CXLVIII. URICASE AND ITS ACTION.

IV. PREPARATION AND PROPERTIES OF OX-KIDNEY URICASE.

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Introduction.

It has been shown in the previous parts of this series that uricase is a contact catalyst, and that this enzyme cannot be obtained in the form of an extract or dialysate. The contact nature of the action of uricase was (Part III) based on an analogy with active charcoal. In this part experiments definitely establishing the non-existence of uricase except in association with insoluble cell-constituents are described.

Experimental.

Ox-kidneys were the source of uricase. Glycerol-chloroform suspensions were made from the minced tissue by the method described in Part I [Przyłęcki, 1928] of this series. Uric acid was in all cases dissolved in saturated lithium carbonate solution and was determined colorimetrically using Folin's reagent. p_H was determined potentiometrically.

1. *Action of various uricolytic preparations in flasks shaken at 38°.*

It was shown in Part III that the rate of oxidation of uric acid in the presence of activated charcoal is 1·7 times as great with shaking as without, whilst in Part II it was shown that the action of dog-liver tissue was 2·5 times as great with shaking as without. In Part III a number of curves were given expressing the dependence of uricolysis on p_H for systems containing glycerol-chloroform extracts of ox-kidney tissue, kept without shaking for 48–86 hrs. Similar curves for the p_H range 6·2–11·9 have now been constructed for a number of glycerol-chloroform extracts, for kidney tissue itself, for the residue obtained on centrifuging the extract, and for extracts prepared without glycerol; in these cases the systems were shaken for various periods of time at 38° in 100 cc. flasks tightly stoppered with rubber corks. The results are given in Fig. 1.

(*a*) *Glycerol-chloroform extracts* (*Exps. 1, 2, 3 and 4*).

These were prepared in the following way. 500 g. of hashed ox-kidney were macerated for 48 hrs. with 50 cc. of chloroform, 150 cc. of glycerol, and 500 cc.

of water. At the end of this time a further 500 cc. of water were added, and the mixture was allowed to stand for 24 hrs., when the supernatant layer was decanted off and taken for experiment.

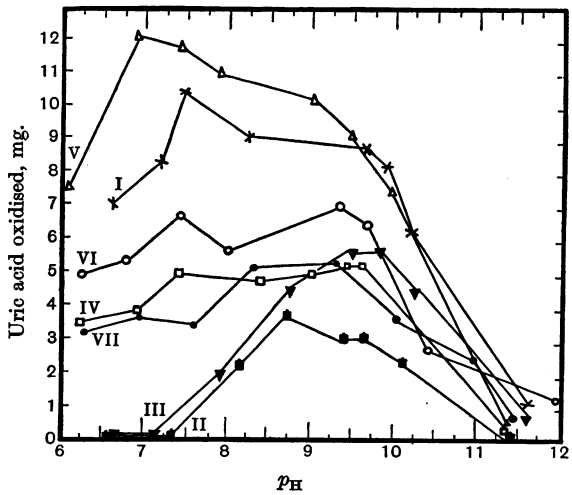

Fig. 1. p_H-uricolysis curves of various ox-kidney preparations.

Curve I. Glycerol-chloroform extract, 3 days old. Shaken 120 mins.
Curve II. Glycerol-chloroform extract, freshly prepared. Shaken 60 mins.
Curve III. Glycerol-chloroform extract, same as used in Exp. 2, 3 days later. Shaken 120 mins.
Curve IV. Glycerol-chloroform extract, freshly prepared. Shaken 90 mins.
Curve V. Aqueous chloroform extract. Shaken 90 mins.
Curve VI. Minced kidney (1 g.). Shaken 60 mins.
Curve VII. Suspension of residue from centrifuged glycerol-chloroform extract. Shaken 35 mins.

All the systems in this section consisted of 10 cc. of uric acid, 20 cc. of buffer solutions (McIlvaine's disodium hydrogen phosphate-citric acid buffers to p_H 8, and Sørensen's glycine-sodium hydroxide buffers for p_H values above 8), and 10 cc. of uricase. The lowest p_H taken was about 6·5, as below this value uric acid crystallised out under the experimental conditions.

Exp. 1. (*Curve I.*) A 3-day-old uricase preparation was taken in this experiment. The flasks were shaken for 2 hrs. at 38°; decomposition of uric acid in the control flasks commenced at p_H 9·90, and amounted at p_H 11·60 to 1·9 mg. The quantity of uric acid oxidised through the agency of uricase was taken as the difference between the uric acid contents of the system in question and of that of the control flask at the same p_H, to which water had been added in place of uricase. Maximum uricolysis was obtained at p_H 7·41. Commencing with p_H 9·60 the solutions were increasingly clearer, until at 11·60 no sediment at all was formed. The systems having a p_H value above 9 smelt strongly of methylamine at the end of the reaction. The same phenomena were observed for all the systems described in this section.

Exp. 2. (*Curve II.*) Here a freshly prepared uricase "extract" was taken; the flasks were shaken for 1 instead of 2 hrs. as in Exp. 1. Maximum oxidation occurred at p_H 8·71.

Exp. 3. (*Curve III.*) The same uricase preparation as in Exp. 2 was taken 3 days later; these systems were shaken for 2 hrs. Maximum oxidation took place at p_H 9·85. Curve III differs from all the other curves in possessing only one peak.

Exp. 4. (*Curve IV.*) A freshly prepared extract was taken in this experiment, prepared by adding 20 cc. of chloroform, 30 cc. of glycerol, and 200 cc. of water to 100 g. of minced ox-kidney, and diluting to 500 cc. after 48 hrs. The systems were shaken for 90 mins. The curve exhibits a first maximum at p_H 7·37, and a second at p_H 9·43–9·65.

Exp. 5. (*Curve V.*) *Aqueous chloroform extract.* This was prepared simultaneously with that used in Exp. 4; the only difference in its preparation was that 30 cc. of water were added in place of glycerol. These systems were, as in Exp. 4, shaken for 90 mins. The action of this preparation is on the whole twice as great as that containing glycerol; no particular significance is, however, attached to this result, as it is not possible, under the given conditions, to obtain different extracts containing comparable quantities of uricase. This will appear more clearly in the subsequent experiments described in this paper. Curve V has a well-defined peak at p_H 6·86.

Exp. 6. (*Curve VI.*) *Fresh kidney tissue.* A number of flasks were prepared containing the usual buffer solutions, 10 cc. of uric acid, and 10 cc. of water. To half the flasks 1 g. of minced kidney tissue was added, and the systems were shaken for 1 hr. Curve VI exhibits two peaks, one at p_H 7·46 and the other at p_H 9·35.

Exp. 7. (*Curve VII.*) *Residue from centrifuged uricase.* The glycerol-chloroform preparation used in Exp. 4 was centrifuged for 5 mins. at 3500 r.p.m., the residue was suspended in water and the suspension was again centrifuged. 10 cc. portions of an approximately 10 % suspension of the washed residue were then added to a number of flasks containing uric acid solution together with the usual buffer solutions, and all systems were shaken for 35 mins. Curve VII has two maxima, a lower one at p_H 6·96 and a higher one at p_H 9·43.

The curves obtained in the above seven experiments are on the whole strikingly similar in general contour. In practically every case two peaks are obtained, one at about p_H 7·4 and the other at about p_H 9·4. All curves fall sharply from p_H 9·6 upwards, until at p_H 11·5 practically no uricolytic action whatsoever is observed. Inactivation is accompanied by dissolution of solid particles present in the enzyme preparation.

Differences in the contours of the curves obtained are undoubtedly to a certain extent due to the small number of p_H values taken at sections of the curve in which abrupt changes in the catalytic action of uricase occur. Thus, in Curve I a striking difference exists between oxidation at p_H 6·62 and 7·23, and in many of the other curves this p_H interval was not taken. For this reason it is not the actual contour of the curves which is of importance for our purpose but their general shape; it is probable that taking narrower p_H intervals many

of the observed discrepancies between the individual curves would disappear, and this will be the subject of a future research.

The curves obtained for shaken systems at 38° are, over the corresponding p_H range, on the whole similar to those found for systems left without shaking at 16° (Part III). The hypothesis advanced in Part III that uricase is a contact catalyst and not a soluble enzyme is supported by these results.

2. *Separation of the active uricolytic factor from inactive tissue extractives.*

The similarity in shape of the p_H-uricolysis curves obtained, using different preparations, suggests that the active agent is the same in all cases, *i.e.* some insoluble cell-constituent. This view was confirmed by centrifuging a number of extracts, and comparing the uricolytic action of the original extract with that of a suspension in an equal volume of water of the residue, and of the centrifugate. Three preparations were taken for this purpose. The first was a 3 day-old glycerol-chloroform extract, which had been allowed to settle, and was then decanted off from the lower layer of kidney tissue; about 130 cc. of the supernatant layer were centrifuged for 5 mins. at 3500 r.p.m. The centrifugate, a brownish yellow, slightly opalescent liquid, had a volume of 120 cc. The combined residues, which consisted of a very finely divided powder, weighing about 2 g. in all, were suspended in 120 cc. of water, giving a stable suspension. A number of flasks were now prepared, each containing 10 cc. of uric acid solution, and 20 cc. of centrifugate, of suspension, or of water were added, and the systems were left with toluene for 43 hrs. at 20°, when the uric acid contents were determined. No decomposition had taken place in the presence of centrifugate, whilst 4·80 mg. had been oxidised in the presence of the suspension. All flasks were now shaken for 3 hrs. at 38°, and the uric acid content was again determined; the uric acid content of the control flasks and of those containing the centrifugate were again identical (14·25 mg.), whilst a further 6·38 mg. had been oxidised in the presence of the suspension (*cf.* Table II).

The above experiment was repeated on the same uricase preparation, with the difference that it was first violently shaken with the kidney tissue and allowed to settle for only 10 mins., after which the upper layer was filtered through cotton-wool and then centrifuged, as before. The amount of residue now obtained was much greater (about 6 g.). The volume of centrifugate was in this case 100 cc., and the residue was accordingly suspended in the same volume of water. In addition to the systems prepared in the preceding experiment, flasks containing 20 cc. of the original extract before centrifuging were prepared, and all flasks were shaken for 2 hrs. at 38°. The results (Table I, Exp. 2) indicate that the centrifugate in this case still retained some slight uricolytic power, 0·75 mg. of uric acid being oxidised, whilst with the suspension of residue 16·13 mg. underwent oxidation, making a total of 16·88 mg. for both centrifugate and residue. During the same time, with the original extract only 11·4 mg. were destroyed, indicating an activation corresponding to nearly

Table I. *Analysis of activity of extracts.*

No. of exp.	Prep. taken	cc. taken of: whole extract	residue suspension	centrifugate	water	uric acid	mg. uric acid after 43 hrs. at 20°: found	oxidised	shaking for a further 3 hrs. at 38°: found	oxidised
1	Glycerol-chloroform extract No. 1, supernatant layer, centrifuged 5 mins. at 3500 r.p.m. Residue (2 g.) suspended in 120 cc. water. Vol. of centrifugate 120 cc.	—	—	—	20	10	15·75	—	14·25	—
		—	20	—	—	10	10·95	4·80	7·87	6·38
		—	—	20	—	10	15·75	—	14·25	—
							Uric acid after shaking 2 hrs. at 38° (mg.)			
							found		oxidised	
2	Same extract, shaken with kidney tissue, filtered through cotton wool, centrifuged 10 mins. at 3000 r.p.m. Residue (6 g.) suspended in 100 cc. water. Vol. of centrifugate 100 cc.	—	—	—	20	10	16·5		—	
		—	—	20	—	10	15·75		0·75	
		—	20	—	—	10	0·27		16·13	
		20	—	—	—	10	5·1		11·4	
							Uric acid after shaking 1 hr. at 38° (mg.)			
							found		oxidised	
3	Chloroform-water extract (no glycerol). Supernatant layer centrifuged 10 mins. at 3500 r.p.m. Residue (1·5 g.) suspended in 70 cc. water Vol. of centrifugate 70 cc.	—	—	—	20	10	14·63		—	
		—	—	20	—	10	13·43		1·2	
		—	20	—	—	10	2·55		12·08	
		20	—	—	—	10	3·83		10·8	

5·5 mg., or 50 % as a result of separation of the suspended particles from the medium. In the third experiment uricase "extract" was prepared as before, with the difference that water was added in place of glycerol. The supernatant layer was centrifuged for 10 mins. at 3500 r.p.m., giving 70 cc. of centrifugate and about 1·5 g. of residue, which was suspended in 70 cc. of water. Similar systems to those used in Exp. 2 were shaken for 1 hr. at 38°, and uric acid was determined as before. In this case 1·2 mg. of uric acid were destroyed in the presence of the centrifugate, 12·08 mg. in the presence of the residue, and 10·8 mg. where the whole extract was added. An activation of 2·48 mg. or about 25 % was thus effected in this case by centrifuging and allowing the products to act separately.

Obviously, the centrifugate must contain some factor retarding the action of uricase, as well as a uricolytic factor, which has only a very feeble action, and is probably residual uricase in very fine particles. The extract differs from water in that it contains chloroform, glycerol, salts, soluble proteins, and other tissue extractives. The centrifugate in Exp. 2 has double the inhibiting action of that in Exp. 3, and differs from it in two respects: firstly, in the possession of glycerol, and secondly, in that it has acted on a much larger (about three times) concentration of suspension, and to these two factors amongst others we would attribute its greater effect. A third possibly inhibitory factor is chloroform, with which the centrifugate and the whole extract are saturated, but not the

suspension of residue. The following experiment showed, however, that chloroform is inactive in this respect. Two suspensions of residue were prepared, each containing 1 % of residue, but one made with ordinary and the other with chloroform water. A number of flasks were prepared, each containing 30 cc. of uric acid solution. To some, 10 cc. portions of the suspension were added, and to others 10 cc. of water—these served as control flasks. All flasks were shaken for 90 mins. at 38°, and uric acid was determined. The control flasks contained 13·8 mg. of uric acid, whilst those with uricase all contained 8·0 mg., irrespective of whether chloroform was present or absent; chloroform is, therefore, under the conditions of this experiment without influence on the action of the residue. Potentiometric determination of the p_H of the whole extracts and of the centrifugates indicated that the inhibiting action of the latter was not due to change in p_H, as both these products had a p_H deviating to only a negligible extent from that of neutrality (6·99, 7·02, 6·85, 6·92). Moreover, the p_H of all the systems after the completion of the experiment was found to vary within very narrow limits round 8·5 (8·42–8·55). The only remaining factors are thus glycerol and tissue extractives, both of which could retard the velocity of reaction in heterogeneous systems by augmenting the viscosity of the medium. That this is the case for glycerol is shown in the following section of this paper.

The results obtained from experiments on centrifuging confirm the view previously expressed in this series that uricase is a contact catalyst and not a soluble enzyme. Further, a method is provided for the preparation of uricase of practically any desired strength, by suspending the washed residue in the required quantity of water—such uricase suspensions retain their activity for many weeks if kept under sterile conditions.

3. *Action of glycerol.*

If it be assumed, on the foregoing evidence, that uricase is insoluble in water and acts exclusively as a contact catalyst, then any increase in the viscosity of the medium in which it acts should retard the velocity of reaction by diminishing the rate of diffusion of uric acid molecules to the active surfaces. For systems allowed to stand without agitation viscosity may, however, also exert an activating influence. If systems containing uricase are allowed to stand, the uricase settles to the bottom of the flask at a rate inversely proportional to the size of the particles, to the viscosity, and to the relative density of the medium. Assuming constant diameter of the particles, increase in viscosity and in relative density should, up to a certain point, accelerate reaction, by retarding the rate of sedimentation of the catalyst, thereby augmenting its relative degree of dispersion. At the same time, diminution of the rate of diffusion of substrate molecules should exert an opposing effect. It should, therefore, theoretically be possible to attain a relative density and viscosity of the medium at which reaction attains a maximum value in unshaken systems.

This postulate was realised experimentally in the following way. A number of flasks was prepared each containing 10 cc. of uric acid, and 10 cc. of 2 % uricase suspension. To each flask 20 cc. of glycerol solution were added, so that the series consisted of solutions containing 0, 1, 2, 5, 10, 20, 30, 40 and 50 % of glycerol. All flasks were left for 24 hrs. at 24°, after which uric acid was determined. The amount of sediment formed at the end of this time diminished progressively with increase in glycerol content; at 50 % practically no sediment at all had formed. The results (Fig. 2) show that oxidation in 1 % glycerol is practically the same as in the absence of glycerol, whilst in 2 % glycerol 0·8 mg. more is oxidised, an increase of about 7 %. The curve then falls, until at 7 % glycerol the velocity of reaction is the same as in its absence. The curve connecting mg. of uric acid oxidised with glycerol content is practically linear between 5 and 50 %, at which latter value only 2·2 mg. of uric acid had been oxidised, as compared with 10·3 mg. in the absence of glycerol.

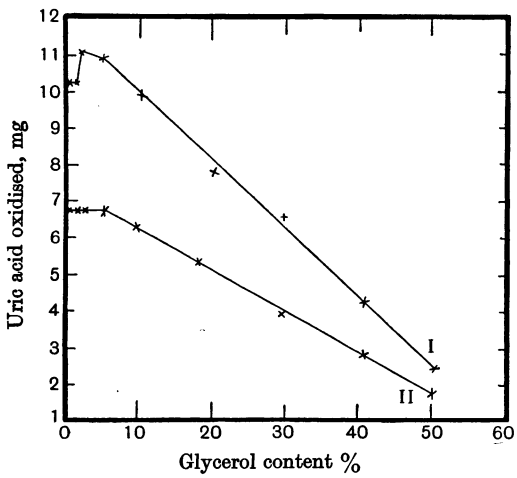

Fig. 2. Action of glycerol on uricase.
Curve I. System left 24 hrs. at 24°.
Curve II. System shaken 1 hr. at 38°.

For shaken systems different results should be obtained. As the glycerol content rises no change in the dispersion of the catalyst takes place, but only a progressive increase in viscosity; the activating factor should therefore be absent from shaken systems.

This postulate was verified experimentally for systems similar to the preceding, which were shaken 1 hr. at 38°. The curve obtained (Curve II, Fig. 2) shows a progressive decline in the action of uricase at concentrations of glycerol above 5 %; below this concentration no effect, either activating or inactivating, is observed.

In the case of activated charcoal, which was shown in the preceding part of this series to possess an action analogous to that of uricase, glycerol has a

similar effect. In this case only one concentration of glycerol was taken, *viz.* 50 %. A number of flasks was prepared each containing 15 cc. of uric acid solution. To some of these flasks 15 cc. of glycerol were added, and to others 15 cc. of water and 0·1 g. of iron-containing charcoal, prepared by the method described in Part III, were added to all but the control flasks. Uric acid was determined in all flasks after 6 hrs. at 30°; in the absence of glycerol the uric acid content had fallen from 15·9 to 6·3 mg., so that 9·6 mg. had been oxidised, whilst in 50 % glycerol, 11·4 mg. remained, corresponding to 4·5 mg. oxidised; inhibition here amounts, therefore, to about 50 %, as compared with 80 % in the case of uricase. The flasks were then left for a further 40 hrs. at 16°, after which time the whole of the uric acid originally present had been oxidised in the absence of glycerol, and only 7·2 mg. in its presence. The greater retardation obtained in the case of uricase is possibly due to the greater magnitude of the diffusion factor in the absence of glycerol for oxidation catalysed by charcoal; in these latter systems the charcoal is present exclusively as a sediment, and not as a suspension, so that the further enhancement of the diffusion factor by increase in viscosity does not relatively retard reaction to such an extent as in systems containing a catalyst in a greater degree of dispersion, and the velocity of reaction in which is initially to a lesser degree governed by velocity of diffusion.

4. *Action of residue after extraction.*

Minced kidney tissue was left with occasional shaking for 72 hrs. with 2 litres of water and 100 cc. of chloroform. The extract was then decanted and the residue was repeatedly shaken with fresh portions of water, until the aqueous layer no longer became turbid. The residue was then left overnight in running water. In this way all easily detachable or soluble cell-constituents were removed, leaving only the more massive structural elements of the tissue. 1 g. of the residue was shaken for 120 mins. at 38° with 30 cc. of uric acid solution at p_H 8·5; at the end of this time 9·45 mg. of uric acid remained in the presence of the residue, as compared with 15·0 mg. in the control flask.

It follows that the whole of the insoluble matter of kidney tissue is associated with the uricolytic agent.

5. *Comparison of activity of various kidney preparations.*

The uricolytic activity per g. hour of a number of ox-kidney preparations was determined and compared, with the object of ascertaining whether any particular fraction is in particular associated with the active factor.

All systems were at p_H 8·5, and contained 30 cc. of uric acid solution, with, in the case of untreated, washed, and ground and washed kidney tissue, 1 g. of substance, and in the case of the residue from the centrifuged aqueous extract, 200 mg. The water content of all preparations was determined, and the results are given in Table II in mg. of uric acid destroyed in 60 mins. (at 30° with shaking) by 1 g. of wet or dry substance.

Table II. *Uricolytic action of various preparations of ox-kidney.*

Prep.	Water content %	Amount taken (g.)	Vol. of uric acid solution (cc.)	Uric acid after shaking 60 mins. at 38° (mg.) in control	in system	oxidised per g. moist substance	oxidised per g. dry substance
Minced kidney	78·8	1·0	30	13·2	7·35	5·85	27·6
Washed residue	88·5	1·0	30	13·8	8·88	5·0	43·5
Washed residue, ground	88·5	1·0	30	15·45	5·25	10·2	88·7
Residue from centrifuged extract	88·6	0·2	30	13·8	8·8	25·0	220·1
Same, 14 days later	88·6	0·2	30	18·6	13·2	27·0	237·4
Same, air-dried	5·0	0·2	30	15·0	9·0	30·0	31·6

The smallest value of 27·6 mg./hr./g. dry substance is given by fresh kidney tissue. Washed residue gives a value of 43·48 mg., whilst the same residue ground in a mortar has over double the activity (88·69 mg.). The residue from centrifuged aqueous extracts oxidises 220·1 mg./hr./g. dry substance; after keeping for 14 days in an atmosphere saturated with water and toluene vapour the activity remains substantially the same. The same residue was allowed to dry overnight and the dry product was powdered in a mortar; its activity was only 31·6 mg./hr./g. dry substance, representing a loss of activity of 85·6 %. The water-content of the above preparations was determined by drying to constant weight at 105°; the fresh tissue contained 78·8 %, the washed residue 88·5 %, the residue from the centrifuged extract 88·6 %, and the air-dried residue 5 % of water.

The above results, on the whole, confirm the conclusions drawn from the previous experiments. The most important factor in determining the activity of uricase is the state of subdivision of the tissue—for this reason the most active product is the residue from the centrifuged extract, the particles of which are so small that they remain in suspension. The next in order of activity is the ground residue, the particles of which are, however, still larger than those of the previous preparation. The whole kidney and the washed residue are both present in large fragments (about 3 mm. cubes); in these cases, during the shaking to which the systems are submitted during reaction, smaller fragments are detached, to which the greater part of the activity of these preparations may in all probability be ascribed. In the case of whole kidney tissue, however, various extractives pass into solution, thereby increasing viscosity and retarding the velocity of reaction. Finally, dried uricase consists of hard, impermeable fragments which cannot develop the same surface as possessed when fresh.

A further conclusion to be drawn is that uricase can be kept without any diminution in activity for at least 2 weeks—it is probable that it would, under appropriate conditions of sterility and moisture, keep indefinitely.

6. *Attempts to dissolve uricase.*

Three methods were applied which might possibly allow the enzyme to be extracted in some soluble form. The first was extraction by alkalis; as was stated in Section I of this paper uricase preparations become increasingly clear as their p_H rises above 9·5. Such solutions deposit a flocculent precipitate on acidification, but, as is shown by the following experiments, this precipitate is uricolytically inactive.

10 cc. of a glycerol-chloroform extract were added to a buffer solution at p_H 11·6, consisting of 16 cc. of 0·1 *N* sodium hydroxide and 4 cc. of 0·1 *N* glycine. The mixture was shaken for 3 mins., and 1 g. of glycine was then added, in order to reduce the p_H to 8·4. A second system was prepared similarly, except that in this case the flask was shaken 1 hr. at 38° before addition of the extra glycine. To a third system the glycine was introduced before the uricase suspension, whilst the control system contained the same constituents with the exception of uricase suspension, in place of which water was added. 10 cc. of uric acid solution were then added to all flasks, which were shaken for 2 hrs. at 38°, after which uric acid was determined. The flasks to which uricase had been introduced after the reduction of p_H to 8·4 contained 10·7 mg. of uric acid; all others contained 16·4 mg., showing that uricase is completely and irreversibly inactivated by even a short contact with solutions of p_H 11·6.

A second experiment was performed as follows. 10 cc. each of Sørensen's glycine and of sodium hydroxide solutions were added to flasks containing 10 cc. of a 1 % suspension of uricase, sodium hydroxide being introduced before glycine to half the flasks, and after to the remainder. Uric acid (10 cc.) was then added to all flasks, as well as to control flasks containing 10 cc. of water and of each buffer. All flasks were shaken for 3 hrs. at 38°, and uric acid was determined; 13·8 mg. being found in the control flasks and in those to which alkali had been added before glycine, and 6·8 mg. in the others. It follows from the above two experiments that although uricase can be dissolved in alkaline solutions its activity cannot be recovered by subsequent reprecipitation; at p_H 11·5 (Fig. 1) uricase is inactive.

Two methods based on the possibility that uricase is present in adsorption on inactive cellular elements were next applied, but without success. The first was an attempt at elution with *n*-butyl alcohol, and the second with buffer solutions at p_H values of 6–11·5.

7. *Action of sodium hydroxide on washed residue.*

The experiments described in the first part of the preceding section showed that uricase is destroyed by the action of strong alkalis. This observation was applied to the elucidation of the question whether uricase is present as a surface coating on inert material, or whether it permeates the whole mass of the solid matter with which it is in association. 2 g. of coarsely divided washed kidney

tissue were shaken for 5 mins. with 20 cc. of $N/40$ sodium hydroxide solution, which should, in the case of finely divided tissue, suffice to destroy the active agent. The fragments of tissue were then washed free of alkali, and their uricolytic power was compared with that of similar tissues not previously treated with alkali. A number of systems were prepared containing 1 g. of tissue and 30 cc. of uric acid solution, and all were shaken for 1 hr. at 38°. The final uric acid content of the control flasks was 15·45 mg., and that of the systems containing kidney tissue, whether alkali-treated or not, was identical, *viz.* 10·83 mg.

The above experiment suggests that uricase is equally distributed throughout the whole mass of tissue, and not only on the surface layer, as the fresh surfaces left exposed by the action of alkali are as active as were the original ones.

8. *Chemical composition of washed extracted kidney tissue and of the residue from centrifuged extracts.*

Elementary analysis established the presence of nitrogen, sulphur and phosphorus in both the above preparations.

Both preparations gave positive reactions in the following tests: biuret, xanthoproteic, Hopkins, Millon, cysteine, and Molisch.

The above results would support the view that the suspension consists of particles of kidney tissue differing from the residue only in their finer degree of subdivision.

Summary.

1. The p_H-activity curves of different uricase preparations are of the same general type for all preparations studied. Two maxima exist, one at p_H about 7·5, and the other at p_H 9·4. The curves all approach zero between p_H 11 and 12.

2. This inactivation is associated with dissolution of the particles in suspension and is irreversible.

3. Centrifugates of uricolytic extracts are catalytically inactive, the residue obtained being more active than the whole extract.

4. This inhibition of the suspended particles by the medium is due, at least partly, to increase in viscosity of the system by dissolved proteins and by added glycerol.

5. The addition of glycerol to suspensions of uricase in water increases the action of the suspension up to 2–3 %, at higher concentrations the activity falls in direct proportion to the glycerol content, until in 50 % glycerol 80 % inactivation is attained.

6. The activating effect is interpreted as being due to retardation of the rate of sedimentation of the particles of uricase, whereby the dispersion of the catalyst is relatively increased; the inactivation at higher concentrations is due to retardation of the rate of diffusion of uric acid to the active surfaces.

7. In shaken systems no activation is achieved by the addition of glycerol, as in this case the sedimentation factor does not exist.

8. The use of glycerol for the preparation of uricase from tissues is thus not only unnecessary but also undesirable.

9. Uricolytic activity is associated with the whole of the insoluble part of kidney tissue.

10. The uricolytic action of kidney tissue is a function of the degree of division of the tissue.

11. The uricolytic activity, measured as mg. of uric acid oxidised in 1 hr. at 38°, with shaking, by 1 g. of substance (dry weight) is as follows, for various preparations: untreated ox-kidney tissue 27·6; washed residue from extracted tissue 43·5; washed ground residue 88·7; residue from centrifuged aqueous extract (fresh) 220·1 mg.; the same, 14 days old, 237·4, and the same, air-dried, 31·6.

12. The following method is proposed for the preparation of uricase.

Minced kidney tissue is shaken violently with water containing chloroform (as an antiseptic) for 2–3 hrs. The suspension is filtered through cotton-wool, and the filtrate is centrifuged (10 mins. at 3500 r.p.m.). The residue is suspended in water, and the suspension is again centrifuged. The residue now obtained can be kept without any loss of activity for at least 2 weeks, and probably indefinitely, in an atmosphere saturated with water and toluene vapours. Drying greatly reduces the activity of such preparations as a result of diminution of the active surface.

13. Uricase does not pass into solution under the influence of either surface-active bodies such as *n*-butyl alcohol or at any p_H between 6 and 12.

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REFERENCE.

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