CX. INFLUENCE OF GUANIDINE AND ITS DERIVATIVES ON THE ACTION OF AMYLASE.

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SHERMAN [1923] and other authors have found that the hydrolysis of starch is accelerated in the presence of amino-acids. Filipowicz [1931] has found that neither the amino-group separately nor the carboxyl group of amino-acids possesses any accelerating influence in this reaction. In the present paper the effect of adding guanidine or its derivatives to the systems amylase-starch or amylase-glycogen are described.

EXPERIMENTAL.

The polysaccharides taken were Merck's soluble starch, and glycogen prepared from rabbit-livers. The enzyme was either Merck's "diastase abs." or salivary ptyalin. The p_H of the solutions was adjusted by addition of Sørensen's phosphate buffers, p_H being determined potentiometrically or colorimetrically by means of Clark and Lubs's indicators. Reducing sugars were determined by Bertrand's method, and residual glycogen was determined nephelometrically according to Rona and van Eweyk [1924].

1. Guanidine.

A number of systems were set up, containing 30 cc. of 1 % starch solution, 40 cc. of Sørensen's phosphate buffers (p_H 6.1), guanidine carbonate solution from 0 to 0.2 %, and water to 90 cc. The flasks were left for 20 mins. at 34° after which 10 cc. of 0.1 % amylase solution were added to all flasks, except the control to which 10 cc. of water were added. Reducing sugar was determined in 20 cc. portions taken from all flasks at 10 min. intervals after the addition of amylase. The maximum yield of maltose calculated from the glucose content of 20 cc. of the control solutions after hydrolysis with HCl amounted to 60 mg., so that the percentage hydrolysis as given in Table I represents a value of about 57 % after 90 mins.

It follows from Table I that guanidine retards reaction to an extent increasing with its concentration, and that percentage retardation diminishes with time.

Table I. *Effect of varying concentration of guanidine.*

Conc. of guanidine carbonate %	20 mins.		30 mins.		60 mins.	
	Maltose	Retardation	Maltose	Retardation	Maltose	Retardation
	mg.	%	mg.	%	mg.	%
0	19.3	—	26.0	—	38.9	—
0.04	18.2	5.7	24.5	5.8	37.0	4.9
0.06	17.6	8.8	24.0	7.7	37.0	4.9
0.08	17.6	8.8	24.0	7.7	37.0	4.9
0.10	14.8	23.3	22.2	14.6	35.4	8.9
0.12	14.8	23.3	22.2	14.6	35.4	8.9
0.14	14.2	26.4	22.2	14.6	35.4	8.9
0.16	14.2	26.4	20.5	21.2	34.2	12.1
0.18	12.5	35.2	20.5	21.2	34.2	12.1
0.20	10.8	44.0	17.6	32.3	34.2	12.1

2. *Action of guanidine in systems containing glycogen and ptyalin.*

Substantially the same results are obtained if glycogen is substituted for starch or ptyalin for Merck's amylase. The conditions of the experiments as well as the compositions of the systems were the same as in the preceding experiments. In systems (p_H 6.1) in which the concentration of starch or glycogen was 0.4 %, of guanidine carbonate 0.1 %, and of Merck's amylase 0.08 %, the retardation of reaction amounted to 19.2 % for starch and 21.8 % for glycogen, after keeping for 1 hr. at 34°. Under similar conditions, but taking saliva (0.02 %) in place of Merck's amylase, the corresponding retardations of the reaction were 25.8 and 26.3 %.

This result was confirmed by experiments in which residual glycogen was determined nephelometrically and the maltose produced was determined by Bertrand's method. The systems consisted of 30 cc. of 1 % glycogen, 80 cc. of Sørensen's buffers (p_H 6.1), 20 cc. of water or 0.9 % guanidine carbonate, and 20 cc. of 0.1 % amylase, added to the preceding solutions after they had been warmed to 34°. Portions of the systems were taken at 10–20 min. intervals for determination of reducing sugars and glycogen. The results (Table II) indicate that the process of hydrolysis of glycogen proceeds parallel with that of production of reducing sugars, the difference between the results given by the two methods lying within the margin of experimental error. Both processes are retarded to the same extent in the presence of guanidine.

Table II. *Comparison of results obtained nephelometrically with those obtained by Bertrand's method.*

Time mins.	Percentage decomposition					
	Without guanidine		With guanidine		Retardation (%)	
	Nephelometer	Bertrand	Nephelometer	Bertrand	Nephelometer	Bertrand
10	6.0	—	3.0	—	50.0	—
20	12.0	—	8.0	—	33.3	—
30	20.0	22.3	16.0	16.1	20.0	27.6
50	27.0	27.5	24.0	24.3	11.1	11.0
70	34.0	34.0	30.0	30.5	11.7	10.2
90	42.0	43.9	38.0	40.1	9.2	8.6

3. *Action of creatine.*

In experiments on the action of amylase in the presence of creatine or creatinine only nephelometric measurements were made, as both of these substances reduce cupric oxide in Bertrand's reagent. The systems consisted of 20–25 cc. of 1 % creatine, 20–25 cc. of 1 % glycogen, 10–20 cc. of Sørensen's buffers (p_H 5.88–7.39), and water to 97 or 99 cc. The systems were warmed to 34°, and 1–3 cc. of a 0.1 % solution of saliva was added, after which glycogen was determined at 10 min. intervals.

Table III. *Effect of creatine.*

No. of exp.	Conc. of gly-cogen %	Conc. of crea-tine %	p_H	Minutes							
				10	20	30	40	50	60	70	
1	0.25	0	5.88	45	68	—	—	—	—	—	% hydrolysis
	"	0.25	"	38	60	—	—	—	—	—	"
	"	"	"	15.5	11.5	—	—	—	—	—	% retardation
2	0.20	0	6.8	30	53	61	—	—	—	—	% hydrolysis
	"	0.20	"	28	51	65	—	—	—	—	"
	"	"	"	6.6	3.7	8.4	—	—	—	—	% retardation
3	0.25	0	7.22	—	14	24	33	—	—	43	% hydrolysis
	"	0.25	"	—	14	24	33	—	—	43	"
	"	"	"	—	0	0	0	—	—	0	% retardation
4	0.2	0	7.39	32	55	67	—	—	—	—	% hydrolysis
	"	0.25	"	31	52	65	—	—	—	—	"
	"	"	"	3.1	5.4	3.0	—	—	—	—	% retardation
5	0.2	0	6.0	20	26	32	40	48	54.	—	% hydrolysis
	"	0.4	"	14	20	26	32	38	46	—	"
	"	"	"	30	23.1	18.8	20.0	20.8	18.6	—	% retardation

The results (Table III) indicate that creatine considerably retards reaction in acid media, and to a much less marked degree or not at all in alkaline media. Its inhibitory effect is, however, much smaller than is that of guanidine.

4. *Action of creatinine.*

Systems similar to those described in the preceding experiment were set up, with the difference that creatinine (in final concentrations of 0.4–0.8 %) was taken in place of creatine. The results (Table IV) indicate that the reaction

Table IV. *Effect of creatinine.*

No. of exp.	Conc. of gly-cogen %	Conc. of crea-tinine %	p_H	Time in minutes							
				10	20	30	40	50	60	70	
1	0.2	0	6.27	25	40	50	60	—	—	—	% hydrolysis
	"	0.4	"	40	58	74	80	—	—	—	"
	"	"	"	60.0	45.0	48.0	33.3	—	—	—	% acceleration
2	0.2	0	6.3	25	42	54	—	—	—	—	% hydrolysis
	"	0.8	"	60	77	90	—	—	—	—	"
	"	"	"	140	83	66	—	—	—	—	% acceleration
3	0.2	0	7.01	14	25	31	40	46	50	56	% hydrolysis
	"	0.4	"	15	30	42	48	55	62	69	"
	"	"	"	7.1	20.0	32.6	20.0	24.0	24.0	23.0	% acceleration

proceeds with a considerably greater velocity in the presence of creatinine than in its absence, and that percentage activation increases with concentration of creatinine.

5. *Action of amylase in the presence of both activating and inhibiting substances.*

The activator taken was glycine, whilst guanidine carbonate was selected as an inhibitory factor. The systems consisted of 20 cc. of 0.5 % starch, 10 cc. of buffer solution (p_H 5.8), 10 cc. of water or 0.5 % guanidine carbonate, 2.5 % glycine in such quantities as to give a range of concentrations of 0 to 1 %, and 2 cc. of 0.1 % amylase, added after the rest of the solution had been warmed up to 27°. Reducing sugars were determined in all systems after keeping for 1 hr. at 27°. The results (Table V) indicate that a retardation of reaction amounting to 29.1 % is brought about by addition of guanidine, whilst glycine accelerates reaction by from 7.6 to 26.8 %, according to its

Table V. *Simultaneous action of guanidine and glycine.*

Conc. of glycine %	Without guanidine		With guanidine	
	Maltose mg.	Acceleration %	Maltose mg.	Retardation %
0	15.8	—	11.2	29.1
0.25	17.0	7.6	12.4	21.5
0.50	18.1	14.5	13.4	15.2
0.75	19.2	21.5	15.2	3.8
1.00	20.0	26.5	16.6	- 5.0

concentration. Retardation due to guanidine diminishes progressively with increase in concentration of glycine, in 1 % solutions of which the reaction is accelerated by 5 %. It appears, therefore, that the processes of retardation due to guanidine, and of acceleration due to glycine proceed independently of each other, and that the resultant effect is the algebraic sum of the effects due to each factor separately. Thus guanidine in the concentration taken retards reaction by 29.1 %, whilst 0.25 % glycine accelerates it by 7.6 %; where the two substances are present together, the resultant retardation amounts to 21.5 %, or 7.6 % less than in the presence of guanidine alone. The same applies to systems containing 0.5 % glycine, whilst with 0.75 and 1 % glycine the summation of effects is not so exact.

SUMMARY.

1. The action of guanidine, creatine, and creatinine in enzymic amylolysis has been studied.
2. Guanidine and creatine retard, and creatinine accelerates, the reaction; the effect obtained is proportional to the concentration of the substance studied.

3. The effects obtained are of the same order in the hydrolysis of starch or glycogen by vegetable or by salivary amylase.

4. When both accelerating and retarding substances, *e.g.* glycine and guanidine, are present together, the resultant velocity of reaction is the mean of the velocities which would be obtained in the presence of each substance separately.

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