Enzyme Formation in Higher-Plant Tissues

DEVELOPMENT OF INVERTASE AND ASCORBATE-OXIDASE ACTIVITIES IN MATURE STORAGE TISSUE OF *HELIANTHUS TUBEROSUS* L.

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1. Washed aerated disks of mature tubers of *H. tuberosus* developed invertase and ascorbate-oxidase activities; this effect was not due to contaminating micro-organisms. 2. The use of specific inhibitors showed that such development was an expression of protein synthesis. 3. There was no visual evidence of cell division during induction of the enzymes. 4. The invertase could not be solubilized; most of the ascorbate oxidase was freely soluble.

Bacon (1961) has shown that, although invertase is hardly detectable in mature storage tissue of sugar beet, washed aerated slices of the tissue progressively develop the enzyme activity. Removal of most of the soluble contents of the slices by treatment with ethyl acetate left the enzyme associated with the insoluble residue.

We have confirmed Bacon's (1961) observations with a different tissue, namely tubers of Jerusalem artichoke (*Helianthus tuberosus*), and the present paper purports to show that the increase in invertase is an expression of protein synthesis and that this synthesis is closely associated with the cell walls. Moreover, the synthesis is induced in mature cells of tissue slices, as histological examination showed that it is not associated with cell division. Ascorbate-oxidase activity is shown to develop similarly to invertase, although this enzyme is largely soluble.

A preliminary account of part of this work was communicated to The Biochemical Society (Edelman & Hall, 1963).

MATERIALS AND METHODS

Plant material. Jerusalem-artichoke tubers were obtained from the University of London Botanical Supply Unit or from Chelsea Physic Garden. The varieties given in Table 2 were given by the Scottish Horticultural Research Institute. Tubers were stored in a cold room (about 3°) in pots in damp sand.

Unsprouted tubers were scrubbed, and sliced transversely on a microtome into slices 1 mm. thick from which disks (1 cm. diam.) were cut with a cork borer. These were rinsed briefly in a large volume of water before being transferred to the appropriate medium. In preliminary experiments disks were agitated in running tap water as they aged, but most experiments were subsequently performed on disks aged at room temperature (about 20°) in a fixed quantity of distilled water during agitation in conical flasks on a transverse shaker.

Assay of invertase activity. Aged disks were rinsed briefly twice, and incubated with unbuffered 25 mM-sucrose (one disk/ml.) in shaken flasks. Controls incubated in water were always included. At intervals 1·0 ml. samples were removed, and that number of disks discarded necessary to maintain a constant ratio of tissue to medium in the residual incubation mixture. The amount of reducing sugar in the samples was determined by the method of Nelson (1944) and Somogyi (1952).

Assay of ascorbate-oxidase activity. Disks were rinsed as above and shaken with 2 mM-L-ascorbic acid in 10 mM-phosphate–citrate buffer, pH 5·6 (two disks/ml.). Reagent and disk blanks were included. After 30 min. 1·0 ml. samples were removed and the amount of dehydroascorbic acid was determined by the method of Schaffert & Kingsley (1955). In some early experiments activity of whole disks (0·5 cm. diam.) was estimated manometrically in a Warburg apparatus.

Anaerobiosis. The washing water was boiled to remove dissolved oxygen, and cooled in a stoppered container; disks were incubated in this water in 150 ml. flasks that were hermetically sealed with Subaseal serum caps, and the flasks were flushed at the onset of the washing period through hypodermic needles for about 1 min. with oxygen-free nitrogen at 5 lb./in.^{2} pressure; flushing was repeated daily where appropriate.

Respiratory rates. These were estimated manometrically in a Warburg apparatus.

Preparation from snail-crop juice of enzymes that hydrolyse cell-wall carbohydrates. Edible snails (*Helix pomatia*) were obtained from T. Gerrard and Co. Ltd., London, N. 1. Crop juice from 34 snails was collected by the method of Bawden & Pirie (1946), and a freeze-dried powder was

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obtained by the method of Burger, Bacon & Bacon (1961). Snail preparations contain a variety of cell-wall carbohydrases (Bawden & Pirie, 1946) but negligible proteolytic activity.

Nitrogen estimation. Fresh disks were weighed and extracted three times, each time with 2 vol. of boiling aq. 60% (v/v) ethanol. Samples of unextracted and extracted disks were dried to constant weight at 80°. Nitrogen was determined in weighed samples of the dried material by the micro-Kjeldahl method by using colorimetric determination of ammonia after nesslerization. The nitrogen content of disks before and after extraction was thus obtained directly, and the soluble nitrogen by difference.

Chromatography of nucleotides. Samples were freed from contaminating substances by using a Dowex 1 (Cl^- form, adjusted to pH9) column (10cm.×0·8cm. diam.); nucleotides were eluted from the column with 2 bed-volumes of 2N-HCl and dried over NaOH and $CaCl_2$ in a vacuum desiccator. For convenience of resolution by paper chromatography, the whole mixture of nucleotides present in 50ml. of incubation medium was treated with N-HCl at 100° for 1hr. to convert guanylic acid and adenylic acid into guanine and adenine; uridylic acid and cytidylic acid are not hydrolysed by this treatment. Paper chromatography of the whole hydrolysate was carried out by using the solvent mixture of Wyatt (1951), and compounds were detected by examination under ultraviolet light.

Histological examination. For microscopic examination tissue was sectioned by hand, or by microtome after being embedded in wax; squashes were also prepared after fixation. Nuclei were examined by staining sections or squashes in leuco-basic fuchsin (as quoted by Darlington & La Cour, 1960).

Cell-free enzyme preparations. (*a*) Invertase. Induced tissue was disintegrated either by hand in a glass pestle and mortar (for about 10g. of tissue) or in a mechanical blender (450g. fresh wt.). The mush was squeezed through three thicknesses of muslin, and the solid residue was washed twice with several volumes of 20mM-phosphate buffer, pH6·5, freeze-dried and stored in a desiccator over $CaCl_2$; the enzyme was stable for many weeks at room temperature or at −25°. This material is referred to below as the cell residue.

(*b*) Ascorbate oxidase. Induced tissue (120g. fresh wt.) was disintegrated in a mechanical blender; a few grams of tissue were added to a few millilitres of 20mM-phosphate buffer, pH6·5, to get the blending process started, and the rest of the tissue was added gradually. The homogenate was strained through four thicknesses of muslin. The liquid was put into dialysis sacs that were suspended in a partial vacuum: in this way a solution of the low-molecular-weight compounds was drawn through the membrane, leaving the enzyme in a smaller volume; washing was effected by the addition of further quantities of buffer. The solution was removed from the sacs, and the protein fraction that precipitated at pH6·5 between 10% and 80% saturated ammonium sulphate was prepared by standard procedures, collected by centrifugation and washed with 80% saturated ammonium sulphate solution. It was dissolved in about 20ml. of 5mM-phosphate buffer, pH6·5, dialysed overnight against tap water at 4°, centrifuged at 3000*g* for 10min. at 0° and the supernatant freeze-dried; the yield was about 0·6g.

RESULTS

Invertase was absent from newly cut disks of artichoke, but appeared in aged disks; increase in activity was found to be a function of time. Results of an experiment demonstrating this relationship are shown in Fig. 1. Prolonged aging showed that

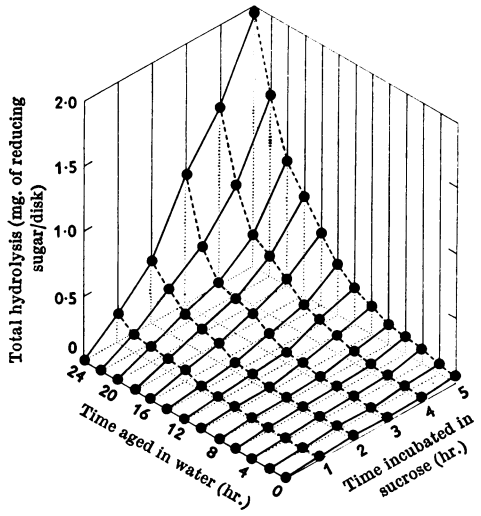

Fig. 1. Development of invertase in disks aged in water followed by incubation in 25mM-sucrose. Experimental details are given in the text.

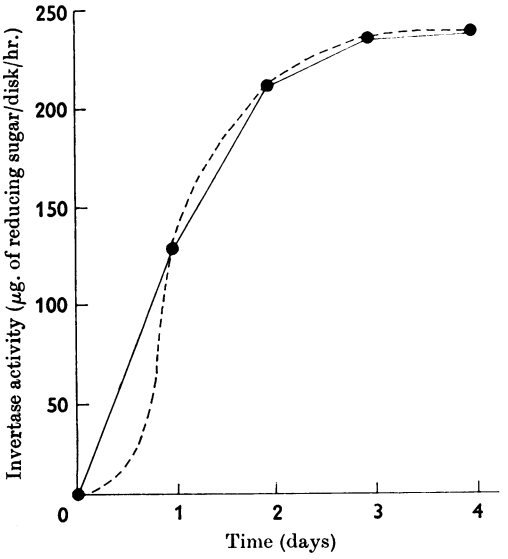

Fig. 2. Time-course of invertase development. Conditions were as given in Fig. 1. The broken line was deduced from the results shown in Fig. 1.

Table 1. *Effect of various methods of aging disks on invertase development*

Disks were aged for 24 hr. Enzyme activity was assayed after 1 hr. incubation in 25 mM-sucrose. Experimental details are given in the text.

Treatment	Invertase activity (μg. of reducing sugar/disk/hr.)	Decrease in invertase activity (%)
25 disks shaken in 50 ml. of distilled water	318	—
25 disks on wet filter paper in a saturated atmosphere; not shaken	250	21
25 disks in 50 ml. of distilled water; not shaken	58	82

Table 2. *Invertase development in tissue of varieties of* H. tuberosus

Disks were aged for 24 hr. and assayed under standard conditions, as described in the text. Unless otherwise stated, the source of seed was the National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

Variety	Invertase activity (μg. of reducing sugar/disk/hr.)
Artichoke	769
Bianke	485
Dagneutral 0976 (Vollebekk, Oslo, Norway)	349
Fuseau (Wallace and Barr)	504
Gigant	330
No. 41 (Statens, Forsogsstation	404
No. 57 Ingsberg, Esbjerg, Denmark)	510
Rozo	474
Spindel	1060
Topine	484
Traube	639

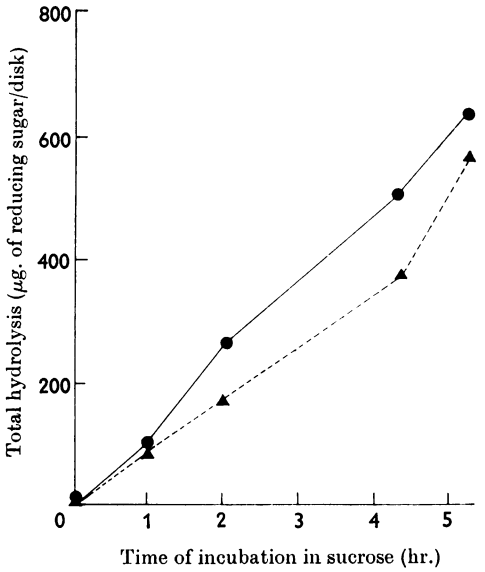

Fig. 3. Effect of imposition of sterile conditions on invertase development. ▲, Disks kept in sterile conditions; ●, control under standard conditions, as described in the text. Tubers were scrubbed, immersed in 0·02% $HgCl_2$ for 5 min., washed three times with sterile water, and disks were cut with a sterile knife and cork borer. They were washed briefly in aq. 60% (v/v) ethanol and rinsed in sterile water. The disks were aged and subsequently incubated in 25 mM-sucrose under aseptic conditions.

maximum enzyme content was reached after about 70 hr. (Fig. 2): the lag period shown by the broken line is justified on the basis of results such as those given in Fig. 1. The effectiveness of agitation on invertase development is shown in Table 1. A comparison of invertase development by several varieties is given in Table 2.

Problem of contamination by micro-organisms

It has been shown by Bacon, MacDonald & Knight (1965) that growth of bacteria up to a content of 3×10^7 organisms/disk normally occurs on sugar-beet tissue aged under conditions comparable with those described in the present work. In view of the shape of the curve given in Fig. 2, an exhaustive study was undertaken to investigate whether the enzyme development was an expression of bacterial growth. The adoption of normal techniques of sterilization as practised in bacteriology had no marked effect on enzyme development (Fig. 3), although the bacterial population was decreased more than tenfold (see below).

Bacteria obtained from scrapes of aged disks were cultured on potato–dextrose–agar until the agar surface was densely covered by a continuous layer of micro-organisms. Disks (1 cm. diam.) were punched from the agar plate and incubated with sucrose under conditions identical with those used for artichoke disks. No significant inversion of sucrose occurred.

Normally treated tissue disks were homogenized in a glass homogenizer, the brei was suspended in a

known volume of sterile water, and measured samples of the liquid were spotted on to agar plates by the surface-drop technique (Ficker, 1898). Counts of the resultant colonies showed that newly cut disks each contained fewer than 10^2, whereas disks aged for 24hr. each contained 5×10^4 bacteria as an upper limit (average for four occasions $3{\cdot}8 \times 10^4$). A similar count on disks aged under sterile conditions gave $2{\cdot}4 \times 10^3$ bacteria/disk. The addition of chloramphenicol as bacteriostatic agent to the medium in which the disks were aged showed that over the range 10–250 μg./ml. no bacterial growth occurred as measured by the surface-drop technique, but invertase development was unaffected (see Fig. 4). Only one species of micro-organism was present in significant amounts: it was identified as a pseudomonad.

From these results it is concluded that enzyme development was not a result of the growth of micro-organisms. Disks were henceforth washed in water containing 50 μg. of chloramphenicol/ml.; results were normally compared with those of controls with pure water.

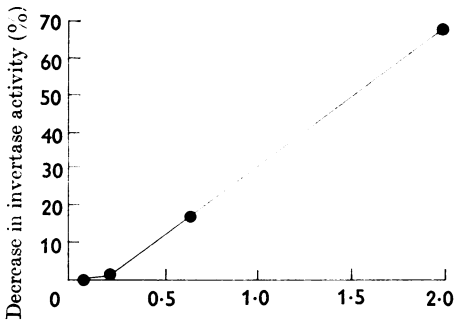

Fig. 4. Inhibition of invertase development by chloramphenicol. Enzyme was assayed under standard conditions, as described in the text.

Effect on enzyme development of inhibitors of protein synthesis

Chloramphenicol. Fig. 4 shows the effect of this substance on enzyme development. There was little or no direct effect on the enzyme (Table 3); the small effect shown when the inhibitor was present during incubation with sucrose can be accounted for by inhibition of enzyme development during that period and not by inhibition of the enzyme action.

Ribonuclease. Table 4 shows that crystalline ribonuclease inhibited invertase development: thus (*b*) and (*c*) show that the inhibition resulted from treatment during induction, and (*d*) shows that it was not merely due to destruction of the enzyme after it had been formed, the smaller inhibition here being accounted for by a shorter effective induction

Table 3. *Effect of chloramphenicol on invertase development*

Conditions of aging and other experimental details are described in the text. Chloramphenicol was used at a concentration of 2mg./ml.

Additions during 24hr. washing period	Additions during incubation in 25mM-sucrose for 1hr.	Invertase activity (μg. of reducing sugar/disk/hr.)	Decrease in invertase activity (%)
None	None	388	—
None	Chloramphenicol	336	13
Chloramphenicol	None	236	39
Chloramphenicol	Chloramphenicol	144	63

Table 4. *Effect of ribonuclease on invertase development*

Conditions of aging and other experimental details are described in the text. Ribonuclease (4 × crystallized; British Drug Houses Ltd.) was used at a concentration of 0·5mg./ml.

Additions during washing period	Additions during incubation in 25mM-sucrose for 1hr.	Invertase activity (μg. of reducing sugar/disk/hr.)	Decrease in invertase activity (%)
(*a*) None (24hr.)	None	409	—
(*b*) Ribonuclease (24hr.)	Ribonuclease	228	44
(*c*) Ribonuclease (20hr.); none (4hr.)	None	239	41
(*d*) None (20hr.); ribonuclease (4hr.)	None	320	22
(*e*) None (20hr.)	None	287	30

Table 5. *Effect of* p-*fluorophenylalanine and thiouracil on enzyme development*

Disks were aged for 24hr. under standard conditions, followed by incubation in substrate. Experimental details are given in the text.

Additive	Additive present during: Washing period	Additive present during: Incubation in sucrose for 1hr. or ascorbic acid for 0·5hr.	Invertase activity (μg. of reducing sugar/disk/hr.)	Decrease in invertase activity (%)	Ascorbate-oxidase activity (μg. of dehydroascorbic acid/disk/hr.)	Decrease in ascorbate-oxidase activity (%)
None	−	−	143	—	61	—
Thiouracil (1·0mM)	+	−	42	70	35	43
	−	+	137	4	56	8
Uracil (1·0mM)	+	−	150	None	52	15
None	−	−	214	—	61	—
p-Fluorophenylalanine	+	−	93	56	32	47
(2·0mM)	−	+	210	2	60	2
Phenylalanine (2·0mM)	+	−	224	None	56	8

period (see *e*). Examination of the water in which the disks had been incubated with ribonuclease showed that considerable quantities of nucleotides had been released; these were not estimated quantitatively, but paper chromatography showed the presence of guanine, adenine, cytidylic acid and uridylic acid; these hydrolysis products were not detectable in controls.

Other inhibitors. Table 5 shows the inhibitory effects of thiouracil and *p*-fluorophenylalanine on enzyme development, and it is presumed that these compounds acted as antagonists of uracil and phenylalanine respectively; the latter compounds used as controls were without effect.

Anaerobiosis. Disks kept in anaerobic conditions for up to 3 days remained turgid and showed no visual evidence of degeneration; those kept in aerobic conditions showed the rise in respiratory activity characteristic of tuber tissue treated in this way (see, e.g., Beevers, 1961), this rise being largely prevented in disks preincubated anaerobically (Fig. 5*a*); Fig. 5(*b*) confirms that during anaerobiosis metabolic activity is relatively low. Under anaerobic conditions, enzyme development was drastically inhibited, but was normal when disks were transferred to aerobic conditions (Fig. 6). Under aerobic conditions, 10μM-2,4-dinitrophenol suppressed invertase development by over 50%.

Attempts to solubilize the invertase

The enzyme was not secreted into liquid medium. The following attempts were made to solubilize it.

Extraction with aqueous solutions. Separate 50mg. quantities of the freeze-dried cell residue were shaken at room temperature with 5ml. quantities respectively of 20mM-phosphate buffer, pH6·5, 10mM-acetate buffer, pH5·0, and 0·1% (v/v) Teepol. Samples were removed at various intervals up to 3hr. At no time was enzyme activity extracted into the liquid, nor was the activity of the residual solid diminished.

Extraction with organic solvents. The procedure was similar to that above, with butan-1-ol, diethyl ether, acetone or ethyl acetate, all at 0°; the solvent was decanted, and the solid residue extracted with 20mM-phosphate buffer, pH6·5, at room temperature; samples were removed at intervals up to 3hr. None of the solvents rendered the activity soluble; the ether and acetone destroyed all the invertase activity, whereas butan-1-ol and ethyl acetate left it unaffected.

Mechanical disintegration. No invertase activity was ever found in the supernatant liquid after grinding induced tissue either by hand in a mortar or by a blender. The cell residue was further disintegrated by ultrasonic treatment: four 25mg. samples of powder were suspended in 7ml. of 20mM-phosphate buffer, pH6·5, maintained in an ice bath, and subjected to 20kcyc./sec. in an MSE–Mullard ultrasonic disintegrator. The samples were centrifuged after 1, 2, 5 and 30min. respectively, and the supernatant liquid and the solid residues assayed for invertase activity, the former also being assayed for protein. In no case did invertase appear in solution, although the soluble protein rose with time and the enzyme activity of the solid fraction fell.

Use of freeze-dried snail-crop juice. Attempts were made to effect solubilization of the cell-residue enzyme by dissolving away the cell-wall polysaccharides. In initial experiments the digestion was carried out in the presence of phosphate buffer, pH5·5, but the acidity produced during the enzyme action necessitated such high concentrations of buffer (0·2M) that both the activity of the snail-crop juice and that of the plant invertase were inhibited. In subsequent experiments no buffer was added,

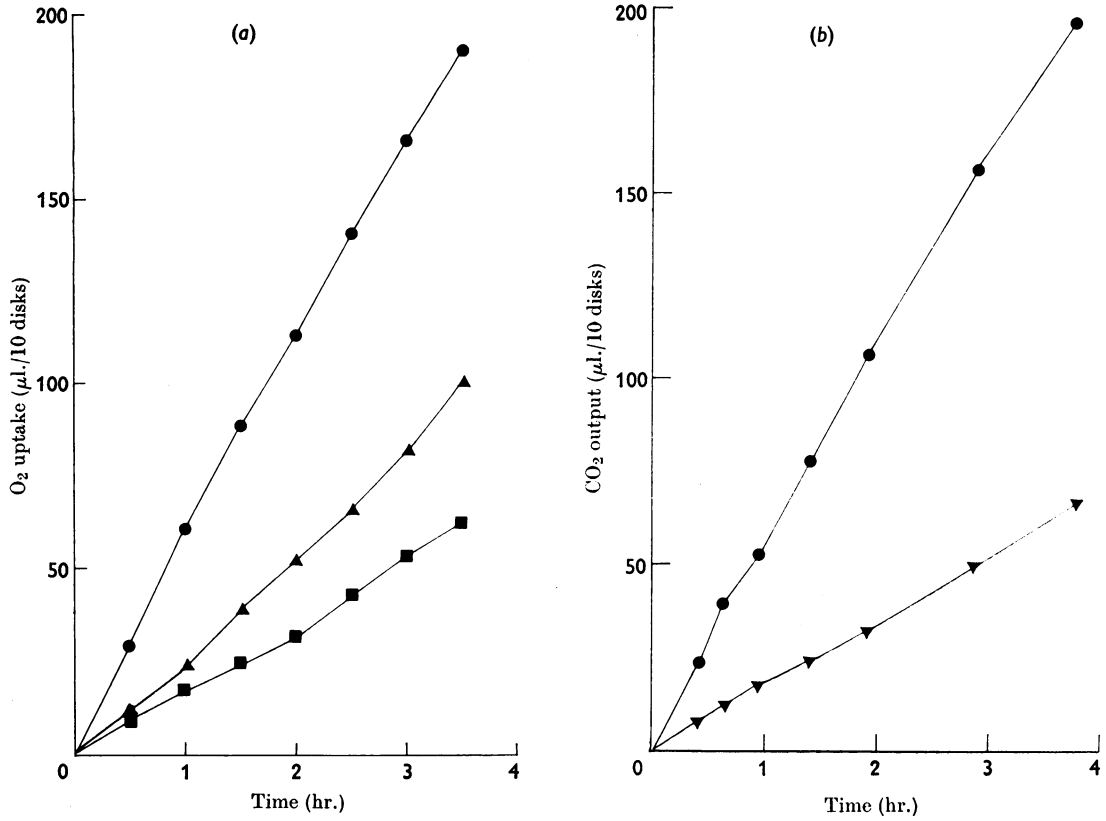

Fig. 5. Effect of anaerobiosis on metabolism as measured by gas exchange. (*a*) O_2 uptake in air by freshly cut disks (■) or disks previously aged in air for 24hr. (●) as compared with disks aged in N_2 (▲). (*b*) Anaerobic CO_2 output by disks previously aged for 24hr. in N_2 (▼) as compared with respiratory output (in air) from disks previously aged for 24hr. in air (●).

the digest being adjusted periodically to pH 5·5 with 10mN-sodium hydroxide. Check was kept on the pH by using a meter with an attached Stadie miniature electrode, the suspension being strained through a piece of muslin before entering the electrode assembly; at no time was the pH allowed to fall below 5. The temperature was normally controlled at 30°. Although the snail-crop enzymes included invertase, the ratio of this to cell-residue invertase was always such that significant solubilization of the latter could have been detected: in no experiment was any solubilization of the enzyme effected, when either a small proportion of the cell-residue preparation or up to 83% of it was brought into solution. As the solid material, but not the enzyme activity, was solubilized, the invertase activity per unit of residue might be expected to rise; although this occurred during the early stages of the digestion in some experiments, there was ultimately a fall in specific activity; results of one such experiment were as follows: the specific invertase activity of a cell-residue preparation was 211 μg. of reducing sugar liberated under standard assay conditions/mg./hr.; after 3·5hr. treatment with snail-crop juice the specific activity rose to 490; after 5·5hr. it fell to 199; after 12·5hr. to 157; by this last time 60% of the cell residue had been solubilized.

Papain. The invertase was gradually inactivated, without solubilization, on treatment with papain (Sigma Chemical Co., St Louis, Mo., U.S.A.).

Changes in weight of the tissue and distribution of nitrogen during enzyme induction

Table 6 shows that there was little change in the fresh weight of disks over a period of 3 days; the total dry weight fell but the dry weight of the extracted tissue rose; there was appreciable conversion of soluble into insoluble nitrogenous

compounds, and, assuming it to be protein, this accounted for about one-tenth of the increase in total insoluble dry weight.

Microscopic examination of the tissue

No cell division was detected in sections of aged disks even after 3 days. Mitotic figures were absent from sections and squashes taken at various times from disks aged up to 3 days.

DISCUSSION

Freshly cut slices of artichoke tuber contained no detectable invertase activity, but when they were suspended in aerated distilled water activity developed that reached a maximum after about 3 days. The course of this development followed a sigmoid curve, with an initial lag of 8–10hr. Such a curve suggests the possibility of the growth of micro-organisms that hydrolyse sucrose, and bacterial counts did in fact show that the population on disks during aging increased several hundredfold. That the enzyme development was not due to bacterial growth, however, was shown by two main observations: (1) chloramphenicol (50μg./ml.) suppressed bacterial growth completely without affecting enzyme development; (2) subcultures of bacteria isolated from disks were shown to be almost devoid of invertase activity. J. S. D. Bacon (personal communication) has also concluded that invertase development in sugar-beet slices is not the result of bacterial growth.

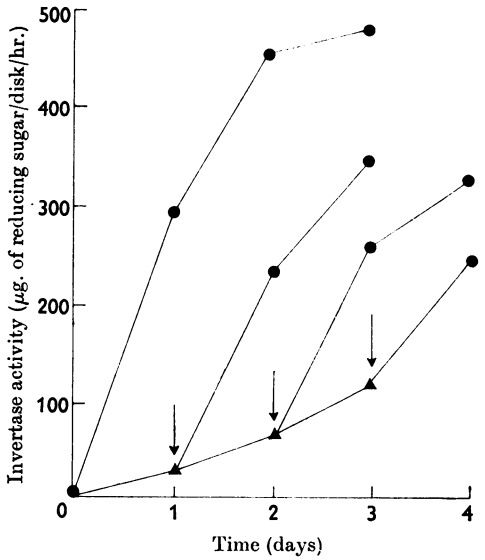

Fig. 6. Effect of anaerobiosis on invertase development. Disks were aged in an atmosphere of N_2 (▲) or air (●). Arrows indicate transfer of disks from N_2 to air. Experimental details are given in the text.

The invertase has so far defied attempts to solubilize it. Burger *et al.* (1961) were able to release invertase from cell walls of yeast into the medium by digestion of the wall carbohydrates with snail-crop juice, but we were not able to solubilize the tuber invertase by digestion of the carbohydrate in the cell walls; beyond a certain point the enzyme was denatured without being rendered soluble. Islam & Lampen (1962) showed that naked protoplasts of yeast will secrete soluble invertase, although normally the enzyme is located in the wall. This soluble yeast invertase appears to be closely bound to a mannan, and it is possible that tuber invertase is bound to an insoluble polysaccharide. Ascorbate-oxidase activity, which showed at least tenfold increase during aging for 24hr., was, however, readily soluble.

Reports of induction of enzyme activities in plant tissues are beginning to appear (see, e.g. the introduction to the paper by Afridi & Hewitt, 1964; Venis, 1964), and it is known that excised plant tissues can synthesize protein; the observations given in Table 6 are relevant here, as, although total nitrogen content of the disks fell with time, the insoluble nitrogen content rose. From other results presented above it seems likely that the development of invertase and ascorbate oxidase in artichoke disks is correlated with protein synthesis, as it is inhibited by known inhibitors of the latter

Table 6. *Changes in weight of disks and distribution of nitrogen during enzyme induction*

Experimental details are given in the text. Samples (100 disks) were treated, and results are given as averages/disk.

Time aged (hr.).....	0	24	48	72
Fresh wt. (mg.)	73	75	78	79
Dry wt. (mg.)	14·1	12·9	12·7	11·8
Extracted dry wt. (mg.)	2·5	3·0	3·2	3·1
Nitrogen content (μg./disk):				
Soluble	108	98·5	96·3	83·3
Insoluble	55·8	63·5	63·2	66·7
Total	163·8	162·0	159·5	150·0

process, namely chloramphenicol, ribonuclease, *p*-fluorophenylalanine and thiouracil; none of these affected the developed enzymes. Slices suspended under anaerobic conditions (nitrogen) developed invertase only slowly (about 13% after 24hr. compared with the control in air), but their full synthetic capacity was not lost, as transfer to aerobic conditions, even after 3 days, initiated normal enzyme development. The uncoupling agent 2,4-dinitrophenol also substantially decreased enzyme development under aerobic conditions.

The number of cells/disk did not change over a period of 2 days, as demonstrated by the absence of cambium initials and of mitotic figures in squashes and sections, by the unchanged number of cells in longitudinal sections, and by the negligible change in fresh weight over the period. The development of invertase, an enzyme that is absent from freshly cut disks, therefore can be considered to be a true induction in mature cells. Absolute induction of ascorbate oxidase is not clear-cut, as some activity is detectable in fresh disks, but the situation here is more obscure as oxidation of ascorbic acid may be effected by a number of enzymes (Butt & Hallaway, 1958): thus the increase in activity may be due to an enzyme that is absent from the fresh tissue. Similar observations have been made in this Laboratory by C. J. Leaver (unpublished work) on phosphatase development in artichoke-tuber slices, and invertase and indolylacetate oxidase in carrot-root slices.

Why is the potential that the tissue has for making these enzymes not fully realized in the intact tuber? Three main changes in conditions when tissue is cut and washed can be considered as possible causes of enzyme induction: (1) there may be an increase in oxygen concentration in the tissue; (2) new compounds may be produced in wounded cells that diffuse into surrounding undamaged cells; (3) volatile or soluble substances may be leached from the tissue. Burton (1950) has shown that the oxygen concentration in the centre of potato tubers is near atmospheric, and this would preclude (1) above; (2) appears unlikely owing to the drastic washing process, which might be expected to remove products of ruptured cells faster than they could diffuse into the rest of the tissue; (3) would appear the most likely, and is in agreement with the current repressor hypothesis of the control of enzyme synthesis. The significant compound could be volatile, the most obvious being carbon dioxide, which has been reported to affect growth via cell division (Mer & Causton, 1963), but we have found that 5% (v/v) carbon dioxide in air did not affect enzyme induction. Alternatively, Payes & Laties (1962) have suggested that β-hydroxy-α-oxoglutarate is lost from washed potato disks.

The development of enzymes described above shows parallels with the stimulation of respiration in storage tuber tissue after cutting (confirmed here in artichoke). Thus Laties (1962) has rejected, as controlling factors for this respiration, changes in oxygen and carbon dioxide concentrations, and invokes the removal of a volatile respiratory product (see above); this respiratory rise is known to be associated with increased oxidative-enzyme activities, and Click & Hackett (1963) have correlated it in potato with synthesis of RNA and proteins; moreover, these workers have commented that such synthesis 'must be released from some kind of repression soon after the slices are cut'.

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