The Synthesis of Sucrose by Extracts of the Root of the Sugar Beet

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There is no doubt that synthesis of sucrose does occur in the leaf of the sugar beet but the root has not previously been shown to possess a sucrose-synthesizing system.

Buchanan *et al.* (1952) and Buchanan (1953), in photosynthetic experiments with $^{14}\text{CO}_2$, demonstrated the formation of sucrose phosphate in the leaves of the sugar beet as well as of other plants. They postulated that the sucrose phosphate was formed according to (1):

Cardini, Leloir & Chiriboga (1955) reported that extracts of wheat germ and other plant materials would bring about the synthesis of sucrose according to (2):

Negative or non-reproducible results were obtained, however, with extracts of sugar-beet leaves or roots.

Burma & Mortimer (1956), using $[^{14}\text{C}]$ glucose 1-phosphate, showed that sugar-beet-leaf homogenates could synthesize uridine diphosphate glucose (UDPG) from uridine triphosphate and glucose 1-phosphate. The same homogenates yielded sucrose and traces of sucrose phosphate from uridine diphosphate glucose and fructose 6-phosphate and the authors considered that the sucrose phosphate was the initial product:

and that the sucrose was formed subsequently by dephosphorylation. Sucrose was not produced if fructose 6-phosphate was replaced by fructose or by fructose 1:6-diphosphate.

More recently, Rorem, Walker & McCreedy (1960) have demonstrated the presence of enzymes capable of catalysing both reactions (2) and (3) in sugar-beet-leaf extracts from which invertase had been partially eliminated.

Kursanov & Pavlinova (1952) and Kursanov (1954) found that sucrose was produced when glucose and fructose were introduced into leaf disks by vacuum infiltration, but they were unable to detect any sucrose synthesis in similar infiltration experiments with root samples, except possibly to a slight extent with young roots.

It is now demonstrated that the necessary precursors and an enzyme system for the synthesis of sucrose are present in the sugar-beet root.

EXPERIMENTAL

Materials. Adenosine monophosphate (AMP), adenosine diphosphate (ADP) and fructose 6-phosphate (F6-P) were obtained from L. Light and Co. Ltd. Adenosine triphosphate (ATP), glucose 1-phosphate (G1-P) and glucose 6-phosphate (G6-P) were obtained from British Drug Houses Ltd. Uridine monophosphate (UMP), uridine diphosphate (UDP), uridine triphosphate (UTP) and uridine diphosphate glucose (UDPG) were obtained from Sigma Chemical Company, St Louis, Mo., U.S.A.

Preparation of extracts. Healthy beet plants, which had been sown outdoors in April, were harvested at intervals from June to November. They were thoroughly washed with cold water and extracted, usually within 1 hr. of harvesting.

The sample of plant material was chopped and 200 g. was macerated in 250 ml. of ice-cold 0.05 M-phosphate ($\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$) buffer, pH 7.0. The macerate was filtered rapidly, first on stainless-steel gauze and then through glass wool, to yield 150 ml. of crude extract, which was immediately centrifuged at 34 000g for 5 min. The precipitate was discarded and the supernatant was saturated with $(\text{NH}_4)_2\text{SO}_4$. The solution was allowed to stand in a refrigerator for 1 hr. and then centrifuged again at 34 000g for 5 min. The supernatant was discarded and the precipitate was taken up in the minimum of water and dialysed against 0.05 M-phosphate buffer, pH 7.0, at 5° for 24 hr. Approx. 10–15 ml. of concentrate, free from sucrose and nucleotides, was obtained. The extract was used within 48 hr. since the activity diminished on storage at 5°. Freezing caused coagulation and complete loss of sucrose-synthesizing activity.

Throughout the extraction, temperatures above 10° were normally avoided except during centrifuging, but temperature control was not critical and active extracts could be prepared without refrigeration.

Detection of sucrose-forming activity. The digests contained: 0.02 ml. of 2.0 M-tris-HCl buffer, pH 7.2; 1.5 μ moles of fructose; 0.25 μ mole of UDPG and 0.25 ml. of extract; total volume 0.3 ml. Two blank digests, one in which fructose was omitted, the other in which UDPG was omitted, were also prepared.

After 30 min. at 37° the reaction was terminated and residual fructose was destroyed, as described by Cardini *et al.* (1955); sucrose was estimated by a modification of the resorcinol method of Roe (1934), as described by Bacon & Bell (1948). After incubation, 0.20 ml. of 0.5 N-NaOH solution was added to each digest and, after careful mixing, the tubes were heated for 10 min. in a boiling-water bath. The tubes were cooled, 0.5 ml. of water and 1.5 ml. of

resorcinol solution (0.15%, w/v, in absolute ethanol) were added, followed 1 min. later by 1.5 ml. of conc. HCl (containing 7.5 mg. of $\text{FeCl}_3/\text{l.}$). The tubes were heated for 30 min. in a thermostatically controlled water bath at 75°. After cooling, the colours were measured at 490 m μ and the sucrose content was calculated from the extinction of concurrent sucrose standards.

Higher concentrations of UDPG and fructose were employed in preparative experiments and also in experiments designed to estimate both sucrose synthesis and changes in nucleotide composition. In these experiments the reaction was terminated by the addition of 1 vol. of ethanol. The clear supernatant obtained after centrifuging was used for determining nucleotides and sucrose. In these extracts, sucrose was separated by paper chromatography, detected with the α -naphthol reagent, and occasionally estimated by visual comparison with the colour developed by known amounts of sucrose on the same chromatogram. Results obtained by this method were consistent with those obtained by the resorcinol method.

Estimation of nucleotides. Nucleotides were estimated spectrophotometrically after separation by paper chromatography and detection by contact photography by using a Hanovia 253.7 m μ ultraviolet source (Hanovia Ltd., Slough, Bucks.). The areas containing ultraviolet-absorbing material were cut from the chromatograms with appropriate blanks and were soaked in portions (5 ml.) of 0.1N-HCl. Nucleotide concentration was determined from the extinction. A molar extinction coefficient of 15×10^3 at 257 m μ was used for adenosine compounds and 10×10^3 at 262 m μ for uridine compounds.

Paper chromatography and electrophoresis. All separations were carried out on Whatman no. 3MM paper. Solvents 1-6 (proportions by vol.) were employed for the separation of sugars: (1) propan-1-ol-ethyl acetate-water (7:1:2); (2) ethylacetate-acetic acid-water (3:1:3); (3) propan-1-ol-aq. NH_3 (sp.gr. 0.88)-water (80:5:15); (4) butan-1-ol-ethanol-water (10:1:2); (5) butan-1-ol-acetic acid-water (3:3:2); (6) propan-1-ol-benzyl alcohol-formic acid-water (50:72:17:23).

Nucleotides were separated by chromatography in the solvents: (7) ethanol-m-ammonium acetate soln., pH 7.5 (5:2); (8) isobutyric acid-aq. NH_3 (sp.gr. 0.88)-water (66:1:33) and by high-voltage paper electrophoresis (100 v/cm.) in citrate buffer, pH 3.3 (0.2M-citric acid-0.2M sodium citrate, 3:1).

Spray reagents. Fructose and sucrose were detected with α -naphthol-phosphoric acid (Albon & Gross, 1950) and urea-phosphoric acid (Wise, Dimler, Davis & Rist, 1955). Glucose and fructose were detected with AgNO_3 -NaOH (Trevelyan, Procter & Harrison, 1950). Glucose and G6-P were detected as brown spots and G1-P as a yellow spot with *p*-anisidine-phosphoric acid (Mukherjee & Srivastava, 1952); UDPG also reacted weakly to give a yellow spot. After electrophoresis in 0.1M-borate buffer, glucose phosphates were detected by spraying with FeCl_3 -salicylsulphonic acid (Wade & Morgan, 1953) modified by addition of 1.75 ml. of conc. HCl/100 ml. of FeCl_3 soln. to counteract the effect of residual buffer.

RESULTS

Distribution of acid-soluble nucleotides. An initial examination was undertaken principally to determine whether UDPG was present in sugar

beet and whether there was any differential concentration between particular sections. The plants were divided into blade, petiole, crown portion of the root and residual main root, and the four sections were extracted by the technique described by Bergkvist (1956) for the isolation of the acid-soluble nucleotides of wheat plants.

UDPG was identified in all the extracts and the concentration, which was similar in all four sections, was of the order of 1 $\mu\text{mole}/100$ g. of fresh plant material. UDP, ADP and ATP were also present in all extracts, but no UTP was detected.

Synthesis of sucrose. When enzyme extracts from the above-mentioned plant sections were incubated with UDPG and fructose, no resorcinol reaction for sucrose was obtained with leaf or stem extracts but crown and root extracts gave positive results. When fructose was replaced by fructose 6-phosphate negative results were obtained with all enzyme extracts. The activity of root and crown extracts when incubated with UDPG and fructose appeared to be similar, so that all subsequent experiments were carried out with extracts prepared from the whole root including the crown.

The resorcinol-positive compound was identified as sucrose by paper chromatography and by hydrolysis to glucose and fructose. Digests, after incubation at 37°, were treated with an equal volume of ethanol and centrifuged. The supernatant solutions were applied to paper chromatograms, which were developed in solvents 1-6. Chromatograms were sprayed with the α -naphthol or the urea reagent. Blank digests containing UDPG plus root extract gave no reaction; those containing fructose plus root extract gave only a fructose spot whereas digests containing UDPG, fructose and root extract gave, together with the fructose spot, a slower-moving spot. This product when mixed with sucrose gave a single spot on chromatograms in all six solvents and, like sucrose, gave a violet reaction with α -naphthol, blue with urea and a very weak reaction with the silver nitrate-spray test.

Digests containing approx. 0.3 μmole of the product were applied as bands to paper chromatograms, which were then irrigated in solvent 1 for 40 hr. The positions of the fructose band and the slower-moving band were established by spraying guide strips with the α -naphthol reagent. The slower-moving band was cut from the unsprayed section of the chromatogram and eluted with water to give approx. 0.5 ml. of eluate. One-half of this eluate was acidified with 0.05 ml. of 0.1N-hydrochloric acid and placed, together with the remaining untreated eluate, in a boiling-water bath for 15 min. The acid-treated eluate was neutralized with 0.1N-sodium hydroxide soln. and both eluates were applied to paper chromatograms,

which were developed in solvents 2, 4 and 6. The sugars were detected by spraying the chromatograms with silver nitrate-sodium hydroxide soln. The untreated eluate was found to contain only sucrose, whereas in the acid-treated eluate sucrose had disappeared and two products had been formed which, when mixed with glucose and fructose respectively, gave single spots.

In a similar experiment one-half of the eluate was treated with 0.01 ml. of invertase concentrate (Sumasuco, The Sugar Manufacturers' Supply Co. Ltd.) and together with the untreated portion of eluate was incubated at 45° for 6 hr. Again paper chromatography showed sucrose in the untreated eluate and glucose and fructose together with a trace of residual sucrose in the invertase-treated eluate.

Although no sucrose was detected if blade or petiole extracts were incubated under the standard conditions, sucrose was detected chromatographically with the petiole extract when the quantity of UDPG in the digest was increased 20-fold. Even with the increased UDPG concentration, negative results were still obtained with blade extracts.

If the sucrose is formed according to the reaction

the nucleotide product, UDP, should also be detectable.

Digests in which sucrose had been synthesized were found to contain an ultraviolet-absorbing compound which was identical with authentic UDP on paper chromatograms and electrophoretograms.

The yield of UDP was always less than the yield of sucrose but UMP and uridine were also formed. Traces of UMP were present as impurity in the UDPG before incubation. It therefore appeared that UDPG and UDP were being degraded, probably by nucleotide pyrophosphatase and nucleotidase. Degradation was found to be particularly rapid with root extracts that had been dialysed against water instead of the recommended phosphate buffer. Although such extracts synthesized sucrose from UDPG and fructose, the yield of sucrose was occasionally so low that it could not be detected by paper chromatography but only by the resorcinol method. In these extracts UDPG was found to be completely degraded, sometimes after incubation for only 30 min. at 37°.

On incubation of the digests without fructose, UDPG was degraded to UMP and not UDP, suggesting the simultaneous production of a glucose phosphate. These digests were applied to paper chromatograms and the products were separated by development in solvent 7. The nucleotides were characterized by ultraviolet

photography and the glucose derivatives were detected with the *p*-anisidine reagent. The glucose phosphate produced initially gave a single spot on chromatograms with, and the same yellow reaction as, glucose 1-phosphate. With increasing time of incubation a second product, running just behind glucose 1-phosphate and giving a red-brown reaction, was observed. This was found to give a single spot on chromatograms with glucose 6-phosphate. The identification of glucose 1-phosphate and glucose 6-phosphate was confirmed by electrophoresis (100 v/cm.) in 0.1 M-borate, pH 9.5. No evidence for the cyclic glucose phosphate, reported by Paladini & Leloir (1952) to arise by alkaline degradation of UDPG, was obtained. The presence of phosphoglucomutase in the extract was confirmed by incubating with either glucose 1-phosphate or glucose 6-phosphate, when their interconversion was detected by paper chromatography and electrophoresis.

Sucrose synthesis ceased with the complete degradation of UDPG in the digests and attempts to synthesize sucrose by incubating root extract and glucose 1-phosphate with fructose or fructose 6-phosphate failed. It was therefore concluded that UDPG was essential for the synthesis and that this occurred according to reaction (2).

A quantitative presentation of the principal reactions described above is recorded in Fig. 1, the formation of sucrose and UDP from UDPG and fructose, together with the degradation of UDPG, UDP and UMP, being shown.

Phosphatase action. The comparative rates of enzymic degradation of various nucleotides were determined.

The results (Fig. 2) demonstrate that the rates of degradation decrease in the order ATP, ADP, UDP, UTP, UDPG, AMP, UMP.

In contrast, chromatography of similar digests containing 10 μ moles of glucose 1-phosphate or 6 μ moles of fructose 6-phosphate showed that no free glucose or free fructose was present after incubation for 4 hr.

Whereas the degradation of UTP, UDP, UMP and AMP occurs by the loss of one phosphate group and in UDPG by the loss of glucose 1-phosphate, the degradation of ADP and ATP was found to be more complex. Dilute digests were prepared, containing only 0.01 ml. of enzyme extract in 0.5 ml., and, on incubation with ADP, approximately equivalent amounts of ATP and AMP were produced, probably owing to the presence of myokinase:

Furthermore, the rate of production of AMP from ATP in these dilute digests was much greater than the calculated rate of production if the source

of the AMP was the ADP yielded by dephosphorylation of the ATP. It was concluded that some of the AMP was produced by apyrase activity:

Synthesis of uridine diphosphate glucose from uridine triphosphate and glucose 1-phosphate. The examination of the acid-soluble nucleotides showed that though UDPG was present in the beet, its concentration was low. It was therefore clear that if this nucleotide were to play an important role in the synthesis of sucrose in the sugar beet, there must be enzymic pathways for its regeneration. The most likely pathway was by the reaction

catalysed by UDPG pyrophosphorylase.

When root extracts were incubated with glucose 1-phosphate and UTP, a nucleotide product giving a single spot on chromatograms with authentic UDPG was obtained. This product was not present in blank digests in which either UTP or glucose 1-phosphate was omitted. The degradation pro-

ducts, UDP, UMP and uridine, were again formed in both active and blank digests containing UTP.

Electrophoresis in citrate buffer showed that the synthesized product had the same mobility as UDPG. Further confirmation of its identity was obtained by hydrolysis. Approximately 1 μ mole of the product was separated from the other nucleotides by developing a band paper chromatogram in solvent 7. This effected only a partial separation between the product and glucose 1-phosphate. The strip containing the synthesized material plus contaminating glucose 1-phosphate was eluted with water and re-applied, as a band, to a second chromatogram, which was developed in 70 % propan-2-ol to give complete separation of the nucleotide from glucose 1-phosphate. The nucleotide band was cut from the chromatogram and eluted with water to give approx. 0.5 ml. of eluate. A portion (0.2 ml.) of eluate was acidified with 0.02 ml. of 0.1 N-hydrochloric acid, heated at 100° for 15 min. and finally cooled and neutralized with

Fig. 1. Synthesis of sucrose from UDPG plus fructose by an enzyme extract of sugar-beet root. Reaction mixtures contained: 5.0 μ moles of fructose, 3.18 μ moles of UDPG (plus 0.29 μ mole of UMP and 0.08 μ mole of UDP as impurities), 0.02 ml. of 2.0 M-tris-HCl buffer, pH 7.2, 0.30 ml. of root extract; total volume 0.60 ml. Temp. 37°. Sucrose was estimated both by the resorcinol technique and by paper chromatography. UDP, UMP, UDPG and uridine were separated by paper chromatography and estimated spectrophotometrically. $\square$, Uridine; $\blacktriangle$, sucrose; $\triangle$, UMP; $\bullet$, UDP; $\circ$, UDPG.

Fig. 2. Degradation of nucleotides by phosphatase in sugar-beet-root extract. Each digest contained 0.01 ml. of 2.0 M-tris-HCl buffer, pH 7.2, 1.0-2.0 μ moles of nucleotide and 0.15 ml. of root extract in a total volume of 0.30 ml. After incubation at 37° the digests were inactivated by the addition of 1 vol. of ethanol. Nucleotide concentrations were determined spectrophotometrically after paper chromatography in solvents 7 and 8. $\square$, AMP; $\blacksquare$, ADP; $\bullet$, ATP; $\circ$, UDPG; $\bullet$, UDP; $\triangle$, UMP; $\blacktriangle$, UTP.

0.1 N-sodium hydroxide. Paper chromatography in solvents 2, 4 and 7 and electrophoresis in citrate buffer showed that the hydrolysate contained glucose, UDP and UMP, whereas only UDPG was present in the initial eluate.

The course of the synthesis of UDPG from UTP, together with the degradation of the nucleotides, is recorded in Fig. 3. Digests were inactivated by the addition of 1 vol. of ethanol. UTP, UDP, UMP, UDPG and uridine were separated by paper chromatography in solvent 7 and estimated spectrophotometrically.

Synthesis of uridine triphosphate from adenosine triphosphate and uridine diphosphate. Though the presence of UDPG pyrophosphorylase in the root extract could be demonstrated, this mode of regeneration of UDPG requires a source of UTP and this nucleotide was not detected in the initial examination of the beet sections. The synthesis of UTP by nucleoside-diphosphokinase activity was demonstrated by incubation of ATP and UDP with the root extract; a product identical with authentic UTP on paper chromatography or electrophoresis was rapidly obtained:

The activity of this enzyme was sufficiently high to permit use of a low concentration (2%) of root extract, whereas a higher concentration (50–67%) was employed to demonstrate the synthesis of sucrose and UDPG. Addition of ethanol was not completely effective in stopping the reaction, which was therefore terminated by immersion of the digests in a boiling-water bath for 5 min.

UTP and ADP were formed in digests containing UDP and ATP, and no UTP was formed in blank digests from which either UDP or ATP was omitted. ADP was, however, produced together with AMP in blank digests containing ATP and root extract.

Fig. 4 illustrates the formation of UTP, together with the partial degradation of the nucleotides to the monophosphate stage. The reaction is also influenced by myokinase (4) and apyrase (5) activity, but, since the concentration of root extract was low, the phosphatase activity was minimized so that nucleosides were not produced to a detectable extent.

Nucleotides were separated for estimation in solvent 8, and whereas all other nucleotides were clearly separated the UMP spot coincided with that of ATP in this solvent. UMP was therefore estimated after separation from the other components in solvent 7 and ATP was then calculated by difference.

Synthesis of uridine diphosphate glucose from uridine diphosphate, adenosine triphosphate and glucose 1-phosphate. After demonstration of syn-

Fig. 3. Synthesis of UDPG from UTP plus glucose 1-phosphate by an enzyme extract of sugar-beet root. Reaction mixtures contained: 10.0 μ moles of glucose 1-phosphate, 1.45 μ moles of UTP (plus 0.32 μ mole of UDP and 0.08 μ mole of UMP as impurities), 0.02 ml. of 2.0 M-tris-HCl buffer, pH 7.2, 0.40 ml. of root extract; total volume 0.60 ml. Temp., 37°. □, Uridine; Δ, UMP; ▲, UTP; ○, UDPG; ●, UDP.

Fig. 4. Synthesis of UTP from ATP plus UDP by an extract of sugar-beet root. Reaction mixtures contained: 3.52 μ moles of ATP (plus 1.10 μ moles of ADP and 0.17 μ mole of AMP as impurities), 2.60 μ moles of UDP (plus 0.55 μ mole of UMP and 0.14 μ mole of UTP as impurities), 0.02 ml. of 2.0 M-tris-HCl buffer, pH 7.2, 0.01 ml. of root extract; total volume 0.50 ml. Temp. 37°. □, AMP; ■, ADP; ○, ATP; ●, UDP; Δ, UMP; ▲, UTP.

thesis of UTP, further experiments showed that this nucleotide could be replaced in the UDPG synthesis (6) by similar concentrations of ATP plus UDP and that the rate of formation of UDPG was not affected. No UDPG was found if any one of the three substrates, glucose 1-phosphate, ATP or UDP, was omitted from the digests. The overall reaction is presumably catalysed by nucleoside diphosphokinase and UDPG pyrophosphorylase:

Glucose 1-phosphate could not be replaced by glucose in the UDPG synthesis. Nor did incubation of glucose and ATP with root extract yield any glucose 6-phosphate, indicating low or no hexokinase activity. Glucose 6-phosphate was, however, shown to be present in the root and the amount extracted by the barium technique of LePage (1957) indicated a minimum concentration of 10 $\mu\text{moles}/100 \text{ g. of root}$.

Synthesis of sucrose from fructose, glucose 1-phosphate, adenosine triphosphate and uridine diphosphate. Since UDPG could be synthesized from glucose 1-phosphate, ATP and UDP, the overall synthesis of sucrose by combination of reactions 2 and 8 was attempted by incubation of a digest containing the root extract with fructose, glucose 1-phosphate, ATP and UDP.

The digest, containing 0.02 ml. of tris-hydrochloride buffer, pH 7.2, 6.5 μmoles of fructose, 6.5 μmoles of glucose 1-phosphate, 1.4 μmoles of UDP, 1.2 μmoles of ATP and 0.3 ml. of root extract in a total volume of 0.6 ml., after incubation at 37° for 3 hr. yielded a detectable sucrose spot on paper chromatograms. A sucrose spot was not present on chromatograms of blank digests from which any single component was omitted.

DISCUSSION

UDPG is shown to be present in all sections of the sugar-beet plant and it is suggested that sucrose may be synthesized, and UDPG regenerated, in the root by the overall reaction (9):

Enzymes capable of effecting reactions (7), (6) and (2) are demonstrably present in the root and in summation the synthesis requires only a source of fructose and ATP, which are readily available, and of glucose 1-phosphate. The root is also shown to contain phosphoglucomutase and glucose 6-phos-

phate, which can provide a source of glucose 1-phosphate. It therefore appears that a complete mechanism for sucrose synthesis occurs in the sugar-beet root.

The conclusions about sucrose synthesis by sugar beet have been conflicting. It has been suggested (Kursanov & Pavlinova, 1952; Pavlinova, 1954; Kursanov, 1954) that sucrose synthesis does not occur in the mature root and that the sucrose is accumulated in the root solely by transfer of sucrose or sucrose phosphate from the blade. This passive role was allocated to the root because sucrose synthesis was readily detected when monosaccharides were introduced into leaf disks but not when the same technique was applied to mature roots.

The synthesis of sucrose from UDPG and fructose reported here was achieved on many occasions and with extracts of roots that were prepared throughout the growing period of the plant. It seems therefore unlikely that failure by others to achieve the synthesis could be due to the fact that they used the plant only at a particular stage of its growth. Differences in technique in preparing the extracts may well be the main cause of confusion. For instance, Cardini *et al.* (1955) dialysed root extracts against water and it is possible to show that UDPG is degraded quite rapidly by extracts prepared in this way. Degradation is slowed down considerably when dialysis is carried out, as described in this paper, against phosphate buffer.

Rorem *et al.* (1960) obtained enzyme extracts from blade which catalysed sucrose synthesis from fructose and UDPG and also sucrose phosphate synthesis from fructose 6-phosphate and UDPG, and Burma & Mortimer (1956) demonstrated sucrose phosphate synthesis from fructose 6-phosphate and UDPG but found no sucrose if fructose 6-phosphate was replaced by fructose. In the present investigations, no sucrose synthesis was detected with blade extracts and neither sucrose nor sucrose phosphate was produced in extracts incubated with fructose 6-phosphate.

It would appear that no particular route of synthesis should be eliminated solely on the basis of negative results.

The blade extracts of Rorem *et al.* (1960) were prepared by a slightly different technique and partial elimination of invertase activity was attempted both during extraction and by incubation of digests at pH 8.7. A high invertase activity in the digest would, however, be required to prevent qualitative detection of sucrose synthesis and, in the present work, addition to the root extract of an equal volume of blade extract did not produce a sensible inhibiting effect on the sucrose synthesis.

The difficulties of interpreting results obtained from extracts of tissue are illustrated by the

apparent distribution of hexokinase and phosphoglucumutase. Kursanov, Pavlinova & Afanasieva (1959) reported the former to be present and the latter absent in petioles and the reverse picture is presented here for root extracts. These observations do not, however, preclude the possibility that both enzymes are present in the root and the petiole of the intact plant. Similarly the present negative findings with blade extract are not to be interpreted as a suggestion that UDPG is not involved in sucrose synthesis in beet leaves.

It is considered that a mechanism for the synthesis of sucrose occurs both in the root and in the blade of the sugar beet and it may be important to consider both potential sources in assessing the overall production of sucrose by the plant.

SUMMARY

1. The root of the sugar beet was found to contain a complete mechanism for the synthesis of sucrose.

2. Uridine diphosphate glucose, uridine diphosphate, adenosine triphosphate and adenosine diphosphate were present at similar concentrations in the blade, petiole, crown and root of sugar beet.

3. Enzyme extracts of the root or crown effected synthesis of sucrose from uridine diphosphate glucose and fructose.

4. The extracts were also shown to possess uridine diphosphate glucose-pyrophosphorylase and nucleoside-diphosphokinase activity, indicating that the mechanism was present in the root for regeneration of uridine diphosphate glucose.

5. The successive syntheses by the above-mentioned enzymes of uridine triphosphate, uridine diphosphate glucose and sucrose were demonstrated on incubation of a single digest containing adenosine triphosphate, uridine diphosphate, glucose 1-phosphate and fructose. The root also contained phosphoglucumutase and glucose 6-phosphate, which can provide a source of glucose 1-phosphate.

6. The kinetics of the synthesis of the sucrose and the interconversion of the nucleotides were

examined. The reactions were complicated by nucleotide-pyrophosphatase, nucleotidase, myokinase and apyrase activity.

7. Contrary to previous views, it is concluded that the root of the sugar beet, as well as the leaf, may play a significant role in the synthesis of sucrose by the plant.

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