CCCCXXI. URICASE AND ITS ACTION.

VIII. EXTRACTION AND PRECIPITATION OF OX KIDNEY URICASE.

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EXTRACTION.

Experimental methods.

Preparation of uricase. Uricase was in some cases prepared according to the method described in Part VII [Truszkowski, 1934] (this will be termed Method I). The following procedure (Method II), which has the merits of economy of material, time and labour, was later adopted. The extracts are more highly coloured than are those of uricase prepared by the previous method, but for many purposes they serve equally well.

Minced ox kidney was placed in a 10-litre bottle, and cold water, entering the bottle through a tube reaching to the bottom, was run through until the supernatant liquid became quite clear and colourless. The residual tissue, freed in this way of its soluble constituents as well as of smaller insoluble particles, was dried at 37° in a current of air, and the dry residue was ground to an impalpable powder in a mill. The yellow powder so obtained exhibits considerable uricolytic activity, and on extraction with aqueous Na₂CO₃ affords deep yellow, active extracts, often possessing a disagreeable odour. The quality of the product is greatly improved by repeated washing on a Büchner funnel with 96 % alcohol at room temperature, followed by ether; the washing is continued until the filtrate becomes colourless. The residue is then washed four times with anhydrous alcohol-free ether, spread in a thin layer and dried at 30° under reduced pressure. The dry uricase so obtained is a light yellow powder, which can be stored for many months without deterioration, provided that traces of solvent (water, alcohol, ether) are absent, and that undue exposure to the air is avoided. The yield is roughly 100 g. per kg. of kidneys.

Solutions of uric acid were prepared by adding uric acid to boiling aqueous NaOH (65 ml. of 0.1 N NaOH per g. of uric acid). The solutions so obtained had p_H 8.6–9.0. Uric acid was determined colorimetrically by the method of Folin and Denis.

Standardisation of conditions of determination of activity of uricase. 10 flat-bottomed test-tubes (of uniform calibre), containing various volumes of a mixture of uric acid and uricase solutions and 0.5 ml. each of toluene, were kept at 37° for 48 hours, the contents of the tubes were mixed once by inversion, and uric acid was determined.

The results, given in Table I, indicate that the apparent activity of the uricase diminishes at first rapidly, and then more slowly, with increasing height of the column of the solution. Since the only difference between the systems consists in the supply of oxygen to the lower layers of solution, it may be concluded that dissolved oxygen in the body of the systems is rapidly consumed, and that further oxidation can take place only at the expense of fresh oxygen diffusing into the

Table I. *Effect of varying the volume of the systems on the apparent activity of uricase.*

Initial concentration of uric acid is 0.11 %.

Height of column of solution cm.	Volume of system ml.	mg. of uric acid after 48 hours at 37° in whole system	
		Initially present	Oxidised
1.2	1.7	1.87	1.87
3.5	5.6	6.16	4.78
5.8	9.5	10.45	4.93
7.3	12.0	12.1	4.76
9.2	15.1	16.61	5.01
11.1	17.6	19.36	5.81
12.7	21.4	23.54	4.28
14.4	23.9	26.29	4.78
16.3	27.7	30.47	5.07
19.6	32.6	35.86	4.71

liquid from the atmosphere. This oxygen is, however, used up in the upper layers of solution as fast as it diffuses into the liquid, with the result that, after a time, a zone forms in which uric acid is completely oxidised, whilst the lower layers, being under practically anaerobic conditions, remain unchanged. Obviously, the thickness of the "oxidised layer" will increase with time, but this process, being dependent on diffusion of uric acid to the upper and of oxygen to the lower layers, is relatively extremely slow.

In the experiment described in Table I it will be observed that, except in the first test-tube, the total amount of uric acid oxidised is approximately constant in all the systems, the mean value being 4.9 mg. This would represent almost entirely uric acid oxidised in the upper zone and corresponds with a depth of 2.72 cm. for the zone in question.

It follows from the above experiments that care should be taken, in comparing the activities of different samples of uricase, that the total volume of solution should be the same in all cases, and that the containers should as far as possible be of the same shape and size. A further consequence of the above mechanism is that, even under strictly comparable conditions, the amount of uric acid oxidised will not necessarily be directly proportional to the concentration of uricase, *i.e.*, that the apparent activity of a given preparation may vary, according to its relative concentration.

The following experiment illustrates this point. 0.5–3 ml. of uricase solution were added to a number of flasks containing 10 ml. of 0.1 % uric acid, and 0.16 % Na_2CO_3 was added to make the volume up to 13 ml. in all the systems, which were then incubated at 37° for 18 hours, and uric acid was determined. The results (Table I *a*) indicate that the number of mg. of uric acid oxidised per

Table I *a*. *Activity of a given preparation of uricase taken in different concentrations.*

All systems contain initially 10.81 mg. of uric acid.

Volume of uricase taken ml.	mg. of uric acid		
	In system	Oxidised	Oxidised per ml. of uricase
0.5	7.26	3.55	7.1
1.0	5.98	4.83	4.83
1.5	4.6	6.21	4.14
2.0	3.45	7.36	3.68
3.0	2.1	8.71	2.9

ml. of uricase solution varies from 7.1 when 0.5 ml. of preparation are taken to 2.9 when 3 ml. are present.

It was shown in Part IV of this series [Truszkowski, 1930] that the activity of insoluble preparations of uricase varies widely with the p_H of the medium, and that the optimum p_H is about 9.0. Identical results were obtained for dissolved uricase.

On the basis of the foregoing, the following standard conditions for the determination of the activity of uricase were adopted. The systems should consist of 10 ml. of 0.1–0.2% uric acid solution, 1 ml. of uricase solution and 0.5 ml. of toluene, in 100 ml. Erlenmeyer flasks, which should be as far as possible of uniform shape. The addition of buffer solution is not necessary, since systems prepared as above are of a suitable p_H . Determinations may be made after any time of incubation at 37°; usually 18 hours will be the most convenient time.

Determination of optimum conditions of extraction.

Concentration of Na_2CO_3 . Ro [1931] found that the most active extracts are obtained using 0.125% Na_2CO_3 . The experiments described below indicate that the optimum concentration of alkali varies according to the preparation of dry uricase taken and should be determined separately in each case (Table II).

0.2 g. portions of dry uricase were kept for 48 hours at 37° with 20 ml. portions of 0.03–0.2% Na_2CO_3 and 1 ml. of toluene, the suspensions were filtered, 1 ml. of filtrate was added to 10 ml. of uric acid solution, and uric acid was determined after 18 hours at 37°.

Table II. *Dependence of activity of uricase solution on the Na_2CO_3 concentration.*

All flasks contain 10 ml. of uric acid, 1 ml. of extract and 0.5 ml. of toluene.

Nature of preparation	Concentration Na_2CO_3 %	p_H		mg. uric acid after 18 hours at 37°	
		Initial	Final	In control	Oxidised
Rabbit liver (Method I)	0.04	—	—	7.7	2.5
	0.08	—	—	7.7	5.6
	0.12	—	—	7.7	4.6
	0.16	—	—	7.7	4.3
	0.2	—	—	7.7	2.0
Ox kidney (Method I)	0.03	9.95	8.28	7.7	3.0
	0.04	10.34	8.48	7.7	5.0
	0.06	10.44	9.55	7.7	5.7
	0.08	10.57	9.69	7.7	6.1
	0.1	10.69	9.95	7.7	5.3
Ox kidney (Method II)	0.04	—	—	8.8	4.4
	0.08	—	—	8.8	6.4
	0.1	—	—	8.8	8.2
	0.12	—	—	8.8	8.56
	0.16	—	—	8.8	8.8
Ox kidney (Method I)	0.2	—	—	8.8	8.6
	0.04	9.5	7.7	5.9	1.7
	0.08	10.1	8.7	5.9	4.2
	0.12	10.34	9.42	5.9	4.2
	0.16	10.38	9.56	5.9	3.6
	0.2	10.51	9.37	5.9	3.5

The results given in Table II indicate that the strength of the extracts first rises as the concentration of Na_2CO_3 increases and then falls. This effect is

interpreted as being the resultant of two opposed processes; dissolution of some substance of an acidic nature, and denaturation by alkali.

It was thought, in view of the above, that more active extracts might be obtained by adding the Na_2CO_3 gradually during the course of the extraction; this possibility was investigated in two ways. In one, the concentration of the Na_2CO_3 was kept constant, but its volume was gradually increased; 0.5 g. of uricase (Method I) was allowed to stand at 37° for 18 hours with 10 ml. of 0.08 % Na_2CO_3 , a further 10 ml. portion of which was added after 25 hours and 20 ml. after 42 hours. 2 ml. of the extract obtained after 48 hours added to 10 ml. of 0.06 % uric acid solution oxidised 1.8 mg. of uric acid after 20 hours at 37° ; under comparable conditions, 2 ml. of the extract obtained when 0.5 g. of uricase was kept for 48 hours with 50 ml. of 0.08 % Na_2CO_3 oxidised 4.5 mg. of uric acid.

The experiment was repeated maintaining the volume of the solution constant, but progressively increasing its concentration; 100 ml. of 0.03 % Na_2CO_3 were added to 1 g. of uricase, five 0.01 g. portions of Na_2CO_3 were added at intervals during 52 hours, to bring the final concentration to 0.08 %; 2 ml. of the filtrate obtained after 48 hours of incubation oxidised 3.0 mg. of uric acid. No advantage is therefore gained by varying the concentration or amount of Na_2CO_3 during extraction.

Extraction by other solutions. The activity of the extracts obtained using solutions of K_2CO_3 , NaOH and glycine-NaOH buffers (Sørensen) was investigated.

1 % solutions of K_2CO_3 , NaOH and Na_2CO_3 were added to water to bring the p_{H} to 9.65, 0.2 g. of uricase (Method I) was added to 20 ml. portions of the solutions, the suspensions were filtered after 48 hours at 37° and their uricolytic activity and final p_{H} value determined. (2 ml. of uricase, 10 ml. of 0.078 % uric acid). A series of Sørensen's glycine-NaOH buffer solutions (30 ml. portions, p_{H} 7.59–10.2) was prepared, 0.3 g. of uricase added to each portion and the activity of the extracts determined. Finally, 2 g. of uricase were extracted as above with 100 ml. of 0.1 % Na_2CO_3 or of 5 % NaCl and the activity of the extracts determined.

Table III. *Activity of extracts prepared from uricase and aqueous K_2CO_3 , NaOH and glycine-NaOH buffer solution.*

All flasks contain 10 ml. of uric acid, 2 ml. of extract or extractive solution and 0.5 ml. of toluene.

Nature of extractive solution	p_{H}		mg. uric acid after 18 hours at 37°		
	Initial	Final	In control	In system	Oxidised
Na_2CO_3	9.65	8.11	7.8	3.6	4.2
K_2CO_3	9.65	7.74	7.8	5.8	2.0
NaOH	9.65	6.23	7.8	7.5	0.3
Sørensen's buffers	7.59	—	6.0	5.9	0.1
	7.96	—	6.0	5.4	0.6
	8.68	—	6.0	5.4	0.6
	9.07	—	6.0	4.1	1.9
	9.35	—	6.0	3.0	3.0
	9.5	—	6.0	1.7	4.3
	9.87	—	6.0	2.9	3.1
	10.06	—	6.0	5.9	0.1
	10.2	—	6.0	6.0	0

The results, given in Table III, indicate that the activity of the extracts depends not only on the initial, but also on the final p_{H} . Thus NaOH is an unsatisfactory solvent owing to the circumstance that the highest concentration

possible without inactivation represents a small amount of base, rapidly neutralised by the acidic constituents of the dry uricase, with the result that the solution becomes actually acid. In conjunction with glycine, NaOH extracts are fully as active as are those with Na_2CO_3 (at p_{H} 9.5). It would follow that the essential conditions for successful extraction are that the initial p_{H} be > 9.5 – 9.6 , and the final p_{H} < 8.0 ; any alkaline solution fulfilling these conditions would be an equally satisfactory solvent.

Optimum proportion of dry uricase. A further conclusion to be drawn from the above experiments is that the relative concentration of dry uricase taken for extraction cannot vary beyond certain narrow limits, determined by the necessity of maintaining the p_{H} between 9.5 and 8.0. As is shown by the following experiment, it is not possible to obtain extracts much more active than those described above by increasing the relative amount of dry uricase.

A number of 0.5 g. portions of uricase (Method I) were extracted with 50, 40, 30, 20 and 10 ml. portions of 0.08 % Na_2CO_3 (p_{H} 9.84) (48 hours at 37°), and the activity of the filtrates was determined.

Table IV. *Effect of varying the relative proportions of dry uricase on the activity of the extracts.*

All flasks contain 10 ml. of 0.075 % uric acid, 2 ml. of uricase and 0.5 ml. of toluene.

Relative proportion of dry uricase (%)	Final p_{H}	mg. of uric acid after 18 hours at 37°		
		In control	In system	Oxidised
0.5	9.36	7.5	6.9	0.6
1.0	8.69	7.5	4.4	3.1
1.5	8.66	7.5	2.9	4.6
2.0	8.5	7.5	2.0	5.5
2.5	8.4	7.5	1.9	5.6

The results (Table IV) show that no advantage is gained by taking more than 2 g. of dry uricase per 100 ml. of solution, and that the final p_{H} falls progressively.

Temperature of extraction.

2 % suspensions of dry uricase (Method II) in 0.16 % Na_2CO_3 were maintained at 37° and 20° , and the activity of the suspensions and of their filtrates was determined after 24 and 48 hours.

Table V. *Activity of suspensions and extracts after incubation at 37° and at 20° .*

All flasks contain 10 ml. of uric acid, 0.3 ml. of suspension + 0.7 ml. of H_2O , or 1 ml. of extract and 0.5 ml. of toluene.

Temperature ($^\circ$)	Time hours	Preparation studied	mg. uric acid after 20 hours at 37°			Oxidised per ml. of preparation
			In control	In system	Oxidised	
—	0	Suspension	8.2	2.1	6.1	20.3
37	24	Suspension	8.7	5.9	2.8	9.3
		Filtrate	8.7	2.4	6.3	6.3
	48	Suspension	8.7	5.7	3.0	10.0
		Filtrate	8.7	3.2	5.5	5.5
20	24	Suspension	8.7	4.8	3.9	13.0
		Filtrate	8.7	2.4	6.3	6.3
	48	Suspension	8.7	4.5	4.2	14.0
		Filtrate	8.7	1.9	6.8	6.8

The results (Table V) indicate that about 50 % of the activity of the suspension is lost after incubation at 37° for 24 or 48 hours, as compared with about 34 % at 20°. The extracts possess equal activities after 24 hours, whilst after 48 hours the 20° extract is the more active.

It follows that extraction at room temperature is more advantageous than at 37°, and that considerable inactivation of uricase takes place during the process of extraction.

Duration of extraction.

A suspension of 2 g. of uricase (Method II) in 100 ml. of 0.16 % Na_2CO_3 was kept at room temperature (10–16°) for six days, and the activity of a sample of the well-mixed suspension and of its filtrate was determined daily.

Table VI. *Variation of activity of suspensions and extracts with time (room temperature).*

All flasks contain 10 ml. of 0.115 % uric acid, 0.3 ml. of suspension + 0.7 ml. of H_2O , or 1 ml. of filtrate or suspension and 0.5 ml. of toluene.

Preparation	mg. uric acid oxidised after 24 hours at 36° per 1 ml. of preparation, taken after					
	0	24	48	72	96	144 hours
Suspension	12.7	7.3	8.6	7.6	6.3	6.1
Filtrate	—	6.0	7.5	7.5	6.3	5.8

The results (Table VI) indicate that the activity of the extracts is at a maximum when the time of extraction at room temperature is 48–72 hours.

Stability of extracts.

An extract (p_{H} 8.7) of 1 g. of dry uricase (Method I) in 0.08 % Na_2CO_3 (p_{H} 9.84) was kept at 37° in a stoppered flask with toluene for one month, and its activity was tested at intervals. The results, given in Table VII, indicate that the activity does not change during the first 4 days, is still considerable after 7 days, and falls to zero after 36 days.

Table VII. *Stability of extracts of uricase.*

All flasks contain 10 ml. of uric acid solution, 2 ml. of uricase and 0.5 ml. of toluene.

Time elapsed after filtration of extract (days)	mg. uric acid after 20 hours at 37°		
	In system	In control	Oxidised
0	1.7	5.8	4.1
4	1.7	5.8	4.1
6	2.2	5.8	3.6
7	2.2	5.8	3.6
12	3.2	5.8	2.6
20	3.1	4.6	1.5
22	7.9	8.8	0.9
29	8.2	8.8	0.6
36	8.8	8.8	0

PRECIPITATION.

Precipitation by acetic acid.

On gradual addition of 1 % acetic acid to the clear extract of uricase the solution becomes progressively more turbid, and, at the point of maximum turbidity a voluminous, flocculent precipitate separates, leaving a clear, light

yellow supernatant liquid when centrifuged or allowed to settle. Further addition of acid leads to the re-dispersion of the precipitate, to yield finally an opalescent solution, addition of 0.2% Na_2CO_3 to which leads to the same changes, in the inverse order.

This preliminary experiment indicates that some amphoteric substance is present, and that its precipitation takes place at the isoelectric point.

Conditions of precipitation of uricase.

Acetic acid. The following experiments show that the activity of the precipitate rises with increase in the quantity of acid added to a maximum, corresponding with the point of maximum turbidity; the activity of the centrifugate falls continuously, whilst both the precipitate and the centrifugate are inactivated by addition of excess of acid.

A 2% suspension of dry uricase in 0.16% Na_2CO_3 was filtered after 24 hours at 37° , 2 ml. of the filtrate were added to 10 ml. of 0.1% uric acid, and uric acid was determined after 20 hours at 37° . Various volumes (0.6–2.0 ml.) of 2% acetic acid were added to 10 ml. portions of the filtrate in 15 ml. centrifuge tubes, 2 ml. of the resulting suspensions were taken for determination of activity as before, the suspensions were centrifuged, the precipitates suspended in the same volume of water as that of the suspensions before centrifuging, and 2 ml. of the suspensions and centrifugates were taken for determination of activity. The N contents of the various fractions were determined, and the p_{H} of the centrifugates. The results, given in Table VIII, are corrected for the volume changes involved in adding acetic acid; thus 2 ml. of the original filtrate are equivalent to 2.2 ml. of the suspension obtained by adding 1 ml. of acetic acid to 10 ml. of filtrate, and the N content and number of mg. of uric acid oxidised were accordingly multiplied by 1.1.

The clear filtrate becomes turbid after addition of 0.6 ml. of 2% acetic acid, but no precipitate forms; for this reason the activity of the centrifugate is equal to that of the suspension. At the same time, it appears that the activity of the preparation is not diminished (apparently even enhanced) by addition of acetic acid to p_{H} 7.89 and 7.48. At p_{H} 7.48 a precipitate forms, of low activity. The most active precipitate is obtained at p_{H} 6.96, but the activity of the suspension is somewhat lower than that of the original filtrate, and that of the centrifugate considerably more so. The activity of all fractions falls rapidly with increasing acidity, approaching zero when 1.0 ml. of 2% acetic acid per 10 ml. of filtrate is present. It will be seen that in this case the activity of the suspension is considerably greater than that of the precipitate, whilst the centrifugate is completely inactive; this apparent discrepancy may be ascribed to the shorter duration of action of acetic acid for the suspension as a whole (13 min.), than for the precipitate and centrifugate before samples were taken for testing activity (about 60 min. necessary for counterpoising and centrifuging the suspensions). Activity is practically nil with 1.4 ml. of acid and is totally absent at higher acidities.

The original N content corresponds to 0.668 mg. per 2 ml. of filtrate; on progressive addition of acid the N distributes itself in the approximate ratio of 3.5 : 1 in the centrifugates and precipitates. The activity, calculated per mg. of N, rises for the precipitates to a maximum for 0.8 ml. of acid (p_{H} 6.96), thereafter falling below the original figure; the greatest increase in activity is almost exactly three-fold. It is of interest that most of the precipitate-N is obtained at p_{H} 7.48; it is probable that this consists largely of inert material together with a small

amount of adsorbed uricase. Precipitation is not complete at the p_H at which inactivation commences.

The chief conclusions to be drawn from the above results are that the filtrates contain a mixture of active and inactive substances; about a third of the protein constituents is precipitable by acetic acid, and the precipitate consists chiefly of originally inactive substances, in addition to those inactivated by the acid. The remaining active constituents are, however, present in a concentration roughly three times greater than in the original filtrate proteins. The active principle is extraordinarily susceptible to acids, slight inactivation being observable at a p_H little different from that of neutrality, and practically total inactivation at $p_H < 5.8$.

Table VIII. *Activity and N content of the products obtained by adding acetic acid to solutions of uricase.*

2% CH ₃ CO ₂ H added per 10 ml. of filtrate ml.	Enzymic preparation taken	p_H centri- fugate	N content of 2 ml. of preparation (corrected) mg.	mg. of uric acid after 20 hours at 37°			No. of mg. uric acid oxidised per mg. of N
				In control	Oxidised	Oxidised (corrected)	
0	Filtrate	9.9	0.668	9.6	9.0	9.0	13.08
0.6	Suspension	—	—	„	8.76	9.29	—
	Precipitate	—	—	„	0	0	—
	Centrifugate	7.89	0.662	„	8.52	9.03	13.64
0.7	Suspension	—	—	„	8.76	9.37	—
	Precipitate	—	0.132	„	1.2	1.28	9.7
	Centrifugate	7.48	0.598	„	6.12	6.55	10.95
0.8	Suspension	—	—	„	7.8	8.42	—
	Precipitate	—	0.150	„	5.52	5.96	39.8
	Centrifugate	6.96	0.532	„	2.4	2.59	4.86
0.9	Suspension	—	—	„	4.92	5.36	—
	Precipitate	—	0.122	„	3.12	3.4	27.8
	Centrifugate	6.14	0.532	„	2.64	2.87	5.39
1.0	Suspension	—	—	„	5.04	5.54	—
	Precipitate	—	0.122	„	1.2	1.32	10.82
	Centrifugate	5.83	0.532	„	0	0	—
1.4	Suspension	—	—	8.4	0.48	0.54	—
1.5 } 1.6 } 2.0 }	Inactive						

A more direct comparison of the activities of the various preparations was effected in the following way. 4 g. of dry uricase (Method II) were suspended in 200 ml. of 0.16 % Na₂CO₃; the activity of the suspension was determined immediately, and after 24 hours at 37°; it was then filtered, 14 ml. of 2 % acetic acid were added to 130 ml. of filtrate, 18 ml. of the resulting suspension were placed in a centrifuge-tube and together with the remainder centrifuged. The precipitate from the first tube, representing one-eighth of the original 130 ml. of filtrate was dissolved in 16 ml. of 0.16 % Na₂CO₃, and the activity of the solution was determined. The remaining residues (representing seven-eighths of the original filtrate) were combined, washed 3 times on the centrifuge with 96 % alcohol and 3 times with dry, alcohol-free ether, and dried in a vacuum oven at 30°, 40 mg. of a white powder being obtained. The powder was dissolved in 28 ml. of 0.04 % Na₂CO₃, and its activity was determined.

The results are given in Table IX. The figures relating to the number of mg. of preparation present in the systems were arrived at as follows: (Exp. 1) 0.3 ml.

Table IX. *Relative activity of various preparations of uricase.*

All systems contain 10 ml. of uric acid solution.

No. of Exp.	Nature and amount of uricolytic prep.	mg. of uricolytic prep. present	mg. uric acid after 20 hours at 37°					Rel. activity	Fraction of original activity
			In control	In system	Oxidised				
					In system	Per ml. prep.	Per mg. prep.		
1	0.3 ml. of original suspension	6	7.21	3.3	3.91	13.3	0.65	1	1
2	1 ml. of final suspension	20	7.15	1.32	—	5.83	0.291	0.45	0.44
3	1 ml. of filtrate	—	7.15	1.98	—	5.17	—	—	0.39
4	1 ml. of solution of precipitate	0.35	7.15	5.28	—	1.87	5.34	8.21	0.14
5	1 ml. of centrifugate	—	7.15	6.27	—	0.88	—	—	—
6	2 ml. of solution of dry precipitate	2.86	6.96	3.24	3.72	1.86	1.30	2	0.035

of suspension was taken containing 6 mg. of powder; (Exp. 2) 1 ml. containing 20 mg. of powder; (Exp. 4) seven-eighths of the filtrate, representing 13/20 of the total extract, yielded 40 mg. of dry precipitate, whence it follows that the whole 200 ml. of solution would have yielded 70.2 mg., and that the 1 ml. of dissolved precipitate contained 0.35 mg. of dry preparation; (Exp. 6) the 2 ml. of solution of the dry residue taken contained $40 \times 2/28 = 2.86$ mg. of preparation. The relative activities are calculated by taking the activity (mg. of uric acid oxidised per mg. of preparation) of the original suspension as 1, and the fractions of original activity by taking the mg. of uric acid oxidised per ml. of the original suspension as the basis of comparison with equivalent amounts of the various derived preparations.

The results are best illustrated by the last two columns, from which it appears that the final product is only twice as active as the original, and that the total loss in activity involved in obtaining this product amounts to 96.5%; 56% of the activity of the original product is lost during the process of extraction, and 11% of the activity of the final suspension remains in the residue. Only 35% of the activity of the filtrate is in the acetic acid precipitate, the remainder being partly destroyed by acid and partly remaining in the centrifugates. Finally, the process of drying with alcohol and ether, together with possible mechanical losses associated with incomplete collection of the precipitates, accounts for the loss of 75% of the activity of the wet precipitate.

It follows that purification of the enzyme by extraction with alkaline solutions, followed by precipitation with acetic acid gives eventually products little more active than is the original uricase at the cost of over 96% of its activity.

Other precipitants. The action of CO_2 , CaCl_2 and $(\text{NH}_4)_2\text{SO}_4$ was investigated. CO_2 at atmospheric pressure did not yield a precipitate and at higher pressures inactivated the enzymes. CaCl_2 added in quantities equivalent to the Na_2CO_3 present yielded a precipitate of CaCO_3 which had no uricolytic activity. Ammonium sulphate added to half-saturation afforded a precipitate containing 64% of the total activity of the solution.

Chemical nature of the products.

The various products obtained by extraction of uricase and precipitation by acetic acid were analysed for N, total P (P_T) phosphoprotein-P (P_P) and nucleoprotein-P (P_N) (eliminated and not eliminated by incubation for 24 hours at 37° with 2% NaOH). 6 g. of kidney powder (Method I; containing N 13.32, P_T 1.1, P_P 0.54, P_N 0.57%) were suspended in 300 ml. of 0.16% Na_2CO_3 , and the suspension was filtered after 48 hours at 37°. 0.6% acetic acid was added to the filtrate to attainment of maximum turbidity, the solution was allowed to stand for 3 hours, the clear supernatant fluid was siphoned off, the voluminous precipitate was centrifuged and washed on the centrifuge 3 times with 96% alcohol and 3 times with dry ether, excess of which was removed in a vacuum oven at 30°; 0.38 g. of a white powder remained (N 13.46, P_T 1.76, P_P 0.65, P_N 1.07%). The residue remaining after filtering the first extract was re-extracted, and the extract treated as above, to yield finally a white powder (P_T 1.28, P_P 0.34, P_N 0.94%). The twice-extracted powder was washed with water, dried as above, and analysed (P_T 0.44, P_P 0, P_N 0.44%).

These results indicate that the dissolved uricase is richer in phosphorus, both labile and non-labile, than was the original product, and that the final, inactive residue contains no labile P and less than half of the original nucleoprotein-P. Since it might be thought that inactivation of uricase above p_H 9 is due to elimination of P_P , determinations of activity and inorganic P were made for a p_H range of 6.77–10.75, but no regularities were established. The question as to whether a connection exists between phospho- or nucleo-protein and uricase thus remains open.

Ro [1931] stated that trypsin does not inactivate uricase. This question was re-investigated, using Merck's "pancreatin", as follows. 0.5 g. of pancreatin was dissolved in 50 ml. of a 2% extract of uricase, 2 ml. of the solution were immediately added to 10 ml. of 0.11% uric acid solution, and uric acid was determined after 18 hours at 37°; 3.7 mg. were found, as compared with 11.0 mg. initially. The activity of the extract was again determined after incubation at 37° for 24 hours (10.5 mg. of uric acid were found). It follows that the activity of uricase is reduced practically to zero by tryptic digestion, pointing to the protein nature of the active principle.

SUMMARY.

1. A new method for the preparation of dry uricase has been described.
2. Standard conditions for the determination of the activity of uricolytic extracts are specified; in particular, the importance of keeping the volume of the systems constant and of conducting the reactions in vessels of the same shape and size is emphasised.
3. The reaction is shown to proceed in three stages, involving oxidation in the entire solution by dissolved oxygen, oxidation in the surface zone by atmospheric oxygen and the gradual extension of this zone downwards as a result of diffusion of fresh uric acid from, or of oxygen to, the lower levels.
4. The most active extracts of uricase are obtained by filtering a 2% suspension of uricase in Na_2CO_3 solution (of an optimum concentration characteristic for each individual preparation, but of p_H > 9.8), after standing for 48 hours at room temperature. No advantage is gained by increasing the relative amount of uricase taken for extraction.
5. The activity of the extract represents about 50% of the original activity of the powder; the remainder is lost as a result of the chemical action of the alkaline solution on uricase.

6. The optimum p_H for the action of dissolved uricase is 8.8.
7. Progressive addition of acetic acid to solutions of uricase leads to the production of an active precipitate, followed by its redispersion.
8. The most active precipitates are obtained by adding acetic acid to p_H 6.99; about one-third of the total nitrogen of the extracts is then in the precipitate and the remainder in the feebly active solution.
9. The enzyme is partly inactivated at p_H 6.99, and totally at $p_H < 5.8$.
10. The most active preparation obtained was 8 times as active as the original one; drying with alcohol and ether reduced its activity to twice the original.
11. The N content of the original kidney powder and of the precipitate obtained from the extract is 13.5 %; the P content of the precipitate is higher than that of the original powder. Approximately half of the P content of the original powder is eliminated by 2% NaOH, pointing to its phosphoprotein character; the labile P contents of various preparations are very roughly proportional to their activities. Inactivation of uricase by alkaline solutions is not parallel with P elimination.
12. Soluble uricase is inactivated by digestion with pancreatin.
13. Ammonium sulphate precipitates uricase from its solutions with comparatively slight inactivation. CO_2 at atmospheric pressure does not precipitate uricase; under higher pressures it inactivates it. Addition of $CaCl_2$ in amount equivalent to the Na_2CO_3 content does not precipitate uricase.
14. The view is advanced that uricase is a substance of protein nature.

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