THE ENZYMATIC CONVERSION OF URIC ACID

SPECTROPHOTOMETRIC ANALYSIS

by

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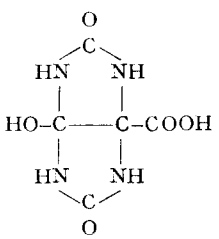
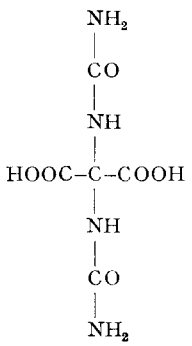
Until recently, the break-down of uric acid by uricase *in vitro* was thought to result in a quantitative formation of allantoin[1, 2, 3] in agreement with the following equation:

$$\text{Uric acid} + O_2 + 2H_2O = \text{allantoin} + H_2O_2 + CO_2 \qquad (1)$$

In 1945 KLEMPERER[4] found, however, that the amount of carbon dioxide developed is, in general, considerably less than one mole per mole of uric acid. He produced some evidence that in addition to allantoin the end products in the enzymatic conversion of uric acid are hydroxyacetylen-diurein-carbonic acid (HDC), and uroxanic acid:



Hydroxyacetylen-diurein-carbonic acid (HDC)



Uroxanic acid

These products are well known from the non-enzymatic oxidation of uric acid in an alkaline medium. HDC, which has been isolated as a tri-silver salt[5] from the alkaline oxidation solution, is an intermediary[5, 6, 7] from which uroxanic acid is formed when the alkaline oxidation solution is evaporated, while allantoin is obtained if the oxidation solution is made weakly acid before the evaporation.

The relative amounts of the end products after completion of the *enzymatic* oxidation depend not only on the p_H but also on the nature of the buffer. For example the formation of allantoin which is augmented as the p_H increases, is less in borate buffer than in other buffers of the same p_H. According to KLEMPERER, the enzymatic reaction occurs as follows:

$$\text{Uric acid} + O_2 + 2H_2O = C_5N_4O_4H_4 + H_2O_2 \qquad (2)$$

and the unknown primary reaction product (which might have a higher water content

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than is indicated by the formula) may give rise to the following non-enzymatic decomposition products:

$$C_5N_4O_4H_4 \begin{cases} + H_2O \rightarrow \text{allantoin} \\ + H_2O \rightarrow \text{HDC} \\ + 2H_2O \rightarrow \text{uroxanic acid} \end{cases}$$

The present study was undertaken to investigate the spectral changes in the ultraviolet which are involved in the conversion of uric acid produced by highly fractionated uricase at various p_H, and with the use of different buffers.

The principles as well as the technique and the methods of enzymatic differential spectrophotometry have been described in a previous communication[8], to which reference must be made ("Optical Methods" and "Determination of uric acid")*.

Owing to the diversity of the present experiments, and their gradually increasing complexity, it was thought advisable to discuss the results separately as they appear, and to give a summarizing conclusion.

Method of plotting. In all figures the directly read extinction difference between a measuring cell and a reference cell is plotted against the time of reaction. The measuring cell contains uric acid in a buffer, the reference cell containing only the buffer solution. At zero time a small volume of uricase suspension[8] is added to the contents of the measuring cell.

RESULTS

On the addition of a small amount of highly active uricase to a solution of uric acid in borate buffer of p_H 8, the extinction gradually decreases at all wave-lengths at which uric acid absorbs. The total decrease at any-wave length higher than 280 mμ equals the extinction of uric acid as measured at the same wave-length by simple spectrophotometry on the uric acid solution previous to the addition of enzyme.

From this it may be concluded that *uric acid is converted into substances with no absorption at wave-lengths higher than 280 mμ.*

Below 280 mμ, $-\Delta E_\lambda$ is less than E_λ, (the deficit being higher, as the wave-length decreases). The end products, therefore, absorb at wave-lengths shorter than 280 mμ. Allantoin, however, does not absorb at 260 mμ or higher wave-lengths. Accordingly, *end products other than allantoin are formed by the enzymatic conversion of uric acid.*

Fig. 1 is representative of such experiments. At the maximum wave-length of the uric acid spectrum (293 mμ) a gradual extinction decrease appears as uric acid is broken down; at the minimum wave-length 260 mμ the decrease is considerably smaller, and at the wave-length 325 mμ, at which uric acid does not absorb, the conversion of uric acid is not observed,—the extinction is not changed. For the sake of simplicity, only the changes at these three characteristic wave-lengths are included in the figure. (Compare the spectrum of uric acid[8]).

The final constant extinction value at any wave-length higher than 280 mμ agrees

* *The uric acid absorption spectrum* in the ultraviolet, which is shown in the above mentioned paper, is independent of the nature of the buffers used; it is only a little varied, when the p_H range is between 7 and 11. The slight variations have no practical influence on the results of the experiments, which are to be reported.

The agreement between the determinations of uric acid, by the *simple*, and by the *enzymatic differential spectrophotometry*[8], has been confirmed at various concentrations of uric acid, at various p_H-values, and, within certain limits, at various wave-lengths, the values of E_λ and of $-\Delta E_\lambda$ being approximately equal.

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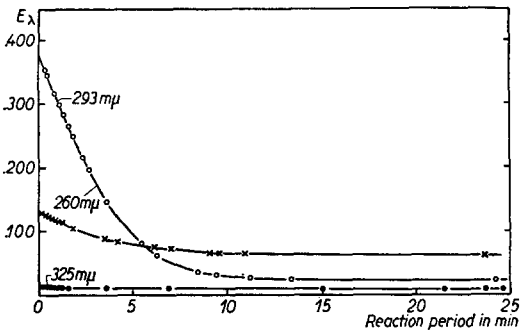

Fig. 1. The extinction at the wave-lengths 293 mμ, 260 mμ, and 325 mμ, as functions of the reaction time. The system contains 4.9 μg uric of acid per ml of M/15 borate buffer of pH 7.9. Uricase is added at zero time. Ordinates: Eλ. Abscissae: reaction period in minutes.

with the extinction of the enzyme. If uricase is put in the reference cell after the determination of the uric acid spectrum, just before the same amount of the enzyme is put in the measuring cell, the extrapolation value at any wave-length (higher than 280 mμ) equals the uric acid extinction, and the final constant value will be approximately zero in any case.

From this it appears that *uricase and uric acid contribute additionally to the total extinction*, which is read as the extrapolation value, when the reference cell only contains the buffer as is the case in the experiment shown in Fig. 1. *The absorption spectrum of the enzyme is the same before and after the enzymatic reaction.*

The period in which an arbitrarily chosen ratio is reached, for instance half of the total extinction decrease is practically independent of the wave-length. It appears, therefore, as if uric acid is directly converted into end products in these experiments. Such simple graphs, however, are obtained only when borate buffer is used, and only when the p_H is not higher than 8.

Fig. 2, which represents a similar experiment to that of Fig. 1, shows altogether different curves when the borate buffer is replaced by a phosphate buffer of the same p_H (7.9). The decrease at the maximum wave-length of uric acid is retarded. At the minimum wave-length, and at the wave-length at which uric acid does not absorb, a gradual increase occurs which is followed by a gradual decrease. *These phenomena always occur together, and occur in any buffer solution except borate buffer of p_H 8 or less.* They are most pronounced when the formation of carbon dioxide (according to KLEMPERER) is high, and least pronounced under conditions at which the carbon dioxide formation, and so the formation of allantoin, is low. (In the case of borate buffer of p_H 7.9 KLEMPERER found a formation of carbon dioxide, which was only 9% of the theoretical according to the reaction 1). In other words, *the phenomena are connected somehow with allantoin formation.*

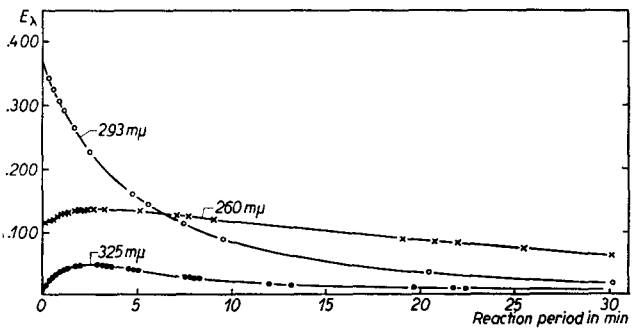

Fig. 2. The extinction at the wave-lengths 293, 260 and 325 mμ as functions of the reaction time. The system contains 4.9 μg of uric acid per ml of M/15 phosphate buffer pf p_H 7.9. Uricase is added at zero time. Ordinates and abscissae as in Fig. 1.

In Fig. 3 the heavy lines show directly that, in the case of a phosphate buffer, the decrease at the maximum wave-length of uric acid, 293 mμ, is retarded as compared to the borate-buffered system. The thin lines show what happens at this wave-length, on the addition of a small volume of a strong solution of

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sodium cyanide, which instantaneously produces a complete inhibition of uricase. In the case of borate buffer, the extinction decrease suddenly ceases, while in the phosphate-buffered system the decrease is at first even accelerated. The final constant value after the addition of cyanide is approximately equal in both.

A spectrum taken when the final values were reached was in both cases identical with the spectrum of uric acid (+ uricase). This indicates that the same amount of uric acid has been converted at the time of inhibition. (The spectrum of uricase is easily eliminated by the addition of uricase also to the reference cell).

The curve in the borate-buffered system, at p_H 8 or less, is practically a true expression of the disappearance of uric acid. No matter of what time cyanide is added, a sudden cessation of the extinction decrease occurs and a spectrum taken immediately afterwards will be identical with that of uric acid.

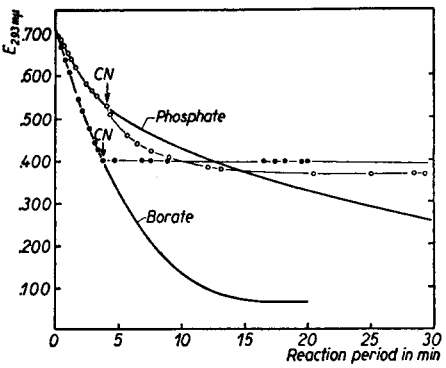

Fig. 3. *Heavy lines*: the extinction at 293 mμ as a function of the reaction time in a system buffered by phosphate and in a system buffered by borate. The pH is in both cases 7.9. The concentration of uric acid is 9 μg per ml. Uricase is added at zero time. *Thin lines*: the extinction on the addition of cyanide at the same time to both systems. Ordinates: $E_{293\,m\mu}$ Abscissae as in Fig. 1.

In the case of the phosphate buffer, however, *it appears as if an absorbing substance is enzymatically formed and spontaneously broken down.*

Fig. 4 is a comparison between borate- and glycine-buffered systems of pH 9.45. In this experiment extinctions are read only at the maximum wave-length of uric acid. The heavy lines represent the changes in systems to which cyanide was not added. The thin lines represent the changes after the addition of cyanide.

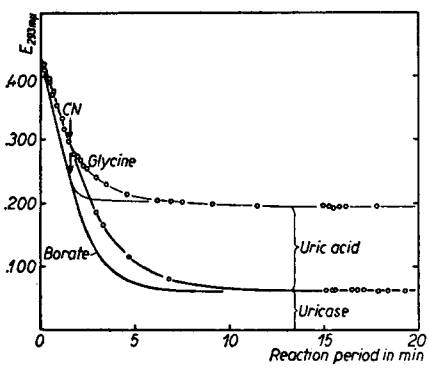

Fig. 4. *Heavy lines*: $E_{293\,m\mu}$ as a function of the reaction time in a system buffered by glycine and in a system buffered by borate. The pH is in both cases 9.45. The concentration of uric acid is 4.9 μg per ml. Thin lines, ordinates and abscissae as in Fig. 3.

On the addition of cyanide to the glycine-buffered system, the decrease is continued for a rather long time, (about 5 min), but in the case of borate buffer only a slight decrease takes place. The final constant level is equal in both cases, indicating that at the time of inhibition the same amount of uric acid has been converted.

A system buffered to the same pH, for instance by a sodium pyrophosphate buffer, will behave exactly like the glycine-buffered system.

Since borate buffers do not produce inhibition or acceleration of the velocity at which uric acid disappears, there is *no reason to believe that borate ions have any effect on the enzyme activity.*

The retardation of the decrease at the maximum wave-length of uric acid (Fig. 2), and the increase at the minimum wave-length and at the wave-length at which uric acid does not absorb, (*i.e.*, the phenomena which altogether are connected with the

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allantoin formation), are now most reasonably interpreted as *a transitory accumulation of an intermediate substance,* the decomposition of which is accelerated in the presence of borate.

From the findings of KLEMPERER it appears, however, that the formation of allantoin is higher in the absence than in the presence of borate. The decomposition of an intermediate substance, accelerated by borate ions, should be a reaction, therefore, in which allantoin is *not* formed.

In order to examine the formation of the intermediate substance and some of its properties, a series of experiments were carried out at the wave-length 325 mμ, at which uric acid does not absorb and at which, therefore, no disturbance will be produced by the *disappearance* of uric acid.

The maximal *level* of the extinction at 325 mμ, which should be an expression of the maximal *concentration* of the intermediate substance, depends on the amount of uricase in the system, on the oxygen tension, and to some extent on the concentration of uric acid, all factors which determine the velocity of the enzymatic uric acid oxidation. In other words, the extinction level at 325 mμ, an so the concentration of the intermediate substance, depends on the velocity of the oxidation of uric acid. This indicates that *the substance is enzymatically formed by the oxidation of uric acid.*

The maximal extinction at 325 mμ is lowered as the p_H increases. That this is due to *an increased decomposition velocity in the alkali medium* and not to a spectral change caused by the higher p_H appears from experiments in which the formation of the substance is stopped by the addition of cyanide.

The duration of the increased extinction at 325 mμ is greater in a neutral than in an alkaline medium owing to the greater stability of the substance at a lower p_H. The duration of the increased extinction at a given value of p_H, depends on the original concentration of uric acid, owing to the continued formation of the substance.

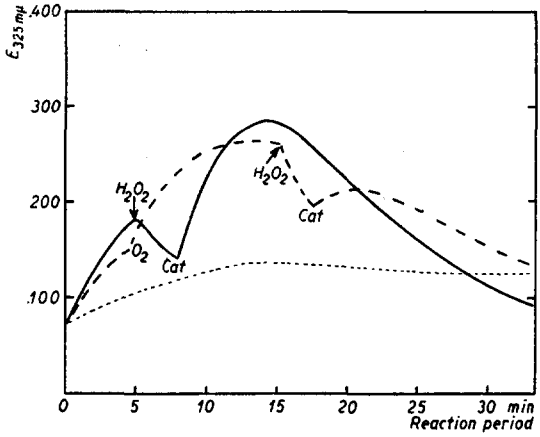

Fig. 5. $E_{325\,m\mu}$ as a function of the reaction time. The system contains 40 μg of uric acid per ml of M/15 phosphate buffer of p_H 7.3. Uricase is added at zero time. Continuous line: the system is saturated with oxygen. Broken line: the system is at equilibrium with air. Dotted line: the system is poor in oxygen. Ordinates: $E_{325\,m\mu}$. Abscissae: reaction period in minutes.

Some of these findings are demonstrated and utilized in the following experiments. Fig. 5 shows the changes of the extinction at 325 mμ, at which wave-length uric acid does not absorb. The concentration of uric acid is rather high, *viz.*, 40 μg per ml of phosphate buffer. The p_H is 7.3.

The continuous line represents a system previously bubbled with oxygen; the broken line a system at equilibrium with air, and the dotted line a system poor in oxygen. The same amount of uricase is present in all systems.

The slope of the initial part of the curves rises with the oxygen tension indicating that the velocity of the formation of the intermediate substance is increased with an increasing velocity of uric acid oxidation.

On the addition of hydrogen peroxide, which produces some inhibition on uricase, the extinction suddenly decreases. When catalase is added, the curve will again rise, and in the system saturated with oxygen even at a higher rate than prior to the inhibition. This is due presumably to an oxygen super-saturation, caused by the decomposition of hydrogen peroxide.

In the system with air the slope of the curve also becomes steeper when the oxygen

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tension is increased, for example when oxygen is bubbled through it. In the oxygen-poor system only a small concentration of the absorbing substance is reached.

It is obvious that *the curves are expressions of a formation and a simultaneous decomposition of a substance which is enzymatically formed and spontaneously broken down.*

If a similar experiment is carried out at the optimum p_H of uricase activity, (9.2–9.4) the peak of the 325 mμ-line is reached in less than 2 minutes (Fig. 6) when the system is saturated with oxygen. In spite of the much more rapid formation of the intermediate product, the maximum is only 50% of the value reached in the previous experiment at p_H 7.3. The more rapid formation cannot compensate for the accelerated decomposition of the substance in the alkaline medium.

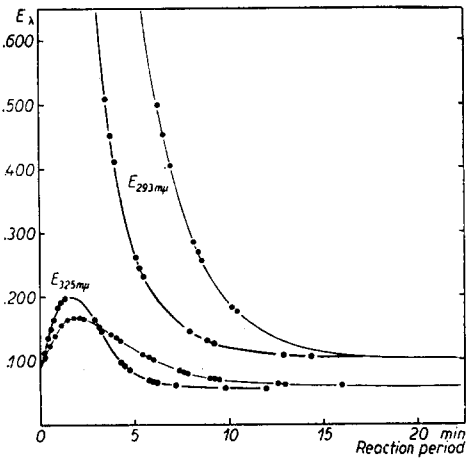

Fig. 6. $E_{293\ m\mu}$ and $E_{325\ m\mu}$ as functions of the reaction time. 40 μg uric acid per ml of M/15 glycine buffer of pH 9.4. o——o saturated with oxygen. •——• equilibrium with air. Ordinates and abscissae as in the previous figures.

In Fig. 7 the effect of borate ions on the intermediate substance is compared to the effect of a p_H change. Both the presence of borate and a high p_H will accelerate the decomposition of this substance. From the findings of KLEMPERER, however, it appears that the formation of allantoin predominates in the absence of borate but is suppressed in its presence. *The decomposition of the intermediate substance, which is accelerated by borate, is therefore most likely to be another kind of reaction than the decomposition, which is accelerated by an increased p_H.*

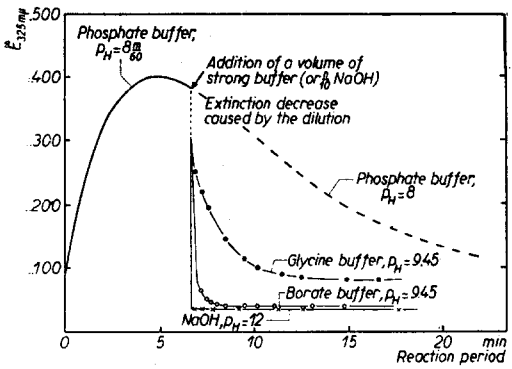

Fig. 7. The effect of borate ions and of pH-change on the decomposition of a 325 mμ-absorbing substance, which is formed by the action of uricase. 125 μg uric acid per ml M/60 phosphate buffer of pH 8. Uricase at zero time. At $6^2/_3$ minutes the addition of $^1/_5$ volume of, respectively, $^2/_3$ M glycine buffer of pH 9.45 •——•, $^2/_3$ M borate buffer of pH 9.45 o——o, and N/10 NaOH x——x. *Continuous line*: From zero time to $6^2/_3$ minutes 4 curves are represented, one of which (corresponding to a system to which nothing is added at $6^2/_3$ minutes) is continued as the *broken line*. *Dotted vertical line*: The effect of the dilution. Abscissae and ordinates as in the previous figures.

In this experiment, again at the wavelength 325 mμ, a phosphate buffer was used (p_H 8).

The initial part of the curve (the heavy line) represents 4 quartz cells, the contents of which were successively examined. The initial part, together with the broken line represent changes of the extinction values in a system to which nothing has been added.

Just after the maximum was passed, a volume of strong glycine buffer of p_H 9.45 was added to one of the cells, strong borate of the same p_H to another, and a solution of sodium hydroxide to a third. The added volume in all cases amounted to $^1/_5$ of the original volume and caused, therefore, a

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dilution on the reaction mixture. The calculated effect of this dilution on the extinction is marked by the dotted vertical line on the figure.

In all cases an abrupt and strong decrease of the extinction takes place, as the reaction becomes more alkaline. It is most rapid with sodium hydroxide. Here the final p_H is considerably higher than in the two cells with glycine and borate buffer. With borate the decrease is much more rapid than with glycine buffer, and this is true, even when a borate buffer of p_H 8 is added instead of one of p_H 9.45. In other words, the effect of borate addition is the same whether the p_H is changed or not. The curve after addition of borate of p_H 8 is *identical* with that of borate buffer of p_H 9.45.

A sodium pyrophosphate buffer gave a curve identical to that of the glycine buffer of the same p_H (9.45).

There is no doubt, therefore, that *the borate ions have a peculiar effect on the intermediate substance, which, in the absence of borate, is most rapidly decomposed at a high p_H.*

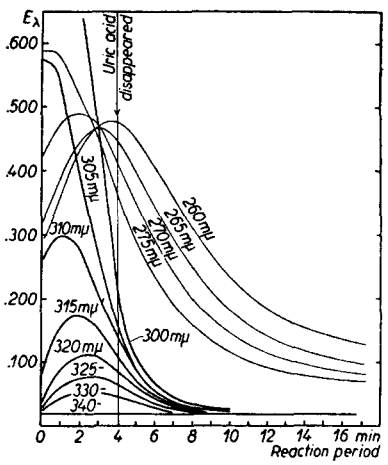

Fig. 8. The extinction changes at various wave-lengths during the enzymatic reaction in a system containing 15 μg of uric acid per ml of glycine buffer of p_H 9.4 saturated with oxygen. Uricase at zero time. No uric acid left at 4 minutes. Abscissae and ordinates as in the previous figures.

The experiment which is represented in Fig. 8 was carried out in order to obtain some information as to the spectrum of the intermediate substance, (or perhaps substances), and with the purpose of making a study of the intermediate spectral changes in connection with the conversion of uric acid.

The same amount of uricase was added at zero time to a number of cells, all of them containing 15 micrograms of uric acid per ml of glycine buffer at p_H 9.4 and saturated with oxygen.

Fig. 8 shows the extinction changes during the enzymatic reaction at various wave-lengths ranging from 260 mμ, the minimum wave-length of the uric spectrum, to 340 mμ. Uric acid does not absorb at wave-lengths higher than 320 mμ.

4 minutes after the start of the reaction, no uric acid was present.

This was established in a quite similar system with borate buffer instead of glycine buffer by readings at the maximum wavelength of uric acid. The final constant level was reached in 4 minutes. The disappearance of uric acid in 4 minutes was confirmed, in the glycine-buffered system, by the addition of cyanide at this time; the curves with and without cyanide were identical.

By a vertical line through the 4-minute point on the axis of abscissae, each curve is divided into two parts, one corresponding to the period of uric acid conversion, the other representing only changes in which uric acid does not participate.

4 minutes is approximately the time at which the lower curves have an inflecting point. These curves are obtained by measuring at 300–340 mμ. The inflection of the other group of curves, representing measurements at 260–275 mμ, occurs later. At 260 mμ, for instance, it is the maximum of the curve which is near to the 4-minute line.

The different shapes of the two groups of curves *after 4 minutes* might be explained by the assumption that *a primary substance which absorbs the long-waved ultraviolet is converted into a secondary intermediate compound with a high absorption of the short-waved, but not of the long-waved ultraviolet.*

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From the final constant values it was calculated (by subtraction of the extinction produced by uricases), that *the end products have only slight absorption, and exclusively in the short-waved region.*

A spectrum of the intermediate substances may be obtained by plotting the extinctions, read on the 4-minute line, against the wave-lengths. After subtraction of the spectrum of the enzyme, the result will be a spectrum of the intermediate substances together. A spectrum obtained in this way when the long-waved absorption has completely disappeared (about 9 minutes after the start of the reaction), is not only very different from the first mentioned, but also different from that of the end products. These findings also seem to indicate, that a primary intermediate substance is converted into a secondary one.

A separation of a primary intermediate substance with absorption in the long-waved ultraviolet from other intermediate products with absorption only in the shortwaved region, should be possible by the addition of borate at the time at which uric acid has completely disappeared, and by extinction readings at a short wave-length, for instance at the minimum wave-length of uric acid (260 mμ), while the disappearance of the primary intermediate might be tested at a long wave-length (325 mμ).

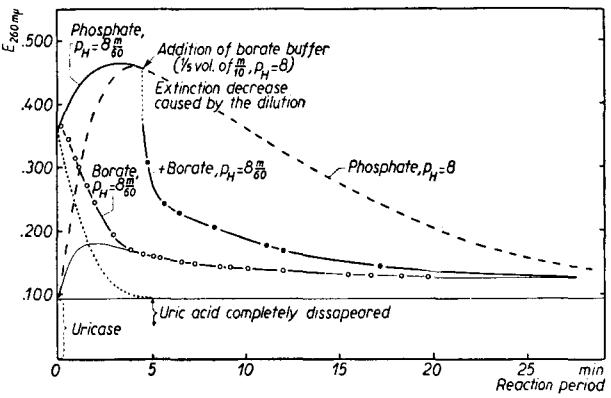

Fig. 9. $E_{260\ m\mu}$ as a function of the reaction time. Uricase is added at zero time to systems containing 15 μg uric acid par ml of, respectively, phosphate buffer, (*heavy line* continued as the *broken line*) and borate buffer (o——o). No uric acid is left at 5 minutes. At this time $^1/_5$ volume of borate buffer is added to the phosphate system. *Dotted vertical line* (at 5 minutes): the effect of the dilution •——• the phosphate-borate system. *Dotted curved line*: (from zero time to 5 minutes): the disappearance of uric acid (see the text). *Total broken line*: Non-uric-acid-change in phosphate buffer. *Thin line* (interrupted by o——o): Non-uric-acid-change in borate buffer. p_H 8 in all cases.

Fig. 9 shows such an experiment with phosphate and borate buffers at p_H 8. The ordinates represent the extinction at 260 mμ, the minimum wave-length of uric acid absorption.

In the phosphate-buffered system, a considerable increase is seen instead of the expected decrease corresponding to the decomposition of uric acid which, previous to the addition of uricase, produces an extinc tion of 0.270. In the borete-buffered system, however, an initial increase is at any rate insignificant.

By measuring at the maximum wave-length of uric acid on the cell containing borate buffer, it was found that the final value of extinction was reached in 5 minutes. At this time, therefore, no uric acid was left.

The dotted curve (from zero time to 5 minutes) represents the disappearance of uric acid as calculated from measurements at the maximum wave-length in borate buffer. If this line is subtracted from the phosphate-curve, the result will be the broken line which, therefore (on and after zero time), represents the non-uric changes at 260 mμ in phosphate buffer.

Likewise the thin curve is the difference between the borate curve and the dotted

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curve. The thin line, accordingly, represents the non-uric acid changes in borate buffer.

If borate buffer is added to the phosphate system, a decrease takes place. The dotted vertical line corresponds to the dilution caused by the addition of borate buffer. The first part of the decrease is very rapid, just as in the corresponding experiment at 325 mμ (Fig. 7).

From these experiments it may be concluded that *the primary intermediate substance is rapidly decomposed in the presence of borate ions into substances with no absorption in the long-waved region and with only small absorption at 260 mμ.* (Compare the borate curve in Fig. 9—the thin line).

In the case of the phosphate-borate system (Fig. 9), the readings at 325 mμ, at which wave-length only the primary substance absorbs, showed that this intermediate has completely disappeared in less than 1 minute after the addition of borate.

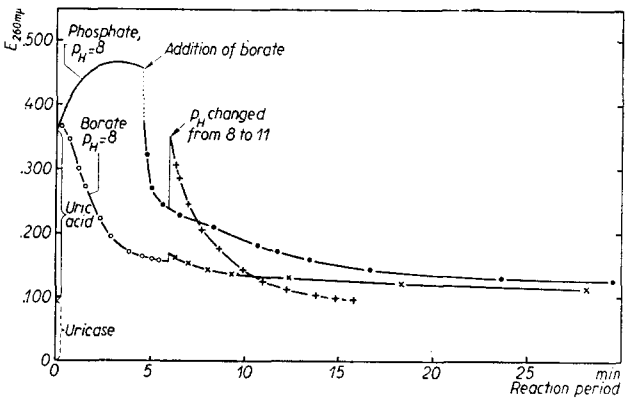

Fig. 10. The effect of p_H-change in the borate-buffered (o——o) and the phosphate-borate-buffered (•——•) system shown in Fig. 9. At 6 minutes the 325 mμ-absorbing substance has completely disappeared. At this time p_H is changed from 8 to 11 (without measurable dilution). *The vertical line*: instantaneous increase of $E_{260\ m\mu}$ caused by the p_H-change. x——x $E_{260\ m\mu}$ of the borate- system after p_H-change. +——+ $E_{260\ m\mu}$ of the borate-phosphate-system after p_H-change.

The difference between the curves representing the phosphate-borate system and the simple borate system, at and after 6 minutes, cannot be due to uric acid or to the primary intermediate substance, since they are not present at this time. The difference between these systems after 6 minutes is due, therefore, to the presence in the phosphate-borate system of compounds which are formed prior to the addition of borate buffer.

If the difference is a qualitative one, this would indicate that the manner of decomposition of the intermediate substance is different in the presence and in the absence of borate. That there is a qualitative difference appears from the following experiment.

When the p_H is changed from 8 to 11 by the addition of a small volume of sodium hydroxide to the simple borate system at 6 minutes, practically no extinction change occurs. If, however, the same p_H-change is produced at the same time in the phosphate-borate system, the extinction is considerably increased. The change occurs instantaneously and should be marked on the figure by a vertical line. The instantaneous increase is followed by a gradual rapid decrease to a constant low level, as is shown in Fig. 10.

A similar phenomenon is observed in the simple phosphate system (Fig. 11).

The phosphate-curve and the phosphate-borate curve are quite similar to those in Fig. 9. Uric acid has completely disappeared at the time of borate addition.

The addition of borate always causes a rapid decomposition of the primary intermediate substance, since a very rapid extinction decrease may be observed at all wavelengths concerned, and at all values of p_H, when borate is added *without a p_H-change.*

In the experiment shown in Fig. 11, however, a p_H-change is produced. The instan-

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taneous increase of the extinction, caused by the addition of borate buffer of p_H 9.45, corresponds exactly to the increase caused by the addition of any other strong buffer of the same p_H. The instantaneous increase is not an effect on the primary intermediate substance, since this is rapidly decomposed by borate ions. A similar extinction increase takes place in a system which does not contain a measurable amount of this primary substance (Fig. 10).

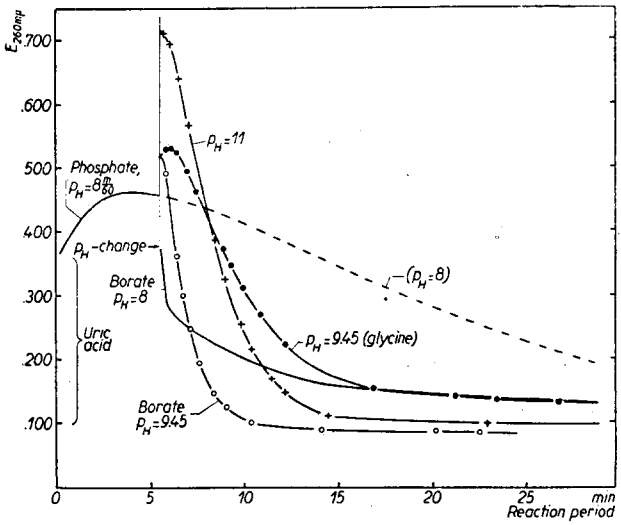

Fig. 11. The effect of p_H-change and of addition of borate (without p_H-change) to the phosphate system of Fig. 9. The original p_H is 8. *Continuous line + broken line*: the unchanged phosphate system. *Dotted vertical line*: the effect of dilution. From the lowest point of this line is measured *upwards*: the instantaneous increase of $E_{260\,m\mu}$ caused by the addition of, respectively, borate buffer of p_H 9.45 o——o, glycine buffer of p_H 9.45 •——•, and NaOH (the p_H becoming 11). *Downwards*: the phosphate-borate system of p_H 8.

The extinction increase (Fig. 11), *which is caused by the p_H-change is an effect on another compound, whose formation seems to be accelerated* not by borate ions but *by a high p_H*. The curved line on the top of the glycine-curve is most probably an expression of a simultaneous formation and decomposition of a secondary intermediate compound.

The extinction coefficient of this secondary intermediate as well as the velocity at which it decomposes is increased as the p_H is raised.

CONCLUSION

According to KLEMPERER, borate ions suppress the decarboxylation of an unknown intermediate product and favour the formation of HDC and uroxanic acid from this intermediate. In the absence of borate, however, the formation of allantoin predominates, and increases as the p_H becomes more alkaline. This might now be formulated as follows:

$$\text{Uric acid} \rightarrow \text{I} \begin{cases} \overset{\text{(a)}}{\nearrow} \text{II} \rightarrow \text{allantoin} \\ \overset{\text{(b)}}{\searrow} \text{HDC} + \text{H}_2\text{O} \leftrightarrows \text{uroxanic acid.} \end{cases}$$

a. velocity increased by a p_H increase;
b. velocity increased by borate ions.

The primary substance (I) is decomposed in two different ways. One reaction is the formation of a secondary intermediate (II) which is converted into allantoin; the velocity of this conversion increases as the p_H becomes higher.

Another kind of decomposition of the primary substance is the formation of HDC, which is accelerated by borate. HDC is partly converted into uroxanic acid; the relative amounts of these two end products depend on the p_H.

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SUMMARY

The disappearance of the light absorption of uric acid in enzymatic experiments is generally not a true expression of the disappearance of uric acid. The spectral changes involved, show that at least two intermediate substances are transitorily accumulated, which cause a retardation of the disappearance of the absorption at the maximum wave-length and the occurrence of a transitory absorption at wave-lengths at which uric acid produces little or no absorption.

The accumulation of the intermediate substances is very slight when borate ions are present and the p_H not above 8. The reason for this is that the primary intermediate substance, which absorbs the long-waved and also the short-waved ultraviolet, is rapidly decomposed in the presence of borate. This decomposition is independent of the p_H.

The end products have only slight absorption and exclusively in the short-waved region.

The disappearance of uric acid may therefore be followed, and the activity of uricase may be estimated, through observations of the disappearance of the absorption at the maximum wave-length of uric acid in systems with borate buffer of p_H 8.

The primary intermediate substance may be examined in the absence of borate and at a higher wave-length at which uric acid does not absorb.

The secondary intermediate compound may be examined separately at shorter wave-lengths, when uric acid has disappeared, and when the primary substance has been excluded by the addition of borate. The absorption of the secondary substance is greater as the p_H increases. Both the formation velocity and the decomposition velocity of the secondary substance is increased with an increasing p_H.

From a comparison of these results with the findings of KLEMPERER it seems probable, that the formation of allantoin is conditioned by a rapid decomposition of a secondary intermediate compound. Under conditions at which this decomposition is relatively slow the formation of other end products from the primary intermediate substance will predominate.

RÉSUMÉ

La disparition de l'absorption de la lumière par l'acide urique, produite lors d'expériences enzymatiques, n'est en général pas en corrélation directe avec la disparition de l'acide urique. Les changements spectraux montrent qu'au moins deux substances intermédiaires sont accumulées transitoirement. Elles produisent un retardement dans la disparition de l'absorption dans la région du maximum d'absorption; en même temps apparaît une absorption transitoire à des longueurs d'onde ou l'acide urique n'absorbe pas.

L'accumulation des produits intermédiaires est minime en présence d'ions de borate et à un p_H ne dépassant pas 8. La première substance intermédiaire, absorbant les rayons ultraviolets longs et courts, est rapidement décomposée en présence de borate. Cette décomposition est indépendante du p_H.

Les produits finaux ont une absorption très faible, se confinant aux régions d'ondes courtes

La disparition de l'acide urique peut donc être étudiée, ainsi que l'activité de l'uricase, par l'observation de la disparition de l'absorption-maximum de l'acide urique, dans des systèmes contenant du tampon borate de p_H 8.

La première substance intermédiaire peut être étudiée dans l'absence de borate, et à une longueur d'onde plus élevée, ou l'acide urique n'absorbe plus.

Le second produit intermédiaire peut être étudié séparément à des longueurs d'onde plus courtes, quand l'acide urique a disparu, et quand la substance primaire est exclue par l'addition de borate.

L'absorption du produit secondaire, ainsi que ses vitesses de formation et de décomposition, augmentent avec le p_H.

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Lorsqu'on compare ces résultats avec ceux de KLEMPERER, il semble probable que la formation d'allantoine est conditionnée par une décomposition rapide d'un intermédiaire secondaire. Dans des conditions ou cette décomposition est lente, il se formera de la substance intermédiaire première surtout d'autres produits finaux.

ZUSAMMENFASSUNG

Das Verschwinden der Lichtabsorption von Harnsäure bei enzymatischen Versuchen ist im allgemeinen kein richtiger Ausdruck für das Verschwinden von Harnsäure. Die dabei auftretenden spektralen Veränderungen zeigen, dass zumindest zwei Zwischenstoffe vorübergehend angehäuft werden, die eine Verzögerung des Verschwindens der Absorption beim Wellenlängenmaximum und das Auftreten einer vorübergehenden Absorption bei Wellenlängen, bei welchen Harnsäure wenig oder nicht absorbiert, verursachen.

Die Anhäufung der Zwischenstoffe ist sehr gering, wenn Borationen vorhanden sind und der p_H nicht oberhalb von 8 ist. Der Grund dafür ist, dass der primäre Zwischenstoff, der im lang-, und auch im kurzwelligen Ultraviolett absorbiert, bei Anwesenheit von Borat schnell zersetzt wird. Diese Zersetzung ist p_H-unabhängig.

Die Endprodukte absorbieren nur schwach und ausschliesslich im kurzwelligen Gebiet.

Durch Messungen des Verschwindens der Absorption beim Wellenlängenmaximum von Harnsäure in Systemen mit Boratpuffer von p_H 8 kann daher das Verschwinden von Harnsäure verfolgt, und die Uricaseaktivität bestimmt werden.

Der primäre Zwischenstoff kann bei Abwesenheit von Borat und bei einer höheren Wellenlänge, bei der Harnsäure nicht absorbiert, untersucht werden.

Der sekundäre Zwischenstoff kann getrennt bei kürzeren Wellenlängen untersucht werden, wenn die Harnsäure verschwunden ist, und wenn der primäre Stoff durch Zufügen von Borat zersetzt worden ist. Die Absorption des sekundären Stoffes ist grösser, wenn der p_H zunimmt. Sowohl die Bildungs- wie die Zersetzungsgeschwindigkeit des sekundären Stoffes nimmt mit zunehmendem p_H zu.

Bei Vergleich dieser Resultate mit den Funden von KLEMPERER wird es wahrscheinlich, dass die Allantoinbildung durch den schnellen Zerfall einer sekundären Zwischenverbindung bedingt wird. Unter Bedingungen, bei denen dieser Zerfall verhältnismässig langsam ist, ist die Bildung anderer Endprodukte aus dem primären Zwischenstoff vorherrschend.

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