THE INFLUENCE OF CERTAIN CHEMICALS UPON AMYLASE ACTIVITY

by

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THE INFLUENCE OF CERTAIN CHEMICALS UPON AMYLASE ACTIVITY.

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Introduction

In a preliminary report of an investigation carried out in this laboratory, Clark, Fowler and Black (1) have shown that potassium thiocyanate, ethylene chlorohydrin and thiourea exert a definitely stimulating effect upon the activity of malt diastase. From the data obtained, it was suggested that the ability of these compounds to induce a renewal of growth in dormant plants (2), (3) was due probably to their action upon the enzymes concerned in the utilization of the food required for the initial growth and germination, and more particularly, the plant amylases.

In this report and in a more recent paper by Denny (4), it is stated however, that no increase in amylase activity was observed when such chemicals were added directly to the freshly expressed potato juice. From this, Denny concludes that the effect of the thiocyanate and chlorohydrin upon dormant plants is indirect in nature rather than a direct stimulation of the potato amylase.

In support of this conclusion, Denny further shows (5) that ethylene chlorohydrin while having no appreciable effect when added to the expressed juice directly, did produce a definite increase in amylase activity when the juice was expressed

from tubers treated with the chemical, several days previously. Potassium thiocyanate exhibited a corresponding effect only in the case of less dormant potatoes. He suggests that the power of such compounds to break dormancy may lie in their ability to stimulate the plant cells into the formation of a greater amount of enzyme, rather than the stimulation of that already present.

In addition to the work cited above, a considerable number of investigations have been carried out in other phases of amylase activity. As early as 1875, Nasse (6) reported that there was an important and specific dependance in the activity of ferments, upon the presence of salts, while Preti (7) reported that amylases from different sources were rendered practically inert by dialysis, but restored to activity by the addition of neutral electrolytes.

A large proportion of the experiments have been confined to the effect of amino acids and other protein products upon diastatic activity. Rockwood (8) has studied the effect of a large number of nitrogen compounds on salivary diastase and noted that the amino acids were accelerating in character while amides were not. Effront (9) also obtained similar results and ascribed this property to their amphoteric character im neutralizing some inhibitory product of hydrolysis. Diastase, generally believed to be protein in nature, rapidly deteriorates in an aqueous solution. This loss in activity is considered by Sherman and Walker (10) to be due to the hydrolysis of the enzyme. They suggest that the favorable influence exerted by

amino acids is due to the protection of the enzyme from hydrolysis. Sherman and Naylor (11) report that benzoic acid, hippuric acid, aniline sulfate and benzamide had little effect upon the activity of salivary and pancreatic amylase. Thus, neither the presence of the carboxyl and amino groups, alone, or together in the same molecule, was sufficient to accelerate the enzyme, but only compounds of the amino type were effective. That there was some truth in Rockwood's theory, was demonstrated by Sherman and Caldwell (12). In their experiments they had found that amino acids exerted a protective influence against the inhibitiom of pancreatic amylase by mercuric chloride. No explanof this was offered, but if, as Euler suggests (13) such toxicity is caused by the combination of the mercuric ion with the enzyme molecule, the amino acids may prevent this by combining with the metallic ion.

Up to the present time however, little experimental work has been carried out in determining the effect of specific chemical groups or ions upon amylase activity. Moreover, a review of the available literature has yielded comparatively little information concerning the effect of the concentration of the chemical 'activators', upon the enzyme activity. It was considered therefore, that such an investigation including a large variety of compounds over a wide range of concentrations, might prove of value in throwing further light on the mechanism of enzyme reactions and the relation of amylase activity to dormancy. In addition, because of the divergence in results presented in (1) and in a recent paper by Denny (14), it was considered necessary to

repeat the experiments with potassium thiocyanate and ethylene chlorohydrin and extending them to a wider range of concentrations.

As a further test of any relation existing between the ability of a compound to break the rest period of dormant plants and its effect upon diastatic activity, those compounds found to be most accelerating in character were employed in an experiment to determine their power in breaking the dormancy of potato tubers.

The usual methods of measuring diastatic activity fall into two general classes: those determining the rate of disappearance of the substrate, and those determining the quantity of reaction products formed. Of the first type, that most commonly used is the Wohlgemuth method (15), based upon the alteration in the color reaction with iodine, occurring during starch hydrolysis. Measurements of this type are an index of the liquefying or amyloclastic power of the enzyme. Of the second type, the usual copper reduction procedure is frequently employed in determining the rate of formation of reducing substances. This is an index of the saccharogenic power of the enzyme.

Whether the results obtained by the two methods are comparable is still a matter of some controversy. Most investigators claim that diastatic hydrolysis of starch is effected by at least two distinct enzyme fractions of diastase. As yet however, all attempts to separate the two fractions have been unsuccessful. Ohlsson (16) announced that by a brief heating of amylase solutions, it is possible to destroy entirely the saccharifying

component of the system while yet retaining the liquefying action. It is possible that in the case of amylase, instead of two distinct enzymes, the single enzyme molecule may contain two specific reactive groups, one concerned in the degradation of starch to dextrins and the other hydrolysing the dextrins to reducing bodies.

Of the two methods of measurement, the determination of the amyloclastic power may be the more basic, since before the formation of reducing sugars can occur, it seems evident that part of the starch at least must first be hydrolysed to the intermediate dextrins. In addition to this, the Wohlgemuth method is much more rapidly executed. Since one of the objects of this investigation was the testing of a large number of compounds, many of which might render the more complex copper reduction inaccurate or even impossible, it was decided to employ the former procedure, modified in some details, as a measure of amylase activity. However, for purposes of comparison with Denny's results (14), those compounds found most accelerating were further tested using the standard copper reduction procedure.

Part I

The Influence of the Concentration of Certain Chemical Compounds upon the Amyloclastic Activity of Malt Diastase.

Experimental Procedure:

As a source of the enzyme, malt diastase, U.S.P. IX, prepared by Eimer and Amend, was used. For each day's experiments, 1.0 gram of the enzyme material was extracted for 30 minutes at room temperature with 500 ccs. distilled water, after which all

solid residue was filtered out as rapidly as possible, through a folded filter.

As substrate, soluble starch (Baker's, C.P.) was employed, one lot being used exclusively throughout the entire investigation. As in the case of the enzyme extract, a fresh solution was prepared each day by mixing 15.0 grams of the starch with 100 ccs distilled water and heating in a water bath till solution was complete. This was finally diluted to a volume of 200 ccs.

The reaction mixture consisted of: 7.5 ccs. of McIlvaine's citric acid - phosphate buffer (17), 1.5 ccs. enzyme extract, 6.66 ccs. soluble starch solution and varying proportions of a solution of the chemical under examination. The whole was then made up to a volume of 25.0 ccs. This mixture after being placed in test-tubes and corked, was incubated in an electric oven at a temperature of 30°C., for the course of the experiment.

Starting approximately two hours after the beginning of the experiment, the rate of disappearance of starch was observed by withdrawing a few drops of the reaction mixture from time to time and testing with N/200 Iodine on a porcelain spot plate. The length of time required for the hydrolysis to reach the achromic point, or the point at which no color other than that of the iodine was apparent, was noted in each case. Sufficient iodine was added to produce a well-defined color reaction, although care was taken in its addition, for an excess obscured the end point.

As Denny (14) and Miller (18) have pointed out, certain

compounds, particularly potassium thiocyanate and thiourea react with the iodine and are likely to render the starch-iodine test inaccurate. In the case of the thiocyanate it was found that by adding a few drops of dilute hydrochloric acid to the mixture before the iodine, little difficulty was experienced. Thiourea however, absorbed iodine under acid and alkaline conditions. It was found necessary to use a larger volume of the reaction mixture and to add sufficient N/50 iodine solution to combine completely with the thiourea before the starch reaction could be observed. Even under these circumstances, it was difficult to obtain accurate readings.

The quantity of enzyme employed was sufficient to carry the hydrolysis to the achromic point in approximately four hours. It was considered more satisfactory to complete each run in a single day, in order to avoid the use of preservatives such as toluene, which, apart from its inhibitory effect (19) might react with certain of the chemicals in use. Preliminary experiments also indicated that constant shaking, particularly when toluene was added, decreased the rate of hydrolysis appreciably, a point noted by Schultz and Landis (20) in their experiments with vegetable amylases.

Chemically pure compounds were used throughout and all glassware after cleaning with chromic acid, thoroughly rinsed with distilled water before use. All pH measurements were made with the quinhydrone electrode in all but a few instances when the colorimetric method was used. Compounds having too great an effect upon the reaction of the medium, even in the

presence of the buffer, were omitted from further tests.

Preliminary experiments indicated that for this sample of malt diastase at least, the optimum reaction range lay between pH 6.0 and pH 6.5, although the optimum range usually given is pH 4.0 - 4.5 (21). It is quite probable however, that the optimum reaction varies with the type of buffer employed, as well as with the temperature. (21). The buffer mixture used, was adjusted toa pH of 6.2. On addition of the starch solution to the reaction mixture, a pH of 6.4 finally resulted. No pH variations were greater than ±.15.

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Results

The values redorded in Table I represent the decrease (acceleration) or increase (inhibition) in time, in minutes, required for the hydrolysis of the starch in the presence of the chemicals listed, as compared to the controls. The average time required for the appearance of the achromic point in the control tubes was approximately 245 minutes. Daily variations in the activity of the enzyme were balanced by comparing each day's experiments with the controls for that day only. All values presented are the mean of several readings. In cases where inhibiting compounds had so delayed the reaction that it was impossible to obtain a value for the achromic point that day, the minimum degree of inhibition is recorded. This was done only where hydrolysis was apparent, as evidenced by the red or purple coloration of the intermediate dextrins with iodine. In cases where the compound was so strongly inhibiting that no

dextrins could be detected by means of the iodine test, but only the blue color of the starch was apparent, the effect is recorded as 'complete inhibition'.

TABLE I

Variation in the activity of Malt Diastase in the presence of certain chemical compounds.

COMPOUND	CONC. %	ACCEL. (Minutes)	INHIB. (Minutes)
Potassium Bromide	0.01	67	
	0.03	100	
	0.10	121	
	1.0	120	
Potassium Chloride	0.0004	0	
	0.001	35	
	0.004	80	
	0.01	98	
	0.03	115	
	0.10	133	
	1.0	140	
	2.0	150	
Potassium Iodide	0.01		14
	0.03		18
	0.10		63
	2.0		180
Potassium Nitrite	0.005	35	
	0.01	55	
	0.05	87	
	0.20	102	
	0.40	120	
	2.0	137	
	3.0	125	
Potassium Nitrate	0.01	30	
	0.03	41	
	0.10	34	
	0.60	15	
Potassium Sulfate	0.01	0	
	0.03	0	
	0.10	2	
	0.60	13	
	1.0	12	

COMPOUND	CONC. %	ACCEL. (Minutes)	INHIB. (Minutes)
Potassium Thiocyanate	0.10	37	
	0.50	80	
	0.75	90	
	1.0	100	
	1.5	43	
	2.0	9	
	3.00		38
Ethylene Chlorohydrin	0.24	17	
	0.60	38	
	1.20	55	
	2.48	71	
	3.72	80	
	4.96	80	
	6.20	53	
	6.80	7	
	7.44		78
Epichlorhydrin	0.80		2
	1.6		16
	2.4		42
Glycerol MoDichlorhydrin (Symmet.)	0.05	31	
	0.22	72	
	0.65	96	
	1.0	120	
	1.64	1322	
Glycerol Monochlorhydrin (Symmet.)	0.26	79	
	1.32	122	
	4.0	149	
	5.30	88	
	8.0	32	
Chloral Hydrate	0.20	0	
	1.0	8	
	2.0	10	
Dichlorethylene	0.025	0	
	0.10	0	
	.0375	2	
Dichlor ethyl ether	0.23		23
	0.46		74
	1.20		>130
Chloroform	0.06	6	
	0.27	0	0
	0.90		39

COMPOUND	CONC. - %	ACCEL.	INHIB.
Ethyl Iodide	0.10	3	
	0.20	11	
	0.30		21
Ethyl Bromide	0.218	9	
	0.58	12	
	1.16	16	
	1.45	22	
n. Propyl Bromide	0.27	6	
	0.54	22	
	1.36		10
Ethylene Glycol	0.44	0	
	0.90	5	
	2.23	7	
Propylene Glycol	0.41	0	
	0.84	2	
	2.10	2	
	3.10	12	
Trimethylene Glycol	0.21	1	
	0.42	10	
	1.02	14	
	2.05	22	
	3.10	29	
Methyl Alcohol	0.47	5	
	1.60	3	
	6.38		34
	15.90		160
Ethyl Alcohol	0.80	1	
	3.16		6
	9.50		13
	15.8		120
Allyl Alcohol	0.56		8
	1.13		15
	3.50		52
	6.90		> 120
	17.10		> 120
Acetone	0.31	0	
	0.80	20	
	1.58	30	
	3.16	23	
	6.32	2	
	9.50		12
	15.80		135

COMPOUND	CONC. %	ACCEL.	INHIB.
Maltose	0.20	15	
	0.50	14	
	1.00	11	
	2.00	5	
	3.00		30
Glucose	0.50	0	
	1.0		5
	2.0		10
	3.0		34
Ethyl Acetate	0.90	2	
	1.80	5	
	3.58		21
	7.20		>120
β Brom Ethyl Acetate	0.30	58	
	0.90	53	
	1.50	47	
	3.0		10
Alanine	0.08	40	
	0.24	77	
	0.40	91	
	1.20	118	
Glycine	0.10	19	
	0.50	24	
	1.50	40	
Tyrosine	0.004	2	
	0.02	10	
	0.03	20	
Acetamide	0.08		9
	0.20		13
	0.40		16
	0.60		16
	0.80		27
	1.20		46
Propionamide	0.08		1
	0.40		6
	0.80		9
	1.20		13
n.Butyramide	0.04	0	0
	0.08		0
	0.20		15
	0.60		21
	1.20		62

COMPOUND	CONC. %	ACCEL.	INHIB.
Oxamide	0.005	3	
	0.010	8	
	0.0375	26	
Urea	0.10	11	
	0.50	3	
	1.00		9
	1.50		20
Thiourea	0.10	20	
	0.50	48	
	1.00	77	
	2.00	63	
Phenyl Urea	0.015	0	
	0.045	6	
	0.225	25	
Phenyl Thiourea	0.005	2	
	0.020	12	
	0.05	23	
	0.075	37	
Tolyl Thiourea	0.01	12	
	0.10	38	
Methyl Urea	0.16	26	
	0.40	39	
	1.20	56	
Creatine Hydrate	0.04	0	
	0.20	10	
	0.60	24	
Pyridine	0.20	12	
	0.80		85
	5.90	Complete	Inhib.
Piperidine	0.070		24
	0.17		140
Pyrrol	0.01	16	
	0.06	22	
	0.09	27	
	0.17	30	
Succinimide	0.02	0	
	0.08	4	
	0.30	20	

COMPOUND	CONCN. %	ACCEL.	INHIB.
Acetonitrile	0.16	0	
	0.60	0	
	1.60	8	
	2.40	15	
Phenol	0.10	12	
	0.20	0	
	0.60		130
	1.00		225
o. Chlorphenol	0.012		3
	0.062		6
	0.124		15
	0.31		100
	0.62	Complete	Inhib.
	0.93	"	"
p. Cresol	0.10		40
	0.40		80
	0.60		>120
	0.80		>120
Hydroquinone	0.10		38
	0.50		>170
	1.00	Complete	Inhib.
Phloroglucinol	0.04		17
	0.20		100
	0.60		>200

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Part II

The influence of certain chemicals upon the saccharogenic activity of Malt Diastase.

Experimental Procedure

The method employed differed from that described above only in decreasing the quantity of enzyme extract added to the

reaction mixture, from 1.5 to0.75 ccs. The tubes containing the hydrolysing mixture were incubated at 30°C. for a period of four hours, rather than the longer period of eighteen hours used by Denny (14) for the reason that any acceleration due to the added chemicals would be more evident in the early stages of hydrolysis than when the reaction had almost reached equilibrium. Preliminary experiments indicated that this was actually the case.

At the end of the four hour period, 5.0 cc. volumes of the reaction mixture were pipetted into 20.0 ccs. Fehling's solution and the reducing sugars determined by the standard Munson and Walker procedure (22). Cuprous oxide was determined by the volumetric permanganate method. From the cuprous oxide values thus obtained were deducted those from blank determinations obtained by the same procedure except that 0.75 ccs. boiled enzyme solution were added.

It was found impossible to apply this method to mixtures containing thiourea however, owing to the decomposition of the latter compound on heating with Fehling's solution with the formation of a copper sulfide. Attempts to use the colorimetric picric acid method were equally unsatisfactory.

The values obtained are presented below.

Results

In Table II Amylase activity is expressed in terms of milligrams of cuprous oxide. Each value given is the mean of several determinations. As a further means of comparison with the Wohlgemuth method, the colors noted on the addition of iodine

to a few drops of the reaction mixture at the end of the four hour period, are recorded.

The effect of the concentration of certain of the compounds upon the activity of the enzyme, is represented graphically in Figures 1 and 2.

TABLE II

COMPOUND	CONC. %	Mgs. Cu_2O	Color Reaction
CONTROL	00	33.8	Blue
Alanine	0.10	36.1	Blue
	0.20	38.9	Blue violet
	0.50	44.5	Violet
	1.00	51.8	Violet
	1.50	52.9	Red violet
	1.80	53.6	Violet red
	3.60	56.4	Red
β Brom ethyl acetate	0.015	38.5	Blue violet
	0.06	40.4	Blue violet
	0.15	42.2	Violet
	0.30	46.5	Violet
	0.45	50.2	Violet red
	0.60	49.6	Violet red
	1.20	45.2	Violet
	1.50	33.4	Blue violet
Glycerol Monochlorhydrin			
	0.33	36.1	Blue
	0.53	48.1	Red
	0.66	49.6	Red
	1.32	50.6	Red
	2.65	50.1	Red Violet
	4.00	46.5	Violet
	5.30	43.7	Violet
	7.80	36.2	Violet blue
	8.50	33.2	Blue
	10.50	28.8	Blue
	13.20	13.1	Blue

COMPOUND	CONC. %	Mgs. Cu_2O	Color Reaction
CONTROL	00.00	33.8	Blue
Ethylene Chlorohydrin	0.62	36.2	Blue
	1.24	38.7	Blue violet
	2.48	39.5	Violet
	3.71	39.5	Violet
	4.95	35.6	Violet
	5.60	35.1	Violet
	6.20	29.7	Violet blue
	7.44	23.9	Blue
	8.66	19.4	Blue
	9.60	13.2	Blue
Potassium Thiocyanate	0.01	33.9	Blue
	0.05	35.2	Blue
	0.10	38.4	Blue violet
	0.20	41.8	Blue violet
	0.50	42.3	Violet
	1.00	41.8	Blue violet
	1.50	38.4	Violet
	2.00	35.6	Blue violet
	2.50	32.1	Blue violet
	3.00	30.2	Blue violet
	3.60	25.9	Blue violet
Potassium Nitrite	0.005	39.2	Violet blue
	0.01	40.0	Violet
	0.05	48.7	Red violet
	0.10	50.3	Red violet
	0.25	50.9	Red
	0.50	52.1	Red
	1.00	52.5	Red
	2.00	52.9	Red
	3.00	54.0	Red
Potassium Chloride	0.00025	37.8	Blue violet
	0.0005	40.5	Blue violet
	0.001	43.1	Blue violet
	0.005	53.1	Violet
	0.01	51.7	Red violet
	0.02	52.4	Red violet
	0.05	54.1	Red
	0.50	54.4	Red
	1.00	55.8	Red
	2.00	55.8	Red
	6.00	60.0	Red
	10.00	54.2	Red violet
	15.00	41.0	Blue violet
	20.00	38.1	Blue
	25.00	35.6	Blue

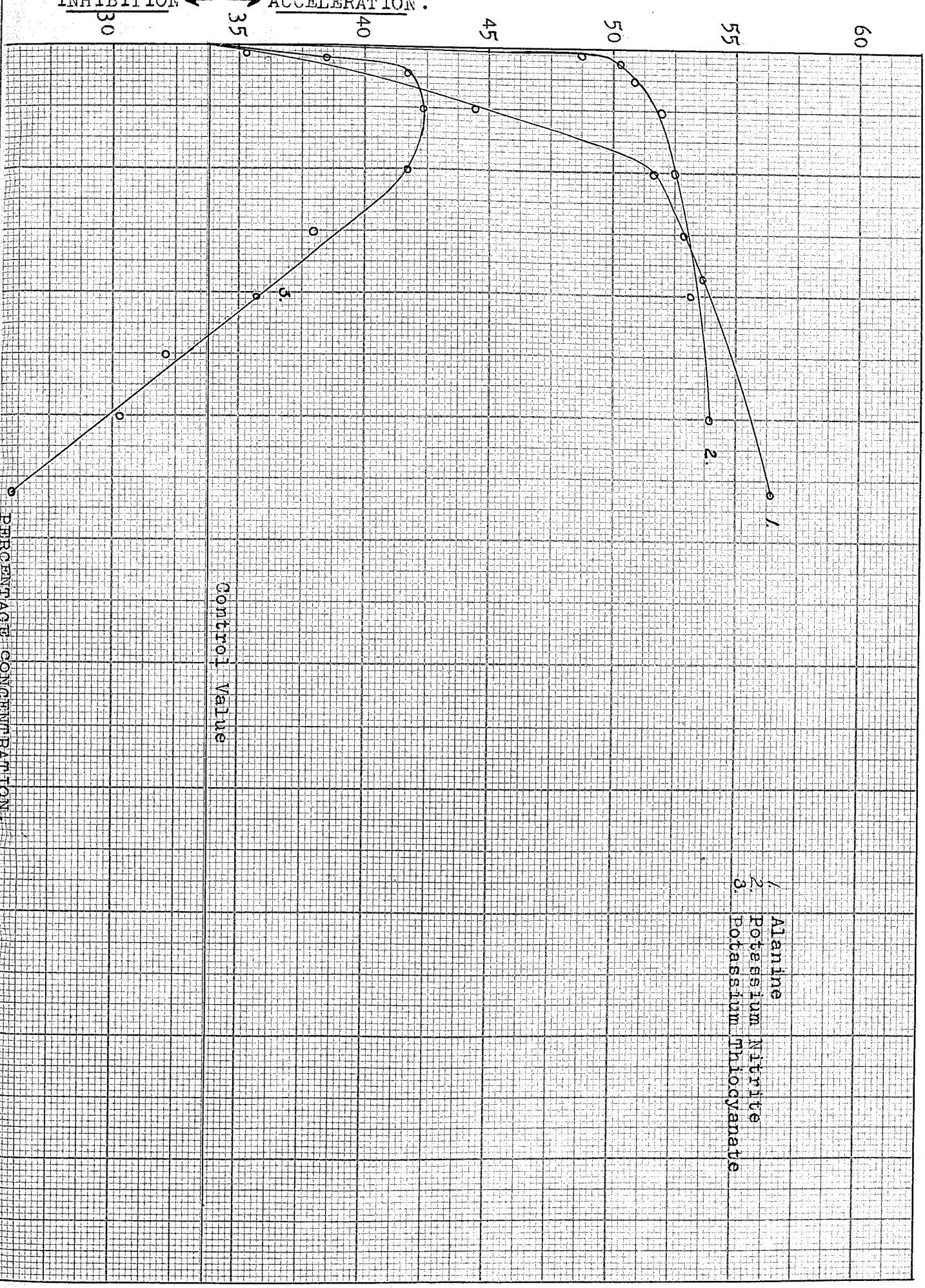
AMYLASE ACTIVITY in Milligrams Cuprous Oxide.
INHIBITION ← → ACCELERATION.
30
35
40
45
50
55
60
Control Value
PERCENTAGE CONCENTRATION
1. Alanine
2. Potassium Nitrite
3. Potassium Thiocyanate

Figure 1.

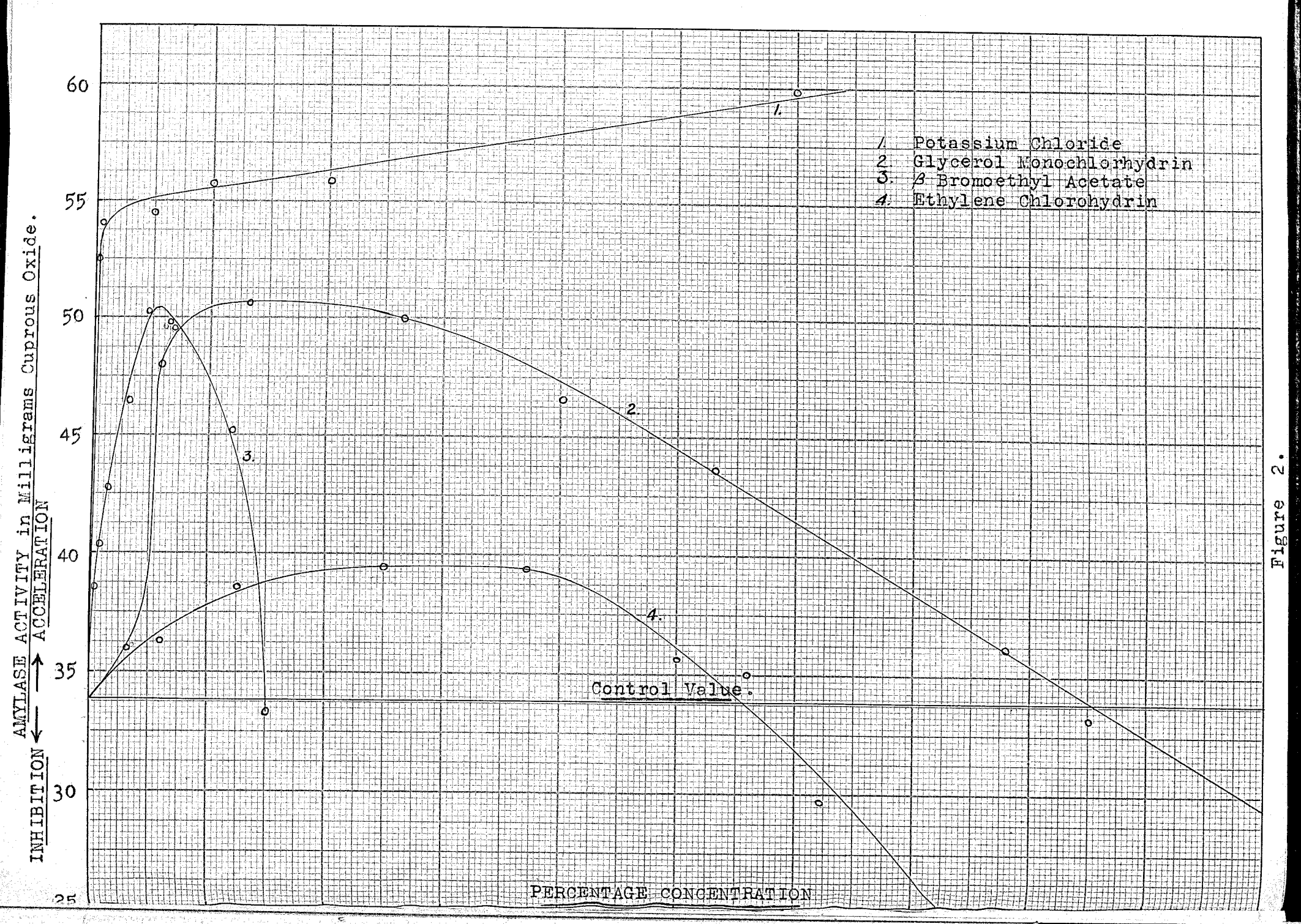
AMYLASE ACTIVITY in Milligrams Cuprous Oxide.
INHIBITION ← → ACCELERATION
60
55
50
45
40
35
30
25
1.
2.
3.
4.
5.
Control Value.
PERCENTAGE CONCENTRATION
1. Potassium Chloride
2. Glycerol Monochlorhydrin
3. β Bromoethyl Acetate
4. Ethylene Chlorohydrin

Figure 2.

Part III

The effect of certain chemicals found to increase amylase activity, upon dormant potato tubers.

In order to examine more closely the possible relationship between enzyme activity and dormancy in plants, certain compounds found to be most active in the stimulation of malt diastase in the experiments described above, were further employed in a series of tests upon dormant potato tubers.

In the case of the non-volatile compounds, particularly the inorganic salts, the soak method described by Denny (2) was used exclusively. With the more volatile organic compounds both the soak and the dip methods were employed. As plant material, Certified Seed Potatoes of the Netted Gem and Up-to-Date varieties, harvested in mid-October and varying in weight from 4 to 6 ounces, were used.

Before treatment, the tubers were first washed to remove all adhering soil. The selected tubers were then cut longitudinally from stem to seed end into seed pieces of approximately 2 ounces in weight. This method of cutting was used in order to avoid any possible variations due to the more rapid germination of the seed end.

Five seed pieces of each variety from as many individual tubers, were selected for treatment. In the soak treatment, these were soaked in approximately one litre of a 1.5 % solution of the salt, in the case of the inorganic compounds and alanine, for a period of one hour at 21°C. After treatment

the seed pieces were removed, drained and planted without rinsing, in sandy loam in flats at a depth of 1½ inches. These were left under greenhouse conditions for the duration of the experiment, water being added to keep the soil sufficiently moist.

In the dip treatment, seed pieces prepared as above were completely immersed in a 1.0 % solution of the chemical under examination and shaken for one minute, after which the excess liquid was drained off, the jars sealed tightly and left at a temperature of 25° for 24 hours. At the end of this time, the seed pieces were planted in greenhouse flats.

Controls were prepared for both the dip and soak treated tubers, using in place of the different solutions, distilled water.

After a period of 70 days from the date of planting, a comparison in the relative amount of growth from treated tubers and controls, was made. For this purpose, the plant tops were cut off at soil level and the green weight of the tops taken as an index of growth.

Results

The values recorded in Table III are the total green weights of all growth from the five seed pieces included in each treatment. As an additional means of comparison, the number of tubers germinated out of the five, is recorded in each case.

TABLE III

The effect of certain Amylase stimulating compounds upon the germination of dormant potato tubers.

COMPOUND	NUMBER GERMINATED	GREEN WEIGHT OF TOPS - GRAMS
NETTED GEM VARIETY		
(Dip Treatment)		
Control	2	44.0
Ethyl Acetate	2	1.0
β Bromoethyl Acetate	5	210.0
Glycerol Monochlorhydrin	2	14.7
(Soak Treatment)		
Control	3	27.4
Ethyl Acetate	3	94.2
β Bromoethyl Acetate	5	235.0
Glycerol Monochlorhydrin	2	73.3
Alanine	4	75.2
Potassium Bromide	2	10.2
Potassium Chloride	4	53.0
Potassium Thiocyanate	5	104.3
Potassium Nitrate	4	76.3
Potassium Nitrite	3	38.7
**********		
UP-TO-DATE VARIETY		
(Dip Treatment)		
Control	2	43.2
β Bromoethyl Acetate	4	75.3
Glycerol Monochlorhydrin	3	97.0
(Soak Treatment)		
Control	1	40.2
β Bromoethyl Acetate	2	97.1
Alanine	4	61.0
Glycerol Monochlorhydrin	3	71.2
Potassium Bromide	3	74.4
Potassium Chloride	4	151.3
Potassium Nitrate	1	2.4
Potassium Nitrite	2	6.5

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Discussion

From the data presented in Table I, it may be seen how diverse are the effects of compounds of various types upon the activity of malt amylase. On the basis of their effects upon diastatic activity, these compounds may be placed in three distinct classes: compounds having a definitely accelerating effect; compounds having an inhibitory action; and compounds exerting little influence in either direction.

This classification however, is subject to one very important restriction,- the concentration employed, a factor which apparently many workers have neglected. It is evident from the results listed, that the effect produced by a chemical compound is largely dependant upon its concentration in the reaction mixture. Thus, many compounds are accelerating at one concentration but inhibiting at another.

Most accelerators exhibited their maximum effect between definite limits of concentration; on either side of this the enzyme activity may be appreciably less. This optimum effect is represented graphically in Figures 1 and 2. Certain of the compounds studied however, such as Potassium Chloride and Alanine, were definitely stimulating at all concentrations employed. Others were either slightly accelerating or had little effect at lower concentrations, but became increasingly inhibitory with the higher concentrations. Of this type were the alcohols and amides. Still others, such as maltose, ethylene glycol and acetonitrile, had little effect at any concentration.

With regard to specific ions or groups, a few generalizat-

ions may be drawn.

Of the inorganic compounds, those containing halide anions were by far the most active. In addition, from the values given in Table I, it is evident that the stimulating properties decreased with the increase in atomic weight of the halogen. The chloride ion was strongly accelerating while the iodide was definitely inhibiting, even at low concentrations.

A similar variation in activity was displayed by the organic halogen compounds. Glycerol monochlorhydrin and ethylene chlorohydrin greatly increased the diastatic activity while the ethyl iodide produced an appreciable decrease. Ethyl acetate in concentrations below 1.0% had little effect but on the substitution of bromine, became strongly accelerating, even below 0.5%.

At high concentrations however, all these latter compounds became inhibiting. The predominance of other more unfavorable properties masks the effect of the halogen constitutent. This may also be the case with chloroform, dichlorethyl ether, dichlorethylene, epichlorhydrin and chloral hydrate, all of which were observed to produce either little effect or a definite inhibition.

In view of the high degree of acceleration brought about by the chloride ion, it seems probable therefore, that the ability of the chloro-organic compounds to stimulate diastatic activity, is due to the same factor. Organic compounds of this type would undoubtedly dissociate sufficiently to liberate the extremely low concentration of chloride ions required. Even a concentration of 0.0005% KCl exerts an appreciable effect upon the rate of starch hydrolysis.

This marked effect of inorganic chlorides has long been known and many investigators claim that traces of such anions are essential for the enzymatic hydrolysis of starch. Waksman and Davison (21) describe the chloride ion as a 'co-enzyme' or specific activator. Chrempinska (23) reports that chlorides produce a definite increase in diastatic activity, particularly at pH's above the optimum range for the enzyme. At pH values below the optimum, a decrease is produced.

In consideration of the investigations cited above and the results presented herein, the explanation of the data recorded by Denny (14) in a recent paper, is extremely difficult. Only in the case of pancreatin do his results indicate any increase in amylase activity upon the addition of NaCl to the reaction mixture. This is true even when the enzyme solution had been previously dialysed, in which case the addition of an electrolyte would be expected to produce an even more marked effect.(7).

Moreover, Denny states that in no case was any increase in enzyme activity observed upon the addition of ethylene chlorohydrin. This is a further divergence from the results recorded in this present paper and in that previously published by Clark et al(1). In Denny's experiments however, the hydrolysis was allowed to continue for the relatively long period of eighteen hours. It seems likely therefore, that his failure to obtain an increase in amylase activity in the presence of NaCl and ethylene chlorohydrin, may be due to the fact that the hydrolysis both in the controls and in the mixtures containing the chemical, had almost reached the equilibrium point. It is probable that any accelerating effect would be more apparent in the early stages of the react-

ion, than toward the end.

In the second part of the latter paper, Denny states that relatively large amounts of NaCl were added to the reaction mixture in order to minimize the influence of small traces of chlorides present in the ethylene chlorhydrin. However, in view of the fact that the stimulating effect of ethylene chlorohydrin and other similar compounds, upon malt diastase, is likely due to their slight dissociation into chloride ions, the addition of NaCl would defeat the object of the experiment. The activity of the chlorohydrin would be entirely masked by the presence of the NaCl. In addition, the latter compound, with its high ionization, would appreciably decrease the dissociation of the chlorohydrin by the common ion effect.

Of the other inorganic anions studied, the sulfate and the nitrate had little effect upon the amyloclastic properties of malt diastase. Potassium nitrite however, was almost as efficient an accelerator as the chloride.

Alanine and glycine were found to be definitely accelerating in their action and tyrosine only slightly so, although its low solubility prevented its use in higher concentrations.

Acetamide and the other amides recorded exerted little influence in the lower concentrations but were appreciably inhibiting at higher concentrations. These observations including amino acids and amides are in accordance with the previously cited work of Rockwood (8).

Urea and phenyl urea had little effect in either direction. It is apparent therefore, as Sherman and Naylor (11) have pointed out, that the presence of the NH_2 group in a compound is

insufficient to produce enzymatic 'activation'. Its position in the molecule is of first importance.

On replacement of the oxygen of the urea compounds, by sulfur, the resulting compounds such as thiourea, phenyl thiourea, and tolyl thiourea, became definitely accelerating. Methyl urea was observed to have a similar effect, but whether this is due to the presence ofbthe methyl group, is uncertain.

Potassium thiocyanate was found to be definitely stimulating in its action upon malt diastase by both the Wohlgemuth and the copper reduction methods. Contrary to the results published in the previous paper by Clark, Fowler and Black (1), concentrations above 2.0% KSCN were appreciably inhibiting. Maximum acceleration, by the copper reduction method appeared to be at approximately 0.5% while by the iodine method, it was nearer 1.0%.

Both Denny (14) and Miller (18) report that the acceleration is apparent only in the alkaline reaction range. Under acidic conditions, inhibition is produced. Denny's results, as do those in Table II, indicate that a concentration of KSCN of 2.5% is definitely inhibitory at a pH of 6.4. However, had Denny carried out his experiments over a wider range of concentrations he would no doubt have obtained an appreciable acceleration with the KSCN at even lower pH values.

Hydroxy compounds in general were found to decrease amylase activity to a great extent. In this respect, members of the aromatic series were considerably more active than those of the aliphatic series. Methyl and ethyl alcohols were inhibiting at concentrations above 8.0% while phenol, cresol, hydroquinone and phloroglucinol were equally effective at concentrations of 0.3%

Waksman and Davison (21) attribute this property of alcohols to their effect upon the degree of dissociation of the enzyme and to the influence upon the colloidal state both of the enzyme and the substrate. It would seem evident therefore, that the inhibition induced by the higher concentrations of ethylene chlorohydrin is due to a similar cause, since the chlorohydrin and ethyl alcohol are such closely related compounds.

A qualitative comparison of the data obtained by the Wohlgemuth and the copper reduction methods of determining diastatic activity, yields no outstanding points of difference. In Table II, only in the case of the three highest concentrations of KSCN are colors observed which do not correspond to the reducing sugar data. The colors noted, in comparison with the blue of the control, would suggest that the hydrolysis had proceeded to a point further in advance of that indicated by the accompanying copper reduction values. Similar observations were made by Johnson and Wormall (24), who thus concluded that the thiocyanate exerted its accelerating effect only in the earlier stages of hydrolysis. It is possible however, that the discrepancies observed may be due to the effect of the thiocyanate upon the starch-iodine color reaction, rather than the preferential stimulation of the amyloclastic activity of the enzyme. The data obtained is insufficient to draw any accurate conclusions regarding this aspect of the problem.

Curve 1. in Figure 2. illustrates clearly, the sensitiveness of amylase preparations to extremely low concentrations of chlorides. The relatively higher concentrations of the chloroorganic compounds required to produce an equivalent effect, is

no doubt due to their much lower dissociation, as well as the parallel inhibitory effect exerted by other constitutents of the molecule. All compounds of this type show a fairly wide optimalconcentration range. β Bromoethyl acetate however, exerts its maximum effect only between very narrow limits.

In Figure 1. the effect of KSCN and KNO_2 is rather more obscure. They may, like the chlorides, have some influence in assisting in the dissociation of the enzyme molecule into the active cation, believed to be directly responsible in starch hydrolysis.

While the data presented in Table III cannot be considered significant, from a statistical standpoint owing to the relatively small numbers of tubers used in the various treatments, they do assist in drawing further conclusions regarding the relation between enzyme activity and dormancy.

In most cases, treatment of the dormant tubers with those chemicals found to exert a marked influence upon malt diastase, did result in much earlier germination of the treated tubers and in increased growth by the end of the experiment.

The two varieties of potatoes used in these tests, showed certain variations with respect to the different chemicals.

With seed pieces of the Netted Gem variety, β bromoethyl acetate was by far the most effective, both in the number of germinated seed pieces and in the total amount of growth produced, The next most effective was KSCN. Alanine, KCl, and KNO_3 all induced germination in four out of the original five seed pieces. Glycerol Monochlorohydrin was equally effective with respect to the green weight of the tops but caused germination in only two

of the treated pieces. KNO_2 while having a marked effect upon the enzyme, had little effect upon the dormant tubers.

Using the Up-to-Date variety, KCl was most effective. Glycerol monochlorohydrin and β bromoethyl acetate were slightly less efficient than the chloride, but were equivalent in their effect. KBr and alanine, as with the Netted Gem tubers, were also capable of breaking the dormancy.

Thus, with the exception of KNO_2, all compounds found to stimulate the activity of malt diastase, produced a corresponding response in dormant tubers.

In view of the failure of chemicals, known to be extremely active in breaking dormancy, to produce a similar increase in the amylase activity of potato juice, Denny concludes that the action of these compounds is essentially indirect in nature. As further evidence for this conclusion, he has shown that ethylene chlorohydrin, while having little influence upon the diastatic activity when added directly to the expressed potato juice, did exhibit an appreciable effect when the tubers were first treated with the chemical and the juice expressed after several days. In addition to this, Denny has been unable to duplicate the results of Clark et al (1) with ethylene chlorohydrin and KSCN and claims therefore, that his previous conclusions require no modification.

As the writers of the latter paper have pointed out, the fact that these compounds have little effect upon the activity of the potato juice, does not necessarily preclude the possibility that a direct as well as an indirect effect may exist. It may be possible that the failure to obtain any increase in amyl-

ase activity is due rather to the parallel activation of antagonistic enzymes or to side reactions of the chemicals employed with other constituents of the potato juice.

As Denny suggests, the relationship between dormancy-breaking chemicals and the various enzyme systems of living cells is undoubtedly very complex. Moreover, the ability of a compound to induce a renewal of growth in dormant plants must certainly be dependant upon a variety of factors.

That the activation of the amylase of resting plants is one important function at least, of the chemicals capable of inducing germination, is obvious from the experiments herein described. Under proper conditions of concentration and reaction, those compounds most efficient in breaking the rest period of plants, bring about a definite increase in the activity of malt amylase.

That a relationship of some kind exists between the two distinct properties of the chemicals in question, is further indicated by the results tabulated in Table III. Alanine, Glycerol monochlorhydrin and β bromoethyl acetate, hitherto untried in their effect upon dormant plants, were found to be equally as efficient in that respect as the ethylene chlorohydrin and KSCN first reported by Denny. These chemicals were selected for the germination tests on the basis of their ability to stimulate malt diastase. It seems apparent therefore, that the effect of chemicals in inducing sprouting is more direct in some phases at least, than Denny's conclusions would indicate.

The foregoing results and their discussion have yielded little information of value concerning the mechanism of enzyme

reactions and their relation to dormancy in plants. It is hoped however, that the data presented may prove of value in suggesting further methods of approach to these and related problems.

SUMMARY

1. The effect of a number of organic and inorganic compounds in varying concentrations, upon the amyloclastic and saccharogenic activity of malt amylase, has been recorded.

2. On the basis of their influence upon amylase activity, the chemicals studied have been classified as: accelerators, inhibitors, or compounds having little effect. Whether a compound is accelerating or inhibiting in nature has been found to be largely dependant upon its concentration in the reaction medium.

3. The high degree of stimulation noted in the case of the inorganic chlorides, has led to the suggestion that the similar effect exerted by chloro-organic compounds is due to their partial dissociation, with the liberation of chloride ions.

4. Of the nitrogen compounds examined, amino acids exhibited the greatest acceleration. Amides were appreciably inhibiting in their action. Urea compounds were effective accelerators only when the oxygen was replaced by sulfur, forming the corresponding thio-compound. Potassium nitrite was exceedingly stimulating in character, while the nitrate was only slightly so.

5. No evidence was obtained which would suggest that any of

the compounds stimulated preferentially either the amyloclastic or the saccharogenic phases of starch hydrolysis.

6. Ethylene chlorohydrin and Potassium thiocyanate, compounds known to be effective in breaking the rest period of plants, have been shown to be accelerating in their effect upon malt diastase. This data is presented as evidence of the possible relationship between amylase activity and the breaking of dormancy.

7. Earlier germination and growth of dormant potato tubers has been brought about by treatment with certain chemicals previously found to exert a stimulating effect upon the activity of malt diastase. This data is presented as further evidence of a direct relation between the ability of a compound to induce germination and its effect upon the plant amylase.

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NOTE: References marked (*) cited in (25).

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