Studies on Enzyme Action. XIX.—*Urease: a Selective Enzyme.* II.—*Observations on Accelerative and Inhibitive Agents.*

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(Received January 1,—Read February 20, 1913.)

In the previous communication* experiments were described which had been made with the Urease present in the Soja bean proving that the enzyme is strictly selective in its action and that whilst its activity is much reduced by ammonia it is increased, in a remarkable manner, by the presence of carbonic acid: in other words, the two products of change affect the activity of the enzyme in opposite ways—a result altogether without precedent. In explanation of these results, the suggestion was made that Urease is a feebly acidic substance.

Though it was obvious that the results were not to be harmonised with the views that were current as to the manner in which enzymes act, we refrained from comment, deeming it desirable to obtain more information before discussing the new situation that was created. In the interval, the behaviour of other enzymes has been under observation by Dr. E. F. Armstrong and ourselves and it is proposed to discuss the general outcome of the work, in a comprehensive communication, at an early date. Meanwhile, we desire to bring forward an account of further observations on Urease carried out with the object of ascertaining the manner in which the activity of the enzyme is affected by the presence of various substances together with the urea.

Experimental Method.—In cases in which the substance to be added was easily soluble in water, solutions were prepared containing either one-half or one-tenth of a molecular proportion of the substance per litre. Having measured out 50 c.c. of a half-molecular solution of urea into each of two 200 c.c. Jena flasks fitted with indiarubber stoppers, 50 c.c. of water were added to the one and to the other 50 c.c. of the M/2 (or M/10) solution of the substance of which the effect was to be determined; each flask received also 25 c.c. of Soja extract (prepared as described in our former communication); all operations were carried out as near as possible at 25°.

As soon as the two flasks were charged, they were placed in an incubator which was maintained at 25°. After intervals of 5, 10, 15, 30, 45, 60, 75, 90 and 120 minutes, samples (10 c.c.) were withdrawn from each flask by means of pipettes previously warmed to 25°; each sample was run into a

* "Studies on Enzyme Action. XV.—Urease: a Selective Enzyme," 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 109.

200 c.c. Erlenmeyer Jena flask containing a measured volume (an excess) of standardised chlorhydric acid. In all the experiments, the carbon dioxide present was removed by bubbling air through the mixture to which the standard acid had been added, after a few drops of olive oil had been introduced in order to prevent the frothing which otherwise occurs. At the end of an hour the excess of standard acid present was determined by titration with standard baryta solution, using litmus as indicator.

When dealing with substances of slight solubility (*e.g.* benzaldehyde, methylic salicylate, etc.), 105 c.c. of a solution were prepared having a concentration of M/4 as regards urea and M/20 as regards the substance to be added; 100 c.c. of the liquid taken out with a pipette were introduced into the 200 c.c. Jena flask and treated with 25 c.c. of Soja extract as before.

In most of the experiments made with the object of studying the action of carbon dioxide on more concentrated solutions of urea (semi-molecular, molecular, twice molecular and pentamolecular), in which samples were taken over a considerable period, the quantity of urea solution used was 200 c.c. together with 50 c.c. of Soja extract.

Influence of Acid Compounds on the Activity of Urease.

Not only strong acids but even the relatively much weaker carboxy-acids prevent the enzyme from acting, if present in appreciable amount. Thus no action took place in solutions of Aspartic and Salicylic acids of M/50 strength.

Boric Acid.—This acid has a remarkable depressant action when present in a solution containing the proportion $B_2O_3/50$ as shown in Table A (see Graph No. 12).*

Phenol.—In M/25 strength phenol itself has little influence but it appears to be sufficiently "acid" in M/5 strength to exercise a marked retarding effect (Table A, Graphs 1 and 2).

The influence of guaiacol and resorcinol is distinct from that of most other substances. At first these compounds retard the rate of hydrolysis but subsequently accelerate it slightly. Apparently, at the outset, they enter into competition with the enzyme and share the urea with it; as ammonia is liberated, however, they also serve to neutralise this base and therefore promote the change (Graphs 4, 5 and 6).

In the presence of quinol, action soon comes to an end (Table B). Only about 2 per cent. of change was effected when the solution was of M/25

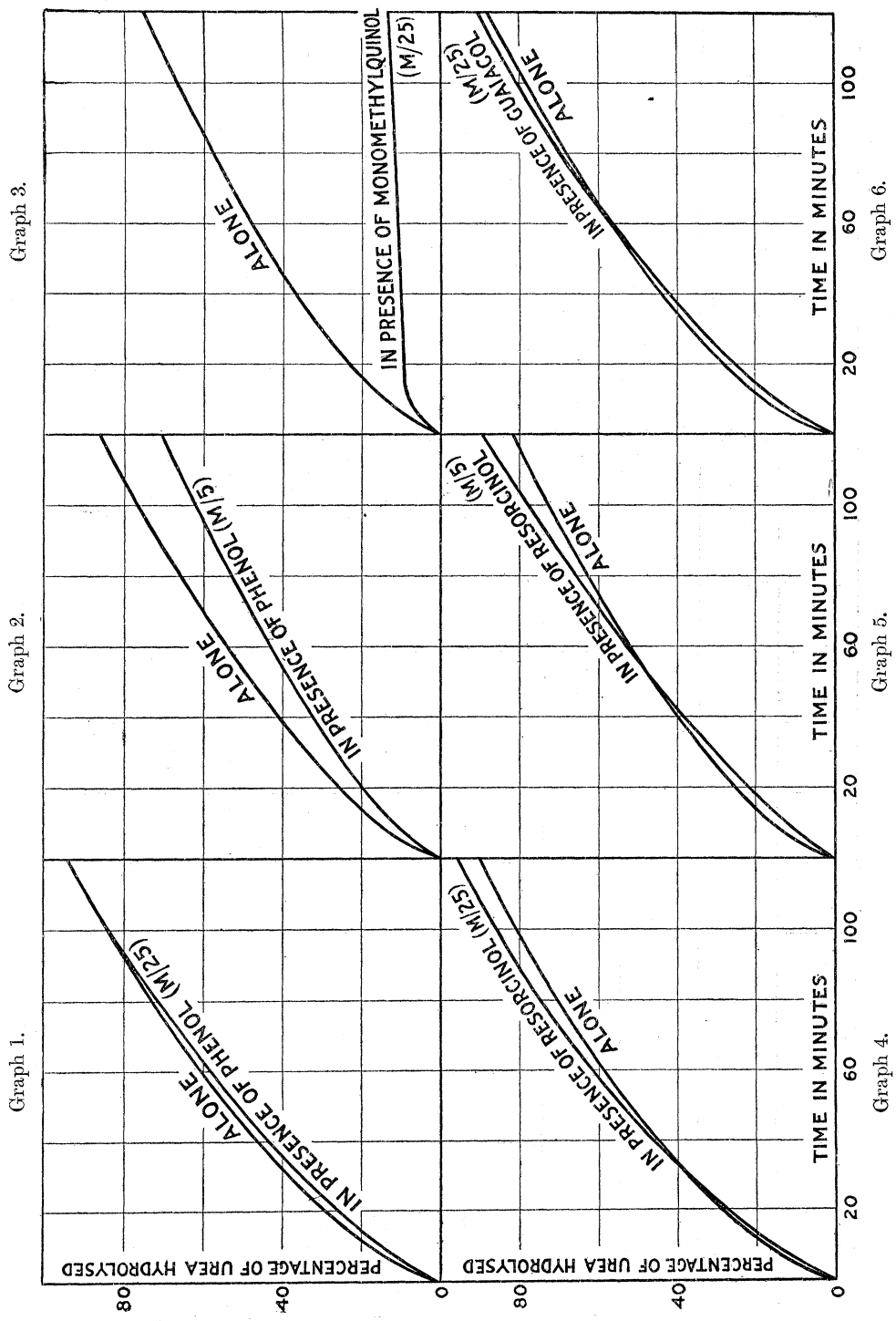
* Apparently boric acid is singular in that it retards the action of urease even in very weak solutions; all other acids, if present in sufficiently small amount, accelerate hydrolysis.

Table A.—Hydrolysis of Urea in M/5 Solutions containing Acidic Substances.

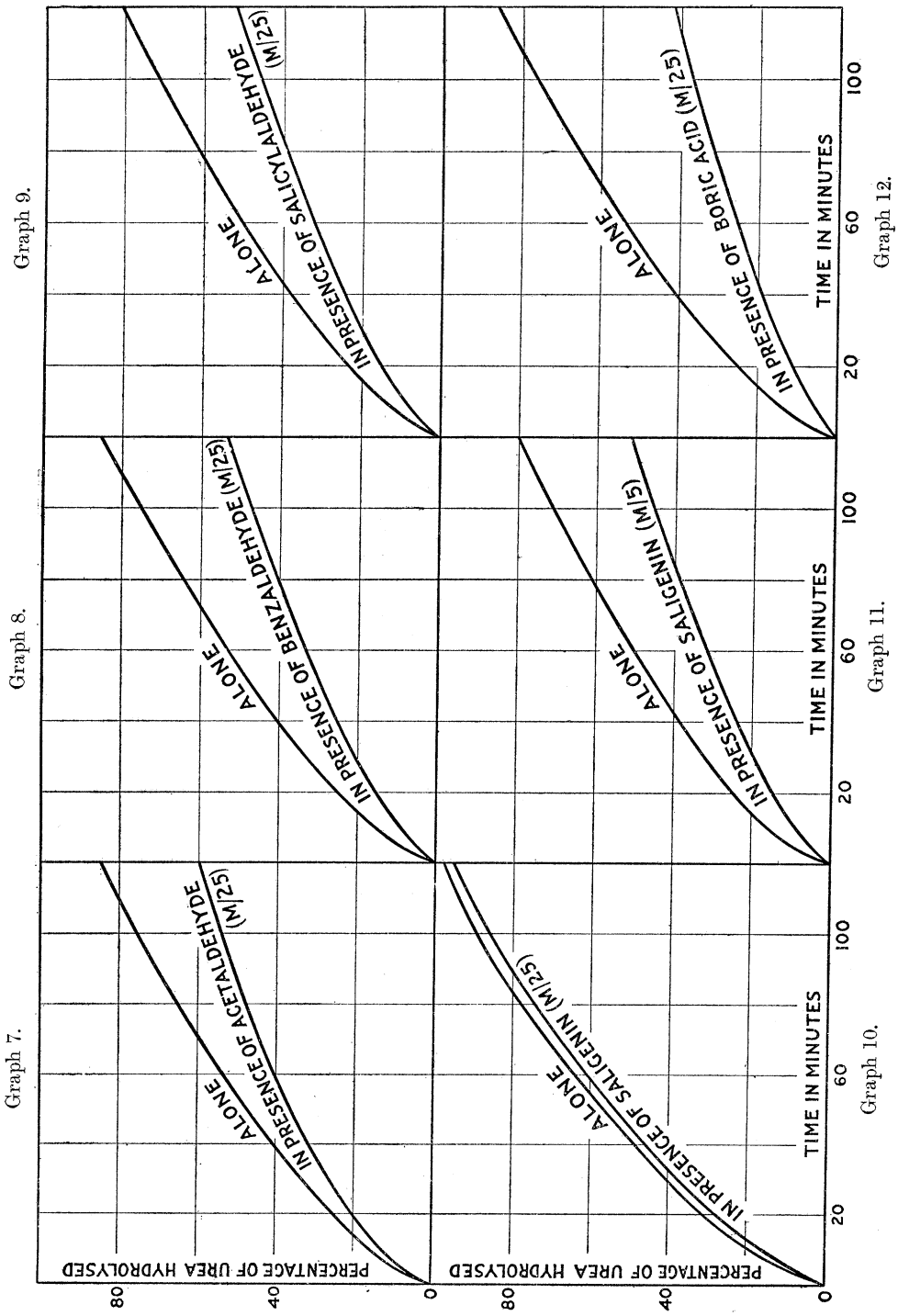
Time (mins.).	Percentage of urea hydrolysed.									
	Alone.	In the presence of phenol M/25.	Alone.	In the presence of phenol M/5.	Alone.	In the presence of resorcinol M/25.	Alone.	In the presence of resorcinol M/5.	Alone.	In the presence of guaiacol M/25.
5	10·8	9·2	9·9	7·1	10·6	9·4	9·5	6·5	11·8	9·2
10	17·7	15·5	15·8	11·9	17·7	16·2	15·3	12·6	17·1	15·3
15	23·8	21·2	20·6	15·9	23·3	22·4	20·8	17·6	22·7	20·6
30	37·4	35·6	33·9	26·5	36·7	37·7	32·9	30·9	36·0	34·8
45	49·5	47·8	44·9	35·3	49·2	50·6	43·2	42·6	47·4	46·2
60	60·8	58·4	54·2	43·4	59·1	61·2	52·5	53·3	56·7	56·9
75	69·7	68·8	63·5	50·7	67·6	71·4	60·5	63·2	65·8	66·4
90	78·2	78·2	71·5	57·7	76·5	81·0	68·3	72·1	74·2	75·1
120	94·4	94·4	86·0	71·2	90·9	95·6	81·6	89·9	88·2	90·1
	Alone.	In the presence of boric acid M/25.	Alone.	In the presence of glycine M/5.	Alone.	In the presence of glycine 2M/5.	Alone.	In the presence of glycine 4M/5.	Alone.	In the presence of asparagine M/5.
5	9·7	3·0	10·5	11·1	9·8	12·0	10·0	10·8	8·3	8·8
10	16·5	6·4	16·5	19·1	16·5	21·4	15·5	21·0	13·4	14·6
15	21·3	8·8	20·5	27·8	21·6	29·0	21·0	29·4	17·2	22·6
30	34·3	15·6	33·2	46·5	33·9	48·1	33·4	49·4	27·7	39·2
45	44·9	20·9	44·4	61·1	44·7	63·7	44·7	65·5	36·2	52·8
60	53·6	25·8	53·5	73·5	53·0	75·9	55·5	78·7	43·9	64·5
75	63·7	30·1	62·2	85·0	64·3	87·7	63·2	91·4	52·2	74·6
90	71·5	35·0	69·9	94·4	72·0	96·5	72·4	101·0	57·4	83·4
120	86·3	41·8	84·2	99·7	87·3	98·9	85·5	101·4	69·8	97·1

Table B.—Hydrolysis of Urea in M/5 Solutions containing Quinol and Monomethylquinol.

Time (minutes).	Percentage of urea hydrolysed.					
	Alone.	In presence of quinol M/500.	Alone.	In presence of quinol M/25.	Alone.	In presence of monomethyl-quinol M/25.
5	10·4	3·8	9·9	2·3	9·0	5·6
10	17·0	4·2	16·9	2·1	14·3	8·1
15	22·5	4·2	22·4	1·9	18·3	8·7
30	35·6	4·2	36·5	1·6	30·3	9·5
45	46·4	4·2	48·7	1·6	39·4	10·1
60	56·0	4·0	58·6	—	48·2	10·9
75	64·7	4·2	67·8	1·9	55·6	11·8
90	72·1	4·0	76·5	1·4	62·8	12·3
120	85·0	3·8	91·9	0·9	75·5	13·3



Graph 1. Graph 2. Graph 3. Graph 4. Graph 5. Graph 6.



Graph 7. Graph 8. Graph 9. Graph 10. Graph 11. Graph 12.

strength and only about 4 per cent. when it was reduced to M/500; the solution rapidly darkened in colour. As no action takes place in presence of quinone (M/50), there can be little doubt that the effect produced by quinol is dependent on the production of this compound directly ammonia is present in sufficient amount to condition the oxidation of the quinol.

A series of experiments were made with the monomethylic-derivative of quinol, $C_6H_4(OH)\cdot OCH_3$, a compound of some interest, as it is formed together with quinol when arbutin is hydrolysed by emulsin.

The material used was that supplied by Kahlbaum. We were inclined at first to attribute its inhibitive power to the presence of quinol; we therefore purified it by distilling it *in vacuo* and made use of the intermediate fraction. The results given in Table B are those obtained with this product. We then digested the compound with ferric chloride, with the object of oxidising any quinol that might be present; after treatment with a little sulphite, to remove quinone, the residue was distilled *in vacuo*. As the substance thus purified was as active as the original material, we are inclined to think that in presence of ammonia and air monomethylated quinol is slowly converted into quinone and that this is the reason why it is so active an inhibitant (Graph 3).

Glycine and Asparagine.—These substances accelerate the rate of change as shown in Table A (Diagram 13).

Though they are "neutral" compounds, they neutralise both acids and bases; their marked accelerative effect is probably due to the fact that they serve to neutralise the ammonia as it is produced by the hydrolysis of the urea. As the positive influence of glycine is no greater apparently in more concentrated solutions, it is not improbable that it acts in two directions, both serving to fix ammonia and combining also to some extent with the enzyme.

Carbonic Acid.—A further series of observations carried out in presence of carbonic acid is given in Table C. The experiments were made in the manner already described (XV, p. 121). The results are represented by graphs in Diagram 15.

The four graphs in Diagram 14 are drawn from data given in XV, Part I. They represent comparable results obtained in experiments carried out simultaneously with the same sample of enzyme. It will be noticed that whilst the products of change taken together have but little influence, taken singly they are relatively very active but in opposite directions.

The set of graphs marked *c* (Diagram 15) show that when the proportion of urea is varied the difference observed in the absence of carbonic acid (XV, p. 117) is again apparent, the amount of change taking place in solutions

Diagram 13.

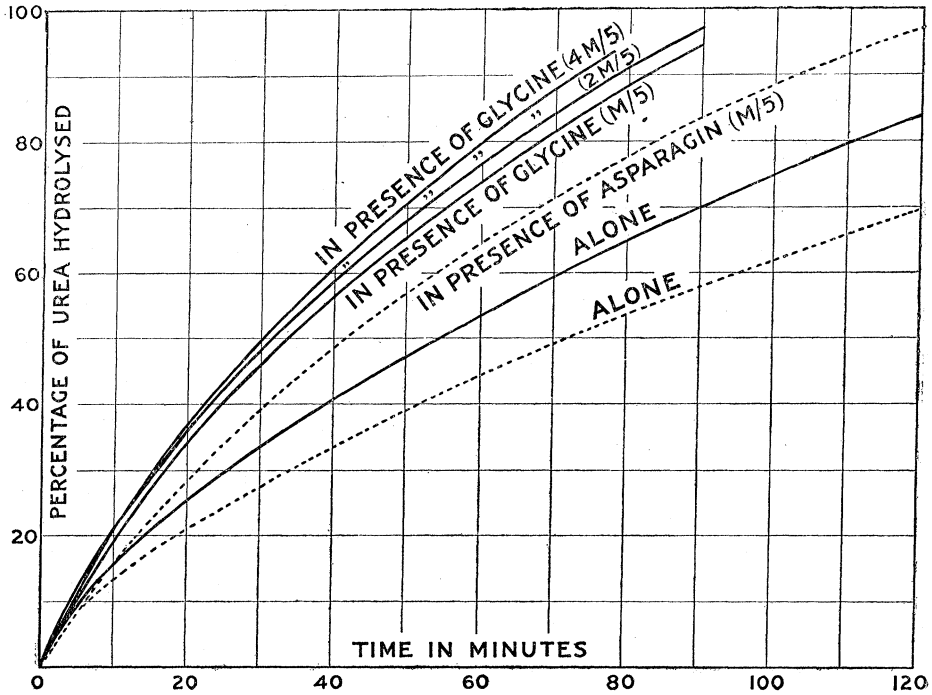


Diagram 14.

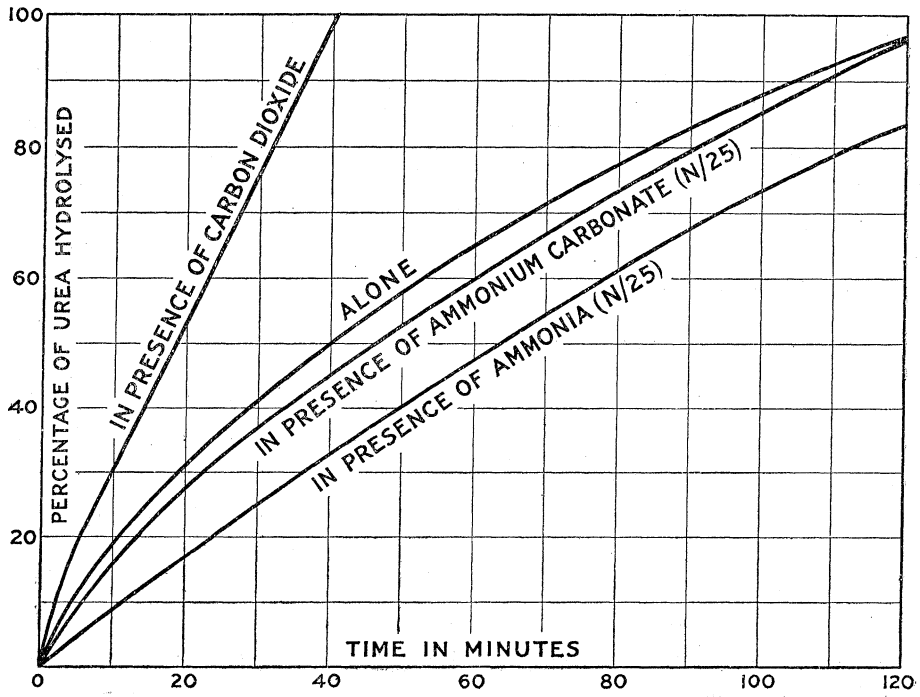


Table C.—Hydrolysis of Urea in Solutions saturated with Carbon Dioxide.

Time.	Volume of decinormal acid neutralised by 10 c.c. sample.										
	1. *Urea in M concentration.	2. Urea in M concentration.	3. Urea in M/5 concentration.	4. Urea in M concentration.	5. Urea in 5M concentration.	6. Urea in M/2 concentration.	7. Urea in M concentration.	8. Urea in M concentration.	9. Urea in 2M concentration.	10. Urea in M concentration.	11. †Urea in M concentration.
5 mins.	3·9	5·8	6·4	5·9	5·3	4·9	5·2	5·5	5·8	5·5	9·3
10	6·0	10·0	11·2	11·3	8·0	8·1	9·1	9·7	9·9	9·7	14·7
15	8·1	13·4	15·2	—	—	11·9	12·7	13·2	12·3	13·0	19·4
20	9·8	16·3	20·4	20·2	15·2	15·0	15·5	16·5	14·8	15·9	24·0
30	13·0	21·7	28·7	29·4	22·2	21·7	22·3	23·8	21·5	22·6	33·8
40	15·5	28·0	34·9	—	—	28·2	29·5	30·5	27·6	28·6	43·4
50	—	—	40·1	—	—	—	—	—	—	—	—
60	20·9	39·1	40·0	52·5	38·5	40·7	43·0	44·5	39·5	41·1	64·7
90	27·2	57·2		70·5	53·1	54·1	57·4	63·3	59·8	58·7	92·3
2 hours	33·2	73·7			—	66·4	70·6	80·0	76·7	75·2	116·1
2·5	—	—			—	79·8	—	—	—	—	—
3	44·3	103·8			100·3	92·8	99·2	107·8	104·7	103·1	155·2
3·5	—	—				99·4	—	—	—	—	—
4	55·5	—				99·2	124·9	131·8	132·0	128·9	185·3
4·5	—	—				99·2	—	—	—	—	196·8
5	64·7	151·3				99·4	146·5	152·9	156·7	—	200·6
6	74·8	169·0					164·4	171·5	181·3	166·1	200·6
7	84·0	184·3					181·1	187·0	—	181·1	
7·5	—	—					187·4	—	—	—	
8	92·7	197·2					193·3	199·1	218·7	193·7	
8·5	—	—					197·6		—	198·9	
9	—	200·4							—		
10	108·9	200·2							249·2		
24	187·6								353·9		

* No carbonic acid was present in this experiment.

† Twice as much enzyme was used in this case.

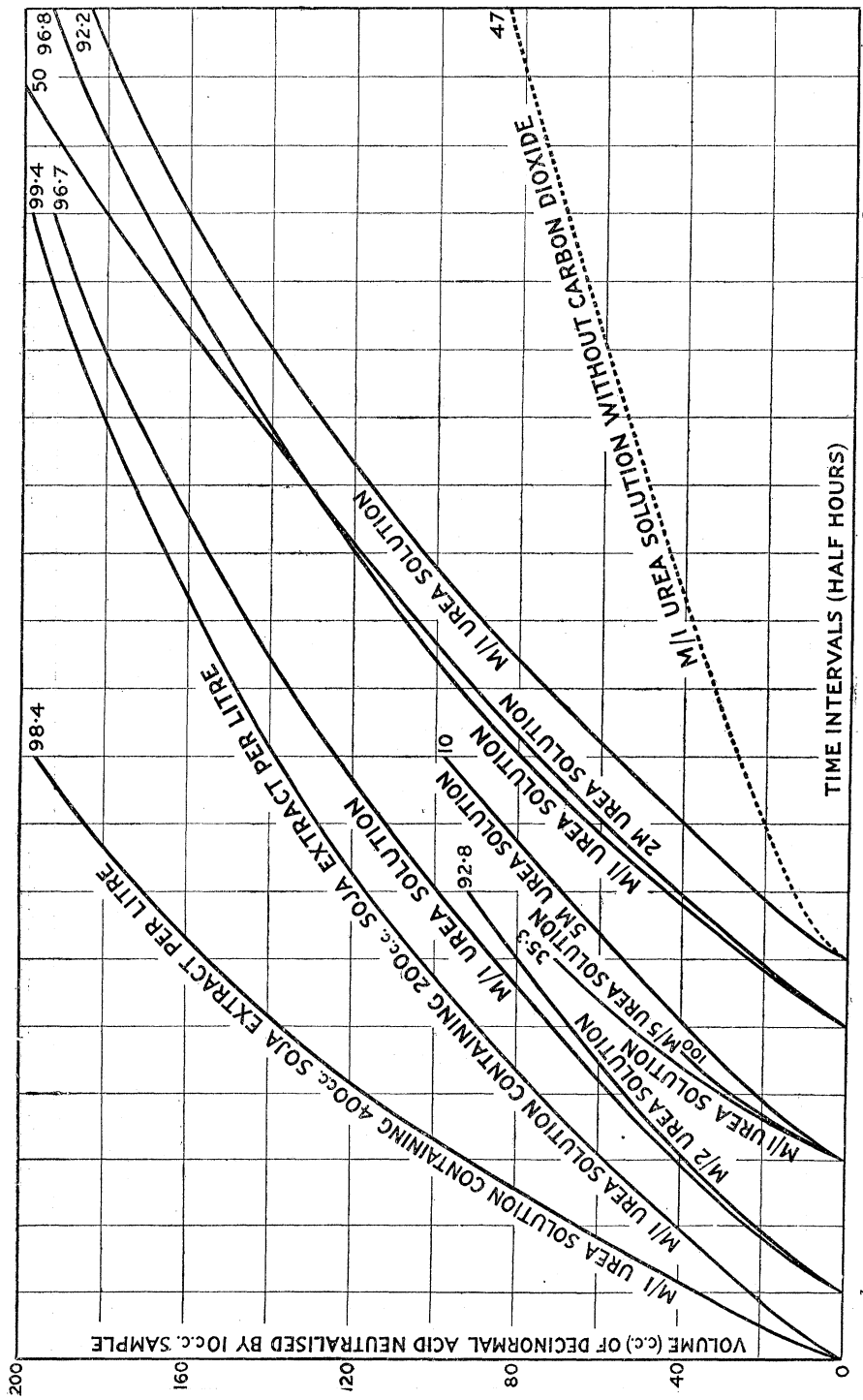

VOLUME (c.c.) OF DECINORMAL ACID NEUTRALISED BY 10c.c. SAMPLE
0
40
80
120
160
200
TIME INTERVALS (HALF HOURS)
a
b
c
d
e
M/1 UREA SOLUTION WITHOUT CARBON DIOXIDE
47
2M UREA SOLUTION
M/1 UREA SOLUTION
99·4
96·7
M/1 UREA SOLUTION
50
96·8
92·2
M/1 UREA SOLUTION CONTAINING 400c.c. SOLA EXTRACT PER LITRE
98·4
M/1 UREA SOLUTION CONTAINING 200c.c. SOLA EXTRACT PER LITRE
M/1 UREA SOLUTION
10
92·8
5M UREA SOLUTION
35·3
100 M/5 UREA SOLUTION
M/1 UREA SOLUTION
M/2 UREA SOLUTION

DIAGRAM 15.

of M/5 and M strength being almost the same, less change taking place in a solution of 5M strength. When change was complete in the weakest solution, only 1/5 of the urea in the solution of intermediate strength was hydrolysed and about 1/23 of that in the strongest solution.

To ascertain the optimum strength of solution, a comparative experiment was made with solutions of M/2 and M strength (the two graphs marked *b*). Again, the solution of molecular strength was found to be slightly the more active.

On contrasting the behaviour of solutions of molecular and twice molecular strength (the two graphs marked *d*), it was found that the change took place at very nearly the same rate in each, being slightly more rapid in the weaker during more than half the period of change.

Two experiments were made with solutions of molecular strength, twice the usual amount of enzyme being added to the one (the two graphs marked *a*); these gave results showing that the use of the larger proportion of enzyme is attended with a slight advantage.

The striking fact brought out in all the graphs representing experiments made in presence of carbonic acid is the approximation of the rate of change to a "linear" character.

To secure a more rigid comparison, smooth curves were drawn carefully to a large scale from the data obtained in the experiments and the rates of change were deduced by finding the value of the tangent at each of a series of points. The results are given in Table D.

It will be noticed that the influence of the acid increases as the action proceeds and that the rates are not far from being constant over considerable intervals. The values of the ratio $\frac{dx/dt}{a-x}$ in no way correspond to those to be expected in the case of a change proceeding at unimolecular rate, which is commonly regarded as the rate to which such actions tend to approximate.

Hydrogen Cyanide.—In view of the fact that hydrogen cyanide is a product of the hydrolysis of a considerable number of glucosides by "emulsin" and other enzymes, as well as on account of its remarkable physiological activity, it appeared to us to be important to study its behaviour towards an enzyme with which, presumably, it is not ordinarily brought into relationship. Our anticipation that it would act merely as a very weak acid and accelerate hydrolysis was proved to be correct. The results of a series of experiments with various strengths of the cyanide are given in Table E and as graphs in Diagram 16.

It will be seen that the accelerative influence increases with the

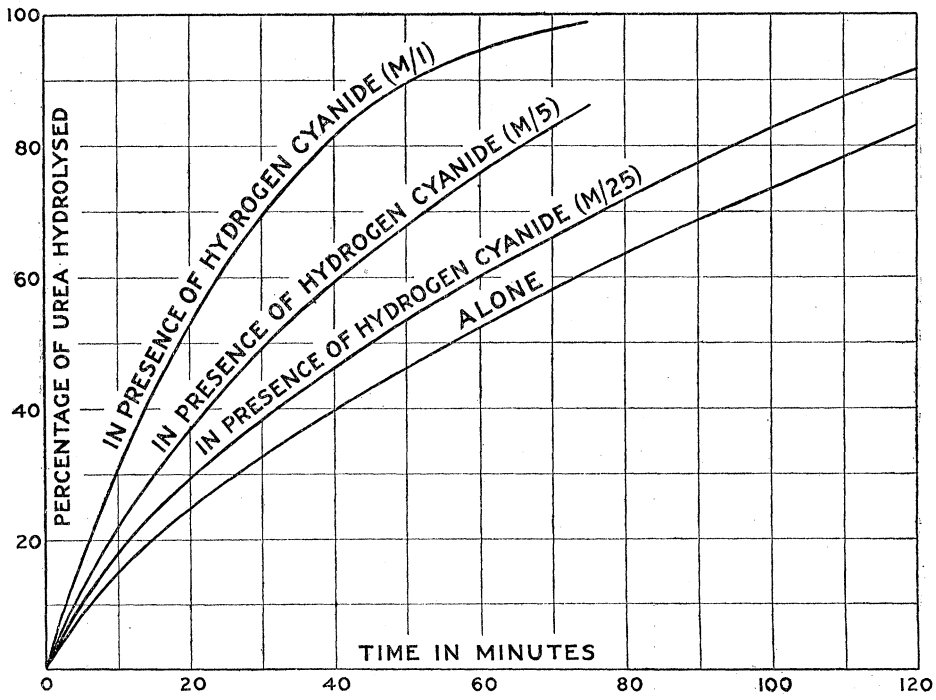
Table D.—Rates of Hydrolysis of Urea and Velocity Coefficients.

Grammes of urea hydrolysed (x).	Grammes of urea hydrolysed per hour (dx/dt)		Velocity coefficient, $\frac{dx/dt}{a-x}$		Grammes of urea hydrolysed (x)	Grammes of urea hydrolysed per hour (dx/dt)		Velocity coefficient, $\frac{dx/dt}{a-x}$	
	M Urea solution (aqueous).	M Urea solution (saturated with carbon dioxide).	M Urea solution (aqueous).	M Urea solution (saturated with carbon dioxide).		M Urea solution saturated with carbon dioxide.		M Urea solution saturated with carbon dioxide.	
						Containing 200 c.c. enzyme per litre.	Containing 400 c.c. enzyme per litre.	Containing 200 c.c. enzyme per litre.	Containing 400 c.c. enzyme per litre.
0·6365	1·623	3·583	0·113	0·250	1·591	2·851	4·436	0·212	0·330
1·273	1·171	2·616	0·085	0·191	3·182	2·730	4·379	0·231	0·370
2·546	0·872	2·558	0·070	0·205	4·774	2·470	4·354	0·241	0·425
3·819	0·795	2·527	0·071	0·226	6·365	2·215	4·086	0·256	0·473
5·092	0·732	2·489	0·074	0·252	7·956	1·973	3·647	0·280	0·517
6·365	0·668	2·329	0·078	0·270	9·547	1·706	3·112	0·312	0·572
7·638	0·605	2·113	0·082	0·288	11·139	1·426	2·762	0·368	0·713
8·911	0·560	1·948	0·092	0·320	12·730	1·177	2·291	0·513	1·004
10·184	0·515	1·687	1·073	0·351	14·321	0·910	1·801	1·318	2·601
11·457	0·458	1·445	1·297	0·409					
12·730	0·401	1·216	1·775	0·539					
14·003	0·337	0·981	3·420	1·058					

Table E.—Hydrolysis of Urea in M/5 Solutions in presence of Hydrogen Cyanide.

Time (minutes).	Percentage of urea hydrolysed.				
	Alone.	In presence of hydrogen cyanide M/25.	In presence of hydrogen cyanide M/5.	Alone.	In presence of hydrogen cyanide, M.
5	8·8	10·9	12·6	9·7	17·0
10	15·6	18·5	21·9	16·5	31·4
15	20·4	24·0	—	21·3	42·2
20	—	—	36·3	—	53·3
30	32·9	38·6	49·6	34·3	69·1
40	—	—	—	—	81·5
45	43·4	49·8	63·1	44·9	—
50	—	—	—	—	89·7
60	52·2	59·7	75·4	53·6	94·3
75	61·4	69·2	86·2	63·7	98·8
90	68·7	77·6	94·8	71·5	—
120	83·1	91·6	100·0	86·3	—

Diagram 16.



concentration and becomes very considerable in solutions of molecular strength, 50 per cent. of the urea in such a solution being hydrolysed after an

interval of about 18 minutes, whilst in the absence of the cyanide this amount is changed only after 55 minutes. In this case it was noted that the solution became brown.

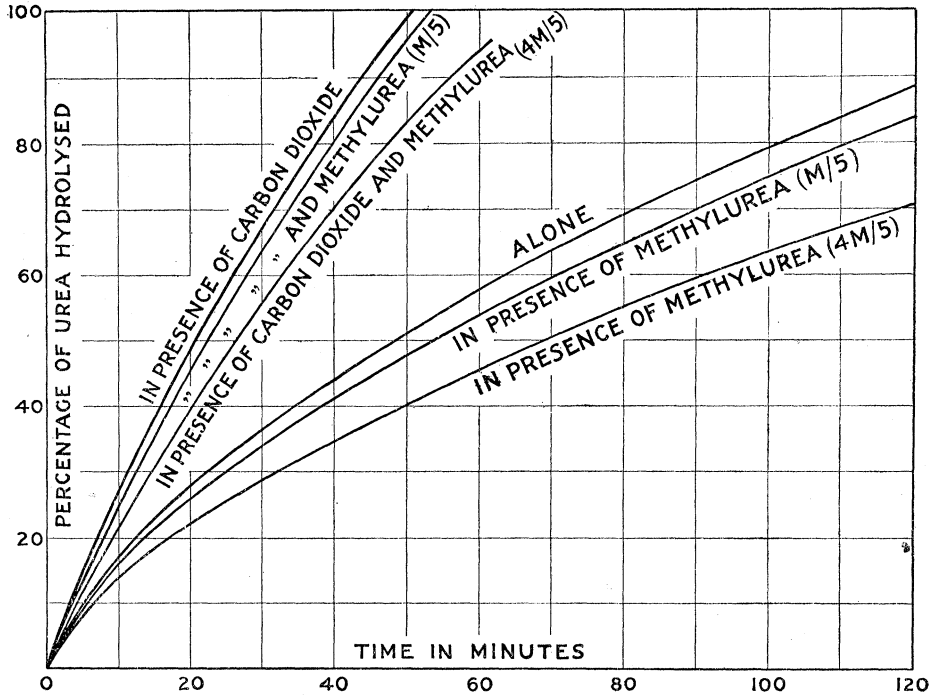
It is noteworthy that hydrogen cyanide has relatively less influence than carbonic acid in the later stages of the change.

Influence of Methylurea.—In Part XV it is shown that methylurea has a definite retarding effect. We have therefore carried out a further series of experiments in which, in one case, only the amount of methylurea present was varied, whilst in the other the action took place in presence of carbonic acid. The results obtained with this substance when used in presence of carbonic acid are of special interest in view of the close relationship of urea and methylurea; they are given in Table F and in Diagram 17. It will be noticed that the addition to the M/5 solution of urea of an equivalent amount of methylurea has a marked depressant effect and that when the urea and the methylurea are present in the ratio 1 : 4 the effect of the neutral substance is considerable. As practically the same alteration in osmotic conditions would be produced by equivalent proportions of urea and methylurea, it is to be supposed that the influence exercised by methylurea is due in part to the fact that it shares the acid enzyme with the urea but it also interferes mechanically. The results obtained in presence of carbonic acid are similar to those obtained in its absence but action proceeds at accelerated rates.

Table F.—Hydrolysis of Urea in M/5 Solutions in presence of Methylurea.

Time (minutes).	Percentage of urea hydrolysed.					
	Alone.	In presence of methylurea M/5.	In presence of methylurea 4M/5.	In presence of carbon dioxide.	In presence of methylurea (M/5) and carbon dioxide.	In presence of methylurea (4M/5) and carbon dioxide.
5	10·0	9·7	8·3	15·9	15·3	11·7
10	16·7	15·9	13·4	27·8	25·0	22·0
15	22·9	21·4	18·0	36·9	35·4	30·9
20	—	—	—	49·0	46·0	39·6
30	36·0	34·3	28·4	66·6	62·8	55·1
40	—	—	—	83·9	80·6	70·8
45	47·9	44·1	37·3	—	—	—
60	57·0	53·4	45·2	97·9	98·6	94·1
75	66·8	62·1	53·0	—	—	—
90	74·0	70·0	59·6	99·6	99·8	99·6
120	88·4	83·9	70·6	—	—	—

DIAGRAM 17.



Influence of Neutral Agents which depress the Activity of Urease.

Alcohols.—Ethylic and propylic alcohols exercise moderate effects which may be attributed to the changes they produce in the osmotic conditions. As in all other cases studied, the less soluble alcohol is the more active (Diagram 18).

Saligenin, $C_6H_4(OH)\cdot CH_2(OH)$, is far more active than either of the paraffinoid alcohols (Graphs 10 and 11).

Aldehydes.—In the presence of formaldehyde (M/25), action comes to an end when about 4 per cent. of change has taken place.

Acetic aldehyde, benzoic aldehyde and salicylic aldehyde are moderately active depressants; the results obtained with these substances and with saligenin are given in Table G and in Graphs 7, 8 and 9.

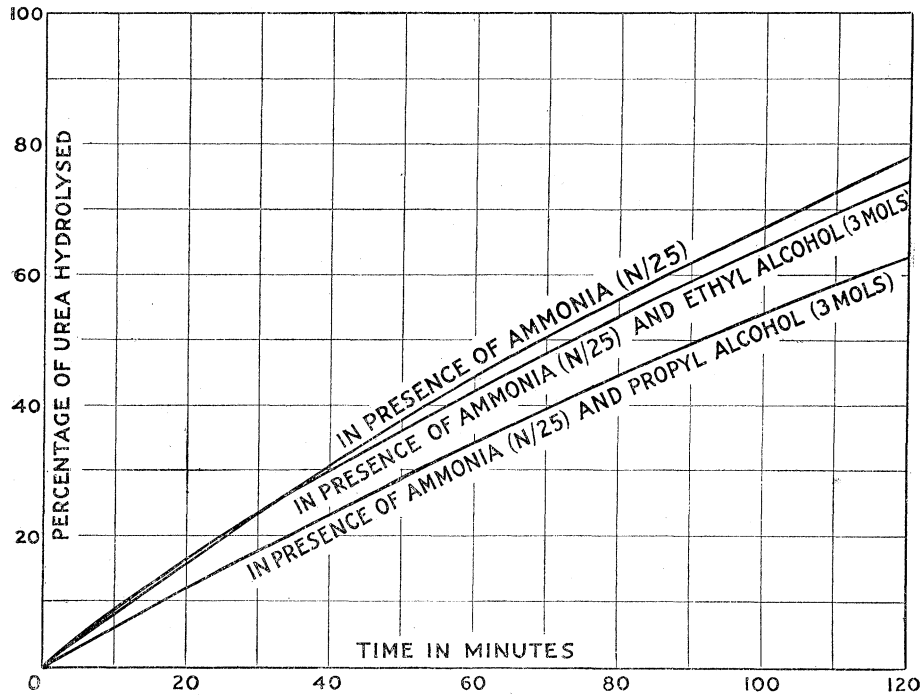
The observation that glucose has a slight retarding effect has been confirmed.

Sodium salicylate and methylic salicylate have practically no action.

We are inclined to think that the aldehydes are all, in some measure, chemically active towards urease and that even saligenin may be credited with slight chemical activity.

It has been customary to regard the action of enzymes as subject to the

Diagram 18.



"law of mass action" and to assume that the rate at which action takes place is such that it is proportional at any moment to the amount of substance left unchanged. It should be possible, therefore, to express the rate of such changes by logarithmic curves but it is recognised that the products of change have a more or less marked retarding influence and that, on this account, the actual curve expressing the rate of change always falls below the theoretical curve. It is further supposed that the action is reversible and that therefore, on this account, the change is never complete, though it may be very nearly so in dilute solutions. Lastly, it is recognised that when the enzyme is present in very small proportion, the action proceeds at a nearly constant rate: also that it is much retarded in very concentrated solutions—a result ascribed by some to the viscosity of such solutions.

It appears to us that our results are not in accord with the views hitherto accepted and that it is to be supposed that enzymic changes would be found to take place at approximately constant rates were it not that they are subject directly and indirectly to considerable retardation by the products of change; indeed it is probable that the products of change have an affinity for the

Table G.—Hydrolysis of Urea in M/5 Solutions containing Alcohols and Aldehydes.

Time (mins.).	Percentage of urea hydrolysed.							
	Alone.	Plus ethylic alcohol 1 mol.	Plus propylic alcohol 1 mol.	Alone.	Plus ethylic alcohol 3 mols.	Plus propylic alcohol 3 mols.	Alone.	In presence of saligenin M/25.
5	2·7	2·9	2·8	4·9	3·7	2·5	11·7	9·8
10	7·0	6·9	6·6	7·9	8·7	6·5	19·9	16·5
15	11·2	11·4	10·9	12·4	12·2	8·8	26·1	23·7
30	22·5	21·5	21·0	23·2	23·5	17·9	40·3	37·4
45	32·6	31·8	30·8	34·8	33·1	26·2	—	—
60	42·0	41·5	40·5	44·6	42·1	34·5	64·3	—
75	51·2	50·4	49·0	53·3	50·9	41·9	74·4	—
90	60·2	57·0	57·3	61·8	58·9	49·6	84·0	80·6
120	75·2	73·3	73·0	78·1	74·3	62·7	97·4	95·5
12 hrs.	98·1	98·7	98·7	—	—	—		
	Alone.	In presence of saligenin, M/5.	Alone.	In presence of acetic aldehyde, M/25.	Alone.	In presence of benzoic aldehyde, M/25.	Alone.	In presence of salicylic aldehyde, M/25.
5	10·4	4·7	10·6	8·5	9·4	5·4	8·5	5·6
10	16·7	9·2	16·1	13·1	16·0	8·9	14·6	9·4
15	20·5	12·2	21·0	17·2	20·5	12·5	19·3	12·0
30	32·5	19·3	33·5	26·1	33·7	21·2	31·1	20·0
45	41·5	26·1	43·9	34·3	44·3	27·8	41·9	26·8
60	50·9	31·5	53·6	40·5	53·9	33·7	50·9	32·0
75	60·3	37·7	63·4	46·4	62·9	39·3	59·3	38·1
90	66·9	42·6	70·8	51·3	70·4	44·0	66·6	43·1
120	80·4	51·8	84·8	60·2	86·0	53·7	81·0	52·5

In the experiments with ethylic and propylic alcohols in weight normal solutions, an amount of ammonia equivalent to one-tenth of that ultimately produced was added initially to the urea solutions.

enzyme which is actually greater than that which obtains between the hydrolyte and the enzyme.

It has often been suggested that the enzymes are colloids. The experiments carried out in the course of this series of studies appear to justify the belief that enzymic action takes place entirely at the surfaces of colloid particles suspended in the solution of the hydrolyte and not between substances which are all in true solution.

The subject will be more fully discussed in the later communication to which we have referred.

[The cost of this investigation has been partially met by a grant for which I am indebted to the Government Grant Fund of the Royal Society.—H.E.A.]