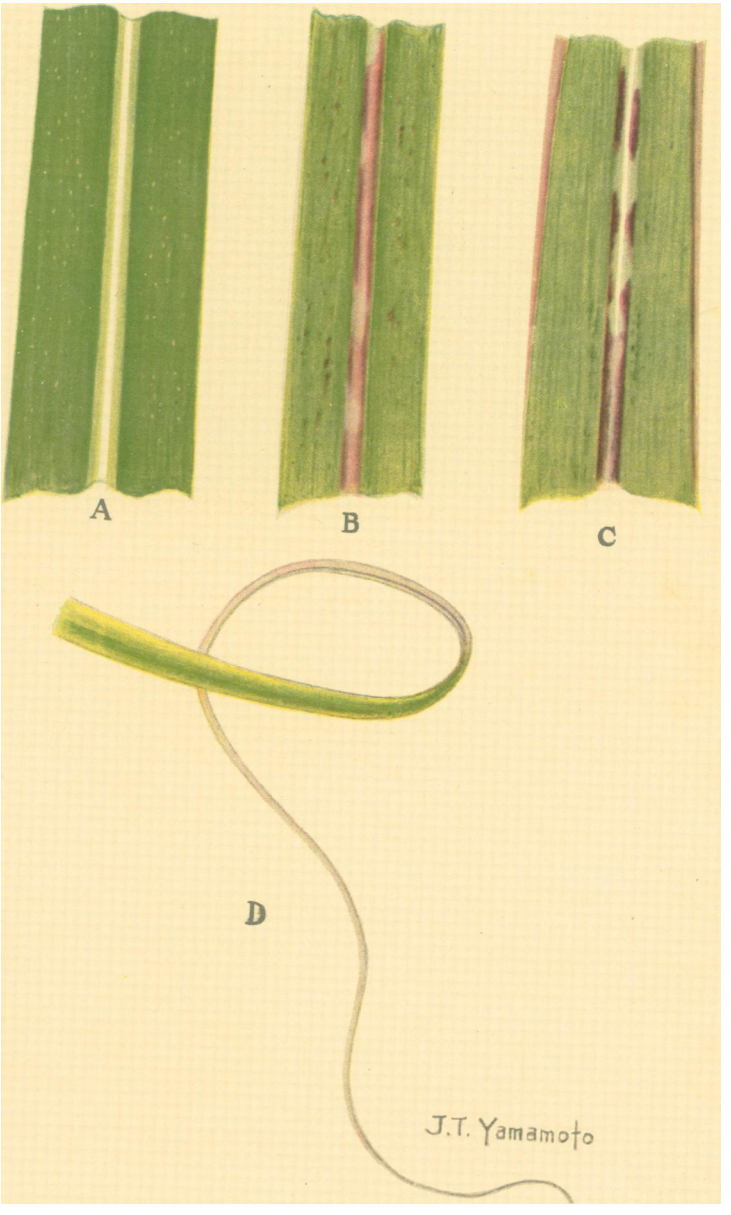
EXPLANATION OF COLOR PLATE

Color symptoms of potassium deficiency in sugar cane: *A*, typical leaf from control plant; *B*, *C*, and *D*, leaves from plants deficient in potassium. *B* shows the color of a midrib soon after it has become red, while *C* shows the darkening of the discoloration as the leaf ages.


A
B
C
D
J.T. Yamamoto

SOME EFFECTS OF POTASSIUM UPON THE AMOUNTS OF PROTEIN AND AMINO FORMS OF NITROGEN, SUGARS, AND ENZYME ACTIVITY OF SUGAR CANE[1]

CONSTANCE ENDICOTT HARTT

(WITH ONE FIGURE)

Introduction

This paper reports the results of determinations of the enzymes invertase, amylase, and ereptase; analyses of total and amino nitrogen, reducing sugars and sucrose; and the hydrogen ion concentrations, titratable acidity, and titration curves of the juices expressed from the leaves, stems, and roots of plants grown with varying amounts of potassium.

METHODS.—Details of the care of the plants during growth and of the methods employed in harvesting them have been presented in the preceding paper (**26**).

Invertase, amylase, peptase, and ereptase, and total and reducing sugars were determined by the methods used in the studies reported in 1929 (**24**). The titration method of WILLSTÄTTER and WALDSCHMIDT-LEITZ (**55**) was also used for the detection of peptase.

Total nitrogen was determined by the Kjeldahl method, while the Van Slyke method was employed for the estimation of amino nitrogen. The nitrogen determinations were performed by the Chemistry Department of this Station.

Results

1. PHYSICO-CHEMICAL STUDIES OF EXPRESSED SAP

Determinations of the hydrogen ion concentration, titratable acidity, and titration curves were performed using two representative plants of series 1 and 3, harvested December 4, 1931, eleven weeks after starting the plants in the nutrient solutions. The results of the electrometric determinations of the hydrogen ion concentration of the sap expressed from frozen tissues are presented in table I. These data show very little difference between the hydrogen ion concentration of the controls and that of the plants deficient in potassium, the former being slightly more acid than the latter, which is the reverse of the results obtained by REED and HAAS (**40**).

Because of the dark color of the juices, it was necessary to dilute 1 cc. of juice with 20 cc. distilled water before titration. N/5 NaOH was used, with phenolphthalein as indicator. The results are given in table I. This shows that the blades of series 1 were a little less acid than those of series

[1] See footnote 1 of preceding paper.

TABLE I

pH AND TITRATABLE ACIDITY OF EXPRESSED SAP

SERIES	pH	N/5 NaOH TO NEUTRALIZE 1 CC. JUICE
		cc.
Blades		
1	5.26	0.32
3	5.32	0.49
Stems		
1	5.17	0.4
3	5.34	0.3
Roots		
1	5.58	0.09
3	5.64	0.09

3, while in the stems the reverse held, and the roots showed no difference.

By adding varying amounts of sulphuric acid and sodium hydroxide to the expressed sap and determining the hydrogen ion concentration electrometrically, the titration curves were obtained. These are shown in figure 1. A slight change in pH upon addition of acid or alkali indicates that the juice is well buffered, while a greater change means a poorly buffered juice. The graphs show that the blades of series 3 seemed to have a slightly better buffer system than those of series 1; that the buffer systems of the stems were very much alike; but that with roots, the plants of series 1 had a slightly better system on the acid side but those of series 3 were better on the alkaline side. On the whole there was very little difference in the way they reacted to additions of acid and alkali. It would seem, therefore, that differences in hydrogen ion concentration, titratable acidity, and buffer systems were insufficient to explain the results of the enzyme determinations here reported.

2. ANALYTICAL DATA

The results of the nitrogen determinations are presented in tables II and III.

In November, there was a higher percentage of amino nitrogen and lower percentage of protein nitrogen in the plants of series 3 than in the controls, while the total nitrogen remained about the same. These differences were more conspicuous in the blades than in the stems. In April, higher percentages of amino nitrogen, protein nitrogen, and total nitrogen were found in the blades of the plants deficient in potassium, while in the stems the opposite relationship was found. When the total amounts of nitrogen contained in the entire plants are calculated, the results presented in table IV are obtained. These figures show that in November the average dry weight

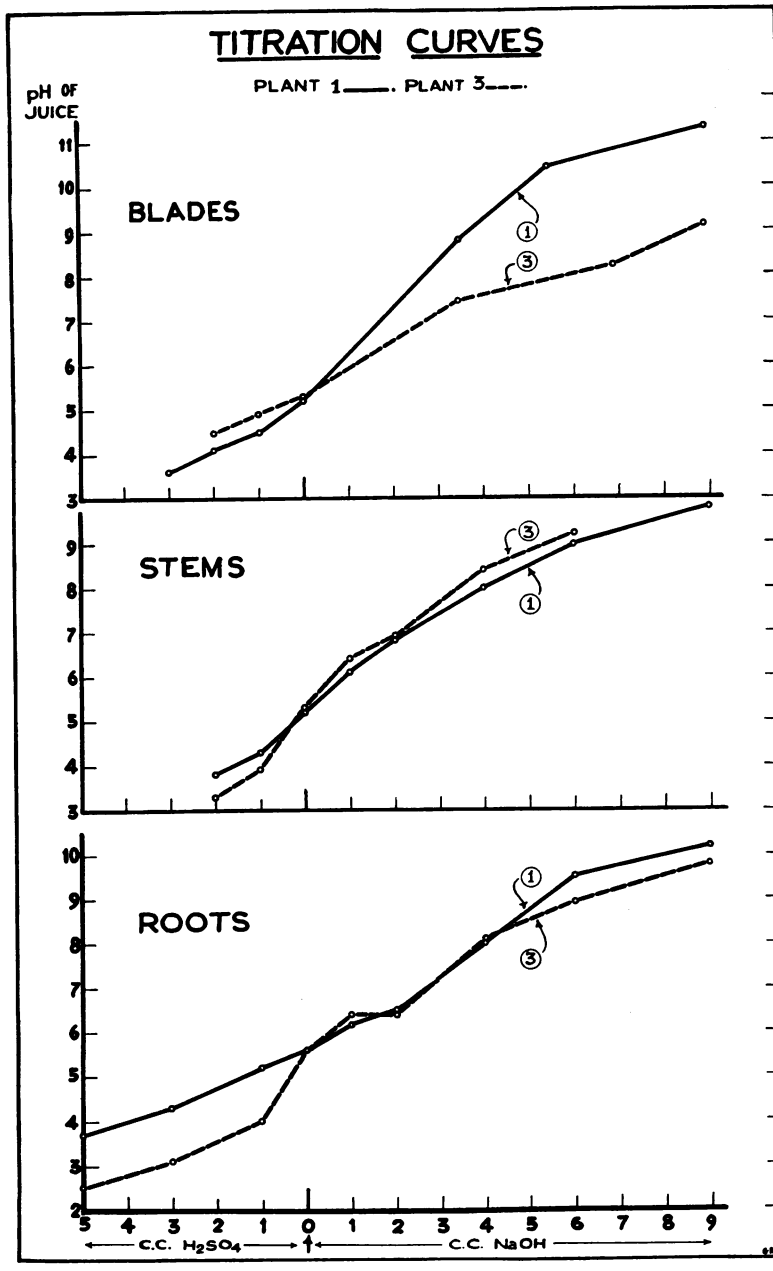


FIG. 1. Effect of addition of varying amounts of acid and alkali upon hydrogen ion concentration of juice expressed from plants 1 and 3.

TABLE II

NITROGEN ANALYSES OF PLANTS HARVESTED NOVEMBER 20, 1931, NINE WEEKS AFTER STARTING THE PLANTS IN THE NUTRIENT SOLUTIONS
PERCENTAGES EXPRESSED ON MOISTURE-FREE BASIS

SERIES	AMINO N	PROTEIN N	NITRATE N	TOTAL N
	%	%	%	%
Blades				
1	0.070 ± 0.011	1.585 ± 0.0004	None	1.655 ± 0.001
2	0.086 ± 0.004	1.605 ± 0.003	None	1.691 ± 0.008
3	0.231 ± 0.001	1.461 ± 0.001	Trace	1.692 ± 0.0004
Stems				
1	0.395 ± 0.006	0.986 ± 0.007	Trace	1.381 ± 0.0007
2	0.463	1.122	Trace	1.585
3	0.441	0.911	None	1.352

TABLE III

NITROGEN ANALYSES OF PLANTS HARVESTED APRIL 27, 1932, 7½ MONTHS AFTER STARTING THE PLANTS IN THE NUTRIENT SOLUTIONS
PERCENTAGES EXPRESSED ON MOISTURE-FREE BASIS

SERIES	AMINO N	PROTEIN N	TOTAL N
	%	%	%
Blades			
1	0.130 ± 0.0009	1.707 ± 0.008	1.837 ± 0.009
2	0.099 ± 0.001	1.573 ± 0.022	1.673 ± 0.020
3	0.148 ± 0.002	1.923 ± 0.008	2.071 ± 0.005
4	0.231 ± 0.007	2.285 ± 0.026	2.516 ± 0.018
5	0.161 ± 0.001	2.139 ± 0.023	2.300 ± 0.024
Stems			
1	0.666 ± 0.019	1.334 ± 0.015	2.000 ± 0.001
2	0.633 ± 0.013	1.294 ± 0.008	1.927 ± 0.022
3	0.344 ± 0.007	1.114 ± 0.002	1.459 ± 0.010
4	0.199 ± 0.002	1.205 ± 0.011	1.404 ± 0.013
5	0.155 ± 0.0007	0.866 ± 0.016	1.021 ± 0.016

of the total blades of series 3 was 36.3 gm., and of series 1, 66.4 gm. At that time the blades of series 1 contained 0.0465 gm. of amino nitrogen per plant, while those of series 3 contained 0.0838 gm. Although weighing only half as much as those of series 1, the blades of series 3 contained almost double the amount of amino nitrogen. This seems to be evidence of a derangement in the synthesis of proteins by the plants deficient in potassium.

TABLE IV

TOTAL AMOUNTS OF PROTEINS WITHIN THE PLANTS

SERIES	AVERAGE DRY WEIGHT OF PLANT		TOTAL PROTEIN N PER PLANT		TOTAL AMINO N PER PLANT	
	NOV.	APRIL	NOV.	APRIL	NOV.	APRIL
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Blades						
1	66.4	189.7	1.052	3.238	0.0465	0.2466
3	36.3	69.7	0.530	1.338	0.0838	0.1032
Stems						
1	13.5	299.4	0.133	3.994	0.0533	2.660
3	4.6	36.0	0.030	0.401	0.0203	0.124

The percentages of sugars in the plants are given in tables V and VI. Table V shows that two months after starting the plants in the nutrient solutions there was no great difference in the percentages of sugars in the three groups of plants studied. A slightly higher percentage of reducing sugars and lower percentage of sucrose is suggested in the blades of the potassium-deficient plants at that time. Although these differences may be within the limits of experimental error, it is possible that they suggest a tendency when compared with the results obtained later. In April the

TABLE V

SUGAR PERCENTAGES OF PLANTS HARVESTED NOVEMBER 20, 1931, EXPRESSED ON MOISTURE-FREE BASIS

SERIES	REDUCING SUGARS	SUCROSE	TOTAL SUGARS
	%	%	%
Blades			
1	3.230 ± 0.094	3.717 ± 0.224	6.947 ± 0.318
2	4.235 ± 0.099	2.487	6.722
3	3.548 ± 0.066	3.183 ± 0.074	6.731 ± 0.005
Stems			
1	4.879 ± 0.079	14.630 ± 0.465	19.509 ± 0.385
2	7.003 ± 0.124	14.163 ± 0.138	21.166 ± 0.013
3	4.155 ± 0.109	13.698 ± 0.011	17.853 ± 0.120

blades of the plants starved for potassium were higher in reducing sugars and lower in sucrose than those of the controls, although the percentages of total sugars remained about the same. The stems of series 2 were higher in reducing sugars and lower in sucrose than the controls, but the stems of

the other potassium-deficient plants were very low in reducing sugars as well as sucrose and there was a positive correlation between the amount of potassium supplied and the total sugar produced. These results indicate derangements in the transformations between the hexoses and sucrose. These derangements were found to be correlated with a weak activity of invertase, which will be discussed later.

3. Invertase studies

In a former paper (**24**) evidence was presented indicating that a deficiency in potassium resulted in a weakened activity of invertase in sugar cane. This has been substantiated and studied in greater detail in the present investigation and a preliminary report of some of the results has already appeared (**25**).

TABLE VI

Sugar percentages of plants harvested April 27, 1932, expressed on moisture-free basis

Series	Reducing sugars	Sucrose	Total sugars
	%	%	%
Blades			
1	4.580 ± 0.050	4.225 ± 0.145	8.810 ± 0.095
2	4.360 ± 0.028	5.622 ± 0.014	9.982 ± 0.014
3	5.617 ± 0.134	2.559 ± 0.212	8.176 ± 0.078
4	5.973 ± 0.188	3.070 ± 0.044	9.044 ± 0.145
5	5.346 ± 0.087	3.647 ± 0.005	8.993 ± 0.081
Stems			
1	8.538 ± 0.045	26.220 ± 0.112	34.758 ± 0.157
2	10.055 ± 0.231	21.431 ± 0.053	31.487 ± 0.117
3	8.024 ± 0.057	23.116 ± 0.257	31.140 ± 0.314
4	3.483 ± 0.049	23.747 ± 0.472	27.230 ± 0.522
5	3.653 ± 0.160	18.980 ± 0.160	22.634 ± 0.000

Determinations were made of the activity of the invertase of the blades and the stems of the material harvested in November and in April, both unbuffered and at the optimum reaction. The buffers used in these experiments were those of McIlvaine which are described by Clark (**8**). These were chosen because they do not contain potassium. Controls were run on the hydrogen ion concentration of the tests before and after inversion, which showed that the buffers were efficacious. It was also found that the reaction of the unbuffered tests did not change materially during inversion.

Determinations were made of the effect of the hydrogen ion concentration upon the activity of invertase of the plants of series 1, the results of which are given in table VII.

The results of all the invertase tests are reported as cc. N/20 $KMnO_4$, the Bertrand titration method having been used. With the exception of the effect of the reaction on the invertase activity of the blades harvested in

TABLE VII

pH AND INVERTASE OF SERIES 1

pH	Inversion by H+	Inversion by invertase			
		November		April	
		Blades	Stems	Blades	Stems
		cc.	*cc.*	*cc.*	*cc.*
3.3	7.77	.....	.....	23.30	.....
3.7	4.71	17.9	52.30	29.91	21.07
4.0	1.83	18.9	59.87	34.09	23.21
4.4	0.90	18.6	62.11	38.27	25.54
4.9	1.39	18.25	49.85	28.87	17.47
5.3	1.15	17.5	.....	20.31	.....
5.9	.....	16.9	.....	.....	.....

November, all the tests were conducted in duplicate, with duplicate controls for each lot of material and for each buffer, and were repeated. Table VII shows that the optimum reaction for the invertase of the controls was about pH 4.4.

Invertase determinations of all the plants are presented in table VIII. The determinations of the activity in the blades and stems, buffered and unbuffered, were performed on separate days. While every effort was made to keep the conditions uniform, identical conditions were impossible of attainment. Consequently the results in table VIII are only roughly comparable vertically. They are intended to be read horizontally.

Table VIII shows that the blades and stems of the plants supplied with potassium had a greater invertase activity than those of the potassium-deficient plants. The difference was greater in the stems than in the blades. Greater differences would have been obtained in the stems of the April material if a distinction had been made between green and dry leaf cane, a point which will be discussed later. When tested at the optimum reaction, the activity was apparently equal in all the blades. The activity of the stems was not equalized at the optimum reaction, however, although the difference between the extremes was less than when tested unbuffered.

Because of the importance of hydrogen ion concentration in invertase activity, which is illustrated in table VII, one might suppose that the dif-

TABLE VIII

INVERTASE ACTIVITY IN SUGAR CANE

TEST	SERIES						
	1	2	3	4	5	6	7
	November material						
Blades (unbuffered)	42.00	.....	39.10	28.25	33.89		
Blades pH 4	21.9	21.3	20.16	22.5	22.5		
Stems (unbuffered)	56.45	33.10	16.68	18.65	19.97		
Stems pH 4.4	50.70	42.04	27.42	26.67	22.62		
	April material						
Blades (unbuffered)	25.00	21.21	22.64	17.11	17.71	.....	.....
Blades pH 4.4	36.08	36.16	40.64	39.43	36.69	35.19	34.77
Stems (unbuffered)	22.16	15.64	24.10	10.52	7.97	.....	.....
Stems pH 4.4	23.04	14.82	19.78	15.78	13.57	.....	.....

ferences in activity shown in table VIII were caused by differences in reaction of the unbuffered plant material. This is not true. Colorimetric determinations of the hydrogen ion concentration of the blade and stem powder were made and no correlation was found between the reaction of the powder and the activity of invertase, as will be seen in table IX. For example, in the April material the blades of series 4 and 5 had equal invertase activity when tested unbuffered but differed in reaction, while series 3 and 4 had the same reaction but differed in activity. In the stems, series 1 had the least favorable reaction but that material was one of the strongest in invertase activity. It is evident that hydrogen ion concentration is not the only factor governing the activity of invertase.

Another factor affecting the activity of invertase in sugar cane is the amount of potassium present in the tissue. The stem powder of the plants of series 1 contained 1.504 per cent. potassium, while that of series 5 had only 0.278 per cent. potassium. An attempt was made in the study reported in 1929 (**24**) to equalize the potassium in the two lots of material; the result was an equal increase in activity in both the control and

TABLE IX

pH OF AIR-DRY POWDER

SERIES	NOVEMBER BLADES	APRIL BLADES	APRIL STEMS
	pH	*pH*	*pH*
1	5.6	5.2	5.7
2	5.6	5.2	4.6
3	5.6	5.4	4.6
4	5.8	5.4	4.8
5	5.8	6.0	4.9
6	.....	5.3	5.2
7	.....	5.4	4.9

the potassium-deficient powder. It was concluded at that time that potassium is essential for the formation of invertase within the plant. Unfortunately no buffered tests were made. In the present study the activity of the stems of series 1 and 5 was determined at pH 4.4, with and without the equalization of potassium. The results are reported in table X. The potassium content of the stem powder of these plants was approximately equalized by the addition of potassium dihydrogen phosphate to series 5 and sodium dihydrogen phosphate to series 1. It will be seen that the addition of sodium phosphate to series 1 had no effect, while the potassium phosphate increased the invertase activity of series 5. These data indicate that potassium is a specific accelerator for the activity of invertase in sugar cane, and that sodium and phosphorus are not.

TABLE X

POTASSIUM AND INVERTASE OF STEMS AT PH 4.4

SERIES	POTASSIUM	ACTIVITY ALONE	ACTIVITY WITH EQUAL POTASSIUM
	%		
1	1.504	17.44	18.07 (+Na)
5	0.278	10.85	13.88 (+K)

When all the tests for stems are averaged it is found that when tested unbuffered, series 1 is 2.78 times as active as series 5; at the optimum reaction, series 1 is only 1.71 times as active as series 5; and when the potassium is equalized at the optimum reaction, series 1 is only 1.31 times as active as series 5. Evidently the invertase is present within the potassium-deficient plants, but it needs the proper conditions for its optimum activity.

The plants deficient in potassium showed derangements in the transformations of the sugars correlated with the weak activity of invertase. This

should be expected since invertase is the enzyme which catalyzes the hydrolysis and synthesis of sucrose. Direct evidence for the latter has recently been obtained by OPARIN and KURSSANOW (**39**). The idea occurred that other factors which curtail the production of sucrose from the hexoses within the plant might operate by their effect upon the activity of invertase.

To obtain additional evidence on this point a supplementary experiment was performed. At the Waipio Substation of the Hawaiian Sugar Planters' Association there was a field of variety P.O.J. 2878 which had received uniform fertilizer and irrigation treatment. For some undetermined reason the juices in the cane in one portion of the field were consistently low in sucrose, while in another part of the same field juices high in sucrose were obtained. The object of the experiment was to determine the juice quality and invertase activity of the plants giving good and poor juices. Five plants of each lot were taken and were subdivided into blades, green leaf cane, and dry leaf cane. The quality of the juices is given in table XI, these data being supplied by the Department of Sugar Technology of this Station.

The invertase activity of these samples is given in table XII. The term green leaf cane is applied to that portion of the millable cane to which green leaves are attached, while dry leaf cane is the part which bears dry leaves or none.

TABLE XI

JUICE QUALITY OF CANE

SAMPLE	BRIX*	POLARIZATION*	PURITY*	QUALITY RATIO*
Good cane (green leaf part)	13.60	9.60	70.6	16.41
Poor cane (green leaf part)	12.89	7.23	56.1	28.73
Good cane (dry leaf part)	22.12	20.30	91.8	6.39
Poor cane (dry leaf part)	20.87	18.84	90.3	6.95

* Purity is the ratio between the polarization (or total sucrose) and the Brix (or total solids measured by the Brix hydrometer), while quality ratio is the number of tons of cane necessary to make one ton of sugar.

It will be seen that when tested unbuffered, the activity of the stems of the cane giving good juices is the same as that of the poor cane. The difference between the activities of the blades of the two lots of plants is not great, but may be significant. The most striking point brought out by this

TABLE XII

INVERTASE ACTIVITY, VARIETY P.O.J. 2878

SAMPLE	CANE GIVING GOOD JUICES	CANE GIVING POOR JUICES
Blades	12.20	9.37
Green leaf cane	12.71	13.99
Dry leaf cane	6.07	6.88

experiment is the fact that the activity in the green leaf part of the stem is double that of the dry leaf portion. This applies to both the good cane and the poor. In the good cane the invertase of the blades is equal in activity to that of the green leaf portion of the stalk. It is evident that wherever sucrose is actively made or stored, invertase activity is great; whereas in the dry leaf portion of the stick, where the storage of sucrose is practically completed, the invertase activity decreases.

4. AMYLASE STUDIES

In the study reported in 1929 (**24**) the activity of amylase was found to be greater in the plants deficient in potassium than in the controls, in both blades and stems. It was suggested that the cause of the increased activity in the plants deficient in potassium might be their greater percentage of sugars, which perhaps constitute the substrate necessary for the formation of starch by amylase, since the amount of substrate has been found in certain cases to affect the amount of enzyme produced. Another possibility suggested was an increase in the absorption of phosphorus in the potassium-deficient plants, since phosphorus is known to favor the activity of amylase, and JOHNSTON and HOAGLAND (**31**) found that tomato plants absorbed an increased amount of phosphorus when the supply of potassium was deficient.

In the present studies, determinations were made of the optimum reaction for the amylase of cane; of the activity in the blades and the stems of the plants supplied with varying amounts of potassium; and of the effects of potassium, phosphorus, calcium, magnesium, dialysis, and sugars upon the activity of amylase. The results are given as color with iodine-potassium iodide after incubation for 20 hours at 36.5° C., using soluble starch as the medium. The following twelve colors are used to represent the course of the digestion of starch, in the order named: blue, purple-blue, purple, red-purple, very dark red, dark red, red, bright red, orange-red, orange, pink-yellow, yellow.

TABLE XIII

pH AND AMYLASE OF SERIES 1

pH	NOVEMBER BLADES	APRIL BLADES	APRIL STEMS
2.2	Purple-blue	.....	.....
4.9	Dark red	Dark red	Red-purple
5.3	.....	Dark red	Red
5.9	Bright red	Bright red	Red
6.3	.....	Red	Red
7.1	.....	Red-purple	Red
8.1	Red-purple	.....	.....

The effect of the reaction of the medium upon the amylase activity is shown in table XIII. The optimum reaction for the amylase of blades was found to be pH 5.9. The amylase of stems did not seem to be influenced appreciably by hydrogen ion concentration.

The amylase activity of all the plants is given in table XIV.

TABLE XIV

AMYLASE OF PLANTS GROWN WITH VARYING AMOUNTS OF POTASSIUM

SERIES	NOVEMBER MATERIAL		APRIL MATERIAL	
	BLADES (pH 5.9)	STEMS (UNBUFFERED)	BLADES (pH 5.9)	STEMS (UNBUFFERED)
1	Very dark red	Orange-red	Red-purple	Red-purple
2	Dark red	.....	Red	Purple
3	Orange	Yellow	Orange	Red
4	Yellow	Yellow	Yellow	Orange
5	Pink-yellow	Yellow	Orange-red	Bright red
6	.....	.....	Dark red	Dark red
7	.....	.....	Dark red	Dark red

The amylase activity of the blades of the November material in decreasing order was $4 > 5 > 3 > 2 > 1$. That of the blades of the April material was $4 > 3 > 5 > 2 > 6$ and $7 > 1$. That of the stems collected in April was $4 > 5 > 3 > 6$ and $7 > 1 > 2$. In every test the plants deficient in potassium had greater amylase activity than the controls.

Studies were made concerning the cause of the active amylase of the potassium-deficient plants:

a. Sugars

Perhaps the formation of starch from dextrose in plants is catalyzed by amylase. If so, and if the production of amylase is regulated by the amount of dextrose, then the plants which have the most active amylase should also contain the largest percentage of reducing sugars, other things being equal. The data for the sugars in the November harvest are insufficient for this comparison. Table VI shows that the percentage of reducing sugars in the blades of the April material decreased in the order $4 > 3 > 5 > 2 > 1$. The amylase of the same blades differed in the same order. The stems of the plants deficient in potassium, however, had the lowest percentage of reducing sugars and the most active amylase. In short, although the principle of the quantitative regulation of enzyme production might apply to the amylase activity in the blades, it could not be applied in the stems.

Before the sugar determinations were performed a supplementary test was conducted to determine the effect of sugars upon the activity of amylase. Cane shoots were taken from cuttings and supported in split corks in Erlenmeyer flasks, and were placed in intense diffused light. One plant was given distilled water, another a 1 per cent. solution of sucrose, and a third a 1 per cent. solution of dextrose. The sugars and water had been boiled and cooled. They were renewed at intervals. After four days the blades were removed, ground and dried, and amylase determinations were performed. At the time of the removal of the blades the three plants appeared about the same, except that the spindle of the plant which had received dextrose was greener than the spindles of the other two plants. It was found that the plant which had received dextrose had the most active amylase, that which had been in water was the least active, while the amylase of the plant receiving sucrose was intermediate in activity. These results are not in agreement with those of SJÖBERG (**43**), who found that bean plants receiving sugars had weaker amylase activity in stems and leaves than those with no organic nutrients. The present results seem to uphold the general theory of the effect of the substrate upon the production of enzymes, although, as explained above, that theory cannot explain the amylase activity in the stems in these potash investigations.

b. Phosphorus

The percentages of phosphorus in the plants were given in tables XV and XVI of the first paper of this series (**26**). The relative amounts of phosphorus, potassium, and amylase are given in table XV, in which the

TABLE XV

RELATIVE AMOUNTS OF PHOSPHORUS AND POTASSIUM, AND AMYLASE ACTIVITY

	NOVEMBER BLADES	APRIL BLADES	APRIL STEMS
Phosphorus	5 > 4 > 3 > 2 > 1	4 > 5 > 3 > 2 > 1	5 > 4 > 1 and 3 > 2
Potassium	1 > 2 > 3 > 5 > 4	1 > 2 > 3 > 5 > 4	1 > 2 > 3 > 4 > 5
Amylase	4 > 5 > 3 > 2 > 1	4 > 3 > 5 > 2 > 1	4 > 5 > 3 > 1 > 2

figures are the series numbers of the plants. From this it will be seen that there is a fairly good positive relationship between the phosphorus content and the amylase activity. There is also a good negative correlation between the potassium content and the amylase activity.

Tests were conducted with the blades of the material collected in both November and April to determine whether equalizing the phosphorus would result in the equality of the activity of amylase and it was found that it did not. Evidence was obtained, however, indicating that the amylase of the potassium-deficient blades is activated by phosphorus more than is the amylase of the control blades, as shown in table XVI.

TABLE XVI

AMYLASE ACTIVATION BY PHOSPHORUS

SERIES	TREATMENT	COLOR WITH IKI
Blades		
1	H_2O	Dark red
1	Na phosphate	Dark red
2	H_2O	Orange-red
2	Na phosphate	Orange

c. Theories more or less disproved

Aspargine is known to be an activator of amylase. A microchemical test was made of the blades of series 1 and 4, using copper acetate. No marine blue crystals of copper asparginate were found, indicating that little or no asparagine was present.

Sodium is one of the ions known to activate amylase, but the plants of series 5 were grown entirely without sodium and their amylase was about equal in activity to the amylase of series 4.

It might be supposed that the potassium content of the control plants was high enough to exert a direct inhibitory action upon amylase. This

was not true because the addition of potassium to the blades of the control plants harvested in November resulted in a slight increase in activity.

OPARIN and DJATSCHKOW (**38**) concluded from studies with wheat that amylase is made in the plant and goes to the grain. Amylase might be made in the blades of the sugar cane plant, its production being controlled by the sugar content of the blades rather than of the stems. But if amylase migrates from the blades to the stems it would supposedly go through the phloem. The phloem necrosis which curtailed the translocation of proteins and sugars from the blades to the stems would also prevent the migration, of amylase. This should tend to result in less amylase in the stems of the potassium-deficient plants than in the controls, which was not found.

d. Activation by salt constituents in general

The blades of the potassium-deficient plants harvested in November were higher in total ash, calcium, and magnesium than were the controls, as shown in the first paper of this series (**26**). These differences held for magnesium in the blades and stems of the April material, and for calcium in the blades, but did not hold for calcium in the other organs or for total ash. Therefore, although the higher ash, calcium, and magnesium content of the potassium-deficient plants might explain their more active amylase in the material of the November harvest, only the magnesium content could apply to that collected in April.

The magnesium contents of the blades of series 1 and 2, harvested in November, were equalized by the addition of magnesium sulphate to the former. It was found that the magnesium sulphate activated the amylase of the blades of series 1 slightly but did not make it equal to that of series 2. Similar results were found when the calcium content was equalized.

To find the effect of the ash constituents in general, the blades of the November harvest were dialyzed in collodion bags before incubation. Pre-

TABLE XVII

AMYLASE ACTIVITY OF BLADES AFTER DIALYSIS FOR 24 HOURS

SERIES	COLOR WITH IKI	
	NON-DIALYZED	DIALYZED
1	Dark red	Red-purple
2	Red	Dark red
3	Pink-yellow	Orange
4	Yellow	Pink-yellow
5	Yellow	Pink-yellow

liminary tests and controls showed that the amylase of sugar cane is non-dialyzable through collodion. The amylase activity of the blades with and without previous dialysis for 24 hours is shown in table XVII.

In every plant, dialysis for 24 hours resulted in decreased activity, indicating the loss of an activator. In another experiment dialysis was continued for three days. Amylase determinations were then performed, the results of which are given in table XVIII.

Dialysis for three days thus decreased the amylase activity of series 2, 4, and 5 but increased that of series 1 and 3. This shows that dialysis

TABLE XVIII

AMYLASE ACTIVITY OF BLADES AFTER DIALYSIS FOR 3 DAYS

SERIES	COLOR WITH IKI	
	NON-DIALYZED	DIALYZED
1	Purple	Red-purple
2	Red-purple	Purple
3	Very dark red	Dark red
4	Pink-yellow	Red
5	Pink-yellow	Dark red

even for three days does not equalize the activity of amylase. It is therefore not the mere presence of the ash constituents which alone determines the activity of amylase. It should not be surprising that dialysis at times results in increased activity of amylase and at other times decreases its action. Among the dialyzable constituents of the plant material it seems only reasonable that there are some which increase and some which decrease the activity of amylase, and that these diffuse at different rates.

Further considerations of the amylase activity will be found in the discussion.

Other tests to be reported in another contribution have shown greater dextrinase and weaker maltase activity in the blades of cane plants deficient in potassium than in the controls. Greater differences in maltase activity were found in wheat and buckwheat than in sugar cane.

5. EREPTASE STUDIES

The optimum reaction for the activity of ereptase was determined and the results are shown in table XIX. The test consisted of the formation of tryptophane from Witte peptone, the amount of tryptophane being de-

termined by the addition of bromine water. This gives a pink color and the deeper the pink the greater the amount of tryptophane, and hence the greater the activity of ereptase.

TABLE XIX

pH AND EREPTASE OF SERIES 1

pH	November material		April material		
	Blades	Roots	Blades	Stems	Roots
2.2	Colorless*	.....	.....	.....	.....
3.3	.....	Colorless	Lightest pink	.....	.....
3.7	.....	Colorless	Lighter pink	.....	Colorless
4.0	.....	Light pink	Light pink	Colorless	Very light pink
4.4	.....	.....	Pink	Colorless	Light pink
4.9	Light pink	Very light pink	Rose	Colorless	Pink
5.3	.....	.....	Light pink	Light pink	Light pink
5.5	.....	.....	.....	Pink	.....
5.9	Colorless	.....	.....	Deeper pink	Colorless
7.0	.....	.....	.....	Light pink	.....
8.1	Colorless	.....	.....	Colorless	.....

* Negative results; blanks indicate no test performed.

The optimum reaction for the blades, November and April, was found to be pH 4.9, stems 5.9, and roots about pH 4.9.

Determinations were made of the activity of ereptase of the blades and roots of the plants harvested in November, and of the blades, stems, and roots of the April material, all the tests being made at the optimum reaction. No difference was found in the activity in the blades. Some slight differences occurred in the stems, but they were not correlated with the amount of potassium supplied nor with any other observed factor. The activity in the roots of the material harvested in April occurred in the order $4 > 3$ and $5 > 2$ and $7 > 1 > 6$. The gradation is not perfect, but it does indicate greater activity in the plants deficient in potassium.

6. Peptase studies

Peptase activity was found in the studies reported in 1929 (**24**) to be greater in the controls than in the plants deficient in potassium. Since

peptase may be important in catalyzing the synthesis of proteins, any derangement in its activity might be expected to reduce the production of protoplasm and hence curtail growth.

It was hoped to repeat the studies of peptase in the present investigations, using a more exact method. To date no evidence of peptase activity has been obtained. Using the carmine fibrin method of GRÜTZNER (**22**), no peptase activity was detected in blades or roots at pH 2.2–5.9. With the titration of WILLSTÄTTER and WALDSCHMIDT-LEITZ (**55**) no proteolytic action was demonstrated at pH 2.2, 3.3, 4, 4.9, 7.1, or 8.1. In these tests water was used as the medium for extraction. According to VINES (**51**) ereptase is generally more readily extracted by water while peptase is extracted more easily by 2 per cent. NaCl. However, where plants have been grown in a concentrated nutrient solution, VINES found that peptase could be extracted with water. Inasmuch as the nutrient solutions used in the Chicago experiments were much more concentrated than those used in the present studies, the idea occurred that the peptase of the former plants might have been more readily soluble in water than that of the present investigation, which might require additional salt for extraction. To determine this point, blade material was extracted for two days in 2 per cent. NaCl. The reaction was then adjusted to pH 5.2–5.3 with sodium dihydrogen phosphate, and a peptase test was performed using carmine fibrin. No sign of digestion occurred. The WILLSTÄTTER-WALDSCHMIDT-LEITZ method was employed at pH 4.9 and 5.4, with negative results.

The conclusion is drawn that under the conditions of the present investigations, no peptase was found. Possibly using a different method of extraction or a different hydrogen ion concentration, positive results would be obtained. Perhaps the more concentrated nutrient solutions used in the former study (**24**) caused the development of peptase strong enough to be detected. Peptase is probably present in cane but in such small amounts that it is often undetectable. For the study of the relation of potassium to the activity of peptase, soy beans or other plants high in proteins might be more desirable.

Notwithstanding the fact that no evidence of peptase was obtained, derangements in the formation of proteins were indicated. Growth was proportional to the amount of potassium supplied. In November the dry weight of the blades of series 3 was about half that of series 1, whereas series 3 contained nearly double the amount of amino nitrogen, as shown in table IV. This would seem to indicate difficulty in some step of the synthesis of proteins. Because the activity of ereptase was equal in all the blades, the indications are that the synthesis of the higher proteins was curtailed by the deficiency of potassium. Whether or not this was due to a weak activity of peptase as shown previously (**24**) could not be ascer-

tained in the present study. The analogy between the nitrogen and sugar data is interesting although inconclusive.

Discussion

Since the chemical transformations in plants are chiefly catalyzed by enzymes, it would seem that quantitative determinations of their activity in connection with chemical analyses in studies of mineral deficiencies might lead to important results. A few studies of the effects of potassium upon the activity of enzymes will be mentioned. DOBY and HIBBARD (**10, 11**) found more active invertase and diastase in sugar beets deficient in potassium than in those supplied with that element. HARTT (**24**) reported derangements in the activities of invertase, diastase, peptase, and catalase in sugar cane plants grown without potassium. ECKERSON (**12**) found that plants low in potassium are weak in reducase activity. In the present studies a decreased activity of invertase and increased activity of amylase were found in the cane plants deficient in potassium. It would seem that these derangements in enzyme action might be closely connected with the disturbance in the protein and carbohydrate metabolism of the plant.

While it is probably not essential to consider that all of the reactions catalyzed by enzymes are reversible, yet this has been proved for several and assumed for many. The supposition that there is a separate enzyme which condenses dextrose and levulose to form sucrose does not seem necessary in view of the recent work of OPARIN and KURSSANOW (**39**), who have demonstrated the synthetic action of invertase. Their report provides a summary of the subject of enzymatic synthesis. In the studies, the views that sucrose is not the first sugar formed in photosynthesis, and that both the synthetic and the analytic reactions are catalyzed by the same enzymes, are taken as working hypotheses. It is realized that further studies may disprove these points, but at present they seem to explain the results obtained.

The enzymes of sugar cane have been insufficiently studied. BROWNE (**4**) mentioned the presence of the following enzymes in cane: diastase, invertase, oxidase, and a reducing enzyme. In addition to these, BROWNE and BLOUIN (**5**) stated that peptonizing enzymes are present in cane stalks. HARTT (**24**) reported the results of quantitative determinations of diastase invertase, peptase, ereptase, and catalase. NEEB (**36**) reported the occurrence of saccharase, amylase, catalase, tryosinase, oxidase, peroxidase, and maltase.

PROTEIN METABOLISM

The subject of the utilization of potassium by plants, with particular reference to the interrelationships between carbohydrate and protein

metabolism in plants deficient in potassium, has been so well summarized by NIGHTINGALE *et al.* (**37**) in connection with studies of the tomato plant that it does not need to be repeated here. In substance, they conclude that where carbohydrates accumulate in potassium-deficient plants, a principal cause of their accumulation is a derangement in the synthesis of proteins. A similar conclusion had previously been reached by HARTT (**24**), who found higher percentages of total sugars and increased formation of lignin correlated with a deficiency in the activity of peptase in sugar cane plants grown without potassium.

In the present studies, derangements in the synthesis of proteins in the plants deficient in potassium were indicated indirectly by the curtailment of growth (**26**) and directly by the nitrogen determinations. Two months after starting the plants in the nutrient solutions, the blades of series 3 had a higher percentage of amino nitrogen and a lower percentage of protein nitrogen than those of series 1, the total nitrogen remaining about the same. Similar though less pronounced differences were found in the stems, as shown in table II. Not only was there a difference in percentages, but the actual amount of amino nitrogen in the blades of series 3 was almost double that in series 1, although the average dry weight of the plants of series 1 was about twice that of those of series 3, as shown in table IV. This accumulation of amino nitrogen in the blades and stems of the plants deficient in potash which occurred at the age of two months, together with the slight decrease in percentage of protein nitrogen, seems to be evidence of the failure of the potassium-deficient plants to synthesize proteins as usual. Attention is called to the fact that the curtailment in synthesis occurred after the formation of amino acids rather than before, indicating that in these plants the reduction of nitrates proceeded as usual. Notwithstanding this point, reducase determinations would be interesting and would have been made had time permitted.

The terminology of proteolytic enzymes has become considerably complicated in recent years. Following VINES (**51**), in this paper the simple distinction between peptase and ereptase is made, the former catalyzing the digestion of complex proteins to peptones and allied compounds, and the latter carrying the digestion from these intermediate products to amino acids. Since there is little evidence of a tryptic enzyme occurring generally in plants, that is left out of consideration. In the studies reported in 1929 (**24**) both peptase and ereptase were found to occur in cane, whereas in the present study ereptase was the only proteolytic enzyme detected. As mentioned in the results, the use of other solvents at other reactions might have shown the presence of peptase. Inasmuch as the activity of ereptase seemed not to be affected by the supply of potassium, with the possible exception of the roots, derangements in ereptase cannot explain the deficiency

in the synthesis of proteins. If the synthesis of proteins follows the same steps as their digestion, would not a weakened peptase activity result in the accumulation of peptase and proteoses rather than amino acids? It is, however, conceivable that both might occur. It is unfortunate that no determinations of the forms of nitrogen were made in the Chicago studies, and that the attempted detection of peptase in the Honolulu studies failed. Since at the age of two months the synthesis of proteins in the potassium-deficient plants had proceeded as usual, as far as the production of amino nitrogen, it seems likely that either some essential amino acids were lacking or that a derangement occurred in the activity of the higher proteolytic enzymes. There is need for further work along these lines.

Although in November the stems of the plants deprived of potash were slightly higher in amino nitrogen and lower in protein nitrogen than were the controls, in April the stems of the potassium-deficient plants had considerably lower percentages of amino, protein, and total nitrogen than the controls. The blades of the plants harvested in April showed higher percentages of amino, protein, and total nitrogen in the plants deficient in potassium than in those supplied with an adequate amount of that element. Thus the proteins of the blades and stems of the plants harvested in November varied in the same way, while in the April harvest the results of the protein analyses of the blades and stems were diametrically opposed. These observations may be explained by assuming that the chief seat of synthesis of amino acids and simple proteins is in the blades; that at least some water and nutrient salts can still move up in the xylem of the potassium-deficient plants; but that a necrosis of the phloem curtailed the translocation of nitrogenous compounds from the blades to the stems. Evidence of phloem necrosis possibly similar to that reported by JOHNSTON and DORE (**30**) has been presented in the first paper of this series (**26**), the evidence consisting of brown discolorations of the sieve tubes and companion cells in the midribs and stems of the plants deficient in potassium. The necrosis might be due either to a disturbance in the synthesis of proteins resulting in abnormal protoplasm in the sieve tubes and companion cells, or to the accumulations of iron at the nodes resulting in the coagulation of the proteins of the phloem, or to both conditions. Whatever the cause, it is apparent that such a necrosis might seriously interfere with the translocation of nitrogenous and carbohydrate compounds.

In short, the results here reported indicate that both the synthesis and the translocation of proteins in the sugar cane plant are decreased by a deficiency in potassium. The derangement in the synthesis of proteins is possibly caused by a weak activity of peptase, as shown by former studies (**24**). The interference with their translocation may be caused by the necrosis of the phloem.

Differences so slight as to be within the limits of experimental error in the November harvest became intensified by the time of the April harvest, when the blades of the plants deficient in potassium had greater percentages of reducing sugars and lower percentages of sucrose than the controls, as shown in table VI. Inasmuch as the activity of invertase was greater in the plants receiving potassium than in those deprived of that element, it would seem that invertase aids in the synthesis of sucrose in the blades of sugar cane.

In the stems of the plants harvested in April a distinction must be made between partial and complete starvation for potassium. The plants of series 2, which were only partially starved for potash, reacted the same way as the blades, having a higher percentage of reducing sugars and lower percentage of sucrose than the plants of series 1. The other plants were more severely affected, the percentages of reducing sugars, sucrose, and total sugars all being lower in series 3, 4, and 5 than in series 1. The activity of invertase was weaker in the plants starved for potash. The supply of dextrose, the essential source of energy for growth and life, was curtailed especially in the stems of series 4 and 5, both by the necrosis of the phloem which would interfere with the translocation of sugars from the blades to the stems, and by the weak activity of invertase which would decrease the inversion of the sucrose already in the stems.

The suggestion is therefore made that a deficiency in potassium in the sugar cane plant may interfere with the transformations between the hexoses and sucrose, both anabolic and catabolic, as well as with their translocation.

From the practical standpoint the object of the sugar planter is to obtain the plant which stores the most sucrose in the stems. Under field conditions it is unlikely that any plants as deficient in potassium as the plants of series 3, 4, and 5 would continue until harvest. Their weakened condition is so obvious that further applications of fertilizer would be made, or else they would probably die. The important distinction for the planter to make is between plants such as series 1 and 2, those plants which appear very much the same in color, size, and general condition but which show a difference in the amount of sucrose in the stems. The stems of series 2 were higher in reducing sugars and lower in sucrose than those of series 1, and the activity of invertase in series 2 was weaker than that of series 1. If plants in the field similar to the plants of series 2 could be recognized in time and given the proper fertilizer, it seems possible that the activity of their invertase could be increased and so increase the amount of sucrose stored in the stems.

The relationship between potash fertilization and yield is obscure. VAN DEN HONERT (**50**) found a low figure for Brix in variety P.O.J. 2878 deficient in potassium, but suggested that this might be caused by the fact that the suckers of the plants starved for potash were younger and less mature than the controls. As mentioned in the discussion of the first paper in this series (**26**), the results of some experiments have shown an increase in yield of sugar following the application of potash, others have shown no response, and still others have indicated a depressing effect, the last condition possibly resulting from a depression in the absorption of phosphorus. It is evident that a delicate relationship exists. While it is by no means suggested that the only factor involved is the activity of invertase, yet the present results show that enzyme to be important, and it is felt that further attention should be paid to the activity of invertase in determining the effects of fertilizer applications and other cultural practices. This matter is discussed more fully below.

For a complete understanding of the carbohydrate metabolism of potassium-deficient plants, certain fundamental principles should be determined. Is sucrose synthesized in the leaves by the enzyme invertase? Is sucrose merely a temporary storage product in the leaves, converted into hexoses before translocation to the stems, or is sucrose the main translocation form of sugar, or are both the hexoses and sucrose translocated? These questions being of general importance are summarized in textbooks of plant physiology. The investigations with sugar cane have been discussed by VISWANATH (**52**), who concluded that the bulk of evidence seems to favor the view that sucrose is built up in the stem from reducing sugars sent into it by the leaf. GEERLIGS (**19**), however, is of the opinion that sucrose, dextrose, and levulose are all continuously supplied by the leaf.

The writer is now conducting studies on these points and the results will be reported later. In the meantime it would seem that the present studies offer indirect evidence that sucrose in the sugar cane plant is synthesized by invertase, as already mentioned in a preliminary report (**25**). The blades of the plants deficient in potassium had the highest percentage of reducing sugars and lowest percentage of sucrose, and they also had the weakest activity of invertase. Since the percentage of total sugars remained about the same in the blades, it would seem that this is evidence of a derangement in the forms of sugars rather than a difference caused by some other factor, such as photosynthesis or protein formation. Interference with translocation may have affected the results in the material harvested in April, since both sucrose and hexoses were low in the stems of the plants deficient in potash, with the exception of the plants of series 2. If the action of invertase is only hydrolytic, then what is the explanation of the greater percentage of reducing sugars and lower percentage of sucrose in the plants

which had the weakest invertase activity? Further evidence of the importance of invertase in the synthesis of sucrose is found in the supplementary experiment using variety P.O.J. 2878, described earlier in this paper. Millable cane may be divided into two physiologically different regions: the green leaf cane (or that portion which bears green leaves) and the dry leaf cane (the part bearing dry leaves or none). It is in the green leaf portion of the stem that the most active storage of sucrose occurs, although some additional storage takes place in the dry leaf cane. As shown in table XII, the invertase activity of the green leaf cane was found to be double that of the dry leaf cane. The invertase of the blades was found to be equal in activity to that of the green leaf cane. It is evident that wherever sucrose is actively formed or stored, the activity of invertase is strong, whereas where the process of storage is practically completed, invertase decreases in activity.

The question of the accumulation of carbohydrates in plants deficient in potassium may next be considered. This has already been discussed with references to the literature in an earlier paper (**24**) and also by NIGHTINGALE (**37**). In the present study no evidence of carbohydrate accumulation was obtained. In the blades the total sugars were about the same in all five groups, while in the stems of the plants harvested in April there was a positive correlation between the amount of potassium supplied and the percentage of total sugars formed. As pointed out by NIGHTINGALE, the accumulation of carbohydrates may be an early condition in plants deficient in potassium, disappearing later. Inasmuch as the plants of the present study grew so much more rapidly than those of the former investigation, it may be that at the time of the first harvest the period of carbohydrate accumulation had already passed, if any occurred at all.

It has been shown so far that the utilization of potassium by the sugar cane plant involves the synthesis and translocation of both proteins and carbohydrates. Potassium may affect the synthesis of proteins through its effect on the enzyme peptase, as shown previously (**24**). Potassium affects the transformations of the sugars through its effect on invertase. The question of how potassium affects invertase may next be considered.

Various possibilities suggest themselves as to how potassium affects the activity of invertase: (1) the relationship may be direct, through its entering into the chemical composition of the enzyme, or through its effect as a specific activator; (2) it may be indirect, regulating the hydrogen ion concentration, or the buffer system; (3) it may be antagonistic, in offsetting the effects of inhibitory factors. In a previous report (**24**) the importance of potassium in the formation of invertase was stressed; those tests, however, were all unbuffered. Inasmuch as the activity of invertase in all the blades was equalized at the optimum reaction, it is now felt that potassium is not

essential for the formation of invertase. Because of the equalization of activity at the optimum reaction, it might be concluded that the differences in invertase activity in the blades are due solely to differences in hydrogen ion concentration. This is erroneous since, as shown in table IX, the blades of series 4 and 5 had equal invertase activity when tested unbuffered but differed in reaction; while those of series 3 and 4 had the same reaction but differed in activity. Regarding the possibility of there being differences in the buffer systems, this matter was considered, and it will be seen from figure 1 that little if any difference was found to exist. The suggestion that potassium may offset the effects of inhibitory factors, particularly salt constituents, deserves consideration since, as shown by FALES and NELSON (**14**), the addition of sodium chloride had practically no effect on the velocity of the hydrolysis of sucrose by yeast sucrase at the optimum reaction; but at all other reactions the salt inhibited the action of the enzyme. The greater ash content of the potassium-deficient plants harvested in November might inhibit invertase, but this explanation could not apply to the April material, because in that harvest the plants of series 1 and 2 had the greatest ash content.

Thus either there was some undetermined inhibitory factor, or potassium is a specific activator for invertase in sugar cane, in which latter case the addition of potassium to the enzyme tests should result in increasing the invertase activity; and equalizing the potassium in material from potassium-deficient and control plants should result in approximately equalizing the activity of invertase. Such an experiment was conducted, the results of which are given in table X. Enough potassium dihydrogen phosphate was added to the stem material of series 5 to equalize the potassium in series 1 and 5, and sodium dihydrogen phosphate was added to series 1 to maintain such conditions as hydrogen ion and osmotic concentrations. The test was conducted at pH 4.4. It was found that the addition of potassium to series 5 did not make the activity equal to that of series 1, but did increase it somewhat. As already mentioned, when tested unbuffered, the stem material of series 1 was 2.78 times as active as that of series 5; but when potassium was equalized at the optimum reaction, series 1 was only 1.31 times as active as series 5 (averages of all tests). Since the addition of sodium phosphate to series 1 did not increase the activity, while the potassium phosphate did activate the invertase of series 5, it would seem that potassium is a specific accelerator for the enzyme invertase. If this is true for other plants which store sucrose then it may help to explain the statement of COLIN and BILLON (**9**) that potassium plays a direct rôle in the formation of sucrose in beets.

The preceding discussion shows that the invertase of stems is affected more severely by potassium deficiency than is that of blades. The differ-

ence between the extremes is much greater in the stems than in the blades (table VIII), and the activity in the blades is equalized at the optimum reaction while that in the stems is not. Although a possible explanation is the higher percentage of potassium in the blades than in the stems, as shown in table XVI of the first paper in this series (**26**), yet the subject is too complex to permit complete understanding at the present time.

The accelerating effect of potassium may help to explain the differences in invertase activity in the green leaf cane as compared with that in the dry leaf cane. Table XII shows that the activity in the green leaf cane is double that of the dry leaf cane. Analyses performed by the Chemistry Department of this Station have shown that the percentage of potassium increases from the bottom to the top of the stalk. While other factors undoubtedly contribute to the differences in the activity of invertase in various parts of the stalk, it is suggested that the differences in percentage of potassium are important. Further studies along these lines are now under way.

Certain data in table VIII require discussion at this point. There it will be seen that the invertase of the stems of series 2, April material, is less active than that of series 1 or 3, whether buffered or not. This puzzled the writer until the distinction between green and dry leaf cane became apparent. The plants of series 1 and 2 were very much larger than the others and consequently had considerably more dry leaf cane. Inasmuch as the entire sticks were compounded, the weak invertase of the dry leaf cane would naturally decrease the figures for the entire stalk. This may also explain why the activity of the stems of the controls is so much less in the April than in the November harvest, there being considerably more dry leaf cane in April than in November. The distribution of invertase throughout the cane plant is now being studied and will be reported in a later contribution.

The sensitivity of the invertase of yeast to hydrogen ion concentration is well known and has been discussed by FALK (**15**). The data presented in table VII show that the invertase of sugar cane is similarly affected, having in these plants an optimum reaction very close to that of the invertase of yeast. The effect of hydrogen ion concentration upon the activity of invertase differs from its direct effect upon inversion: in the former case there is a definite optimum around pH 4.4, the activity falling off at other reactions; in the latter case inversion increases with increasing acidity as far as has been tested. The significance of this situation has been examined by FALK, who suggests that the hydrogen ion and the enzyme may attack sucrose differently.

The hydrogen ion concentration of the expressed sap of the blades and stems of the plants harvested December 4 was pH 5.2 to 5.3 (table I), while

the optimum reaction for the invertase of the plants harvested November 20 was pH 4 to 4.4 (table VII). The question arises as to the effect of the hydrogen ion concentration of the expressed sap of cane upon the quality of the juice. In Trinidad, FOLLETT-SMITH (**18**) found the sap of cane leaves to be pH 5.1–5.3. So far as the writer is aware, these are the first determinations to be made in Hawaii of the hydrogen ion concentration of the expressed sap of sugar cane. It is therefore impossible at present to compare these results with others in Hawaii. Some data are available for the reaction of crusher juices at the Honolulu Plantation Company, where the average of 50 determinations is pH 5.17 for crusher juice and pH 5.29 for mixed juice. Although these figures are close to those obtained in the present study, probably little value should be assigned to them in this connection for two reasons: (1) the crusher juice is contaminated with soil and fertilizers; and (2) the crusher juice is composed largely of the juice from dry leaf cane, which may or may not have the same reaction as green leaf cane. There is need, therefore, for further studies concerning the hydrogen ion concentration of the expressed sap, invertase activity, and juice quality. Several questions arise. Is the optimum reaction for the formation of sucrose the same as the optimum for the analytic activity of invertase? If the synthesis of sucrose is as sensitive to reaction as the activity of invertase is shown to be in this paper, which seems likely from the work of OPARIN and KURSSANOW (**39**), then studies should be made of the possibility of controlling the hydrogen ion concentration of the cell sap of sugar cane.

The invertase studies reported here show that the following factors affect its activity: potassium, hydrogen ion concentration, and location in the plant. Inasmuch as enzymes are very sensitive to conditions, it seems likely that many other climatic and edaphic factors may affect its activity. The evidence presented in this paper would seem to indicate that invertase in the sugar cane plant synthesizes sucrose, and that where invertase is most active the greater synthesis of sucrose occurs. For the past 20 years the increase in sugar percentage within the cane plant has not been commensurate with the increase in the applications of fertilizers, although the increase in the total sugar produced per acre has been large because of the greater size of the plants, a point which has been discussed by MARTIN and MCCLEERY (**35**). An important problem in the sugar industry today, therefore, is the improvement of the quality of the juices. Juice quality is known to be affected by weather, fertilizers, etc., but the mechanism is not fully understood. It would seem that both external and internal factors may affect the activity of invertase and that a study of these may aid in controlling the production of sugar. Such studies are now under way.

Another important effect of potassium deficiency in sugar cane is the increase in the activity of amylase. A relationship between potassium content and amylase activity has been suggested by several investigators. ROBBINS (**42**) studied the secretion of diastase by *Penicillium camembertii* and concluded that there was no relationship between potassium and diastase formation. ENGLIS and LUNT (**13**) reported on the diastatic activity of the nasturtium; they found that the activity of diastase decreased with increased potassium in sand, but in peat the medium application of potassium gave the highest activity. DOBY and HIBBARD (**10, 11**) found increased diastase activity in sugar beet plants deprived of potash. JAMES (**28**), from his studies of the effect of potassium upon photosynthetic efficiency in the potato, postulates that potassium increases the activity of diastase. Although for other plants the results are not always in agreement, a more active amylase in potassium-deficient plants seems now to be well established for sugar cane, since the present results are in accord with the former (**24**).

For the sake of clarity, most of the discussion of the amylase determinations was incorporated with the presentation of results and the reader is referred to that section at this point. The following possibilities arose as explanations of the increased amylase activity in the potassium-deficient plants: the quantitative regulation of amylase production by sugars; the stimulating effect of phosphorus; a direct regulatory or protective effect exerted by potassium. Certain theories almost certainly disproved were mentioned in the results and will not be repeated here.

Although some evidence was obtained in favor of the theory of the quantitative regulation of enzyme production by the substrate, that theory is not of general application in the present study because of the negative relationship between the amylase activity and percentage of reducing sugars in the stems of the plants harvested in April, whereas the correlation should be positive to uphold the theory.

The greater amounts of phosphorus in the potassium-deficient plants may play a rôle in their amylase activity, since in both the November and April material the largest amounts of phosphorus occurred in the plants deficient in potash, as shown in tables XV and XVI of the first report in this series (**26**). The correlation between phosphorus content and amylase activity is not perfect, however, and the equalization of the phosphorus in the blades of the November and April material did not result in equal activity of amylase. It is interesting, on the other hand, that the amylase of the potassium-deficient plants was activated by the addition of more phosphorus, while that of the control was not. The plants deficient in potassium contained more phosphorus and their amylase was more readily activated by phosphorus. This is indeed an important factor.

On the whole the evidence at hand seems to favor most strongly the idea that potassium acts as a regulator or protector of the enzyme amylase. It must be considered that the activity in the control plants is "normal" while that in the plants deficient in potassium is "abnormal." The suggestion of GOODWIN and HANGER (**20**) is pertinent, that amylase is a negatively charged ion. If so, it might readily unite with the positively charged potassium ion and this might explain the dialysis results. Dialyzing one day might remove the inorganic activators and thus decrease activity, but further dialysis (3 days) might cause or allow the amylase-potassium compound to dissociate, the potassium going through the membrane, leaving the amylase unprotected and thus more active. Possibly the negatively charged amylase is more readily hydrated than the amylase-potassium compound, since the charging of proteins is known to run parallel with hydration. This might aid in the digestion of starch, the first step of which is hydration. Further evidence of the protective effect of potassium is found in the fact that the amylase activity of the potassium-deficient plants was more readily activated by phosphorus than was that of the controls. DOBY and HIBBARD (**10, 11**) also found that the diastase of sugar beets deficient in potassium was activated by salts more readily than that of beets supplied with an adequate amount of potash. There is a negative relationship between potassium and amylase in both the blades and the stems of the material harvested in November as well as in April (table XV). Changing representative plants from solutions lacking potassium to the control solution in January resulted in a decrease in their amylase activity (table XIV). In short, the evidence points to the view that one function of potassium in the sugar cane plant is the regulation or "protection" of the enzyme amylase. Leaf diastase is supposed to consist of an inactive pro-enzyme and an activating co-enzyme, according to WAKSMAN and DAVISON (**53**). Possibly potassium exerts a protective action on the pro-enzyme, thus preventing its activation by the co-enzyme or other accelerators.

The condensations of dextrose in the formation of dextrins and starch tie up the sugar in undesirable forms which may result in decreasing the yield of sucrose. Starch occurs in cane as a temporary storage product in the starch sheaths of the leaves. This starch is probably for the most part digested to sugar and translocated to the stems and stored as sucrose, in the ordinary course of events. In certain varieties, however, a deposition of starch occurs in the stems, particularly in the nodes. This is especially true of Uba canes, a condition which has been studied by FEUILHERADE (**16, 17**), HADDON (**23**), VON STIEGLITZ (**44**), and WELLER (**54**). One is led to wonder whether the amylase activity of Uba is different from that of other varieties.

The production of too much starch and the like in cane is undesirable not only because of the utilization of dextrose which should go to the formation of sucrose, but also because the presence of certain gums, dextrins, and other higher carbohydrates in the juices leads to difficulties in the process of clarification, as brought out by FEUILHERADE and STIEGLITZ.

Amylase and invertase differ in several respects and a consideration of these differences is interesting. Amylase was more active in the potassium-deficient plants whereas invertase was less active. Amylase was found to be much less sensitive to hydrogen ion concentration than was invertase. Invertase was activated by potassium but not by sodium, whereas amylase was activated by everything tested. The two enzymes behaved similarly in that both were more readily activated in the potassium-deficient plants than in the controls.

RÔLE OF POTASSIUM

The question of the rôle of potassium in the nutrition of plants has interested plant physiologists for years, and men have sought to assign one particular function to the element, *e.g.*, photosynthesis, protein synthesis, translocation, or others. The research of recent years, (**3, 21, 24** and **26, 28** and **29, 37,** and others) has shown that the problem is not so simple, but that potassium probably affects directly or indirectly many if not all of the cellular activities of plants. Thus the present studies show that sugar cane plants deficient in potassium may develop the following characteristics: low percentage of moisture; high total ash content when young, followed later by low ash percentage; high percentages of calcium, magnesium, phosphorus, and iron at certain ages; derangements in the synthesis and translocation of proteins and sugars; phloem necrosis; accumulations of iron at the nodes; weak activity of invertase; and strong amylase activity. Undoubtedly other derangements occur. The total result of these conditions is the cessation of growth of the entire plant, discoloration of the leaves, and dieback of the leaf tips, usually mentioned as the symptoms of potash starvation. The writer is still of the opinion that these derangements are concatenated, as proposed previously (**24**). One of the earliest occurrences in potassium starvation is the increased absorption of other ash constituents, notably phosphorus, calcium, and magnesium, which may enter the plant more readily in the absence of the more mobile potassium. Because enzymes are sensitive to conditions, being readily activated and inactivated, they are soon affected by the lack of potassium. Amylase, not being protected by potassium, is more readily activated by the greater amount of phosphorus, which may cause the accumulation of starch which has been found in some plants. Invertase, peptase, reducase, and probably other enzymes either fail to develop or lose their activity. In the present study

invertase activity was weak, possibly due to the lack of activation by potassium. The weak activity of invertase led to derangements in the transformations between the reducing sugars and sucrose. Derangements in the formation of proteins were also found. The greater amount of iron which entered the potassium-deficient plants accumulated at the nodes, possibly because of a more sluggish transpiration stream. Necrosis of the phloem, which occurred probably both because of the accumulations of iron and because of the derangements in the synthesis of proteins, led to a decrease in the translocation of both sugars and nitrogenous compounds. This eventually caused the stems to be low in reducing sugars, sucrose, total and amino nitrogen. The decrease in the absorption of ash constituents found at the end of the experiment was only to be expected, the poor absorbing capacity of the roots being shown by their weak and discolored condition. Probably all of these factors contributed to the development of the external symptoms.

The properties of potassium by which it functions are probably not one but several. ANDRÉ and DEMOUSSY (**1, 2**) have stressed its mobility which, as was shown in the first paper of this series (**26**), may help explain its migration out of the dying leaves. Also the decrease of the mobile potassium may cause the increase in the absorption of other ash constituents. The suggestion of LOEB (**33**) that the selective absorption of potassium by plants is due to its being built up into complex compounds has been disproved by KOSTYTSCHEW and ELIASBERG (**32**), who found that potassium could be extracted *in toto* by water.

LOEW (**34**) found that potassium has special condensing abilities which sodium lacks. Considerable work has been done by STOKLASA and coworkers (**45–49**) as well as by ZWAARDEMAKER (**56**) to show that the radioactive properties of potassium are important physiologically, whereas BURKSER, BRUN, and BRONSTEIN (**7**) found no evidence of bioradio-activity in the plants they studied.

The writer cannot subscribe to the suggestion that the photoelectric effect of potassium is of physiological significance, as recently proposed by BRUNO (**6**) and JACOB (**27**). Although it is true that potassium is one of the few elements which exhibits the photoelectric effect in ordinary daylight and that it is slightly photoelectric even at 700 μμ (**41**) (thus throughout the region of the spectrum which is chiefly absorbed by plants and where photosynthesis occurs), yet a serious difficulty prevents this from being of significance in the physiology of plants. In order for potassium to exhibit the photoelectric effect in plants it is essential that some of the element be present in colloidal metallic form, because it is the valence electrons which are emitted in the photoelectric effect. If these are already oxidized in a bond with an acid radical none will be free to be emitted photoelectrically. Be-

cause all parts of plants contain water in which potassium readily oxidizes, the presence of colloidal metallic potassium in plants seems impossible.

Unless some way is found of surmounting this difficulty, it appears that the photoelectric properties of potassium cannot be used in explaining its physiological activities in plants. The fact that symptoms of potash deficiency develop in bright light more quickly than in dim light (**26**) may be just a question of limiting factors.

Because of its mobility, its special condensing properties, its radioactivity, and probably also other properties, it seems likely that potassium affects either directly or indirectly most of the activities of plants, and it is at present impossible to assign one particular process as the special rôle of potassium in the physiology of plants.

Summary

1. This paper reports the results of determinations of the enzymes invertase, amylase, and ereptase; analyses of total and amino nitrogen, reducing sugars and sucrose; and certain physico-chemical determinations of the juices expressed from the leaves, stems, and roots of sugar cane plants supplied with varying amounts of potassium.

2. Very little difference was found between the hydrogen ion concentration, titratable acidity, and buffer systems of the expressed juices of the controls and the plants deficient in potassium.

3. The effect of potassium deficiency upon the relative amounts of protein and amino nitrogen in the plants varied with their age.

4. Two months after starting the plants in the nutrient solutions there was a higher percentage of amino nitrogen and a lower percentage of protein nitrogen in the blades and the stems of the plants starved for potassium than in the controls.

5. Seven months after starting the plants in the nutrient solutions higher percentages of amino, protein, and total nitrogen were found in the blades of the potassium-deficient plants, while in the stems the opposite relationship was found to occur.

6. The conclusion is drawn that both the synthesis and the translocation of proteins are diminished by potash starvation.

7. The curtailment in the synthesis of proteins was found to occur after the formation of amino acids rather than before, indicating that in these plants the reduction of nitrates proceeded as usual.

8. The suggestion is made that the interference with the translocation of proteins and carbohydrates is caused by a necrosis of the phloem.

9. The blades of the plants deficient in potassium contained higher percentages of reducing sugars and lower percentages of sucrose than the controls. The percentages of total sugars in the blades remained about the same.

10. The relationships between the sugars in the stems depended upon the degree of potassium deficiency. The stems of the plants partially starved for potassium (series 2) contained higher percentages of reducing sugars and lower percentages of sucrose than the controls; while the stems of the plants more completely deprived of potassium (series 4 and 5) were very low in reducing sugars as well as sucrose. There was a positive correlation between the amount of potassium supplied and the total sugar stored.

11. The conclusion is drawn that potassium deficiency results in derangements in the transformations between the hexoses and sucrose, as well as in curtailment of their translocation.

12. No evidence of the accumulation of carbohydrates in the plants deficient in potassium was obtained in this study.

13. The optimum reaction for the activity of invertase in the blades and the stems was found to be around pH 4.4.

14. The invertase activity of the plants deficient in potassium was weaker than that of the controls, in both blades and stems, when tested unbuffered. The invertase activity in stems was affected more severely by potassium deficiency than that in blades.

15. When tested at the optimum reaction, the invertase activity was equal in all the blades. The activity in the stems was not equal at the optimum reaction, although the difference between the extremes was less than when tested unbuffered.

16. Because the invertase activity in all the blades was equalized at the optimum reaction, it seemed that potassium is not essential for the formation of invertase.

17. Because the addition of potassium phosphate to the stem material of series 5 increased the activity of invertase while the addition of sodium phosphate to series 1 had no effect, it is concluded that potassium is a specific activator for invertase in sugar cane.

18. In a supplementary experiment, in which variety P.O.J. 2878 was used, the invertase activity of the green leaf portion of the stalk was double that of the dry leaf cane.

19. These studies offer indirect evidence that sucrose in the sugar cane plant is synthesized by invertase.

20. The suggestion is made that determinations of the activity of invertase might aid in distinguishing between plants in the field which, like the plants of series 1 and 2, are very much the same in color, size, and general condition but which show a difference in the amount of sucrose in the stems.

21. The optimum reaction for the amylase of blades was found to be pH 5.9. The amylase of stems seemed not to be much affected by the hydrogen ion concentration.

22. Amylase activity was greater in the plants deficient in potassium than in the controls, in both blades and stems.

23. Studies were made of the effects of potassium, phosphorus, calcium, magnesium, dialysis, and sugars upon the activity of amylase.

24. Several possible causes of the increased activity of amylase in the plants deficient in potassium are examined and the conclusion is suggested that potassium deficiency removes the protective action of potassium from amylase, thus allowing the enzyme to be activated more readily by phosphorus, which is present in potassium-deficient plants in greater amounts than in the plants supplied with an adequate amount of potash.

25. The increased activity of amylase in the plants starved for potash is an undesirable characteristic because under the conditions of condensation in the stems it might lead to the formation of starch. This is undesirable both because of the utilization of dextrose which should go to the formation of sucrose and because the presence of dextrins and certain other higher carbohydrates in the juices leads to difficulties in the process of clarification.

26. The optimum reaction for the activity of ereptase in the blades and roots was pH 4.9, and in stems, pH 5.9.

27. Potassium seemed to have no effect upon the activity of ereptase, with the exception of the roots, in which the greater activity occurred in the plants deficient in potassium.

28. Attempts were made at several reactions to determine the activity of peptase, using the carmine fibrin method of GRÜTZNER and the titration method of WILLSTÄTTER and WALDSCHMIDT-LEITZ. No peptase activity was detected.

29. The writer is still of the opinion that the derangements in the physiology of sugar cane deficient in potassium are concatenated, as proposed previously. An outline of the concatenation of these derangements is suggested.

30. The properties of potassium by which it functions are considered. Regarding the suggestions recently appearing in the literature that the photoelectric properties of potassium may be of physiological importance in plants, the difficulty is pointed out that this would require the presence of colloidal metallic potassium in plants, whereas the universal distribution of water in plant tissues would render this impossible owing to the ready oxidation of potassium.

31. It seems likely that potassium affects either directly or indirectly most of the activities of plants, and it is at present impossible to assign one particular process as the special rôle of potassium in the physiology of plants.

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LITERATURE CITED

1. ANDRÉ, G., and DEMOUSSY, E. La répartition du potassium et du sodium dans les végétaux terrestres. Bull. Soc. Chim. Biol. **9**: 861–866. 1927.
2. ————, and ————. Sur la répartition du potassium et du sodium chez les végétaux. Compt. Rend. Acad. Sci. (Paris) **184**: 1501–1503. 1927.
3. BARTHOLOMEW, R. P., and JANSSEN, G. The rate of absorption of potassium by plants and its possible effect upon the amount of potassium remaining in soils from applications of potassium fertilizers. Arkansas Agr. Exp. Sta. Bull. 265. 1931.
4. BROWNE, C. A., JR. Results of investigations at the Sugar Experiment Station Laboratory for 1903. Louisiana Planter and Sugar Mnf. **32**: 47–51. 1904.
5. ————, and BLOUIN, R. E. The chemistry of the sugar cane and its products in Louisiana. Louisana Agr. Exp. Sta. Bull. 91. 1907.
6. BRUNO, A. Hypothèses nouvelles sur le mode d'action de potasse. Rev. Viticulture **69**: 60–62. 1928.
7. BURKSER, E., BRUN, I., and BRONSTEIN, K. Zur Frage der Bioradioaktivität der Pflanzen und der Existenz von Radiumelementen in denselben. Biochem. Ztschr. **181**: 145–148. 1927.
8. CLARK, W. M. The determination of hydrogen ions. 3d ed. Williams and Wilkins Co., Baltimore. 1928.
9. COLIN, H., and BILLON, P. Potasse et betterave. Rev. Gén. Bot. **44**: 5–23. 1932.
10. DOBY, G., and HIBBARD, R. P. Verhalten, insbesondere Ionenaktivierung von Pflanzenenzymen in Abhängigkeit von der Ernährung. I. Mitteilung: Über die Amylase kalihungriger Zuckerrüben. Biochem. Zetschr. 176: 165–177. 1926. II. Mitteilung: Über die Saccharase kalihungriger Zuckerrüben. Biochem. Ztschr. **178**: 139–151. 1926.
11. ————, and ————. Nutrient ions of plants and the ion activation of plant enzymes. Jour. Biol. Chem. **73**: 405–416. 1927.
12. ECKERSON, SOPHIA H. Conditions affecting nitrate reduction by plants. Contrib. Boyce Thompson Inst. **4**: 119–130. 1932.
13. ENGLIS, D. T., and LUNT, H. A. Effect of the concentration of potassium salts in soil media upon the carbohydrate metabolism of plants. The diastatic activity of the nasturtium. Soil Sci. **20**: 459–463. 1925.

14. FALES, H. A., and NELSON, J. M. The effect of sodium chloride upon the action of invertase. Jour. Amer. Chem. Soc. **37**: 2769–2786. 1915.
15. FALK, K. G. The chemistry of enzyme actions. 2d ed. Chem. Catalogue Co., New York. 1924.
16. FEUILHERADE, L. The presence of starch in the Uba cane. Occurrence and conditions favorable for its presence: Its effect on sugar manufacture and advantages derived from its removal. South African Sug. Jour. **11**: 739, 741. 1927.
17. ————. Starch in Uba juices. Proc. South African Sug. Tech. Assn. pp. 71–74. March, 1929.
18. FOLLETT-SMITH, R. R. The effect of some growth conditions on chemical composition and metabolism in the sugar-cane leaf. Minutes and Proc. of Froghopper Invest. Com. Par. X. Trinidad Gov. Print. Of. Port-of-Spain. pp. 331–347. 1928.
19. GEERLIGS, H. C. P. Cane sugar and its manufacture. 2d rev. ed. Norman Rodger, London. 1924.
20. GOODWIN, T. C., and HANGER, I. C. The ionic nature of amylase. Proc. Soc. Exp. Biol. Med. **23**: 261–263. 1926.
21. GREGORY, F. G., and RICHARDS, F. J. Physiological studies in plant nutrition. I. The effect of manurial deficiency on the respiration and assimilation rate in barley. Ann. Bot. **43**: 119–161. 1929.
22. GRÜTZNER, P. Über eine neue Methode Pepsinmengen kolorimetrisch zu bestimmen. Pflüger's Arch. ges. Physiol. **8**: 452–459. 1874.
23. HADDON, E. A problem for the factory working Uba juice. Effects of starch and methods of elimination. South African Sug. Jour. **10**: 629, 631. 1926.
24. HARTT, CONSTANCE E. Potassium deficiency in sugar cane. Bot. Gaz. **88**: 229–261. 1929.
25. ————. Studies of the invertase of sugar cane. A preliminary report. Hawaiian Pl. Record **37**: 13–14. 1933.
26. ————. Some effects of potassium upon the growth of sugar cane and upon the absorption and migration of ash constituents. Plant Physiol. **9**: 399–452. 1934.
27. JACOB, A. Investigations on the rôle of potassium. (Unpublished). Cited by THOMAS, W. Composition of current and previous season's branch growth in relation to vegetative and reproductive responses in *Pyrus malus* L. Plant Physiol. **7**: 391–445. 1932.
28. JAMES, W. O. Studies of the physiological importance of the mineral elements in plants. I. The relation of potassium to the properties and functions of the leaf. Ann. Bot. **44**: 173–198. 1930.
29. ————. Studies of the physiological importance of the mineral elements in plants. II. Potassium: its distribution, movement,

and relation to growth in the potato. Ann. Bot. **45**: 425–442. 1931.

30. JOHNSTON, E. S., and DORE, W. H. The influence of boron on the chemical composition and growth of the tomato plant. Plant Physiol. **4**: 31–62. 1929.
31. ————, and HOAGLAND, D. R. Minimum potassium level required by tomato plants grown in water cultures. Soil Sci. **27**: 89–109. 1929.
32. KOSTYTSCHEW, S., and ELIASBERG, P. Über die Form der Kaliumverbindungen in lebenden Pflanzengeweben. Ztschr. physiol. Chem. **111**: 228–235. 1920.
33. LOEB, J. The dynamics of living matter. On the specific difference between the nutritive solutions for plants and animals. pp. 71–77. Macmillan, New York. 1906.
34. LOEW, O. The physiological rôle of mineral nutrients. U. S. Dept. Agr. Div. Veg. Phys. and Path. Bull. 18. 1899.
35. MARTIN, J. P., and MCCLEERY, W. L. Juice quality and cane ripening. 51st Ann. Meeting, H. S. P. A. pp. 411–504. 1932. Advertiser Pub. Co., Honolulu.
36. NEEB, JR., A. P. De enzymen van het suikerriet. Arch. Suikerind. in Nederlandsch-Indie **38**: Deel II: 1001–1003. 1930.
37. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull. 499. 1930.
38. OPARIN, A., and DJATSCHKOW, N. Über die Fermentbildung in reifenden Samen. Biochem. Ztschr. **196**: 289–293. 1928.
39. ————, and KURSSANOW, A. Über die enzymatische Synthese des Rohrzuckers. Biochem. Ztschr. **239**: 1–17. 1931.
40. REED, H. S., and HAAS, A. R. C. Studies on the effects of sodium, potassium, and calcium on young orange trees. California Agr. Exp. Sta. Tech. Paper 11. 1923.
41. RICHARDSON, O. W., and YOUNG, A. F. A. The thermionic work-functions and photoelectric thresholds of the alkali metals. Proc. Roy. Soc. London A **107**: 377–410. 1925.
42. ROBBINS, W. J. Influence of certain salts and nutrient solutions on the secretion of diastase by *Penicillium camembertii*. Amer. Jour. Bot. **3**: 234–260. 1916.
43. SJÖBERG, K. Beiträge zur Kenntnis der Amylase in Pflanzen. I. Über die Bildung und das Verhalten der Amylase in lebenden Pflanzen. Biochem. Ztschr. **133**: 218–293. 1922.

44. VON STIEGLITZ, C. R. The starch contents of some sugar canes in Queensland. Queensland Soc. Sug. C. Tech., Proc. 3d Ann. Conf. pp. 63–68. 1932.
45. STOKLASA, J. De l'influence de l'uranium et du plomb sur la végétation. Compt. Rend. Acad. Sci. (Paris) **156**: 153–155. 1913.
46. ————. Über die Radioaktivität des Kaliums und ihre Bedeutung in der chlorophyllosen und chlorophyllhaltigen Zelle. Biochem. Ztschr. **108**: 109–139. 1920.
47. ————. Der Mechanismus der physiologischen Wirkung der Radiumemanation und der Radioaktivität des Kaliums auf die biochemischen Vorgänge bei dem Wachstumprozess der Pflanzen. Biochem. Ztschr. **108**: 140–172. 1920.
48. ————, and PĚNKAVA, J. Über den Chemismus des Zuckerabbaues in der lebenden Pflanzenzelle und den Einfluss der Radioaktivität auf die anaerobe Atmung der Pflanzenorganismen. Ztschr. techn. Biol. **12**: 362–433. 1926.
49. ————, SEBOR, J., and ZDOBNICKY, V. Sur la synthèse des sucres par les émanations radioactives. Compt. Rend. Acad. Sci. (Paris) **156**: 646–648. 1913.
50. VAN DEN HONERT, T. H. Onderzoekingen over de voedingsphysiologie van het suikerriet. Archief Suikerind. in Nederl.-Ind. **40**: Deel III: 1539–1608. 1932.
51. VINES, S. H. The proteases of plants (VI). Ann. Bot. **23**: 1–18. 1909.
52. VISWANATH, B. Studies in the chemistry of sugar cane. Agr. Jour. India **14**: 440–475. 1919.
53. WAKSMAN, S. A., and DAVISON, W. C. Enzymes. Williams and Wilkins Co., Baltimore. 1926.
54. WELLER, D. M. Accumulations of starch in the stalks of Natal Uba cane grown on acid soil. Hawaiian Pl. Record **33**: 294–299. 1929.
55. WILLSTÄTTER, R., and WALDSCHMIDT-LEITZ, E. Alkalimetrische Bestimmung von Aminosaüren und Peptiden. Ber. deutsch. chem. Ges. **54**: 2988–2993. 1921.
56. ZWAARDEMAKER, H. On physiological radio-activity. Jour. Physiol. **53**: 273–289. 1920.