
Alloxan	-182,600	6.70	9,140	-173,460	....	....	....
		8.66	11,810	-161,650	pK_A		
		pK_w-pK_B					
l-Leucine	-81.460	9.70	13,230	-68,230	1.60	-2,180	-83,640

SUPPLEMENTARY DATA

Certain data in addition to those thus far presented are required before the final calculations can be made. The most reliable values have been selected from the literature.

The free evergy of formation of gaseous oxygen at one atmosphere and 25°C is by definition taken as zero.

The free energy of formation of H^+ in solution at one molal activity and 25°C was also taken as zero.

The free energy of formation of liquid water in its standard state was taken as -56,720 calories (46).

The free energy of formation of aqueous ammonia in its standard state was taken as -6,300 calories. (47)

The standard free energy of formation of NH_4^+ aq. is taken as -18,930 calories (47).

The standard free energy of formation of gaseous carbon dioxide was taken as -94,240 calories (48).

From this the standard free energy of formation of aqueous carbonic acid at 25°C was calculated according to Lewis and Randall (39) using their values for the molal solubility of carbon dioxide in water at 25°C at a partial pressure at one atmosphere. The result obtained was -149,150 calories.

The free energy of formation of solid urea was taken as -47,200 calories (45). Following the example in Lewis

and Randall (39) the standard free energy of formation of urea in aqueous solution was calculated. The activity coefficient of .5366, and the solubility of 20.00 molal (38) were used. The standard free energy of aqueous urea was found to be -48,440 calories.

For convenience these are tabulated below.

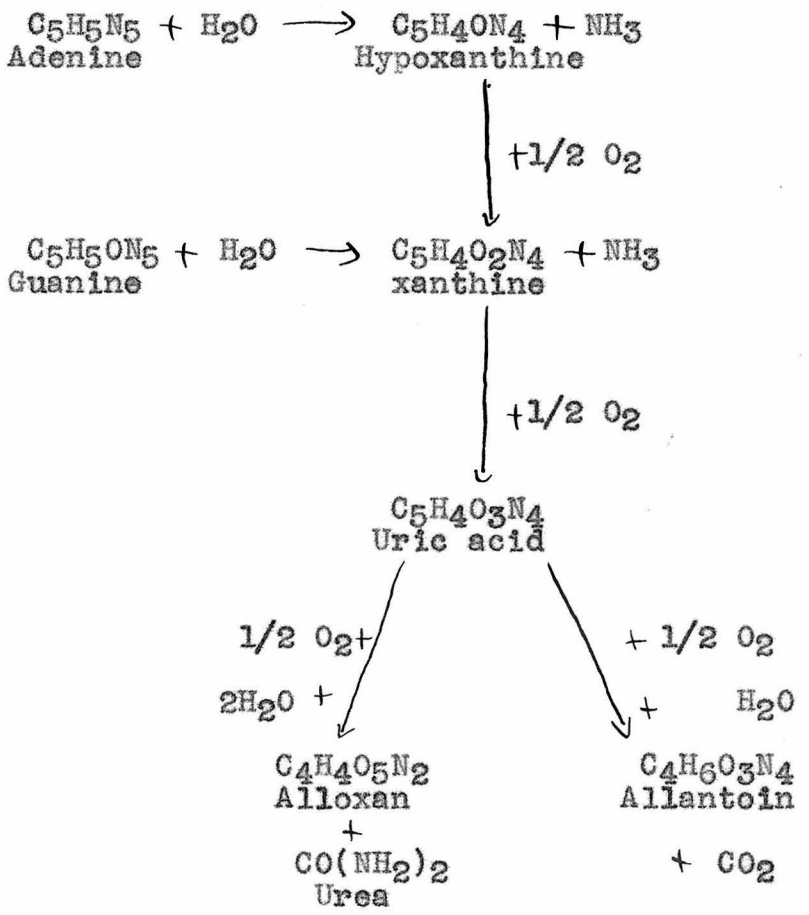
TABLE LIX

SUPPLEMENTARY DATA

Substance	$\Delta F^{o}_{f(a=1,aq.)}$ 298.1°K Calories
Oxygen (gas)	0
H^+	0
$H_2O_{(l)}$	-56,720
NH_3	-6,300
NH_4^+	-18,930
$H_2CO_3^-$	-149,150
$CO(NH_2)_2$	-48,440

EQUILIBRIA OF PURINES AND THEIR DEGRADATION PRODUCTS

The course of the degradation of purines may be formulated as ,



This series of reactions may be divided into six individual reactions. Complete thermodynamical data are now available to compute the standard free energy change in solution for each reaction. From the free energy changes the equilibrium constants and the oxidation reduction potentials can be calculated. Each individual reaction will now be considered.

1. Adenine,(aq.,a=1)+H_2O(liq.) ⟶ Hypoxanthine(aq,a=1)+NH_3(aq)

The standard free energy change in this reaction i.e., when all

the products and reactants are at unit activity in aqueous solution, is the difference between the sum of the free energies of formation of the products and the reactants. Thus for the above reaction

$$\Delta F^\circ_{298.1} = (21,800 - 6,300) - (74,800 - 56,720)$$
$$= -2,580 \text{ calories.}$$

From the relation $\Delta F^\circ = -RT\ln K$, the equilibrium constant for this reaction as written comes out to be 77.5.

The deamination of adenine may be written in a slightly different manner,

$$H^+ + \text{Adenine}_{(aq.,a=1)} + H_2O(liq.) \longrightarrow \text{Hypoxanthine}_{(aq.,a=1)} + NH_4^+(aq.,a=1)$$

$$\Delta F^\circ_{298.1} = (-18,930 + 21,800) - (74,800 - 56,720)$$
$$= -15,210 \text{ calories}$$

2. Similarly for the reaction

$$\text{Guanine}_{(aq.,a=1)} + H_2O(liq.) \longrightarrow \text{Xanthine}_{(aq.,a=1)} + NH_3(aq.)$$

$$\Delta F^\circ_{298.1} = (-33,880 - 6,300) - (18,050 - 56,720)$$
$$= -1,510 \text{ calories}$$

The equilibrium constant for the reaction from right to left is 12.8.

For the reaction written

$$H^+ + \text{Guanine}_{(aq.,a=1)} + H_2O(liq.) \longrightarrow \text{Xanthine}_{(aq,a=1)} + NH_4^+(aq.,a=1)$$

$$\Delta F^\circ_{298.1} = -14,140 \text{ calories}$$

3. The oxidation of hypoxanthine may be written

$$\text{Hypoxanthine}_{(aq.,a=1)} + \tfrac{1}{2}O_{2(g)} \longrightarrow \text{Xanthine}_{(aq.,a=1)}$$

$\Delta F^{o}_{298.1}$ = -33,880 -21,800 = -55,680 calories.

4. Xanthine in turn may be oxidized to uric acid. The reaction may be written

$$\text{Xanthine}_{(aq.,a=1)} + \tfrac{1}{2}O_{2(g)} \longrightarrow \text{Uric acid}_{(aq.,a=1)}$$

$\Delta F^{o}_{298.1}$ = -35,150 + 33,880 = -51,270 calories.

Uric acid may be oxidized in two ways

$$\text{Uric acid}_{(aq.,a=1)} + \tfrac{1}{2}O_{2(g)} + 2H_2O_{(liq)} \longrightarrow \text{Allantoin}_{(aq.,a=1)} + H_2CO_{3(aq.,a=1)}$$

$\Delta F^{o}_{298.1}$ = -149,150 - 104, 480 -(-2x56,620 -85,150)

= -55,040 calories.

6. $$\text{Uric acid}_{(aq.,a=1)} + \tfrac{1}{2}O_{2(g)} + 2H_2O_{(liq.)} \longrightarrow \text{Alloxan}_{(aq.,a=1)} + \text{Urea}_{(aq.,a=1)}$$

$\Delta F^{o}_{298.1}$ = -48,440 -182600 + 2x56,720 +85,150

= -32,450 calories.

7. The dismutation of xanthine to hypoxanthine and uric acid may also be considered in this connection. It may be considered as the sum of reactions in 3 and 4 above and may thus be obtained from the same data.

The reaction is

$$2\text{Xanthine}_{(aq.,a=1)} \longrightarrow \text{Hypoxanthine}_{(aq.,a=1)} + \text{Uric acid}_{(aq.,a=1)}$$

$\Delta F^{o}_{298.1}$ = 21,800 -85,150 +2x33,880

= 4,410 calories.

The equilibrium constant for this reaction or the dismutation constant comes out to be .000588. From this it may be readily calculated that the amount of xanthine which may undergo dismutation is 2.4%.

The above dismutation constant refers to the undissociated products and reactants. In a similar way the dismutation constant for the ions may be obtained. In practice however one deals with the total unionized and ionized participants. This dismutation constant is more useful as will be seen later. Calculations have therefore been made of the free energies of formation of the total participants in a manner similar to that in table LVII. We thus have for the reaction

$$2\ \text{Xanthine}_{(\text{total})} \rightarrow \text{Hypoxanthine}_{(\text{total})} + \text{Uric acid}_{(\text{total})}$$

$$\Delta F^{o} = 21{,}800 - 85{,}200 + 2 \times 33{,}100$$

$$= 4{,}400 \text{ calories.}$$

The result is essentially the same as before. The dismutation constant comes out to be .000595.

8. It is useful to express the results of 3 and 4 above in terms of the oxidation reduction potentials. In accordance with the usage adopted in biochemical literature, all the oxidation reduction reactions are written in the direction

$$\text{Reductant} \longrightarrow \text{Oxidant.}$$

Suppose we consider first the hypoxanthine-xanthine half cell reaction, which may then be written as

$$\text{Xanthine}_{(aq.,a=1)} + 2H^{+}_{(a=1)} + 2e \longrightarrow \text{Hypoxanthine}_{(aq.,a=1)} + H_2O_{(liq.)}$$

From the standard free energy values we obtain

$$\Delta F^{o}_{298.1} = 23,800 - 56,720 + 33,880$$

$$= -1,040 \text{ calories.}$$

The value of the molal electrode potential may then be obtained from the relation

$$\Delta F^{o} = -E_o nF$$

where n is the number of electrochemical equivalents involved in the reaction, F is the value of faraday which is taken as 23,068 calories per volt per electrochemical equivalent and E_o the molal electrode potential.

For the neutral molecules reacting in the direction as written above we have

$$E_o = \frac{1,040}{2x23,068} = .0225 \text{ volts.}$$

Similarly we may consider the anions to take part in the reaction

$$\text{Xanthine}^{-}_{(aq.,a=1)} + 2H^{+}_{(a=1)} + 2e \longrightarrow \text{Hypoxanthine}^{-}_{(aq.,a=1)} + H_2O_{(liq.)}$$

$$\Delta F^{o} = -56,720 + 34,040 + 24,200 = 1,520 \text{ calories.}$$

$$E_o = \frac{1,520}{2x23,068} = .0329 \text{ volts.}$$

In the above reactions the activity of H is one molal. Experimentally however the potentials may be determined in solutions at any pH. The calculation of E'_o at pH 7 is as follows,

$$\text{Xanthine}_{(aq.,a=1)} + 2H^{+}_{(a=1)} + 2e \longrightarrow \text{Hypoxanthine}_{(aq.,a=1)} + H_2O_{(liq.)}$$

$$\Delta F^{o}_{298.1} = 1,040 \text{ calories.}$$

Also

$$2H^{+}_{(a=10^{-7})} \longrightarrow 2H^{+}_{(a=1)}$$

$$\Delta F^{o}_{298.1} = -2RT\ln 10^{-7} = 19{,}096 \text{ calories}$$

Adding these reactions we obtain

$$\text{Xanthine}_{(aq.,a=1)} + 2H^{+}_{(a=10^{-7})} + 2e \longrightarrow \text{Hypoxanthine}_{(aq.,a=1)} + H_2O_{(liq.)}$$

$$\Delta F_{298.1} = -1{,}040 + 19{,}096 = 18{,}056 \text{ calories.}$$

$$E'_o = \frac{-18{,}056}{2x23{,}068} = -.391 \text{ volts at pH 7.}$$

Similarly for the reaction involving anions,

$$E'_o = \frac{-20{,}616}{2x23{,}068} = -.447 \text{ volts at pH 7.}$$

We shall next consider the xanthine-uric acid half cell reaction. The calculations involved are similar to those above. For the reaction involving the undissociated species we have

$$\text{Uric acid}_{(aq.,a=1)} + 2H^{+}_{(a=1)} + 2e \longrightarrow \text{Xanthine}_{(aq.,a=1)} + H_2O_{(liq.)}$$

$$\Delta F^{o} = 85{,}150 - 56{,}720 - 33{,}880 = -5{,}450 \text{ calories.}$$

Corresponding to this

$$E_o = \frac{+5{,}450}{2x23{,}068} = .118 \text{ volts.}$$

Similarly for the reaction involving anions

$$\text{Uric acid}^{-}_{(aq.,a=1)} + 2H^{+}_{(a=1)} + 2e \longrightarrow \text{Xanthine}^{-}_{(aq.,a=1)} + H_2O_{(liq.)}$$

$$\Delta F^{o} = 77{,}290 - 56{,}720 - 24{,}200 = -3{,}630 \text{ calories.}$$

$$E_o = \frac{+3{,}630}{2x23{,}068} = .079 \text{ volts.}$$

Now for the corresponding reactions taking place at pH 7

$$\text{Uric acid}_{(aq.,a=1)} \rightarrow 2H^{+}_{(a=10^{-7})} + 2e \longrightarrow \text{Xanthine}_{(aq.,a=1)} + H_2O_{(liq.)}$$

ΔF = 13,646 calories.

$E'_o = \frac{-13,646}{2x23,068}$ = -.295 volts at pH7.

also for the ionic reaction

$$\text{Uric acid}^{-}_{(aq.,a=1)} + 2H^{+}_{(a=10^{-7})} + 2e \longrightarrow \text{Xanthine}^{-}_{(aq.,a=1)} + H_2O_{(liq.)}$$

ΔF = 15,466 calories.

$E'_o = \frac{-15,466}{2x23,068}$ = -.335 volts at pH 7.

Experimentally it is more convenient to measure the potential of the hypoxanthine-uric acid instead of the hypoxanthine-xanthine system. The oxidation reduction potentials may be readily derived from the above data. Thus we have

$$\text{Uric acid}_{(aq.,a=1)} + 4H\ (a=1) + 4e \longrightarrow \text{Hypoxanthine}_{(aq.,a=1)} + 2H_2O_{(liq.)}$$

ΔF^o = -6,490 calories

E_o = .0703 volts.

E'_o = -.343 volts at pH 7

And for the reaction involving ions

$$\text{Uric acid}^{-}_{(aq.,a=1)} \quad 4H_{(a=1)} \quad 4e \quad \text{Hypoxanthine}^{-}_{(aq.,a=1)} + 2H_2O_{(liq.)}$$

ΔF^o = -2,110 calories.

E_o = .0228 volts.

E'_o = .391 volts at pH 7.

DISCUSSION

The reactions for which the standard free energy changes have been derived above may be divided into three groups.

Adenine and guanine undergo a hydrolytic deamination to yield hypoxanthine and xanthine respectively. From the magnitude and the sign of the free energy changes it may be concluded that these reactions may proceed spontaneously. On the other hand the amination of hypoxanthine to adenine or of xanthine to guanine would not take place to any extent. The synthesis of adenine and of guanine in vivo is probably coupled with another energy yielding reaction or it takes a different route to accomplish this purpose.

In the second category are the oxidative reactions of hypoxanthine to xanthine and of xanthine to uric acid. These reactions have been shown to proceed either aerobically or anaerobically(49). When written in such a manner as to involve molecular oxygen the energetic relationships indicate that the equilibrium is in favor of complete oxidation and that the oxidative reactions are irreversible. On the other hand if the anaerobic process involving hydrolytic oxidation is considered a different picture is obtained. This will be further discussed in connection with the oxidation reduction potentials.

The further degradation of uric acid involves a simultaneous or consecutive hydrolysis and oxidation.

In more alkaline solutions uric acid is known to be broken down to allantoin and carbon dioxide. Allantoin is the final metabolic product of purine metabolism in many animals. It has also been found in plants. In an acid medium uric acid can be broken down to alloxan and urea. Alloxan is only occasionally found in biological materials.

From the standard free energy changes in the uric acid-allantoin and the uric acid-alloxan degradation in aquaous solutions it may be seen that the equilibrium lies in favor of practically complete degradation. These reactions may therefore be considered irreversible unless coupled with other energy yielding reactions.

Another type of reaction which has briefly been considered is the dismutation of xanthine to yield hypoxanthine and uric acid. This has been shown to take place(50)(51)(52). From the dismutation constant we have calculated that the amount of xanthine which may undergo dismutation is 2.4%. This figure is somewhat smaller than those reported but is significant. The reverse reaction has also been observed experimentally. The dismutation reaction is a complicating factor in the calculation of oxidation reduction potentials. This reaction may also be of importance in the in vivo synthesis of purines.

The oxidation reduction potentials of hypoxanthine-xanthine-uric acid systems has been determined experimentally. These will be compared with the results derived from free energy data.

Before making a comparison of our results with those in the literature it is essential to understand clearly the salient features involved in each of the methods employed by the different investigators and the basis to which their results refer. It is further necessary to find a common basis on which the results derived by different methods may be compared.

We shall consider briefly the three different methods as exemplified first, by our method based on free energy data and represented by the work outlined in this thesis; second, the method employed by Filitti(20;53;54) based on potentiometric measurements; and last, that employed by Green(51) which though experimentally the same as Filitti's differs in the method and the basis of calculation.

The derivation of electrode potentials from free energy data has already been illustrated in the calculations given above. The fundamental relation between the free energy change in an oxidation reduction reaction and the electrode potential is given by

$$\Delta F = -EnF \qquad (1)$$

where ΔF refers to the free energy change of the electrode reaction specified and E represents the voltage of the corresponding cell. When the electrode equation is referred to the standard hydrogen electrode potential the potential of the cell is represented by E_h hence

$$\Delta F = -E_hNF \qquad (2)$$

When all the reactants and products in the electrode equation are at unit activity as for example when the standard free energy of the reaction is known then

$$\Delta F^{0} = -E_{o}nF \qquad (3)$$

where E_o is the standard molal electrode potential. Here H^+ is at unit activity. It is sometimes necessary to calculate the electrode potential at some other H^+ activity. There will therefore be included in equation(3) an additional term representing the free energy change involved in diluting H^+ from unit activity to the specified activity. This is given by

$$\Delta F = -nRT \ln[H^+] \qquad (4)$$

The value of the electrode potential at a given pH when the logarithm of ratio of oxidant to reductant is zero is represented by E_o'. This is sometimes referred to as the apparent molal potential. Combining equations (3) and (4) we obtain a general equation relating E_o, E_o', and H^+

$$E_o' = E_o + \frac{RT \ln[H^+]}{F} \qquad (5)$$

In equations (3) and (5) the ratio of oxidant to reductant is such that the logarithm of this ratio is zero. If it is required to calculate the potential of an oxidation reduction reaction corresponding to any ratio of oxidant to reductant at any pH a general equation must include a term representing the dilution or concentration of the oxidant and the reductant from unit activity

to the desired activity. This is given by

$$\Delta F = RT \ln \frac{[Red]}{[Ox]} \qquad (6)$$

Including this term in equation (5) we obtain

$$E_h = E_o - \frac{RT}{nF} \ln \frac{[Red]}{[Ox]} + \frac{RT}{F} \ln [H^+] \qquad (7)$$

In order to avoid confusion when the potential of any oxidation reduction system is given the corresponding electrode equation should also be given indicating clearly the species referred to as well as the conditions.

From the free energy data we know directly the standard free energy of formation of the various species of oxidant and reductant. One can therefore readily write the reactions corresponding to any of the above equations.

The method of deriving the fundamental constants E_0 or E_o' for the oxidation reduction reactions from potentiometric data will be considered next. The general equation relating the observed potential to the ratio of oxidant and reductant and to the H^+ activity has already been given in (7) above. The data employed however are different. Instead of being given ΔF^o of ΔF and calculating the values of E_o, E_o', E_h etc., the procedure is reversed. Here from the observed E_h values, the pH and the ratio of the oxidant to reductant the E_o and the E_o' values for the given system are calculated.

The electrode reaction is generally written in such a way as to refer to a single type of oxidant and reductant e.g., the undissociated molecule the concentration

of which is not directly measurable. The concentration of the undissociated oxidant or reductant according to this scheme is therefore dependent on the dissociation constants and on the pH of the solution. Equations must therefore be developed to include these variables as well as to express the ratio of the oxidant to reductant in terms which are readily measurable.

The following electrode equation for the xanthine-uric acid system is modified from Filitti's. Only one dissociation constant is used for each compound. The oxidant and reductant are considered to be the undissociated species.

Briefly, then, for uric acid we have

$$UH \rightleftarrows U^- + H^+$$

from which

$$K_u = \frac{[H^+][U^-]}{[UH]}$$

also

$$UH_{total} = UH + U^-$$

and combining these

$$UH = UH_t \cdot \frac{H^+}{K_u + H^+}$$

Similarly for xanthine

$$XH = XH_t \cdot \frac{H^+}{K_x + H^+}$$

Substituting these values of UH and XH for [Ox] and [Red] in equation (7) we obtain

$$E_h = E_o - \frac{RT}{2F}\ln\frac{[XH]_t}{[UH]_t} - \frac{RT}{2F}\ln\frac{K_u + H^+}{K_x + H^+} + \frac{RT}{2F}\ln[H^+] \qquad (8)$$

This equation is in a more useful form than equation (7) since the oxidant and reductant are expressed in readily measurable terms. Experimental values may then be substituted for the various terms and E_o thus evaluated for the given system.

Similar equations may be derived for any other oxidation reduction system.

We shall finally consider the method used by Green for the evaluation of electrode potentials. He determined the observed potential, the pH and the concentrations of the total oxidant and reductant. When the ratio of the total oxidant to total reductant is unity the observed potential E_h then becomes E'_o at a given pH. This E'_o is not the same as that derived for the dissociated or the undissociated species of the oxidant and reductant. Its relation to the electrode equation will be made clear by referring again to equation (8). It will be recalled that this equation was derived for the undissociated species of the oxidant and reductant. Furthermore the second and third terms in the equation were derived by the expansion of the second term of equation (7).

If the ratio of the total oxidant to the total reductant is unity equation (8) becomes

$$E'_o = E_o - \frac{RT}{2F} \ln \frac{Ku+H^+}{Kx+H^+} + \frac{RT}{F} \ln [H^+] \qquad (9)$$

where E'_o refers to the system involving the total oxidant and the total reductant and E_o represents the molal electrode potential for the undissociated system.

If the ratio of the total oxidant to the total reductant is unity equation (8) becomes

$$E'_o = E_o - \frac{RT}{2F} \ln \frac{KU + H^+}{Kx + H^+} + \frac{RT}{F} \ln [H^+] \qquad (9)$$

where E'_o refers to the system involving the total oxidant and the total reductant and E_o represents the molal electrode potential for the undissociated system.

The symbol E'_o as used by Green has been interpreted to be that defined by equation (9). This equation makes it possible to evaluate E_o for the undissociated system and thus provides a common basis on which Green's results may be compared with ours and with those of Filitti.

In a similar way equations may be derived for the evaluation of electrode potentials for other systems.

In this connection we shall recalculate Green's data. Green has determined experimentally the E'_o values for the hypoxanthine-xanthine and xanthine-uric acid systems at 30°C and pH 7. He gives the E'_o values of -.371 and -.361 volts respectively for these two systems.

Using Green's values for E'_o , pH and temperature and our results for the dissociation constants and substituting these in appropriate equations corresponding to equation (9) the following results were obtained. At 30°C for the hypoxanthine-xanthine system the E_o value referred to the undissociated oxidant and reductant comes out to be .057 volts. This corresponds to the free energy change of -2,630 calories. In a similar way E_o for the xanthine uric acid system referred to the undissociated species is .090 volts, the corresponding standard free

energy change being-4,150 calories. By summation of these two systems the molal electrode potential E_o for the hypoxanthine-uric acid system comes out to be .0735 volts, with a corresponding standard free energy change of-6,780 calories.

For the purpose of a direct comparison of our results with those of Green and Filitti we have recalculated our values for the electrode potentials at 30 and 38°C. The essential data are given in table LIX. The values of the heat contents were taken from Stiehler and Huffman (2), and the heats of solution from table LV. The following example for hypoxanthine will illustrate the method of calculation involved.

$$5C + 4H + 4N + O \rightarrow Hy(s); \quad \Delta H = -27,630$$

$$Hy(s) \rightarrow Hy(aq) \quad ; \quad \Delta H = 7,780$$

Adding

$$5C + 4H + 4N + O \quad Hy_{(aq)} ; \quad \Delta H = -19,850$$

The ΔH so obtained represents the heat content of the aqueous reactant at one molal activity. The difference between the heat contents of the products and the reactants gives the heat of the reaction. This may be used directly in the van't Hoff equation together with the free energy change of the reaction at a given temperature to calculate the free energy change of that reaction at another temperature. From the free energy changes at 30 and 38° calculated in this manner, the E_o values at these two temperatures were calculated in the same way as before.

A comparison of our results with those of Green and Fititti for the different systems is summarized in table LX.

TABLE LIX

Heat Content Data in Calories per Mole

Compound	$\Delta H_{298.1(S)}$	ΔH Solution	$\Delta H_{(aq.a=1)}$
Hypoxanthine	-27,630	7,780	-19,850
Xanthine	-91,810	10,760	-81,050
Uric Acid	-148,980	8,030	-140,950
Water	-68,310		

TABLE LX

System	E_o (volts) at 30°		E_o (volts) at 38°		
	Green		Fillitti		
	Corr. Dism.	Free Energy Data	Corr. Dism.	Uncorr.	Free Energy Data
Hypoxanthine xanthine (Undiss.)	.057	.020	.009	.013	.017
Xanthine Uric Acid (Undiss.)	.090	.117	.127	.123	.115
Hypoxanthine Uric Acid (Undiss.)	.073	.069	—	.068	.066

In making the above comparison several observations should be made. The estimated uncertainty in the free energy data would lead to a maximum error of about .025 volts in the E_o values. In applying the free energy data to chemical reactions it is likely that the errors are not all in the same direction and will therefore tend to cancel out. In fact the good agreement of our results with those of Filitti throughout, and at least for the hypoxanthine-uric acid system with Green's data, indicates that the probable error in the free energy data is less than this. We should further like to point out that the potentiometric method of obtaining electrode potentials is subject to several sources of error. From the potentiometric measurements one is not certain whether the potential measured actually refers to the reaction postulated or whether it refers to some other reaction. Furthermore in enzymatic reactions one is dependent on the enzyme acting as a perfect catalyst. On the other hand the free energy data are limited only by the accuracy with which determinations can be made.

There appears to be one other complicating factor, namely dismutation. The free energy data are derived for pure compounds and therefore the results obtained from them are independent of dismutation. The direct measurement of electrode potentials of the purine systems are subject to this complication for which a correction must be applied. In fact Green's paper is almost entirely devoted to the mathematical treatment of adequately taking into account

the dismutation reaction in the calculation of oxidation reduction potentials. Filitti on the other hand disregards dismutation in her work making a correction in the addendum to her long paper.

Just to what extent dismutation takes place under the experimental conditions of measuring oxidation reduction potentials is not clear nor are there strictly quantitative data available on the magnitude of the dismutation constant. We have calculated the dismutation constant from free energy data. We have further recalculated this constant at 30° and at 38°, the values being .000617 and .000652 respectively.

We shall next proceed to develop appropriate equations for the electrode potentials taking into account the dismutation. We shall then calculate from our molal electrode potentials and the dismutation constants electrode potentials corresponding to Green's and Filitti's observed potentials.

Let us consider the general equation for the hypoxanthine-uric acid oxidation reduction system

$$E_h = E_o - \frac{RT}{4F} \ln \frac{\{Hy\}_T}{\{UH\}_T} - \frac{RT}{4F} \ln \frac{Ku + H^+}{K_{Hy} + H^+} + \frac{RT}{F} \ln H^+ \qquad (10)$$

The ratio Hy/UH may be varied, the remaining terms being constant for a given pH. The dismutation of hypoxanthine and uric acid to form xanthine will also change this ratio and thus change the value of E_h in the above equation. The effect of dismutation will be to decrease both hypoxanthine and uric acid by equal amounts which may be evaluated from the amount of xanthine formed. The equilibrium

or final ratio of the oxidant to reductant will then be given by $\frac{HY - x}{UH - x}$ where x is defined by

$$x^2 = \frac{[Hy - x][UH - x]}{Km}$$

Km being the dismutation constant. Solving for x we obtain

$$x = \frac{Hy + UH - \sqrt{Hy^2 + UH^2 - 2\,Hy\,UH(1-2Km)}}{2(1-Km)}$$

The ratio $\frac{Hy - x}{UH - x}$ Now becomes

$$\frac{Hy(1-2Km) - UH + \sqrt{Hy^2 + UH^2 - 2Hy\,UH(1-2Km)}}{UH(1-2Km) - Hy + \sqrt{Hy^2 + UH^2 - 2HY\,UH(1-2Km)}}$$

This ratio may now be substituted in equation (10) giving a general electrode equation which takes into account the effect of dismutation. This equation is

$$E_h = E_o - \frac{RT}{4F} \ln \frac{Hy(1-2Km) - UH + \sqrt{Hy^2\ UH^2 - Hy\,UH(1-2Km)}}{UH(1-2Km) - Hy + \sqrt{Hy^2\ UH^2 - Hy\,UH(1-2Km)}}$$

$$- \frac{RT}{4F} \ln \frac{Ku + H^+}{K_{Hy^+} H^+} + \frac{RT}{F} \ln [H^+] \qquad (11)$$

Assuming that dismutation takes place equation (11) may be used to calculate the electrode potentials of the hypoxanthine-uric acid system. It is to be observed that when the ratio of hypoxanthine to uric acid is unity no correction for dismutation is required and equation (10) may be used directly.

In the same way an equation including among its variables the dismutation constant may be developed for the electrode potential of the xanthine-uric acid system.

The general equation for this system has already been given in (8) above. We shall consider the effect of dismutation on the ratio XH/UH. For each molecule of uric acid formed by the dismutation reaction two molecules of xanthine are used up. The ratio of the oxidant to reductant will thus be altered to $\frac{XH - 2a}{UH + a}$. The value of a may be considered in terms of hypoxanthine formed. We thus have from the value of the dismutation constant

$$a = Hy = \frac{Km\,[XH - 2a]^2}{[UH+a]}$$

or

$$a = \frac{-UH-4Km\,XH + \sqrt{UH^2 + 8Km\,UH\,XH + 4Km\,XH^2}}{2(1-4Km)}$$

The ratio $\frac{XH - 2a}{UH + a}$ now becomes

$$\frac{2(XH+UH - \sqrt{UH^2 + 8Km\,UH\,XH + 4Km\,XH^2}}{UH - 8Km\,UH - 4Km\,XH + \sqrt{UH^2 + 8Km\,UH\,XH + 4Km\,XH^2}}$$

The final electrode equation for the xanthine-uric acid system taking into account the dismutation is

$$E_h = E_o - \frac{RT}{2F} \ln \frac{2(XH + UH - \sqrt{UH^2 + 8Km\,UH\,XH + 4Km\,XH^2}}{UH - 8Km\,UH - 4Km\,XH + \sqrt{UH^2 + 8Km\,UH\,XH + 4Km\,XH^2}} - \frac{RT}{2F} \ln \frac{Ku + H^+}{Kx + H^+} + \frac{RT}{F} \ln [H^+] \qquad (12)$$

The electrode potential of the hypoxanthine-xanthine system is not experimentally measured directly due to the fact that the oxidation of hypoxanthine does not stop at xanthine but proceeds to the uric acid stage. The oxidation reduction potential of this system is therefore obtained indirectly from the two other systems which have already been discussed. An electrode equation might however be developed for this

system in a manner similar to the others.

With the aid of equation (11) and (12) it is now possible to make a more detailed comparison of our results with those of Green and Filitti. In table LX Fillitti's observed potentials for the hypoxanthine-uric acid system are given together with the calculated E_h values based on our data. Since she uses a 1 to 1 ratio of hypoxanthine to uric acid no correction for dismutation is necessary. It will be seen that the agreement is extremely good. In table LXI we have calculated the E_h values for the xanthine uric acid system by means of equation (12). The corresponding observed potentials reported by Filitti are also given. Again Filitti uses a 1 to 1 ratio of oxidant to reductant. For this ratio the effect of dismutation was found to be entirely negligible. The discrepancy between the observed potentials and our calculated results is somewhat larger than that already given on p. 166. This is partly due to the fact that dissociation constants used in our calculations were different from those used by Filitti.

Table LXII shows Green's experimental values for the hypoxanthine-uric acid compared in the same way. Green uses several different ratios of hypoxanthine to uric acid. We have calculated E_h values corresponding to Green's using both equation (10) and (11). It will be observed that the potentials calculated by means of equation (10) agree more closely with Green's data. than those obtained by the use of equation (11). It appears from this comparison that

TABLE LXI

COMPARISON OF FILITTI'S OBSERVED ELECTRODE POTENTIALS FOR THE HYPOXANTHINE-URIC ACID SYSTEM AT 38°C WITH CALCULATED POTENTIALS DERIVED FROM FREE ENERGY DATA

pH	E_h observed	E_h calculated
7.03	-.387 volts	-.387 volts
7.08	-.390	-.391
7.08	-.378	-.391
7.21	-.400	-.404
7.29	-.410	-.407
7.30	-.410	-.408
7.31	-.409	-.409
7.31	-.410	-.409
7.42	-.418	-.417
7.47	-.420	-.421
7.67	-.437	-.436
7.67	-.436	-.436
7.68	-.435	-.436

TABLE LXII

COMPARISON OF FILITTI'S OBSERVED ELECTRODE POTENTIALS FOR THE XANTHINE-URIC ACID SYSTEM AT 38°C WITH CALCULATED POTENTIALS DERIVED FROM FREE ENERGY DATA

pH	E_h observed volts	E_h calculated volts
7.47	-.409	-.383
7.50	-.410	-.385
7.52	-.410	-.386
7.60	-.424	-.392
7.60	-.420	-.392
7.60	-.415	-.392
7.62	-.420	-.393
7.64	-.420	-.395
7.64	-.419	-.395
7.65	-.420	-.395
7.65	-.424	-.395
7.66	-.420	-.396
7.68	-.420	-.397
7.70	-.420	-.399
8.35	-.464	-.441
8.37	-.463	-.442

little or no dismutation takes place under the conditions of Green's experiments. Finally in table LXIII Green's results for the xanthine uric acid are compared with calculated results based on free energy data. By the use of equation (12) as well as equation (8) it was found that the effect of dismutation on the xanthine uric-acid potentials is negligible. The agreement between the measured and the calculated potentials is not satisfactory and is probably outside the error in the free energy data. The good agreement of our results for the hypoxanthine-uric acid system with those of Green and Filitti makes us inclined to believe that the free energy data are reliable also for the xanthine-uric acid system. It must also be remembered that the oxypurines have a limited solubility in water and the manipulation of relatively high concentrations of these substances as in Green's and Filitti's experiments introduces an uncertainty in the results. Furthermore the addition of a relatively large amount of the enzyme preparation introduces other substances which may affect the electrode potentials. On the whole the usefulness of thermodynamic data in interpreting the results obtained from equilibrium measurements and in providing an independent method by which the results may be checked has been illustrated.

From the values of the oxidation reduction potentials of oxypurines it may be concluded that they are mutually oxidizable and reducible. However the medium in which these reactions may take place must be strongly reducing.

TABLE LXIII

COMPARISON OF GREEN'S OBSERVED ELECTRODE POTENTIALS FOR THE HYPOXANTHINE-URIC ACID SYSTEM AT 30°C WITH CALCULATED POTENTIALS DERIVED FROM FREE ENERGY DATA

pH	Hy/UH	E_h obs. volts	E_h calc. volts	E_h calc. corr. volts
7.65	9/1	-.454	-.433	-.494
8.07	"	-.484	-.464	-.525
8.43	"	-.497	-.490	-.551
6.93	4/1	-.382	-.375	-.429
7.32	"	-.419	-.405	-.459
6.93	1/1	-.366	-.366	"
7.32	"	-.400	-.396	"
7.65	"	-.425	-.419	"
8.07	"	-.456	-.450	"
8.43	"	-.469	-.476	"
9.10	"	-.497	-.523	"
7.32	1/4	-.380	-.387	-.333
7.65	"	-.406	-.410	-.356
8.07	"	-.432	-.441	-.387
8.43	"	-.446	-.467	-.413

TABLE LXIV

COMPARISON OF GREEN'S OBSERVED ELECTRODE POTENTIALS FOR THE XANTHINE-URIC ACID SYSTEM AT 30°C WITH CALCULATED POTENTIALS DERIVED FROM FREE ENERGY DATA

pH	Hy/UH	E_h obs. volts	E_h calc. volts
6.93	4/1	-.362	-.338
7.32	"	-.390	-.365
8.07	"	-.452	-.416
8.43	"	-.465	-.439
6.93	1/1	-.349	-.330
7.32	"	-.380	-.359
7.65	"	-.415	-.380
8.07	"	-.440	-.407
8.43	"	-.454	-.430
9.10	"	-.476	-.470
6.93	1/4	-.335	-.363
7.32	"	-.370	-.350
7.65	"	-.400	-.371
8.43	"	-.437	-.420

Under ordinary conditions oxidation reactions would predominate.

In concluding this study of equilibrium relations of purines and their degradation products we should like to point out that the standard free energy changes in aqueous solutions do not represent the actual conditions in vivo, although certain broad conclusions may be drawn. It is hoped that the information presented in this thesis will find further application to the biochemistry of purines.

SUMMARY

The following experimental data for adenine, hypoxanthine, guanine, xanthine, uric acid, allantoin, alloxan, and d-l- Leucine have been presented: Solubilities at 25°C and 50°C; dissociation constants; vapor pressure of alloxan-alloxan trihydrate system; evidence for non existence of hydrates of adenine and xanthine; activity coefficients of alloxan,--all referred to 25°C.

The experimental methods used for the determination of the above physical chemical constants have been described.

Utilizing the experimental data the standard free energies of formation of undissociated molecules, and ions in aqueous solution at 25°C have been derived, and the methods of calculating these have been indicated.

The energy relationships of the different processes involved in the degradation of purines have been discussed.

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