inhibition of DNA synthesis in eggs deposited by treated insects. It is not clear, however, whether this chemical has a direct or indirect effect on DNA synthesis in the developing eggs. A direct effect could occur by transfer of the chemical from the ovaries to the eggs. On the other hand, ovarian damage, hormonal imbalance, or any one of a number of metabolic abnormalities caused by the chemical, could have an indirect effect on the synthesis of DNA in the developing eggs. It is clear, however, that the increased amount of acid-soluble deoxyribosidic compounds in eggs of apholate-treated insects was not high enough to account for the quantity of DNA that would have been incorporated into the acid-insoluble fractions under normal conditions.

The absence of DNA synthesis in the developing eggs caused by apholate was also accompanied by the loss of the ability of the eggs to form lactate dehydrogenase. However, the activity of glucose 6-phosphate dehydrogenase was unaffected by the chemical, as the amount of enzyme present initially in both types of eggs remained constant during the incubation period. This again suggests that the latter enzyme could have been incorporated into the eggs by the ovaries and was not synthesized to any appreciable extent during the incubation period. It also suggests that the effects on DNA and lactate-dehydrogenase synthesis may not be significant unless the eggs deposited by flies given apholate have other more general metabolic activity.

SUMMARY

1. After an initial lag period, the DNA content in normal housefly eggs increases rapidly during incubation at 37°, whereas the RNA content remains almost constant.

2. The synthesis of lactate dehydrogenase in normal eggs parallels the synthesis of DNA during incubation. However, the activity of glucose 6-phosphate dehydrogenase in the eggs remains unchanged during the same period.

3. Non-viable eggs deposited by insects given a diet treated with the chemosterilant apholate do not synthesize any significant quantities of DNA during incubation.

4. The absence of DNA synthesis in the non-viable eggs deposited by flies given apholate is accompanied by the loss of the ability of the eggs to form lactate dehydrogenase. On the other hand, the enzyme glucose 6-phosphate dehydrogenase is unaffected.

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The Partial Purification and some Properties of Two Sucrases of *Phaseolus vulgaris*

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Some members of the raffinose family of oligo-saccharides have been known for many years

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(Johnson, 1843; Planta & Schultze, 1890; Bourquelot & Bridel, 1910), but, since early workers in the field found the isolation and characterization of these sugars difficult, they were thought of as rarities. Recent surveys with chromatographic techniques have shown that they occur more

generally (French, 1954; Dupéron, 1955; Courtois, 1957) than had been previously thought. They occur in seeds, roots, stems and underground tubers of many higher plants.

Plants capable of both producing and utilizing these saccharides include the Leguminosae and Labiatae, which therefore present suitable material for the study of their biosynthesis and degradation.

The utilization of the raffinose-type oligosaccharides by micro-organisms seems to depend on the appropriate hydrolytic enzymes, α -galactosidase and β -fructofuranosidase. It is possible, therefore, that plants may degrade the raffinose-type oligosaccharides by similar means.

Ungerminated pine seeds, as well as broad-bean and French-bean seeds, are known to contain α -galactosidase, though no β -fructofuranosidase has been detected until germination has occurred (Hattori & Shiroya, 1951; Dupéron, 1955; Pridham, 1958; Cooper & Greenshields, 1961a). A report on the occurrence of the carbohydrases in this plant (Cooper & Greenshields, 1961a) indicated the presence of two sucrase activities and an α -galactosidase. Further work on these enzymes has now been completed; a β -fructofuranosidase has been purified and some of its properties have been measured and compared with those of an alkaline sucrase. A preliminary report of this work has been published (Cooper & Greenshields, 1961b).

MATERIALS

The seeds used were of the dwarf French bean, *Phaseolus vulgaris* L. var. Improved Canadian Wonder.

Germination. Seeds were germinated in sterile horticultural-grade vermiculite contained in a large plastic photographic tray (22 in. $\times$ 18 in.). About 250 seeds were planted in such a tray and grown at room temperature in a dark cabinet. Before being planted, the seeds were polished with a cloth to remove many of the seed-coat bacteria and moulds (Harris, 1960). The seedlings required no other attention save an occasional watering and removal of any infected seeds. By this procedure approximately 90% germination was achieved.

Enzyme substrates. The purity of sugars was checked by paper-chromatographic analysis. Verbascose was a gift from Dr J. B. Pridham. Stachyose was obtained from L. Light and Co. Ltd. through the courtesy of Mr S. G. Smith. 1-Kestose was a gift from Dr J. Edelman. Raffinose was obtained from Kerfoot Ltd., and analytical-grade sucrose from Hopkin and Williams Ltd.

METHODS

Determination of saccharides. Reducing sugars were estimated colorimetrically by using the copper reagent of Somogyi (1945) with the arsenomolybdate reagent of Nelson (1944). Total carbohydrate was measured by the anthrone method of Trevelyan & Harrison (1952).

Determination of protein. Total nitrogen was determined by the micro-Kjeldahl method. With clear solutions, protein was estimated spectrophotometrically (Warburg & Christian, 1941) after thorough dialysis.

Paper chromatography. Butan-1-ol-acetic acid-water (12:3:5, by vol.) or propan-1-ol-ethyl acetate-water (7:1:2, by vol.) was employed as a solvent in one-dimensional descending chromatography. Saccharides were detected with benzidine-trichloroacetic acid reagent (Bacon & Edelman, 1951). Ketoses were detected with the phloroglucinol-trichloroacetic acid reagent, and reducing sugars with 3,5-dinitrosalicylic acid-sodium hydroxide reagent (Smith, 1960).

Deproteinization. Samples (0.5 ml.) withdrawn from enzyme digests were run into 1 ml. of 5% (w/v) ZnSO₄ solution contained in a graduated centrifuge tube and were titrated with 0.3N-Ba(OH)₂, with phenolphthalein as indicator. When the solution was faintly pink, it was made up to 5 ml., well shaken and centrifuged. If citric acid-sodium phosphate buffers were used, it was essential to use freshly prepared Ba(OH)₂ or NaOH solutions for the neutralization step.

Diethylaminoethylcellulose chromatography. DEAE-cellulose (3-4 g.), prepared by the method of Peterson & Sober (1956), was washed four times with 200 ml. portions of 5 mM-sodium phosphate buffer, pH 7.5. A slurry in buffer was poured into a glass column (2 cm. diam.) fitted with a coarse sintered-glass disk at its base, allowed to settle under gravity and then packed by the application of pressure (10 cm. Hg). Buffer was run through the column until the pH and extinction ($E_{280\text{ m}\mu}^{1\text{ cm.}}$) of the eluate were identical with those of the original buffer.

Assay of enzyme activities. Alkaline sucrase was assayed by mixing equal amounts (usually 0.4 ml.) of 4% (w/v) sucrose in 0.2M-sodium phosphate buffer, pH 7.9, and enzyme, to give a final pH value of 7.7. After incubation for 30 min. at 25°, a sample was withdrawn from the digest and deproteinized as described above. A 2 ml. sample of the supernatant was used in a determination of reducing sugar.

β -Fructofuranosidase was assayed in a similar manner to the alkaline sucrase except that 0.2M-citric acid-sodium phosphate buffer (McIlvaine, 1921) was used to give a final pH of 4.9-5.0. Equal amounts (0.10 ml.) of enzyme and 4% (w/v) sucrose in 0.2M buffer were mixed in a test tube (15 mm. $\times$ 120 mm.) and incubated at 25° for 10-30 min. Then Somogyi reagent (2 ml.) was added followed by water (1.8 ml.), and the determination carried out in the usual manner. The final concentration of citric acid (1.25 mM) had no effect on the sugar estimation (cf. Folin, 1926).

In these assay systems, the initial rate of sucrose hydrolysis was constant up to 10% hydrolysis for alkaline sucrase and 40% hydrolysis for the β -fructofuranosidase. The initial rate of production of reducing sugar was stoichiometrically related to the amount of enzyme added in both cases (at least over a tenfold range of enzyme concentration). No reducing sugars were produced if the sucrose was omitted or if the enzyme preparation had been boiled.

Unit of enzyme activity. A unit of sucrase activity was defined as the amount of enzyme liberating 1 μ mole of reducing sugar (glucose plus fructose)/min. at 25° in a reaction mixture containing buffered 2% sucrose at the optimum pH (cf. Fischer & Kohtès, 1951).

Preparation of alkaline sucrase. All preparative steps were carried out at 0-4° unless otherwise stated.

Step 1: preparation of the crude extract. About 600 embryos (11–12 g.) from 12 hr. water-steeped seeds were ground in a cold mortar with 5 g. of sand and 3 vol. of 10 mM-sodium phosphate buffer, pH 7.0. The mixture was squeezed through muslin and then centrifuged at 35000g for 20 min. (in a Spinco model L centrifuge). A clear yellow-brown supernatant was obtained with a large amount of fatty material at the surface. The fatty material could easily be left on the side of the tube and the supernatant poured off.

Step 2: ammonium sulphate precipitation. Further purification was carried out by fractionation with saturated $(\text{NH}_4)_2\text{SO}_4$ solution at pH 7.0. A twofold purification was achieved by taking the fraction precipitated at between 20% and 40% saturated $(\text{NH}_4)_2\text{SO}_4$. This fraction had 72% of the original activity.

No further purification could be achieved, although other techniques including protein precipitation at pH 5.0, calcium phosphate-gel treatment and DEAE-cellulose chromatography were attempted. The high content of storage protein in extracts of embryos at steeping probably contributed to the fact that the enzyme was not separated by these techniques. The twofold purified alkaline sucrase was used in all subsequent experiments involving enzyme properties.

Preparation of β -fructofuranosidase. All preparative steps were carried out at 0–4° unless otherwise stated.

Step 1: preparation of crude extract. Hypocotyls and roots of 10-day-old seedlings were chopped into small pieces and homogenized in 10 mM-sodium phosphate buffer, pH 7.0 (30 ml. of buffer/100 g. of plant material), for three 30 sec. periods in an Ato-Mix blender. The mixture was squeezed through muslin and the extract centrifuged at 25000g (in a MSE high-speed 17 centrifuge) for 30 min. An almost-clear brownish yellow extract was obtained.

Step 2: acid precipitation of inactive material. Much inactive material was removed by adjusting the crude extract to pH 5.0 with 0.2N-acetic acid. The solution was centrifuged at 10000g for 10 min. and the precipitate discarded. The supernatant was readjusted to pH 7.0 with 0.5N-NaOH and then dialysed for 6–8 hr. against 10 mM-sodium phosphate buffer, pH 7.0.

Step 3: calcium phosphate-gel treatment. The extract at pH 7.0 was treated with calcium phosphate gel (Keilin & Hartree, 1938). The amount of gel (27 mg. dry wt./ml.) required to adsorb the enzyme was determined with a small sample of the preparation. The adsorbed enzyme was washed with deionized water and eluted from the gel by successive treatments with 0.2M-sodium phosphate buffer, pH 7.0.

Step 4: DEAE-cellulose chromatography. The gel eluate was thoroughly dialysed against 5 mM-sodium phosphate buffer, pH 7.5, and then loaded on to a DEAE-cellulose column (10 cm. $\times$ 2 cm.) previously equilibrated with buffer. After development of the column with 5 mM-sodium phosphate buffer, pH 7.5, stepwise elution with NaCl was carried out at 0.05, 0.1, 0.2, 0.4 and 0.6M final concentration in 5 mM-sodium phosphate buffer, pH 7.5. Most of the enzyme activity eluted was in the 0.1M-NaCl peak, though some was eluted after the 0.05M-NaCl peak (Fig. 1). The total recovery of enzyme was only 37% of that applied, but one or two of the fractions from the column contained the enzyme at a high specific activity. This preparation was used for the experiments involving enzyme properties. The scheme of purification is shown in Table 1.

RESULTS

Presence of the sucrase activities in the seedling

Preliminary qualitative experiments (Cooper & Greenshields, 1961a, b) with extracts of cotyledons and embryos from ungerminated seeds indicated that the type of sucrase activity present in the ungerminated seeds and at the very early stages of germination was different from that present in the embryos at later stages of germination. Further evidence for this was obtained by estimating the

Fig. 1. Elution of β -fructofuranosidase from DEAE-cellulose. Experimental details are given in the text. Protein was eluted with NaCl in 5 mM-sodium phosphate buffer, pH 7.5, and 9 ml. fractions were collected. ●, Protein concentration measured as $E_{280\text{ m}\mu}$; ○, β -fructofuranosidase activity.

Table 1. Summary of purification of β -fructofuranosidase

Step	Vol. (ml.)	Units	Total protein (mg.)	Total carbohydrate (mg.)	Specific activity	
					(units/mg. of protein)	(units/mg. of carbohydrate)
1. Crude extract	380	185	704	65	0.26	2.9
2. Supernatant from acid treatment	422	159	165	36	0.97	4.4
3. Supernatant from $\text{Ca}_3(\text{PO}_4)_2$ -gel absorption	80	93	38	1.9	2.5	48.6
4. Fraction from DEAE- cellulose column	9	9.3	0.32	—	29.0	—

Experimental details are given in the text.

reducing sugar produced when extracts of various parts of the seedling were incubated with sucrose over a range of pH values (Fig. 2). In steeped seeds the sucrose activity of embryo extracts showed a single pH optimum at pH 8.0, the specific activity in μ moles of reducing sugar formed/min./mg. of nitrogen being 0.14. At pH 5.0 the specific activity was only 0.006. With 3-day-old seedlings, the embryo extracts gave both an acid and alkaline pH optimum, the specific activities being 0.29 at pH 5.0 and 0.14 at pH 8.0. With 10-day-old seedlings, embryo extracts gave a single pH optimum at pH 5.0 with a specific activity of 2.1. At pH 8.0 the specific activity was 0.23. Since the enzyme with the pH 5.0 optimum probably has some activity at pH 8.0, the specific activity at pH 8.0 given by 10-day-old seedlings together with the value of 3-day-old seedlings suggests that alkaline sucrose does not appreciably increase in amount during germination. Extracts prepared from cotyledons at the steeped stage had specific activities at pH 8.0 of about 0.005. The enzyme with the pH 5.0 optimum did not develop in the cotyledons during germination.

Distribution of the sucrase activities in the seedling

It was not possible to measure the amount of alkaline sucrose after germination owing to interference from the β -fructofuranosidase. The β -fructofuranosidase was not present in ungerminated seeds but appeared on germination and increased

in amount up to 9–10 days' growth (Fig. 3). Thereafter a decrease was shown with a fall to 55 % of the maximum after 15 days and 5 % of the maximum at 20 days' growth under etiolated conditions. Distribution of the β -fructofuranosidase in the various parts of the seedling was measured at 10 days' growth. Table 2 shows that the roots contain the highest activity of enzyme expressed either on a unit nitrogen or on a dry weight basis.

Some properties of the purified sucraes

Stability and pH-dependence. The partially purified alkaline sucrose was stable for at least a month at -14° , whereas purified β -fructofuranosidase lost 40 % of its activity in 1 week at -14° . The pH-dependence of both enzymes acting on sucrose is shown in Fig. 4. The alkaline sucrose has a sharp optimum at pH 7.7, the activity at pH 7.3 and 8.2 being 54 and 66 % of the maximum activity respectively. β -Fructofuranosidase has a broad optimum between pH 4.7 and 5.3 on sucrose and a similar broad pH optimum at 5.0 on raffinose (Fig. 5).

Specificity. Both sucraes were examined with various substrates to determine their specificity. Enzyme digests were assayed for the production of reducing sugars and further examined by paper-chromatographic analysis.

(a) Alkaline sucrose. Although this enzyme was only a partially purified preparation, it was possible to gain enough knowledge of its specificity

Fig. 2. pH-Activity curves for sucrose hydrolysis by dialysed extracts of steeped embryos and 10-day-old seedlings. 0.1 M-Citric acid-sodium phosphate buffers were used for pH 3–6 and 0.1 M-sodium phosphate buffers for pH 6–9. Enzyme assays were carried out as described in the Methods section but with incubation times of 2 hr. for the 10-day-old-seedling extract (○) and 4 hr. for the steeped-embryo extract (●). The pH values are those of the actual digests.

Fig. 3. Assay of the β -fructofuranosidase in embryos during germination. Standard amounts (200 mg.) of ground freeze-dried embryo samples were homogenized in 5 ml. of distilled water. The whole homogenate was washed into dialysis tubing and dialysed against distilled water overnight. The volume was made up to 10 ml. with water and 0.5 ml. of the homogenate incubated at 25° with 0.5 ml. of 4 % sucrose buffered at pH 4.9. After 1 hr., 0.5 ml. of the incubation mixture was removed and deproteinized. Samples (1 ml.) of the deproteinized solution were assayed for reducing sugar.

Table 2. Distribution of β -fructofuranosidase in the seedling

The 10–12-day-old seedlings were divided into four parts: primary leaves plus epicotyls; cotyledons; hypocotyls; roots. The weights of the various parts were noted and each sample was extracted with a known volume of 10 mM-sodium phosphate buffer, pH 7.0, in a small Waring Blender. All samples except the cotyledon sample were squeezed through muslin and then all were centrifuged at 30 000g for 30 min. at 2°. Samples of the supernatant were dialysed against the phosphate buffer for 14 hr. The enzyme activity and nitrogen content of each sample were measured.

Part	Total activity (units)	Total nitrogen (mg.)	Dry wt. (g.)	Specific activity	
				(units/mg. of nitrogen)	(units/g. dry wt.)
Primary leaves + epicotyls	6.6	25.4	0.95	0.30	6.9
Cotyledons	0.26	14.0	3.9	0.02	0.07
Hypocotyls	30.7	9.1	1.9	3.4	16.1
Roots	44.6	5.8	1.1	7.7	40.5

Fig. 4. pH-Activity curves for β -fructofuranosidase and alkaline sucrase. β -Fructofuranosidase (○) was incubated with 2% sucrose in 0.1M-citric acid-sodium phosphate buffers for 15 min. at 25° over the pH range 4.5–5.5. Alkaline sucrase (●) was incubated with 2% sucrose in 0.1M-sodium phosphate buffers for 30 min. at 25° over the pH range 7.2–8.2. The pH values quoted are those of the actual incubation mixtures.

for comparison with β -fructofuranosidase. Sucrose, raffinose, stachyose, melezitose, maltose and 1-kestose were used as substrates under identical conditions at pH 7.7. No significant action was found on melezitose, i.e. it was less than 1 % of the rate on sucrose. No action on 1-kestose could be detected by chromatographic analysis. The action on raffinose and stachyose was usually 3–5 % of the rate on sucrose. However, chromatographic analysis showed that galactose and glucose were present in apparently equal amounts and melibiose was never detected. These results were consistent with the production of galactose and sucrose by an α -galactosidase known to be present in the alkaline-sucrase preparation. The sucrose produced by this hydrolysis was further hydrolysed by the alkaline

Fig. 5. pH-Activity curves for β -fructofuranosidase acting on sucrose (○) and raffinose (●). The enzyme was incubated with 2% substrate in 0.1M buffer for 10 min. at 25°. Citric acid-sodium phosphate buffers were used over the pH range 3–6 and sodium phosphate buffers over the pH range 6–9. The pH values quoted are those of the actual incubation mixtures.

sucrase. Although the crude and partially purified extracts produced glucose from maltose, they did so at different rates with respect to their rate of action on sucrose. This indicates a separation of individual enzyme activities acting on maltose and sucrose. Hence the alkaline sucrase was probably contaminated with maltase.

(b) β -Fructofuranosidase. The enzyme had no action on melezitose. Fructose was produced by the action of the enzyme on sucrose, raffinose and stachyose; glucose, melibiose and manninotriose respectively were produced in these digests. The enzyme hydrolysed 1-kestose producing glucose and fructose.

Transferase activity. When the enzymes were allowed to act on 20 % (w/v) sucrose at the appropriate optimum pH values, transferase action was observed with the β -fructofuranosidase, two oligo-saccharides, with R_{sucrose} values 0.33 and 0.65 respectively (in butan-1-ol-acetic acid-water sol-

vent), being obtained (cf. Bacon & Edelman, 1950). No transference activity was detected with the alkaline sucrase.

Inhibition. The comparative action of enzyme inhibitors on both enzymes was measured. At the appropriate optimum pH values, both enzymes were sensitive to heavy-metal inhibitors. In each case Ag^+ ions (0.1 mM) caused 90–95% inhibition but relatively high concentrations of iodoacetamide

(10 mM) caused only 5–10% inhibition. A difference between the two enzymes became apparent with respect to the action of tris. At pH 7.7, 10 mM-tris inhibited the alkaline sucrase 92%. The same concentration of tris inhibited the β -fructofuranosidase only 6% at pH 5.0 and 38% at pH 7.7. Tris is known to exhibit a high affinity for the active centre of yeast maltase and acts as a powerful competitive inhibitor (Halvorsen & Ellias, 1958). Its mode of action on alkaline sucrase has not yet been determined. A further difference between the sucraes was indicated by the effect of glucose and fructose on the initial rates of reaction of the enzymes. With 2% (w/v) sucrose and 1% (w/v) final concentration of glucose plus fructose, a 50% diminution of reaction rate was found for alkaline sucrase whereas the corresponding diminution of rate for the β -fructofuranosidase was only 6%.

Effect of substrate concentration on the sucrae activities. With sucrose as substrate, the effect of substrate concentration on initial rates of reaction was measured for both enzymes. The reaction rates with 0–30 mM-sucrose were measured and from the initial velocities it was shown that both enzymes followed simple Michaelis–Menten kinetics. From a plot of $1/v$ against $1/s$ (Lineweaver & Burk, 1934), the K_m was determined for each enzyme. The values were 8.9 mM at pH 7.7 and 25° for alkaline sucrae and 2.4 mM at pH 5.0 and 25° for β -fructofuranosidase. With raffinose (0–80 mM) as substrate for the β -fructofuranosidase, the K_m was 14 mM at pH 5.0 and 25° (Figs. 6 and 7).

DISCUSSION

Two distinct sucrae activities have been demonstrated in extracts of the germinating French bean. One sucrae appeared to be unique in having an alkaline pH optimum (at pH 7.7) and a high specificity for sucrose. The trivial name 'alkaline sucrae' has been adopted for the purpose of this work and in previous publications (Cooper & Greenshields, 1961a, b) to distinguish this enzyme from the other bean sucrae, which was shown to have a pH optimum at pH 4.8–5.2 and to act generally on β -fructofuranosides, and thus classifiable as a β -fructofuranosidase (EC 3.2.1.26). Hatch, Sacher & Glasziou (1963) have demonstrated the presence of two invertases in sugar-cane storage tissues, an acid invertase with optimum pH 5.0–5.5 and a neutral invertase with optimum pH 7.0. The acid invertase was present in the immature tissue and decreased in amount as the tissue matured. Conversely, the neutral invertase was absent from the immature tissue and developed as the tissue matured.

Little work has been carried out on the purification of plant sucraes, and in the present work the

Fig. 6. Effect of sucrose concentration on the initial rates of reaction catalysed by β -fructofuranosidase ($\bullet$) and alkaline sucrae ($\circ$). Various sucrose concentrations up to 30 mM were used at the appropriate optimum pH and 25°. Reaction times were such that sucrose hydrolysis did not exceed 10%. The initial reaction velocity is expressed as μ moles of reducing sugar formed in 30 min.

Fig. 7. Effect of raffinose concentration on the initial rates of reaction catalysed by β -fructofuranosidase. Various raffinose concentrations up to 80 mM were used at pH 4.9 and 25°. Reaction times were such that raffinose hydrolysis did not exceed 10%. The initial reaction velocity is expressed as μ moles of reducing sugar formed in 30 min.

110-fold-purified bean β -fructofuranosidase probably represents the purest yet for the plant enzyme. Its specific activity is lower than the best preparations of yeast β -fructofuranosidase, but the crude yeast β -fructofuranosidase used in the work of Fischer & Kohtès (1951) had a specific activity ten times as high as that of the crude enzyme used in the present work.

The bean β -fructofuranosidase had K_m values of 2.4 mM on sucrose and 14 mM on raffinose. Although alkaline sucrase was specific for sucrose it had a lower affinity for this substrate than had the β -fructofuranosidase, since its K_m (8.9 mM) was four times as great as that of the β -fructofuranosidase. The K_m values on sucrose are similar to those obtained by Ward (1953) for the invertase of wheat leaf (4 mM) and by Hellebust & Forward (1962) for the β -fructofuranosidase of maize radicles (6 mM). Hatch *et al.* (1963) report higher values, 13 and 25 mM respectively, for sugar-cane acid and neutral invertases. The K_m for the bean β -fructofuranosidase acting on sucrose is about sevenfold smaller than that for the yeast enzyme (Kuhn, 1923). The ratio of the K_m values on sucrose was 6 for the bean β -fructofuranosidase compared with 15 for the yeast β -fructofuranosidase (Kuhn, 1923). There is also a difference in the pH-dependence curves of yeast and bean β -fructofuranosidase acting on sucrose. Although both have the optimum at pH 4.5–5.2, the bean enzyme showed a sharp decrease in activity below pH 4.0 which was not found with the yeast enzyme (Adams, Richtmyer & Hudson, 1943).

Both bean sucraes resembled yeast β -fructofuranosidase in their sensitivity to inhibition by Ag^+ ions and their insensitivity to inhibition by iodoacetamide. Tris had a greater inhibitory effect on alkaline sucrase than on the bean β -fructofuranosidase. Alkaline sucrase was also much more sensitive to inhibition by the products of its action than was the β -fructofuranosidase. This may have been one of the reasons for the failure of alkaline sucrase to exert transferase activity such as was shown by the β -fructofuranosidase acting on 20% (w/v) sucrose.

It has been reported that some plant invertases are insoluble and associated with cell-wall material (Straus, 1962). However, many plant invertases, including the bean sucraes reported above and the acid and neutral invertases of sugar-cane tissue (Hatch *et al.* 1963), appear to be freely soluble.

The metabolic function *in vivo* of carbohydrases, such as the sucraes and galactosidases, would appear to be the production of monosaccharide units from the appropriate oligosaccharides. Support for this idea can be obtained from studies on micro-organisms where the production of such carbohydrases is often under cellular control

(Monod & Cohn, 1952). In the present work, however, no clear demonstration of the function *in vivo* of the alkaline sucrase could be demonstrated. The alkaline sucrase was present in both the embryo and cotyledons at early stages of germination together with its substrate sucrose, but Greenshields (1960) has shown that these parts do not contain the products of its action. On the other hand the appearance of glucose and fructose in the embryo of the germinating seeds coincided with the appearance of β -fructofuranosidase. The cotyledons, which lacked reducing sugars but contained sucrose, did not develop β -fructofuranosidase. It is possible, therefore, that this enzyme acts as a hydrolase *in vivo*. It had been shown many years before that the appearance of reducing sugars in developing plants often coincided with the appearance of a sucrase (Brown & Morris, 1890).

Although the presence of the sucraes was not directly related to the presence of their substrates, it was conjectured that they might play a part in the breakdown of the raffinose-type oligosaccharides. However, the β -fructofuranosidase does not appear in the embryo until the raffinose-type oligosaccharides have almost disappeared, and does not appear at all in the cotyledons, where the raffinose-type oligosaccharides persist in large amounts until late in germination (Greenshields, 1960). It is therefore unlikely that β -fructofuranosidase is involved in the breakdown of the raffinose-type oligosaccharides. An α -galactosidase, known to be present in the germinating embryo (Cooper & Greenshields, 1961*a, b*) before the β -fructofuranosidase has appeared, could be involved in the breakdown of the raffinose-type oligosaccharides. The more specific alkaline sucrase might then function under these conditions, producing reducing sugars from the sucrose that remained after the action of the α -galactosidase on the raffinose-type oligosaccharides. However, no free galactose was observed in this plant, suggesting that α -galactosidase was not active *in vivo*, unless it was coupled with a very active metabolic system that could utilize the free galactose as quickly as it was formed (cf. Pazur, Shadaksharaswamy & Meidell, 1962). Only a weak galactokinase activity (1.25% of the activity of glucokinase) could be detected in the germinating embryo (Cooper, 1961). The sucraes reported in the present paper are probably not involved in the breakdown of the raffinose-type oligosaccharides.

SUMMARY

1. Two sucraes present in *Phaseolus vulgaris* were easily differentiated, one, apparently specific for sucrose, with optimum pH 7.7 (named alkaline sucrase) and the other (shown to be a β -fructofuranosidase) with optimum pH 5.0.

2. Alkaline sucrase was present in the ungerminated seed. β -Fructofuranosidase was absent from the ungerminated seed but appeared in the embryo on germination, reaching a maximum (under etiolated conditions) after 9–10 days. Much of the activity appeared in the roots.

3. The alkaline sucrase could be purified only twofold, whereas the β -fructofuranosidase was purified 110-fold.

4. The K_m for the β -fructofuranosidase was 2.4 mM on sucrose and 14 mM on raffinose. Alkaline sucrase had K_m 8.9 mM on sucrose.

5. Both enzymes were inhibited by Ag^+ ions (0.1 mM), though iodoacetamide (10 mM) had little effect. Alkaline sucrase was strongly inhibited by tris. Transferase activity was observed with the β -fructofuranosidase but not with alkaline sucrase.

6. The function of the sucraes *in vivo* and their relation to the metabolism of raffinose-type oligosaccharides during germination is discussed.

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The Effect of Sex and Site on the Composition of Skin in the Rat and Mouse

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It is necessary to observe a number of precautions if consistent values are to be obtained for the composition of skin (Rothman, 1954). The samples of skin must be taken from specified sites, and from animals of similar age and sex. It is also known that the composition of skin is affected

by the nature of the diet (Haldi, Giddings & Wynn, 1941–42), by the state of hydration of the animal (Skellton, 1926; Hamilton & Schwartz, 1935; Flemister, 1941–42), by undernutrition (Widdowson, Dickerson & McCance, 1960; Dickerson & McCance, 1964) and by a low-protein diet (Hark-