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**EFFECT OF VARIOUS CONCENTRATIONS
OF THIAMIN HYDROCHLORIDE ON THE DIASTASE
CONTENT OF CIGER ARISTIFOLIUM**

by
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TABLE OF CONTENTS

	PP.
I. Introduction and Choice of Problem	1-2
II. Review of Literature	3-5
III. Experimental Methods	6-19
A. Culture of Plants	
1. Culture Media	
2. Effects of Light and Darkness	
a) Direct sunlight	
b) Diffuse light	
c) Darkness	
3. Germination and Concentrations of Thiamin Used.	
B. Extraction of Diastase	
1. Methods of Extraction	
Method a - By precipitation with alcohol alone.	
" b - By precipitation with $(NH_4)_2 SO_4$	
and alcohol.	
2. Measurement of Activity of Diastase Containing	
Extract.	
IV. Experimental Results	20
V. Interpretation of Results	22
VI. Conclusion	25
VII. Bibliography	24-25

ABSTRACT

EFFECT OF VARIOUS CONCENTRATIONS OF
THIAMIN HYDROCHLORIDE ON THE
DIASTASE CONTENT OF CICER ARIETENUM

Newly germinated seeds of *Cicer arietinum* (chickpea) were grown in water culture with various concentrations of thiamin hydrochloride. Nine days after germination the effect of the various concentrations of thiamin hydrochloride on the length, and fresh and dry weights of roots; and fresh weight of shoots was investigated. There was practically no effect of the chemical on the shoots. There was a definite inhibition of the growth in length of roots with high concentrations and a definite promotion with low concentrations of thiamin hydrochloride. There was also a definite decrease of fresh and dry weights with high concentrations and a definite increase with low concentrations.

As for the effect of thiamin hydrochloride on the diastase content, which was the main theme of the work, there was practically no detectable effect with lower concentrations, whereas with a high concentration, namely 1/20,000, there was a definite decrease in amount and power of activity of diastase.

I. INTRODUCTION AND CHOICE OF PROBLEM

There have been several views about the mechanisms of action of growth promoting substances in plants. Some of these are: effects of these substances on the cell wall by producing a change in its plasticity; effects on the rate of respiration; effects on the protoplasmic streaming; effects on the uptake of water, etc. (10).

Hydrolytic enzymes are claimed to promote growth through hydrolysis of pectin (10).

Seibert (1925) was able to prove that agar containing saliva, diastase, and malt extract caused a promotion of growth. On boiling these preparations for 15 minutes, most of the activity remained, but she still concluded it was connected with enzymic activity(17).

Gerter (1927) also found growth promoting activity in numerous enzyme preparations (17).

Bouillenne and Went (1938) found that when diastase (which was also active after being boiled) or extract of rice polishings was applied to the cuttings of *Acalypha* root, promotion was increased (18). However, Gouwentak and Hellinga (1935) were afterwards unable to obtain rooting by the application of diastase (18).

The application of auxin to young tissues, specially in high concentrations, results in the swelling of the tissue. In woody cuttings these swellings have their counterpart in the

formation of callus at the basal cut surface (19). Such callus formation, which involves both cell enlargement and division, was observed in *Acalypha* cuttings by Bouillenne and Went (1933) after application of diastase or extract of rice polishings (19). They concluded that the callus was caused by a special substance, analogous to, but probably not identical with the root-forming hormone (19).

From the above mentioned findings one is inclined to think that there is some relation between growth promoting substances (or a growth promoting substance) and diastase. This relation might be;

a) The diastase preparations might contain some growth promoting substances. The heat stability, in part, of the growth promoting property of diastase preparations indicates this.

b) The diastase itself might have something to do with promotion of growth by itself or in conjunction with the growth promoting substance concerned.

According to the findings of Doby, G. and Ssladits, as abstracted in Biol. Abstracts (1935) by G.A.B. (1), diastase is more abundant in young growing plants than in old aging plants. One can infer that it has to do something with growth.

The purpose of this work has been, as the topic indicates, to show the relation, if there is any, of one of the growth promoting substances, namely thiamin hydrochloride, on the diastase content of *cicer arietinum*. - the chickpea.

II. REVIEW OF LITERATURE

Several papers have been published showing the effect of certain chemicals, among which some plant growth promoting or regulating substances, in vivo or in vitro, on the diastase activity of certain plants.

Englis, D.T., and Lunt, H.A., (1925) (7) working on the effect of the concentration of potassium salts in soil media upon the carbohydrate metabolism of plants, found that diastatic activity in the leaves of nasturtium decreased with increased rates of application of potassium to sand cultures, while an intermediate rate of application of potassium to peat cultures gave the highest activity of diastase (7). This might indicate a correlation of diastatic activity with different growing conditions rather than with the presence or absence of any element (7).

Englis, D.T., and Louis Garber (1929) (4,7), treated soybeans grown in pot cultures with different amounts of acid phosphate fertilizers. They found out that even though the dry weight and phosphorus absorbed increased markedly with increasing applications of the fertilizer, the changes in diastase activity were slight for moderate amounts. With large amounts the activity seemed to fall off even though the dry weight and phosphorus taken in still increased.

An investigation of the saccharogenic amylase activity of leaves of potato plants grown on acid moor soil and those

fertilized with K on the one hand and K, P, and Ca on the other was done by Dohy, G. and Sladits (1). Regardless of the media, the amylase decreased with the aging of the plant, but was highest in poorly nourished plants.

Haylor, Nellie M. and Vera L. Dawson (8) treated oats with carbon di-sulphide, ethylene oxide - CO_2 , etc. They found out that extracts from carbon-disulphide treated grain contained saccharogenic and dextrinogenic enzymes of high activity. The ethylene oxide - CO_2 treatment caused slow germination and delayed enzyme production or permanent injury to the enzyme.

Neely, W.B., Ball, C.D., Hanner, C.L., and Sell, H.M. (9), considering the fact that 2,4-dichlorophenoxyacetic acid results in a reduction of carbohydrates and an accumulation of nitrogen, worked on the effect of this compound on the alpha and beta amylase activity in the stems and leaves of red kidney bean plants. They found out that this compound lowers considerably the activity of both the alpha and beta amylase in the stems of bean plants. The treatment of the leaves of the plants with the same compound had no effect on the beta amylase activity (9).

Commenting on the above mentioned report, Volker, J.F. and Murray, B.P. (16) stated that, they were able to demonstrate that indole nucleus containing plant hormones, including indole acetic acid, indole butyric acid, and indole propionic acid, were

effective anti-amylases when added to refined starch in concentrations as low as 0.01 %. They also stated that comparable anti-amylolytic ability was also shown for similar concentrations of 2,4 - dichlorophenoxy-acetic acid, trichlorobenzoic acid, naphthalene acetic acid, naphthoxy acetic acid, and nicotinic acid. In view of their observations they suggested that, in addition to 2,4 - dichlorophenoxyacetic acid, others of the above-mentioned compounds may be capable of plant amylase inhibition.

Hyster, H.C. (5), commenting on the above, stated that the anti-amylolytic ability of these auxins is due entirely to pH effect, and that in vitro auxin retardation of diastase is correlated with pH. However, he thought that there might be in vivo inhibition of amylase that is not dependent upon pH effect.

There have been other investigations on the effect of some growth regulating substances on other enzymes as well.

In this investigation thiamin hydrochloride was chosen because some previous work was done on the effect of several growth promoting substances, among which thiamin hydrochloride, on the growth of peas and beans. The effect of thiamin hydrochloride was more pronounced on the differential growth of the roots. Furthermore, thiamin hydrochloride has a role in carbohydrate metabolism and perhaps in other metabolic changes as well, and all plants seem to require Vit. B₁ (5).

III. EXPERIMENTAL METHODS

A. The Culture of Plants

Plants were grown in water culture. The purpose of this was to have better conditions of control. Several kinds of nutrient solutions with thiamin hydrochloride were used in order to find out the medium in which the effect of the latter would be seen best.

1. Culture Media

Knop's solution

The constituents of this solution are as follows:

- (1) 4 gms. KNO_3 in 1 liter
- (2) 6 " NH_4NO_3 in 1 liter
- (3) 10 " $MgSO_4 \cdot 7H_2O$ in 1 liter
- (4) 4 " $Fe_2(SO_4)_3 \cdot 9H_2O$ in 1 liter
- (5) 10 " Colloidal $Ca_3(PO_4)_2$ } in 1 liter
- 5 " KCl }

This solution did not work favorably because of the persistent precipitation of the colloidal phosphate when plants were grown in it. Consequently it was neglected for fear of not supplying uniform nutrition to the plants.

2. Hoagland's solution

- (1) $Ca(NO_3)_2 \cdot 4H_2O$ 1.8109 gms. in 1 liter 0.005 M.
- (2) KNO_3 0.5056 " " " " 0.005 M.

- (3) $MgSO_4$ 0.2408 gms. in 1 liter 0.002 M.
(4) NH_2PO_4 0.1361 " " " " 0.001 M.
(5) Iron tartrate 1 cc. 0.5 % solution in 1 liter.

The above solution supplies mineral elements of the "classical" list. To supply elements recently shown to be necessary in small amounts, or which may be essential, a so-called "A-Z" solution was also added. The composition of the "A-Z" solution is as follows:

$Al_2(SO_4)_3$	1.0 gm.
KI	0.5 "
KBr	0.5 "
TiO_2	1.0 "
$SnCl_2 \cdot 2H_2O$	0.5 "
LiCl	0.5 "
$MnCl_2 \cdot 4H_2O$	7.0 "
H_3BO_3	11.0 "
$ZnSO_4$	1.0 "
$CuSO_4 \cdot 5H_2O$	1.0 "
$Na_2SO_4 \cdot 6H_2O$	1.0 "
$Co(NO_3)_2 \cdot 6H_2O$	1.0 "

These were dissolved separately and mixed and the resulting solution was diluted to 18 liters and 1 cc. of the latter was added to each liter of the "classical" list.

This solution was used with a control and 9 different

concentrations of thiamin hydrochloride, namely, 1/5,000; 1/10,000; 1/20,000; 1/40,000; 1/80,000; 1/160,000; 1/320,000; 1/640,000; 1/1,280,000. About twenty plants were grown in each solution.

Practically there was no noticeable effect of thiamin hydrochloride on the growth of these plants. The conclusion was that the latter was incompatible with one or more of the constituent compounds.

Since it would have been quite time consuming and impractical to use individual elimination, the "A-Z" part was not added and only the classical list was used. To this 0.005 M. KCl was added to supply chloride ions. However, the results were not very much different from the preceding one. So finally it was decided to use simple tap water concentrations of thiamin hydrochloride, since this was tried before and tangible results were noticeable. Naturally it is in order to give the composition of the Beirut tap water. An analysis made in August 1952 by the Public Analysis Laboratory of the American University of Beirut indicated the following results:

	<u>Parts per 100,000</u>
Total solids at 100°C.	15.6
Total hardness	12.5
Permanent Hardness	5.0
Temp. hardness	7.5
Carbonates (CO ₃)	8.20
Chlorine (Cl)	1.20
Calcium (Ca)	3.49

	<u>Parts per 100,000</u>
Magnesium (Mg)	0.98
Sulfates (SO ₄)	0.99
PH = 8.1	

Probable Combinations

Calcium Carbonate (CaCO ₃)	8.71
Magnesium Carbonate (MgCO ₃)	3.55
Sodium Carbonate (Na ₂ CO ₃)	1.15
Sodium Sulfate (Na ₂ SO ₄)	1.46
Sodium Chloride (NaCl)	1.98
	<u>-----</u>
	16.61

2. Effects of Light and Darkness.

The plants cultured in various nutrient solutions with thiamin hydrochloride, and tap water with thiamin hydrochloride were grown in two different conditions of light and in darkness.

a) Direct Sunlight

The plants were placed in a glass chamber located outdoors and opened partly from the top. Although the solutions were changed every other day, it was impossible to control the growth of a kind of unicellular green algae which made its appearance consistently. It first developed in the higher concentrations and gradually appeared in lower concentrations and it prospered quickly in all concentrations.

b) Diffuse Light.

Another batch was grown in a room with two open northern windows. No plant was exposed to direct sunlight. Here fungi appeared but it was easily controlled by changing the solutions daily. In this group there was a noticeable difference in the rate of growth of roots.

c) Darkness.

The best results were seen when the plants were grown in the dark*. The fungi created a problem which was completely solved by preparing and changing daily the culture medium for each group of plants.

In this connection it is interesting to note the findings of Benner and Greene (1958) (11), Ryts Jr. (11), and Ondratschak (1940) (11).

J. Benner and J. Greene working with pea seedlings, found that the roots of the plants grown in light contained two and a half times more thiamin than those grown in the dark whereas the leaves and terminal bud contained three times that amount. Similar observations have also been made by Ryts Jr. (11).

Ondratschek working with the green alga *Chlorella* *pluvialis* also found that light favors the synthesis of thiamin (11).

From these findings one can easily deduce that the more noticeable difference in the rate of growth in the dark was actually

* In this case all the shutters of the windows were closed. But some diffuse light was admitted in while opening the door and also while changing the solutions an electric bulb was lighted for about one hour.

due to the fact that the control plants as well as the plants in low concentrations of thiamin were deprived of this chemical.

Another advantage of culturing the plants in the dark is the uniformity of conditions which is indispensable for the type of work done.

5. Germination and Concentrations of Thiamin Used.

In the final culturing of plants the following precautions were taken:

Seeds were selected for uniformity of size and seed coat color and soaked in a 0.1 per cent Semesan Bel* solution for two hours. Then they were thoroughly washed in tap water and left soaked in it overnight. Subsequently, they were spread on glass trays covered with tightly stretched gauze and filled with pure tap water and allowed to germinate. After germination they were left to grow until the radicles reached about one and a half centimeters in length. At this stage again there was selection of uniform seedlings taking into account the length of radicles and epicotyls. Beakers to include the culture media were covered tightly with gauze and autoclaved. This precaution was taken in order to minimize the possibility of contamination. The seedlings

* This is a dip disinfectant prepared by Du Pont Semesan Company. Its contents are:

Active Ingredients:	
Hydroxymercurinitrophenol	12.0 %
Hydroxymercurichlorophenol	5.2 %
Inert Ingredients	84.8 %

were transferred to the following concentrations of thiamin hydrochloride*:

- 1.- Control
- 2.- 1/1,000,000
- 3.- 1/500,000
- 4.- 1/250,000
- 5.- 1/125,000
- 6.- 1/62,500
- 7.- 1/31,250
- 8.- 1/20,000
- 9.- 1/10,000

The seeds were germinated and plants grown in early August. The temperature range was around 25°C. to 35°C. between the period of germination and harvesting of plants.

The above given solutions were freshly prepared and changed every day. There were three reasons for doing this:

1. To keep the concentrations as constant as possible.
2. To prevent or more correctly to minimize, as far as possible, the growth of fungi.
3. To renew the supply of dissolved oxygen.

After nine days, the plants were harvested, the roots immediately separated from the shoots and each group was weighed fresh accurately. The roots were measured as to length, dried as described later under "Extraction of Diastase" and weighed.

* Preparation of; Eastman Organic Chemicals, Distillation Products.

The attached sheet indicates in a tabulated form the length, the fresh weights, the dry weights, the average per cent increase of the dry and fresh weights of the roots as compared with the control. Also the fresh weights of the shoots were taken.

As seen from the results, the change in the growth of the shoots of thiamin treated plants was negligible. Consequently it was considered superfluous to extract and test the diastase from the shoots.

Figure 1 is the photograph of the plants just before harvesting. The numbers are congruent with the numbers given in Table I.

B. Extraction of Diastase.

The roots were divided into two groups. A different method of extraction was used for each. The purpose of this was to determine the relative diastase content by both methods. The choice of one single method was quite difficult. In all the very extensive literature about the extraction of diastase not a single reference was found on the extraction from roots, specifically.

Method a.

Group 1.- This group included the following:

No. 1	Control		
" 3	1/500,000	Thiamin hydrochloride	
" 5	1/125,000	"	"
" 7	1/31,250	"	"
" 9	1/10,000	"	"

T A B L E I

THE EFFECT OF VARIOUS CONCENTRATIONS OF THIAMIN HYDROCHLORIDE ON THE LENGTH, FRESH WEIGHTS AND DRY WEIGHTS OF ROOTS AND THE FRESH WEIGHT OF SHOOTS OF CIGER ARISTETUM GROWN IN WATER MEDIUM

Lot No.	No. of Plants	Conc. of Thiamin	Fresh Shoot Weight		Average age in days	Fresh Root Weight		Dry Root Weight		% Increase or Decrease in Root Wt.
			Total	Unit		Total	Unit	Total	Unit	
1	21	-	38.089	1.811	13.5	13.122	0.625	0.6972	0.0332	Control: Control
1a	15	-	27.713	1.847	13.5	9.485	0.632	0.4905	0.0327	Control: Control
2	18	1/1,000,000	34.211	1.902	16.5	12.911	0.717	0.6198	0.0361	+ 14.2
3	26	1/500,000	47.972	1.845	20.0	22.142	0.851	1.0504	0.0404	+ 35.5
4	24	1/250,000	45.856	1.911	17.5	18.897	0.787	0.9384	0.0391	+ 25.5
5	23	1/125,000	41.920	1.823	17.5	17.809	0.774	0.8671	0.0377	+ 23.2
6	28	1/62,500	50.729	1.812	16.5	19.678	0.703	1.0192	0.0364	+ 11.9
7	23	1/31,250	40.784	1.773	10.5	13.417	0.583	0.7590	0.0330	+ 7.2
8	24	1/20,000	39.972	1.665	7.0	12.426	0.518	0.7584	0.0316	- 17.6
9	25	1/10,000	41.338	1.652	6.5	12.104	0.484	0.7225	0.0289	- 22.9

Miller, Edwin C., in his Plant Physiology book (1938) refers to the method used by Bartholomew (1914) in extracting diastase from the red algae as a method for the preparation of diastase from any plant parts that contain a relatively small amount of the enzyme (6). So this method was used for "Group 1" with some modifications:

The roots were immersed in 95 % alcohol for about 30 minutes and then in acetone for 30 minutes. The amounts of each of the above reagents used were just enough to cover the roots. They were then dried in a ventilated oven at 37°C. Total drying required about two days. After pulverization the material was placed in six times* its volume of cold 20 per cent alcohol and left standing for one day in the refrigerator with occasional stirring. The mixture was then centrifuged and the supernatant liquid was filtered. To the filtrate was added two and one-half times its volume of 95 % alcohol.

The residue after centrifuging was treated with an equal volume of cold 20 per cent alcohol and left for five hours in the refrigerator with occasional stirring. This was centrifuged, the supernatant liquid filtered and two and one-half times 95 per cent alcohol was added to the filtrate. The precipitate in each case

* The method described in Miller's Textbook of Physiology gives three volumes of alcohol. But this amount used with pulverised dried roots was almost completely absorbed, thus leaving practically no liquid extract.



Fig. 1

A photograph of the thiamin treated plants just prior to harvesting. The numbers are congruent with the numbers given in Table I.

was taken after centrifuging, washed with equal parts ether and alcohol and dried in a desiccator over sulphuric acid.

Method b.

Group 2.- This group included the following:

No. 1a	Control		
" 2	1/1,000,000	Thiamin hydrochloride	
" 4	1/250,000	"	"
" 6	1/62,500	"	"
" 8	1/20,000	"	"

The method used here includes the major steps given in the methods used by Sherman and Schlesinger (7), and Haylor, Spencer, and House (7), for the preparation of diastase from germinating grains.

The roots were dried as in "Method a" in a ventilated oven at 37°C. for about two days. The material was then pulverized and soaked for 3 hours with occasional stirring in five times its weight of cold distilled water. With ground germinated grains two and one-half times the weight of cold distilled water is used, but with roots this amount will give practically no extract. The entire mass was then placed in dialyzing bags and dialysed in tap water for 24 hours in the refrigerator. The dialysis for each lot was done separately. Each one was placed in a three liter beaker in the refrigerator and every 2 hours during the day was transferred to a container with fresh tap water so that dialysis was practically done in running tap water. The material was then filtered, again

in the refrigerator and the residue was washed with very small amounts of water. The filtrate was then treated with solid ammonium sulphate (42 g. to 100 cc. of filtrate) and the precipitate was separated by centrifuging, dissolved in as small a quantity of water as possible and dialysed against tap water and then against distilled water until free from sulphates. After the dialysis there was some deposit of insoluble material which had likely precipitated with the removal of dialysable substances from the solutions. This was removed by centrifuging and discarded. The solutions were then placed in new dialysing bags and the volume reduced to about one half by evaporation in the refrigerator. The concentrated solution was then treated with an equal volume of cold 95 per cent alcohol. The precipitate formed by the addition of alcohol was removed and discarded by centrifuging. To the liquid cold 95 per cent alcohol was added to make the final alcohol content 80 per cent. The precipitate thus obtained was separated by centrifuging and dried over sulphuric acid in a desiccator placed in the refrigerator.

Every step was done as far as possible in the cold. The importance of this is emphasized by Sherman, H.C., Caldwell, M.L., and Doebbeling S.E., (12) Unfortunately a mechanically cooled centrifuge was not available. However, centrifuge cups were always kept in ice. This helped somewhat in keeping the temperature cold while centrifuging and furthermore, centrifuging was done intermittently.

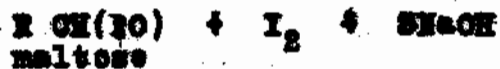
The diastase prepared by this method, as well as the preceding method, could further be purified but the amounts of material, especially in the second method was so small that it would not have been very wise to attempt further purification.

2. Measurement of Activity

The optimal pH of amylase preparations from different sources varies. For example, the amylase of *Aspergillus oryzae* is most active at pH 5.3 to 5.5 in acetate buffer (2) (15). Malt amylases have optimum pH at 4.3 to 4.6 in 0.01 M acetate buffer (15), and pancreatic amylase at a pH of 7.1 in phosphate buffer (15). Even the optimal pH of amylase from the same source depends on other factors. Quoting H.C. Sherman(15) "The optimal pH for malt amylase depended not only on the temperature and length of period of hydrolysis but also on the buffer mixture used to regulate the pH of the substrate. The optimal pH for pancreatic amylase was also influenced to a slight extent by temperature and length of period of hydrolysis."

The amylase preparation of the control - Method "b" - was tested on a pH range of 3.6 - 5.6 having buffer solutions of even ending numbers. pH 4.4, 4.6, 4.8, and 5.0 showed uniform and higher results at 37°C after 30 minutes of hydrolytic action of the amylase. The method used for the estimation of activity was the method of Willstatter, et al. (14). The same method was also used for the comparison of activity of the various extracts. The method is as follows:

25 cc. of freshly prepared 1 per cent soluble starch was put in each of ten glass stoppered bottles and 10 cc. of 0.1 N. acetate buffer of pH 4.6 was added to each. The solutions were put in an incubator at 37°C. After the temperature reached this mark, 1 cc. solution containing 1 mg. of Method "a" preparation was added to each of the bottles of one group, and 1 cc. solution containing 0.2 mg. of the Method "b" preparation was added to each of the bottles of the other group. After two hours* 2 cc. of N hydrochloric acid was added to each bottle to stop digestion. For every mg. of maltose expected 0.6 cc. of 0.1 N iodine was added to each bottle. In the case of Method "a" preparation 20 cc. were added after having done some preliminary work, and with preparation Method "b" 15 cc. were added. Then an excess of 0.1 N sodium hydroxide was added. After 15 minutes an excess of dilute sulphuric acid was added and the excess of iodine was titrated with 0.05 N thiosulfate. The chemical reaction involved in the Willstatter and Schüdel method is:



Na Salt of maltobionic acid

A control was run adding the enzyme only after dilute sulphuric acid had been added. 1 cc. of 0.05 N thiosulfate corresponds to 8.575 mg. of maltose.

* In the original method the time here is 10 minutes. Since the power of activity was not so high it was preferred to hydrolyze for 2 hours.

IV. EXPERIMENTAL RESULTS

On quantitative testing of activity, the results of diastatic power shown by the extract of preparations by Method "a" were very small per unit weight. Probably this was due to the inhibitory action of some of the impurities found in the extract. The ratio of the extract to the dried weight was very big, about 0.75 %. Consequently it was considered unnecessary to interpret these results.

The results gotten by Method "b", namely precipitation by ammonium sulphate, dialysis, and fractional precipitation by alcohol were more substantial. Again here the power of diastase was quite low. Further purification might have given higher results but this was not attempted due to the very small amounts of the extracts. The results are given in Table II.

T A B L E II

EFFECT OF VARIOUS CONCENTRATIONS OF THIAMIN - HCl ON
 THE DIASTASE CONTENT - BY WEIGHT AND ACTIVITY -
 OF THE ROOTS OF CIGER ARIETINUM.

Lot No.	No. of Roots	Concentration of Thiamin HCl	Dried Extract in mg.		Power per mg. of Extract in terms of mg. of Maltose Produced
			Total	Per Root	
1 a	15	Control	7.7	0.52	119
2	18	1/1,000,000	10.1	0.56	121
4	24	1/250,000	13.4	0.55	125
6	28	1/62,500	15.4	0.54	112
8	24	1/20,000	9.3	0.37	88

V. INTERPRETATION OF RESULTS

As can be seen from Table I, concentrations of thiamin of 1/1,000,000 to 1/62,500 had a marked effect on the length of roots, on the fresh and the dry weights of roots. Fig. 2 shows the relation of thiamin HCl concentrations and the percent increase or decrease, as compared with the control, of the dry and fresh weights. Concentration of 1/31,250, 1/10,000, and 1/20,000 have had a definite inhibitory action on the length and the fresh weights. Concentration of 1/31,250 however, had practically no effect on the dry weight.

The results of the diastase content both by weight and activity are seen in Table II. For interpretation of the results more extensive data would have been desirable. Unfortunately, the method with alcohol extraction did not prove to be an effective method for the extraction of diastase from roots grown in water culture. The results, as they are, however, point out clearly that concentrations of 1/1,000,000 to 62,500 inclusive have very slight effect, if at all, on the diastase content both by weight and activity. As for the concentration of 1/20,000, the effect is very clear and obvious in that both in amount and activity there is a definite reduction. In case of the weight of extract per root the reduction is about 30 % while in case of the activity per mg. of extract the reduction is about 26 %.

VI. CONCLUSION

One can conclude from the experimental results that the thiamin hydrochloride, in concentrations of 1/62,000 and lower to 1/1,000,000, in this case, has practically no effect on the diastase content, even though there is a marked effect on the length, the fresh weight and the dry weight of roots. In a concentration of 1/20,000 it effectuates a definite reduction in the diastase content.

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