SUMMARY

1. The incorporation of [2-¹⁴C]acetate into β -carotene by *P. blakesleeanus* is decreased by DL-mevalonate and by L-leucine.

2. DL-[2-¹⁴C]Mevalonate is incorporated into β -carotene by *P. blakesleeanus* some fifteen times more efficiently than [2-¹⁴C]acetate.

3. The C-2 of mevalonate retains its individuality when incorporated into β -carotene and occurs only in C-*w* of the isoprenoid residue.

4. *P. blakesleeanus* cultured on our basal medium, with asparagine as the nitrogen source, does not fix CO₂ into β -carotene; however, when leucine is present in the medium ¹⁴CO₂ appears in the β -carotene. An explanation of this observation is offered.

5. Labelled β -carotene is synthesized by washed suspensions of *P. blakesleeanus* grown in the presence of diphenylamine and DL-[2-¹⁴C]mevalonate.

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Inhibition of Amylase Activity of Human Body Fluids by Citrate

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McGeachin, Dougherty, Hargan & Potter (1957) used the method of Van Loon, Likins & Seger (1952) to compare the amylase activities of plasma and serum

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prepared from the same blood sample. With human blood they found that, when oxalate or citrate was used as anticoagulant, the amylase activity of plasma was slightly lower than that of serum. With rat and dog blood they found no such difference.

During studies of the activity of amylase in the blood serum of patients suffering from diabetes mellitus (Street, 1958) the writer noted (unpublished work) that citrate had an inhibitory effect on amylase activity, but oxalate had no effect.

In view of the claims of McGeachin *et al.* (1957), it was decided to investigate more fully the action on amylase activity of citric acid and substances of close chemical relationship to citric acid.

MATERIALS AND METHODS

Sources of amylase. Human blood was collected by venepuncture in a clean, dry tube and allowed to clot. Unless otherwise stated, 1 vol. of the separated serum was diluted with 9 vol. of 0.85% NaCl. Human urine was diluted 1:5 (or 1:2) with 0.85% NaCl. Human saliva was mixed with an equal volume of water and centrifuged. The clear supernatant liquid was diluted 1:500, 1:1000 or 1:2000 with 0.85% NaCl.

0.2M-Phosphate buffer, pH 7.0. 0.2M-NaOH (29.54 ml.) was added to 50 ml. of 0.2M-KH₂PO₄ and diluted to 200 ml. with water.

0.2M-Phosphate buffer, pH 7.6. 0.2M-NaOH (42.74 ml.) was added to 50 ml. of 0.2M-KH₂PO₄ and diluted to 200 ml. with water.

0.2M-Borate buffer, pH 7.0. Boric acid (1.237 g.) was dissolved in about 50 ml. of warm water. The solution was cooled and diluted to 100 ml. The pH of this solution was adjusted to 7.0 by dropwise addition of 0.2M-NaOH.

Urine and saliva were from 'normal' healthy adults. Blood was taken from 'normal' adults and from adult patients suffering from various diseases. When a serum of high amylase activity was required, the blood was taken from a patient with acute pancreatitis.

Determination of amylase activity. Amylase activity was determined by the method of Street & Close (1956), modified by using 0.2M-phosphate buffer, pH 7.0. In several tests, this method and that of Close & Street (1958) were compared with the same sample. Unless otherwise stated, the digestion mixture was adjusted to pH 7.0 before addition of the amylase sample. Measurements of pH were made with a glass electrode and pH meter.

RESULTS

Effect of variation of concentration of citrate on amylase activity. Aqueous solutions of trisodium citrate dihydrate of the required molarity were prepared. A portion (1 ml.) of each solution was added to each of a series of 100 ml. volumetric flasks containing 5 ml. of 0.2M-phosphate buffer, pH 7.0. With these mixtures (6 ml.) in place of 5 ml. of phosphate buffer amylase was determined by the method of Street & Close (1956) with (a) serum, (b) saliva and (c) urine. The addition of 1 vol. of M-sodium citrate solution to 5 vol. of 0.2M-phosphate buffer solution, pH 7.0, did not measurably alter the pH of the solution. The results of this experiment are illustrated in Fig. 1.

Determination of optimum pH for activity of serum amylase in the presence of citrate. Phosphate buffers (0.1M) were prepared in the range pH 6.0–8.0 in steps of 0.2 pH unit. Amylase activity was determined in the absence of and in the presence of 1 ml. of M-sodium citrate solution. A 1:40 dilution of the same serum sample of high amylase activity was used throughout. The presence of 1 ml. of M-sodium citrate solution had no effect on the extinction of the standard solution. The results are illustrated in Fig. 2.

Comparison of effect of citrate on amylase activity at pH 7.0 and 7.6. To a series of 100 ml. volumetric flasks were added 5 ml. of mixtures of 0.2M-phosphate buffer (pH 7.0) and 0.2M-sodium citrate (adjusted to pH 7.0 with 0.2M-citric acid) in varying proportions (see legend to Fig. 3). These 5 ml. mixtures were used in place of 5 ml. of phosphate

Fig. 1. Effect of variation of citrate concentration on amylase activity. O, Serum diluted 1:10; Δ, urine diluted 1:5; ●, saliva diluted 1:2000.

Fig. 2. Effect of citrate on the pH optimum for serum amylase activity. ●, Citrate present; O, without citrate.

buffer to determine the activity of a dilution of serum by the method of Street & Close (1956). The experiment was then repeated with mixtures of phosphate buffer at pH 7.6 and citrate solution at pH 7.6. Fig. 3 shows the results of this experiment.

Effect of amylase activity of various compounds other than citrate. The previous experiment was repeated (at pH 7.0 only) with 0.2M-solutions of the following in place of citrate: isocitrate, malate, lactate, glycollate, glycine, tartrate, *cis*-aconitate, glutarate, β -hydroxy- β -methylglutarate, tricarballoylate and oxalate. None of these compounds had any effect on amylase activity.

Fig. 3. Comparison of the effect of citrate on amylase activity at pH 7.0 and 7.6. The test solutions (final volume 100 ml.) contained 5 ml. mixtures of 0.2M-phosphate and 0.2M-citrate buffers. The volume of citrate in each mixture can be read from the abscissa. ○, pH 7.0; ●, pH 7.6.

Fig. 4. Effect of borate on amylase activity at pH 7.0. The test solutions (final volume 100 ml.) contained 5 ml. mixtures of 0.2M-phosphate and 0.2M-borate buffers. The volume of borate in each mixture can be read from the abscissa.

When 0.2M-borate buffer, pH 7.0, was added in place of citrate, results were obtained as illustrated in Fig. 4.

Effect of amylase activity of borate in the presence of citrate and phosphate. To a series of 100 ml. volumetric flasks were added 0.2M-solutions of phosphate, citrate and borate in various proportions. The results illustrated in Fig. 5 were obtained.

DISCUSSION

The results show that citrate ions inhibit the activity of serum amylase. Similar results have been obtained with salivary and urinary amylase. This inhibition is real and not an apparent one caused by a shift of the pH optimum in the presence of citrate. It will be seen from Fig. 2 that the citrate moves the pH optimum from 7.0 to 7.6. However, the results of the experiment in which the amylase activity of a serum was determined in the presence of citrate at both pH 7.0 and pH 7.6 show that inhibition by citrate is similar at the two pH values.

Oxalate does not inhibit whether it is added directly to the serum or to the digestion mixture

Fig. 5. Effect of borate in presence of citrate on amylase activity. The test solutions (final volume 100 ml.) contained 5 ml. mixtures of 0.2M-phosphate, 0.2M-borate and 0.2M-citrate. The proportions of phosphate, borate and citrate can be read from the abscissae.

(H. V. Street, unpublished work). This is not in agreement with the work of McGeachin *et al.* (1957). These workers used the method of Van Loon *et al.* (1952), which is similar to the method of Street & Close (1956), the main difference being that starch is used as substrate instead of amylose as in the present method.

When the method of Van Loon *et al.* (1952) was applied with starch as substrate the results of McGeachin *et al.* (1957) could still not be confirmed, although citrate still inhibited amylase activity.

SUMMARY

1. The effect of citrate on the amylase activity of blood serum, urine and saliva has been investi-

gated and it has been shown that it inhibits the enzyme.

2. *iso*Citrate, malate, lactate, glycollate, α -aminoacetate, tartrate, *cis*-aconitate, glutarate, β -hydroxy- β -methylglutarate, tricarballoylate and oxalate have no effect on amylase activity.

3. Borate ions activate amylase activity.

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Oligosaccharides and Associated Glycosidases in Aspen Tissues

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The low-molecular-weight carbohydrates present in the saps of various trees have recently been reviewed by Stewart (1957). Relatively few reports on carbohydrases and transferase activities in tree tissues are to be found in the literature, however.

This paper describes studies with quaking (*Populus tremuloides*) and bigtooth (*P. grandidentata*) aspens, where, in addition to glucose and fructose (and a trace of xylose) the main sugar present in both xylem (1-year-old-xylem elements) and 'soft xylem' (newly differentiated tissue consisting of fibres, vessels and some cambial cells) is sucrose. Photomicrographs and a full description of these tissues have been given by Kremers (1957).

Small amounts of raffinose are also present, particularly in the xylem, and *O*- α -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*- β -D-fructofuranosyl-(1 $\rightarrow$ 2)- β -D-fructofuranoside (1^F- β -fructosylsucrose) has been identified as a component of the 'soft xylem'. Protein preparations from the cambial region of aspen show glycosidase and transferase activities.

MATERIALS AND METHODS

Healthy trees were selected in an experimental forest at the start of the growing season; they were in full leaf. Within 10–15 min. of felling, the tissues required for sugar analyses were collected and quickly placed in absolute

methanol. 'Soft-xylem' material was obtained by removing the bark from the bole and then slicing off the tissue with a knife blade held flat against the surface of the wood. Xylem was subsequently removed with a spokeshave.

All concentrations are expressed as g. of solute/100 ml. of solution unless otherwise stated.

Isolation and characterization of sugars. The collected tissues were filtered off from the methanol and re-extracted with hot methanol. The two methanolic solutions were then combined and concentrated to syrups by distillation under reduced pressure. These were examined on paper chromatograms (Whatman no. 1 paper) with ethyl acetate-acetic acid-water (A; 9:2:2, by vol.), butan-1-ol-pyridine-water (B; 6:4:3, by vol.) and propan-1-ol-ethyl acetate-water (C; 7:1:2, by vol.) solvents. *p*-Anisidine-HCl and resorcinol-HCl (Hough, Jones & Wadman, 1950) were used as spray reagents.

Small quantities of suspected sucrose and raffinose were isolated from xylem extracts (*P. grandidentata*) by partition chromatography on Whatman no. 3 MM paper with solvent A. Total hydrolysis of the raffinose was effected by heating at 95° with N-H₂SO₄ for 3 hr. The acid was then neutralized with BaCO₃ and the hydrolysate examined on paper chromatograms (solvent A). Yeast invertase (35°; 20 hr.) was used for a partial hydrolysis and the resulting products were again examined on paper chromatograms (solvents A and B).

1^F- β -Fructosylsucrose was obtained by passing an aqueous solution of the methanol extract of 'soft xylem' (*P. tremuloides*) through a small charcoal-Celite column (Andrews, Hough & Powell, 1956). The monosaccharides were eluted from the column with water and sucrose with aqueous 2.5% (v/v) ethanol. Elution with aqueous 50%