⊀ EFFECTS OF POTASSIUM DEFICIENCY AND EXCESS UPON CERTAIN CARBOHYDRATE AND NITROGENOUS CONSTITUENTS IN GUAYULE[1] ɣ

BRUCE J. COOIL[2] AND M. C. SLATTERY[2]

(WITH TWO FIGURES)

The nature of the carbohydrates of translocation and the reserve carbohydrates in guayule has only recently been elucidated. McRARY and TRAUB (28) isolated a fructosan, tentatively identified as inulin, and HASSID et al. (19) definitely characterized this substance as inulin. Later, TRAUB and SLATTERY (41) showed that in addition to inulin, levulins are also present in guayule tissue, and in relatively greater amounts. TRAUB and SLATTERY (41) also indicated the importance of relating the 89 per cent. ethanol soluble and insoluble levulins to plant functioning. TRAUB, SLATTERY and WALTER (42) have shown that the carbohydrates of translocation in guayule are apparently the free reducing sugars since they are always present in relatively small amounts in the roots, stems and branches but predominate in the leaves. These workers have also shown that of the total free reducing sugars, the amounts of free non-fructose sugars are always greater than the amounts of free fructose in spite of the fact that the chief reserve carbohydrates in guayule are polymers of fructose, levulins and inulin. The conditions of the present experiment were not conducive to large accumulations of inulin, or higher polymer levulins, insofar as the experiment consisted of a phase of rapid growth.

So far as the writers are aware, no detailed study has been made of the nitrogenous fractions in guayule.

Effects of potassium supply upon growth responses and mineral contents of guayule plants and upon cation and inorganic and organic anion contents of the leaves have been reported (8, 9). In this paper are considered carbohydrate and nitrogenous fractions in tissues of the same plants. Carbohydrate fractions in plants grown with and without potassium are also reported.

Methods

The methods employed in growing the plants have been described (8). Treatments designated K_1, K_2 and K_3 comprised series 1. The nutrient

solutions for these treatments contained 0.5, 3 and 12 milli-equivalents K per liter, respectively. These solutions each contained 10 m.e. Ca per liter and no Na except as impurities. Treatments designated NaK_1, NaK_2 and NaK_3 comprised series 2. The solutions supplied to these plants contained the same concentrations of K as the corresponding solutions of series 1, but each solution contained 4 m.e. Ca and 6 m.e. Na per liter. In a concurrent experiment, nursery-grown plants from the same lot received solutions with and without potassium. The solutions employed were the complete solution containing nitrate as the only source of nitrogen and the minus potassium solution suggested by HOAGLAND and ARNON (21). The treatments are correspondingly designated + K and – K. Plants of the latter experiment received nutrient treatments from December 23, 1944, until April 24, 1945. The methods of growing and harvesting the plants were otherwise the same as in the main experiment.

Reducing sugars, levulins and inulin were determined as described by TRAUB and SLATTERY (40). Total nitrogen was determined in dried samples by a modification of the procedure of PEPKOWITZ and SHIVE (32) described by KELLEY et al. (23). Ammonia, α-amino and total amide nitrogen were estimated by the procedures of SCHLENKER (37). For total soluble nitrogen, a 5-ml. aliquot of sap was heated to 70° C., cooled, centrifuged and the precipitate washed twice with 5 ml. portions of water, centrifuging after each washing. The supernatant liquid and washings were made to a volume of 50 ml. A 10-ml. aliquot of the dilute sap was transferred to a 100-ml. volumetric flask. The nitrate was carefully reduced with reduced iron powder essentially as described by PUCHER, LEAVENWORTH and VICKERY (33). Three ml. of sulphuric acid (1 plus 1) were added. After mixing, 1 gram of reduced iron powder was introduced. The flask was shaken for ten minutes, then gently heated to boiling on a gas hot plate, boiled five minutes and cooled. Subsequent digestion was accomplished by a modification of the procedure of PEPKOWITZ and SHIVE (32) as adapted to analysis of guayule tissue by KELLEY et al. (23). Five ml. of concentrated sulphuric acid containing 2.4 gm. selenium oxichloride per liter were introduced. The flask was heated gently until the water was expelled and finally digested until clear. The solution was then cooled and made to volume with distilled water. Aliquots of the digested solution were made alkaline with sodium hydroxide, aspirated into dilute sulphuric acid and nesslerized. The Nessler's reagent was prepared by the procedure of FRAPS and STERGES (11). The residue from the sap which had been heated, cooled and centrifuged, was dissolved in concentrated sulphuric acid and digested until clear. The digest was cooled, made to volume, and an aliquot nesslerized. In all samples this fraction was very low and no significant variations resulted from nutrient treatments. Therefore, in presenting the results, the sap soluble coagulable nitrogen has been added to the insoluble nitrogen fraction. Soluble organic nitrogen was calculated from the total soluble nitrogen by correcting for nitrate and ammonium nitrogen.

Results

CARBOHYDRATE FRACTIONS

Total carbohydrate contents of the plants are shown in table I.   When expressed in terms of grams per plant, no significant variation in total carbohydrates resulted from treatment variations within a series.   The contents in series 1, however, were consistently higher than those in series 2. In part, these differences resulted from the smaller plants in any given potassium treatment of series 2.   In terms of milligrams per gram of dry weight, the K_1 treatment had a significantly higher concentration of total carbohydrates than did any of the other treatments.   Other differences between individual treatments were not statistically significant, although these values were generally lower in series 2 than in series 1.

TABLE I

TOTAL CARBOHYDRATE CONTENTS* OF PLANTS RECEIVING VARIOUS NUTRIENT TREATMENTS

TREATMENT	GRAMS PER PLANT	MG. PER GRAM OF DRY WEIGHT
K_1	0.262	19.0
K_2	0.275	16.1
K_3	0.266	15.9
NaK_1	0.182	13.6
NaK_2	0.207	14.1
NaK_3	0.180	13.8
DRFS† 0.05	0.043	2.6
0.01	0.062	3.7

* Carbohydrates of leaves, stems and roots.
† Difference required for significance.

The carbohydrate compositions of individual tissues are shown in table II.   In both expanding and mature leaves, total carbohydrates were highest in the low potassium treatments (K_1 and NaK_1).   In the expanding leaves these accumulations are accounted for entirely in terms of reducing sugars. In the mature leaves of the K_2 plants inulin was higher than in the mature leaves of plants of other treatments.   In the young stems total carbohydrates and reducing sugars were significantly higher in K_1 than in other treatments.   In these tissues the relatively high variability in levulins does not permit close comparison.

In the old stems, total carbohydrates were evidently decreased by either low or high potassium treatments in series 1 (i.e., lower in K_1 or K_3 than in K_2).   Analogous differences in series 2 were not statistically significant. In the roots, total carbohydrates were lower in K_1 than in K_2.   In the old stems and roots, total carbohydrates were generally lower in series 2 than in series 1 at any given level of potassium.   In these tissues, in contrast to the leaf and young stem tissues, variations in total carbohydrates resulted almost wholly from variations in levulins.

In column 5 of table II, reducing sugars are expressed as percentage of

TABLE II

CARBOHYDRATE FRACTIONS*

TREATMENT	TOTAL CARBOHYDRATES	INULIN	LEVULIN	REDUCING SUGARS	REDUCING SUGARS
	PERCENTAGE OF DRY WEIGHT				PERCENTAGE OF TOTAL CARBOHYDRATES*
EXPANDING LEAVES					
K_1	1.38	0.18	0.36	0.83	60.1
K_2	0.82	0.16	0.28	0.38	46.3
K_3	0.97	0.11	0.48	0.37	38.1
NaK_1	1.13	0.20	0.30	0.64	56.6
NaK_2	0.90	0.19	0.34	0.36	40.0
NaK_3	0.79	0.11	0.38	0.30	38.0
DRFS† 0.05	0.22	n.s.	n.s.‡	0.19	....
0.01	0.31	....	....	0.26	....
MATURE LEAVES					
K_1	1.97	0.19	0.44	1.35	57.9
K_2	1.25	0.34	0.35	0.57	45.6
K_3	1.22	0.21	0.59	0.42	34.4
NaK_1	1.53	0.21	0.46	0.86	56.2
NaK_2	1.25	0.22	0.51	0.51	40.8
NaK_3	1.12	0.14	0.55	0.43	38.4
DRFS† 0.05	0.27	0.11	0.21	0.34	....
0.01	0.39	0.15	0.30	0.49	....
YOUNG STEMS					
K_1	2.41	0.13	1.32	0.96	39.8
K_2	1.66	0.16	0.98	0.53	31.9
K_3	1.96	0.10	1.60	0.26	13.3
NaK_1	1.38	0.07	0.90	0.41	29.7
NaK_2	1.67	0.12	1.24	0.31	18.6
NaK_3	1.49	0.13	1.05	0.31	20.8
DRFS† 0.05	0.68	n.s.	n.s.	0.29	....
0.01	0.96	....	....	0.40	....
OLD STEMS					
K_1	1.95	0.19	1.32	0.43	22.1
K_2	2.40	0.25	1.71	0.44	18.3
K_3	1.87	0.15	1.29	0.43	23.0
NaK_1	1.27	0.15	0.77	0.35	27.6
NaK_2	1.67	0.14	1.17	0.36	21.6
NaK_3	1.51	0.12	1.05	0.35	23.2
DRFS† 0.05	0.42	n.s.	0.31	....	....
0.01	0.60	....	0.45	....	....
ROOTS					
K_1	1.89	0.03	1.22	0.63	33.3
K_2	2.62	0.09	1.96	0.57	21.8
K_3	2.26	0.01	1.66	0.59	26.1
NaK_1	1.44	0.03	0.94	0.47	32.0
NaK_2	1.68	0.08	1.14	0.46	27.4
NaK_3	1.76	0.16	1.10	0.49	27.8
DRFS† 0.05	0.60	n.s.	0.48	0.12	....
0.01	0.85	....	0.68	0.17	....

* Inulin, levulins and reducing sugars.
† Difference required for significance.
‡ No significant difference.

total carbohydrates. The interesting aspect of these data is the fact that in each plant part of the reducing sugars represent a higher percentage of the total carbohydrates in low potassium treatments than in intermediate potassium treatments in each series. The high proportion of reducing sugars found in the leaves of low potassium plants was due to increased concentrations of these sugars, rather than to variations of other fractions. In the old stems and roots of these plants, the high proportion of reducing sugars resulted from decreased concentrations of levulins.

TABLE III

CARBOHYDRATE CONTENT OF PLANTS GROWN WITH AND WITHOUT POTASSIUM*

TREATMENT	TOTAL CARBOHYDRATES†	INULIN	LEVULIN	REDUCING SUGARS	REDUCING SUGARS AS PER CENT OF TOTAL CARBOHYDRATES
PERCENTAGE OF THE DRY WEIGHT					
EXPANDING LEAVES					
− K	1.79	0.10	0.40	1.29	72.1
+ K	1.22	0.26	0.42	0.54	44.3
MATURE LEAVES					
− K	3.50	0.08	0.74	2.69	76.9
+ K	1.42	0.09	0.55	0.79	55.6
YOUNG STEMS					
− K	3.39	0.02	1.79	1.59	46.9
+ K	3.11	0.11	2.54	0.46	14.8
OLD STEMS					
− K	1.59	0.25	0.89	0.44	27.7
+ K	2.53	0.31	1.76	0.46	18.2
ROOTS					
− K	1.88	0.32	1.01	0.55	29.3
+ K	2.77	0.08	2.20	0.49	17.7
FLOWERS AND FRUITS					
− K	4.57	0.03	0.93	3.62	79.2
+ K	5.11	0.08	1.04	4.00	78.3

* 17-month nursery plants received nutrient treatments from December 23, 1944, to April 24, 1945.
† Inulin plus levulins plus reducing sugars.

A concurrent experiment consisted of treatments where potassium was supplied or withheld (cf. methods). Carbohydrate contents of these plants are shown in table III. As in the previous experiment, the results reported are mean values from three replications.

The main effects of the low potassium treatments noted above were accentuated by withholding potassium. In the leaves greater total carbohydrate concentrations were found in the − K treatment than in the + K treatment. These differences may be largely attributed to higher reducing sugar concentrations in the leaves of − K plants. In the young stems of

TABLE IV

NITROGENOUS FRACTIONS

PERCENTAGE OF DRY WEIGHT

TREATMENT	TOTAL N	SAP INSOLUBLE N	SAP SOLUBLE ORGANIC N	TOTAL ORGANIC N	AMIDE N	AMINO N	AMMONIUM N	NITRATE N
MATURE LEAVES								
K_1	4.12	3.03	0.47	3.50	0.037	0.082	0.009	0.612
K_2	4.05	2.73	0.47	3.20	0.014	0.048	0.015	0.837
K_3	3.68	2.34	0.45	2.79	0.007	0.033	0.015	0.877
NaK_1	4.06	3.04	0.46	3.50	0.020	0.059	0.009	0.551
NaK_2	3.96	2.76	0.48	3.24	0.024	0.075	0.008	0.708
NaK_3	3.78	2.64	0.31	2.95	0.018	0.047	0.012	0.821
DRFS*								
0.05	0.24	0.29	0.10	0.28	0.009	0.021	0.004	0.150
0.01	0.35	0.41	0.14	0.40	0.013	0.029	0.005	0.213
EXPANDING LEAVES								
K_1	5.15	3.82	0.61	4.43	0.035	0.119	0.009	0.713
K_2	5.04	3.74	0.53	4.27	0.017	0.056	0.008	0.766
K_3	4.45	3.10	0.40	3.50	0.013	0.047	0.009	0.944
NaK_1	5.14	3.87	0.55	4.42	0.027	0.068	0.008	0.714
NaK_2	4.86	3.63	0.48	4.11	0.019	0.054	0.009	0.739
NaK_3	4.27	3.01	0.29	3.30	0.012	0.061	0.008	0.966
DRFS*								
0.05	0.21	0.41	0.15	0.39	0.013	0.025	n.s.†	0.119
0.01	0.29	0.59	0.21	0.55	0.018	0.036	....	0.168

* Difference required for significance.
† No significant difference.

−K plants reducing sugars were higher and levulins lower than in the +K plants. In the old stems and roots −K plants had lower concentrations of total carbohydrates resulting almost entirely from lower levulin contents. In each plant part the reducing sugars when expressed as percentage of total carbohydrates were higher in the −K than in the +K plants. Except in the flower samples these differences were relatively great. In the latter samples reducing sugars were very high in both treatments.

NITROGENOUS FRACTIONS OF THE LEAVES

In table IV are shown the total nitrogen contents of the leaves, together with several nitrogen fractions expressed as percentage of the dry weight.

TABLE V

NITROGENOUS FRACTIONS GIVEN AS PERCENTAGES OF TOTAL NITROGEN

TREAT-MENT	SAP INSOLU-BLE N	SAP SOLUBLE ORGANIC N	TOTAL ORGANIC N	AMIDE N	AMINO N	AMMO-NIUM N	NITRATE N
			MATURE LEAVES				
K_1	73.5	11.4	84.9	0.90	2.00	0.22	14.8
K_2	67.4	11.6	79.0	0.35	1.19	0.37	20.7
K_3	63.6	12.2	75.8	0.19	0.90	0.41	23.8
NaK_1	74.9	11.3	86.2	0.49	1.45	0.22	13.6
NaK_2	69.7	12.1	81.8	0.61	1.89	0.20	17.9
NaK_3	69.8	8.2	78.0	0.48	1.24	0.32	21.7
DRFS*							
0.05	4.4	3.41	4.13	0.23	0.56	0.10	3.6
0.01	6.2	4.84	5.87	0.32	0.80	0.15	5.2
			EXPANDING LEAVES				
K_1	74.2	11.8	86.0	0.68	2.31	0.17	13.8
K_2	74.2	10.5	84.7	0.34	1.11	0.16	15.2
K_3	69.7	8.9	78.6	0.29	1.06	0.20	21.2
NaK_1	75.3	10.7	86.0	0.53	1.32	0.16	13.9
NaK_2	74.7	9.9	84.6	0.39	1.11	0.19	15.2
NaK_3	70.5	6.8	77.3	0.28	1.43	0.19	22.6
DRFS*							
0.05	3.5	3.31	2.64	0.27	0.30	n.s.†	2.5
0.01	5.0	4.71	3.76	0.39	0.42	....	3.6

* Differences required for significance.
† No significant difference.

These fractions are also presented in table V, where they are expressed as percentage of the total nitrogen content. The total nitrogen content of mature leaves was significantly lower in K_3 than in other treatments except .NaK₃. The latter had significantly less total nitrogen than K_1, K_2 or NaK_1. In the expanding leaves lower total nitrogen contents were found in the two high potassium treatments than in others. In addition, NaK_2 was significantly lower in nitrogen than NaK_1.

Variations in sap insoluble nitrogen followed a trend similar to that of the total nitrogen content. In the mature leaves this fraction was signifi-

cantly lower in K_3 than in other treatments. In addition, NaK_3 was lower than K_1 or NaK_1. In the expanding leaves the sap insoluble nitrogen contents of K_3 and NaK_3 were lower than those of other treatments. When expressed in terms of total nitrogen content (table V), these same variations in sap insoluble nitrogen were evident except for the comparison between NaK_3 and K_1 in the mature leaves.

Sap soluble organic nitrogen was significantly lower in the mature leaves of NaK_3 plants than in those of the other treatments, when this fraction was expressed on the dry weight basis. In the expanding leaves NaK_3 was lower in this fraction than was K_1, K_2 or NaK_1. In addition, K_3 was lower than K_1 or NaK_1 in sap soluble organic nitrogen. However, when based on the total nitrogen, these variations in sap soluble organic nitrogen were not statistically significant. It may be noted that including the sap soluble organic nitrogen fraction with the sap insoluble fraction did not significantly alter the direction of the variations. Hence, variations in total organic nitrogen were essentially those of the sap insoluble fraction.

Amide nitrogen was significantly higher in the mature leaves of the K_1 plants than in those of other treatments. It might also be noted that the amide content of mature leaves was significantly lower in K_3 than in any of the series 2 treatments. Amides were lower in K_2 than in NaK_2. In the expanding leaves amides were significantly higher in K_1 than in any other treatment except NaK_1. These variations were common to both bases of expression.

Amino nitrogen was higher in the mature leaves of K_1 than in any treatment except NaK_2. In the latter treatment this fraction was higher than in K_2, K_3, or NaK_3. In the expanding leaves, the higher content of amino acids in K_1 than in any other treatment was the outstanding result. When expressed as percentage of the total nitrogen, amino acids in NaK_3 were higher than in K_2, K_3, or NaK_2.

Ammonium nitrogen was relatively low in all cases. In the mature leaves, this fraction was evidently higher in K_2 and K_3 than in K_1, NaK_1, or NaK_2. In the expanding leaves there was no significant variation. Nitrate nitrogen contents of mature leaves were significantly lower in the low potassium treatments than in intermediate or high potassium treatments of a given series. In the expanding leaves, K_3 and NaK_3 had higher nitrate contents than had other treatments.

Discussion

There can be no doubt that condensation of carbohydrates was restricted in the low potassium plants. In an effort to determine whether this effect was more closely related to the deficiency of potassium per se or to the accumulation of soluble calcium in these plants the ratio: levulins + inulin/ reducing sugars has been employed as an index of condensation. This ratio does not strictly denote a definite proportion of combined to free fructose, since only a portion of the reducing sugar fraction is fructose (42).

Neither does it take into account variations in polymer size as between frac-
tions of levulins and between levulins and inulin (**41**). However, this
ratio may be used as an empirical index, if it is assumed that where poly-
merization of fructose is restricted, an accumulation of the reducing sugar
fraction will result. Values for the ratio: levulins + inulin/reducing sugars
obtained for leaf tissues were plotted against leaf tissue concentrations of
potassium and calcium and against the several cation ratios. The best in-
dication of a continuous relationship was found when the ratio: potassium/
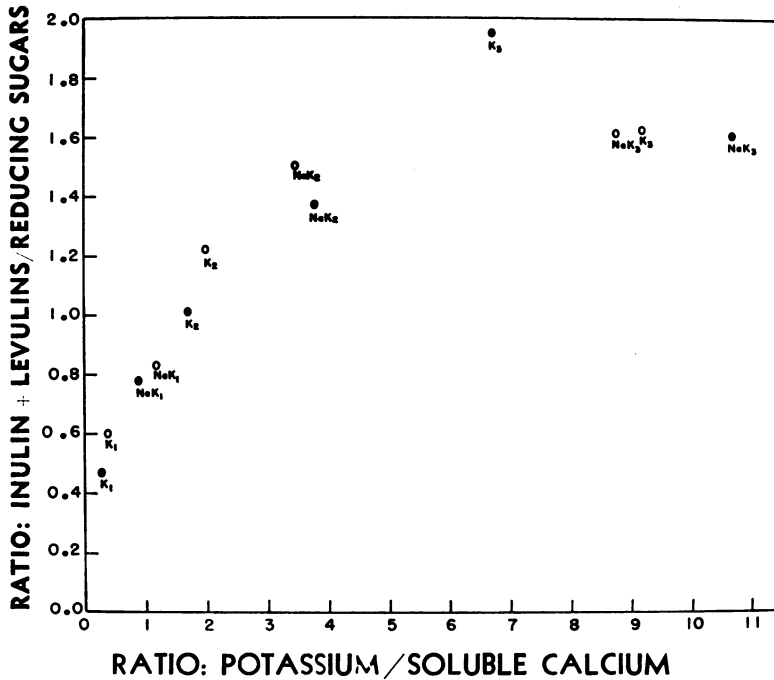calcium was employed. In figure 1 are plotted treatment means for ma-

FIG. 1. Relationship between the ratio: m.e. K/m.e. soluble Ca and the ratio: levu-
lins plus inulin/reducing sugars in mature and expanding leaves. Mean values for ma-
ture leaves (dots) and expanding leaves (circles) for all treatments are shown.

ture leaves and expanding leaves. No consistent departure from the gen-
eral trend is associated with series or with tissue age groups. These plots
appear to represent a trend concave to the axis defining the cation ratio.
However, the curvilinear portion of the relationship seems confined to the
range of excessive potassium. It is reasonable to suppose that in this range
the condensation ratio has suffered secondary alteration resulting from re-
duced nitrogen assimilation (cf. nitrogen fractions) and reduced activity
of the stem tip meristems (**8**).

Of greater interest is the range where condensation appears particu-
larly limited by the cation ratio. This portion of the trend (Fig. 2) ap-
pears nearly linear. The correlation coefficient (+ 0.771) is relatively high

considering the variability between replicates for the carbohydrate fractions as indicated by the differences required for significance between treatment means (table II).

The data presented give good evidence of decreased polymerization of fructose to levulins and inulin in guayule plants deficient in potassium. This effect appears more closely related to the decreased ratio of potassium to soluble calcium than to the concentration of either element singly. It is of interest to recall that REED (34) found that in all green plants studied
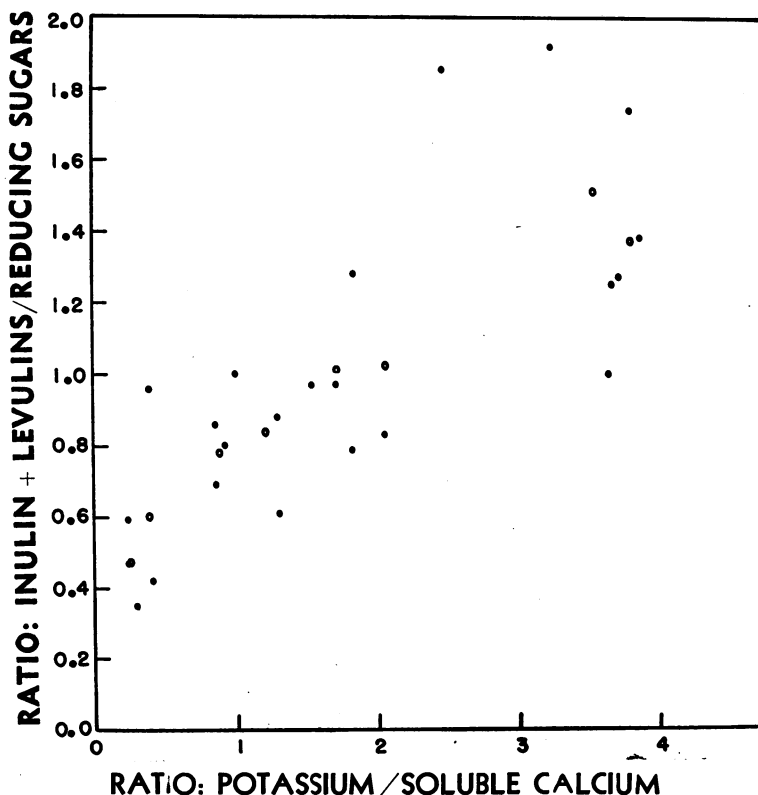

FIG. 2. Relationship between the ratio: m.e. K/m.e. soluble Ca and the ratio: levulins plus inulin/reducing sugars in mature and expanding leaves. Only low and intermediate potassium treatments are included. Dots represent individual samples. Circles denote treatment means for mature or expanding leaves. Coefficient of correlation = + 0.771. Correlation coefficient required for significance = 0.515 (n = 24, p = 0.01).

by him, potassium was essential for starch formation. SIDERIS and YOUNG (38) in reviewing the literature pertaining to effects of potassium upon carbohydrates noted that plants supplied with small amounts of potassium contained in their leaves higher concentrations of reducing sugars than those with high potassium. High reducing sugar values in low potassium plants were usually accompanied by low values in disaccharides and polysaccharides, although exceptions were noted. This type of response was

distinguished from that in plants totally deficient in potassium where the amounts of reducing sugars were low (14, 20). SIDERIS and YOUNG obtained high values for reducing sugars and low values for sucrose and starch in low potassium pineapple plants. They noted that their data emphasize the necessity of adequate amounts of potassium for condensation of reducing sugars to sucrose and starch. CATTLE (7) floated detached leaves of normal and potassium deficient bean plants on glucose solutions in the dark. Greater synthesis of starch was found in normal leaves. KURSSANOV (25) cited data showing increased synthesis of sucrose from leaves infiltrated with potassium chloride. RUSSELL (36) found that in barley plants potassium deficiency resulted in low total sugar concentrations, but the concentrations of fructosan polysaccharides were decreased relatively more than were those of other sugars. These results were obtained in both high calcium and high sodium series. In connection with the present study the findings of Russell are of the greater interest since the fructosan of barley is characterized by linkage of carbon atoms 2 and 6, whereas the inulin linkage is between 1 and 2 positions (1). Of importance is the fact that similar effects upon polymerization of carbohydrates have been found to result from potassium deficiency in cases where the metabolism of the particular plant includes synthesis of fructosans of the cereal type, in plants where fructosans of the inulin type are formed, as well as those in which sucrose and starch are polymerization products.

The enzyme systems involved in synthesis of fructosans have not yet been isolated. However, as noted above, condensation of reducing sugars to sucrose and starch are also affected by potassium deficiency. It is known that starch is formed from glucose-1-phosphate with aid of a phosphorylase (15, 16). DOUDOROFF, KAPLAN and HASSID (10) have obtained from *Pseudomonas saccharophila* a preparation of a phosphorylase which catalyses the formation of sucrose from glucose-1-phosphate and fructose. Each of these reactions requires that phosphate first be transferred to the hexose molecule. The similarity between the mechanism of starch synthesis and breakdown in higher plants and that of glycogen in animal tissues and yeast has been pointed out by HASSID (18) and JAMES (22) who have recently reviewed the literature dealing with phosphorolysis in plants. According to current schemes of phosphate transfers in animal tissues high energy bond phosphate generated in the course of respiration is required for formation of hexose phosphates (27). Necessary for these transfers is the formation of adenosine triphosphate, which arises in part from the reaction: phosphopyruvate + adenosine diphosphate = pyruvate + adenosine triphosphate. LARDY and ZEIGLER (26) employing an enzyme preparation from rat muscle have shown this reaction to be reversible. The reaction is accelerated by potassium. Sodium could not replace potassium in this acceleration (4). Calcium was antagonistic to the action of potassium (4, 5). These findings suggest a scheme for the role of potassium in carbohydrate

condensation. If potassium can be shown to be necessary for this or similar reactions in plants, many of the conflicting views on the function of potassium can be rationalized, since the transfer of high energy phosphate is supposed to be a connecting link whereby energy released in respiration is transferred to various centers of anabolism.

In the low potassium treatments (K_1 and NaK_1) the expanding leaves had approximately normal contents of nitrate and total organic nitrogen. Total sap soluble organic nitrogen and insoluble nitrogen were not significantly altered by potassium deficiency in these leaves, although amides and amino acids accumulated in K_1. The results obtained for the mature leaves of the potassium deficient plants differ in several respects from those of mature leaves of the higher potassium treatments. The proportion of total organic nitrogen was higher than that of nitrate lower in the low potassium treatments. In addition the proportion of sap insoluble nitrogen was significantly higher in K_1 and NaK_1 than in other treatments. It is of interest that SIDERIS and YOUNG (39) have very recently reported similar results for leaves of *Ananas comosus*. Leaves of low potassium plants had lower nitrate, higher soluble organic nitrogen and higher protein concentrations than did high potassium plants. These authors attribute the lower nitrate concentrations in their low potassium plants to decreased nitrate absorption, since when expressed as milligrams per plant total nitrogen contents were lower in low potassium plants. However, the size of these plants was reduced. In the present experiment there was no reduction in absorption of nitrogen by the low potassium plants until after the size of these plants was reduced. Thus, although at final harvest the total milligrams of nitrogen absorbed by low potassium plants were less, the total weights of these plants were also less at this time. However, on February 12, when differences in weights were not yet apparent, low and intermediate potassium treatments resulted in absorption of practically identical quantities of nitrogen in each series (4). It appears that nitrate absorption was not directly effected by low solution concentrations of potassium.

RICHARDS and TEMPLEMAN (35) have emphasized the necessity of studying leaves of comparable "physiological age" when dealing with potassium deficiency. Thus where all "mature leaves" are composited, the result is to confound various stages of maturation and senescence. However, selection of criteria of physiological age proves very difficult where the metabolism of all stages is altered to the degree found in potassium deficiency. In the present case, it may be noted that the mature leaves of the potassium deficient plants were more similar to the expanding leaves with respect to proportions of nitrogen fractions than were the mature leaves of plants supplied with adequate potassium. Thus, the percentages of total nitrogen contributed by sap insoluble nitrogen, sap soluble nitrogen, total organic nitrogen, α-amino nitrogen, ammonium nitrogen, and nitrate nitrogen were similar in mature and expanding leaves in each of the treatments K_1 and NaK_1 (table V). The mature leaf samples obtained from K_1 plants were

on the average younger in actual age than mature leaves in K_2 plants inasmuch as a higher mortality of the oldest leaves had resulted in K_1. In NaK_1, the mortality of the old leaves was no greater than in NaK_2, so between these two treatments there was no great difference in actual age of the mature leaf samples. It therefore appears that in leaves of guayule plants deficient in potassium, one or more of the processes associated with maturation is altered.

The higher contents of a-amino and amide nitrogen in leaves of K_1 are in agreement with observations of some other investigators (6, 17, 30, 39). GREGORY (13) has held the view that such accumulation in the older leaves results from protein degradation, since these accumulations have sometimes been accompanied by lower protein contents. WALL (45) found that the ratio of soluble organic nitrogen to protein nitrogen was higher than normal in various parts of tomato plants which had been supplied with a solution lacking potassium. He concluded that potassium was necessary for synthesis of proteins from amino acids. SIDERIS and YOUNG (39) have found protein nitrogen contents of older leaves in pineapple plants supplied with low potassium to be equal to or greater than those from corresponding leaves of high potassium plants. The latter results are in agreement with the present findings. These authors have pointed to the fact that accumulations of soluble organic nitrogen fractions may be brought about by translocation of protein degradation products from dying tissues and parts to tissues still healthy in appearance. They, however, believe that no definite decision can be made as to whether these products are of synthetic or proteolytic origin. These arguments apply with equal force to the guayule leaves of this experiment. Thus, the K_1 plants in which amides and a-amino nitrogen accumulated had the greatest mortality of leaves which perhaps provided the greater supply of proteolytic products to leaves which were still living. On the other hand the nitrate content of the mature leaves of these plants was low, possibly indicating greater synthesis of soluble organic nitrogen fractions.

The high insoluble nitrogen contents of potassium deficient guayule leaves would not indicate that in these tissues protein synthesis was curtailed. However, it has been found by several workers (6, 17, 39) that although the protein content of potassium deficient leaves may be relatively high, the protein contents of the stems of these plants is frequently reduced. The protein concentrations in the guayule stems were not estimated, but it seems pertinent to note that growth in diameter of the low potassium stems was curtailed, whereas growth in length was not (8, table IV).

The conclusions of various investigators concerning the function of potassium in nitrogen metabolism are not in accord. Many of the differences in results obtained may arise from differences in degree of potassium deficiency, or from differences in the environmental conditions under which the experiments were conducted. In the present experiment with guayule

plants, potassium deficiency was not extreme, as indicated by the fact that only moderate reduction in growth of these plants resulted during the experimental period. The plants employed were relatively young. The experiment was conducted in the greenhouse during the winter months. It is quite possible that a different distribution of nitrogenous fractions would result in older plants, in other seasons, or where deficiency was more severe.

In the high potassium treatments (K_3 and NaK_3) expanding leaves had significantly less assimilated nitrogen and significantly more nitrate than expanding leaves of other treatments. These leaves, therefore, had not only a lower total nitrogen content, but in addition a less rapid rate of nitrate reduction. Although similar results were found in the mature leaves, these were not statistically significant. When it is considered that plants of these treatments were likely deficient in calcium at the time of final harvest (8), a lower rate of nitrate reduction conforms to the results of BURRELL (6). NIGHTINGALE et al. (29) noted low protein contents in calcium deficient plants and ascribed these results to a very limited ability of these plants to absorb nitrate and to restricted reduction of the nitrate absorbed. GAUCH (12) has since shown that the limited absorption of nitrate was associated with high magnesium contents of the minus calcium nutrient solutions employed. However, the restricted nitrate reduction is in agreement with the results reported here.

A relationship between nitrate reduction and malic acid accumulation has been suggested by other workers (31, 44). When guayule leaves from the intermediate potassium treatments are used as a basis for comparison, high potassium leaves were relatively high in citric acid (9). Malic acid did not accumulate in high potassium leaves. This may indicate that transformation of citric acid to malic acid was inhibited in these leaves, since these acids are known to be interconvertible in some plant tissues (43, 22). The tricarboxylic acid cycle of KREBS (24) indicates a possible course for oxidative formation of malic acid from citric acid. In minced breast muscle of pigeon dicarboxylic and tricarboxylic acids have been shown to catalyse oxidative respiration. In higher plants, such catalysis has not been demonstrated (22), but most of the organic acids and a number of the enzyme systems necessary for the reactions in Krebs cycle are known to occur (2, 3, 22). Whether or not these acids act as catalysts, it seems plausible that their oxidation in plants is brought about by a series of reactions similar to those of the tricarboxylic acid cycle. Partial inhibition of one of these reactions might result in accumulation of citrate as observed in guayule leaves from the high potassium treatments. A possible explanation for the accumulation of nitrate in these leaves is obtained, if it is further assumed that the reduction of nitrate is coupled with one of the oxidations in this series (i.e., dehydrogenation of isocitrate, a-ketoglutarate, or succinate) through the action of specific dehydrogenases and the appropriate phosphopyridine nucleotide.

Summary

1. The relative amounts of certain carbohydrate and nitrogenous fractions were determined in the tissues of guayule plants which had received low, intermediate, or high levels of potassium supply.

2. Reducing sugars in the leaves were consistently higher in low potassium treatments than in others. In old stems and roots levulins were consistently lower in low potassium than in intermediate potassium plants. In all tissues of the low potassium plants reducing sugars were high when expressed as percentage of the total carbohydrates. These results indicate that adequate potassium is required for condensation of fructose to fructosans of the inulin type in guayule. Condensation of fructose in the leaves of low potassium cultures appears more nearly related to the ratio: potassium/soluble calcium in the leaf tissues than to the tissue content of potassium per se. The significance of these results is discussed in relation to results from other plants where polysaccharides other than levulins and inulin are the principal products of hexose condensation.

3. Amide-nitrogen and α-amino nitrogen fractions were highest in mature and expanding leaves of the plants which contained the least potassium. Insoluble and sap soluble organic nitrogen fractions were at least as high in the leaves of potassium deficient plants as in those which had received an adequate potassium supply.

4. Leaves of high potassium plants contained less total organic nitrogen and more nitrate nitrogen than did plants of other treatments. The altered proportion of individual organic acids found in the high potassium leaves is considered in relation to limited nitrate reducton.

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