THE METABOLISM OF THE APPLE DURING STORAGE

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Summary

A survey has been made of changes in amounts of certain metabolites and possible respiratory intermediates in the flesh of Australian Granny Smith apples during storage at 0°C.

Respiratory activity shows two peaks and carbohydrates form the predominant substrate for respiration. Evidence indicates that a carbonyl compound of low molecular weight may be an intermediate in carbohydrate metabolism. Fluctuations in total organic acids, malic acid, and citric acid suggest that the tricarboxylic acid cycle of Krebs may operate in carbohydrate oxidation in the apple. Ascorbic acid, which decreases during storage, and oxalic acid, which remains constant, do not fluctuate significantly with the respiration rate. Total nitrogen remains approximately constant during storage but there is an appreciable synthesis of protein. This synthesis appears to be related to the large reserves of available carbohydrate and is dependent on the level of respiratory activity.

I. Introduction

Although a number of chemical and physiological studies on the apple have been carried out, there is no detailed knowledge of the respiratory mechanisms of the fruit even though such information is of fundamental interest and of considerable practical importance. Several workers (e.g. Haynes and Archbold 1928; Onslow, Kidd, and West 1931; Hulme 1932) have followed chemical changes in the apple during storage and this approach has been extended in the present investigation by carrying out a comprehensive survey of changes in a number of substances, including respiratory substrates and possible intermediates. Similar techniques, using starving leaves (e.g. Vickery *et al.* 1939; Wood, Cruickshank, and Kuchel 1943) have proved successful in providing an integrated metabolic scheme for certain leaf tissues, and the formulation of such a scheme for the apple is a desirable preliminary to a detailed investigation of the biochemistry of the fruit.

The Granny Smith apple, a variety of major commercial importance in Australia and the subject of previous physiological work in this laboratory (e.g. Trout *et al.* 1942), was used in this investigation. The present paper describes studies on flesh tissues only.

II. Experimental

(*a*) *Material and Method*

A selected Granny Smith apple tree at Orange, New South Wales, was stripped when the crop was at normal commercial maturity, and unblemished

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fruit from three adjacent size classes placed in a storage room maintained at 0°C., the temperature usually employed in commercial practice. The apples were wrapped in oiled paper (to prevent superficial scald), kept in darkness, and an adequate air circulation around them was maintained. Samples, each consisting of 30 fruits with proportional representation from each size class, were taken at random at approximately monthly intervals for determination of respiration rate and for subsequent chemical analysis. The mean weight of each sample of 30 fruits was approximately constant at the beginning of storage; in all, 15 samplings were taken. The analyses carried out included starch, alcohol-insoluble residue, reducing sugars, non-reducing sugars ("sucrose"), total organic acids, malic acid, citric acid, oxalic acid, total carbonyl compounds, ascorbic acid, total nitrogen, and protein nitrogen. Changes in fresh and dry weight were followed.

All the fruits were initially free from blemish, skin injury, and mould infection. During the later stages of storage life (after 320 days in store) approximately one-third of the fruit was suffering from "lenticel spot," which is a non-parasitic disorder confined to the skin tissue and is unlikely to have any effect on the respiration rate of the flesh. In the later storage samples, up to 10 per cent. of the fruit was suffering from mould infection. The affected fruits were replaced with sound specimens of similar initial weight from the same storage population before any determinations were carried out; it was not considered advisable to prolong the experiment further as wastage became increasingly severe. The significance of the random sampling would then be doubtful, with the population consisting only of the longer-lived individuals. These may differ in respiration rate and chemical composition from the random population and it is difficult to assess the probable extent of such a variation.

(*b*) *Analytical Methods*

Fresh Weight.—Changes in fresh weight were followed by weighing the whole fruit. Each fruit was weighed separately at the beginning of storage, placed in a labelled wrap, and weighed again when taken for sampling. As the weight of the non-flesh tissue of the Granny Smith apple is a relatively small proportion (approximately 5-6 per cent.) of that of the whole fruit, it is considered that this method gives a sufficiently accurate indication, for the purposes of this experiment, of the changes in fresh weight of the flesh region. The changes in dry weight have been calculated on this assumption.

Dry Weight.—The fruits were peeled, cored, and quartered, care being taken to ensure complete removal of all chlorophyll-containing tissue from the outer cortex and all carpel wall tissue etc. from the pith. The flesh from opposite quarters was grated at 0°C. and aliquots of the grated material dried in a draught-oven at 70°C. for 3-4 hours and then in a vacuum oven at 70°C. for 6 hours.

Respiration Rate.—The rate of carbon dioxide production was determined on whole fruits by drawing a stream of CO_2-free air over the samples enclosed in glass containers. The entire sample of 30 apples was used. From three to five

readings, spread over three days, were taken for each sample. The respired carbon dioxide was estimated by the Pettenkofer method by absorption in 0.2N $Ba(OH)_2$ and titration with 0.2N HCl, using phenolphthalein.

Carbohydrates.—Opposite peeled quarters of the fruit were grated and 100 g. of this fresh grated material blended in a Waring Blendor with 100 ml. of water. Aliquots of the blended suspension were removed immediately for the determination of starch and sugars.

Starch.—The starch in the aliquots was subjected to preliminary solubilization with perchloric acid and estimated colorimetrically by the method of Nielsen (1943), as modified by Nielsen and Gleason (1945). Light absorption due to the starch-iodine complex was measured at 6600Å. The method was standardized with a pure starch prepared from immature Granny Smith apples.

Sugars.—Aliquots of the blended material were diluted with water and heated in a boiling water bath in the presence of calcium carbonate (to prevent hydrolysis of the sucrose present). The suspension was cleared with zinc hydroxide and reducing sugars estimated using the copper reagent of Somogyi (1937). The increase in reducing power after hydrolysis with 0.1N HCl at 100°C. was termed "sucrose." It has been found in this laboratory (F. E. Huelin 1945, unpublished data) that non-sugar-reducing substances in apple flesh amount to only 1.3 per cent. of the total sugars. For practical purposes, it may be assumed that only sugars react in the above method.

Ascorbic Acid.—Slices of flesh were cut, dropped immediately into 5 per cent. metaphosphoric acid, and blended in the Waring Blendor. Ascorbic acid was determined in filtered aliquots of the blended material by titration with 2, 6-dichlorphenol-indophenol.

Total Carbonyl Compounds.—Fresh grated material was blended at 0°C. with 10 per cent. trichloracetic acid and filtered. Determinations of total carbonyl compounds in the filtrate were made by the direct method of Friedemann and Haugen (1943), light absorption by the resultant 2, 4-dinitrophenylhydrazones in alkali being measured at 4000, 4200, 5200, and 5400 Å.

Total Nitrogen.—Peeled opposite quarters of the fruits were sliced and then dried in the draught and vacuum ovens as described in the determination of dry weight. The dried material was finely ground and used in all the following estimations. Total nitrogen was determined in dried tissue by a modified micro-Kjeldahl method.

Protein Nitrogen.—Dried tissue was extracted for eight hours in a Soxhlet apparatus with 75 per cent. alcohol. This solvent has been shown by Hulme (1932) to effect a very good extraction of non-protein nitrogen from apple flesh. The nitrogen in the extracted residue was estimated by digestion as for total nitrogen.

Alcohol-insoluble residue was estimated by drying and weighing the residue after the extraction of the non-protein nitrogen with alcohol.

Organic Acids.—The preparation of an organic acid extract was carried out by extraction of dried tissue with peroxide-free ether as described by Pucher,

Vickery, and Wakeman (1934*a*), using the modifications of Pucher, Wakeman, and Vickery (1941).

Total organic acids were determined in the organic acid extract by a modification of the titrimetric method of Pucher, Wakeman, and Vickery (1941). It has been found that interference by sulphuric acid (which is used to acidify the dried tissue prior to ether extraction and which is itself partly extracted) can be avoided by the addition of excess barium nitrate. The separate gravimetric estimation of sulphuric acid and the application of an appropriate correction factor as described by Pucher, Wakeman, and Vickery (1941) can thus be avoided and the time taken for the determination considerably decreased. The following method has been found suitable:

2 ml. 0.5N barium nitrate, 2 ml. 1N nitric acid, and 3 drops of brom-cresol purple are added to 10 ml. of the organic acid fraction contained in a tall 50 ml. beaker with a graduation mark at 25 ml. The solution is boiled for 1 minute, cooled, made alkaline with 1N NaOH (CO_2-free), and adjusted to pH 8.0 with 0.05N nitric acid. The titration to pH 2.60 is carried out with 0.05N HNO_3 as described by Pucher, Wakeman, and Vickery (1941). The method is standardized with a pure sample of malic acid.

The presence of barium nitrate itself has no significant effect on the titration, but the non-removal of sulphuric acid can introduce errrors in excess of 25 per cent.

Malic acid was estimated by a modification of the method of Pucher, Vickery, and Wakeman (1934*b*). Light absorption was measured at 5800 A.

Citric acid was determined by the method of Speck, Moulder, and Evans (1946), using *n*-hexane as solvent for the pentabromacetone.

Oxalic acid was estimated by the method of Pucher, Wakeman, and Vickery (1941).

All the analytical data are expressed as g. (unless stated otherwise) per 100 g. of the dry weight at the beginning of storage.

(*c*) *Statistical Methods*

Analysis of variance has shown that the differences in respiration rate between various times in storage (Fig. 1) are too great to be accounted for by sampling and/or experimental error. It was found that the trend for respiration rate could be represented by a quadratic regression on time and the deviations in respiration rate from this regression line were then correlated with the corresponding deviations of the metabolites from their respective quadratic regression lines on time. The method adopted followed the original procedure of Fisher (1924), using the modified computation of Davis and Pallesen (1940). This method requires that in correlating two series of observations both dependent on time, the same power of regression correction on time should be applied to both series.

The deviations from the quadratic regression lines were subjected to analysis by the method of Kendall (1946) to determine if these residuals could be regarded as a random set. The small number of observations in any one series, however, preclude any conclusion being drawn.

The coefficients of correlation obtained between respiration rate and certain of the metabolites are given in the appropriate sections.

III. Results and Discussion

(*a*) *Respiratory Activity*

(i) *Introduction*

It has been found in this laboratory that Granny Smith apples stored at low temperatures (0-5°C.) have a respiratory quotient closely approximating to 1, so that determination of carbon dioxide production is a reasonably accurate measure of respiratory activity and oxygen consumption. Although ethyl alcohol may be present in very small amounts, alcoholic fermentation does not take place to any appreciable extent in apples stored at these temperatures, and in the present experiment it may be assumed that aerobic conditions hold throughout the course of storage life, even during advanced senescence.

Respiratory activity has been estimated by using whole fruits, as accurate determinations on the flesh tissue alone are impracticable. The total CO_2 production of the whole fruit during the period from day 28 (when sugar concentration was at its maximum) until day 361 was 29,400 mg. per 100 g. dry weight. From the analytical data on the flesh tissue, assuming a similar rate of loss holds throughout the fruit, it can be calculated that the CO_2 output due to losses in sugars, alcohol-insoluble residue (calculated as $C_6H_{10}O_5$), and organic acids (calculated as malic acid) was 28,460 mg. per 100 g. dry weight or 97 per cent. of the total CO_2 production. As the weight of the non-flesh tissue is only 5-6 per cent. of that of the whole fruit, it is thought, on the basis of the above evidence, that the respiration rate determined with the whole fruit is a reasonably accurate estimate of the rate in the flesh region itself.

This interpretation, which does not agree with the suggestions of Hackney (1946) that the respiration of apple skin tissue alone may account for one-third to one-half of the CO_2 production of the whole fruit, is supported by the following experiments. A sample of 24 fruits was taken immediately after picking, when the internal oxygen concentration was high and would not limit the respiration rate. Respiration readings at 20°C. were taken separately on each fruit over a period of 4 days. After this period, during which the rate remained steady, the skin was carefully removed from 12 fruits with a sharp razor. Subsequently, the respiration rate of the whole fruits remained steady. That of the peeled apples (with the exception of four which showed evidence of fungal infection and were rejected) rose rapidly at first (perhaps due to wounding) but fell after 1-2 days to a value almost exactly that of the same fruit before peeling, and therefore remained constant for 7 days, when the experiment was concluded. Further investigations in this laboratory (M. J. Wilkins, unpublished data), with Granny Smith apples after 5 months in storage at 0°C., have also demonstrated that the respiration rates of peeled fruits are very close to those of the whole apples; in these experiments the high initial respiration rate after peeling was not so pronounced. The skin tissue removed from these fruits showed a very high initial respiration rate which fell rapidly during the next 4-5 days.

On the basis of these experiments, it has been concluded that, although the respiration rate per unit weight of skin tissue is higher than that of the flesh, it does not make such a large contribution to the total respiration of these apples as the results of Hackney would suggest, and that it is sufficiently accurate for the purposes of present investigation to accept major fluctuations in the respiration rate of the whole fruit as being largely due to changes in the respiration rate of the flesh region. In view of the observations on wounding effects and their possible implications in relation to the respiration of skin and flesh slices, it seems desirable that a more critical investigation of the respiratory rates of skin and flesh should be carried out.

(ii) *Respiration Rate Changes*

The form of the respiration rate/time graph in this experiment in shown in Figure 1. During the initial weeks of storage there is a fall in respiration rate followed by a small rise at day 81. A steady fall in rate then occurs with a minimum at day 165; this is followed by a rise with a maximum about day 228, another falling trend, and a final pronounced rise with a maximum at day 333. This is the highest level of respiration attained during the period of estimation

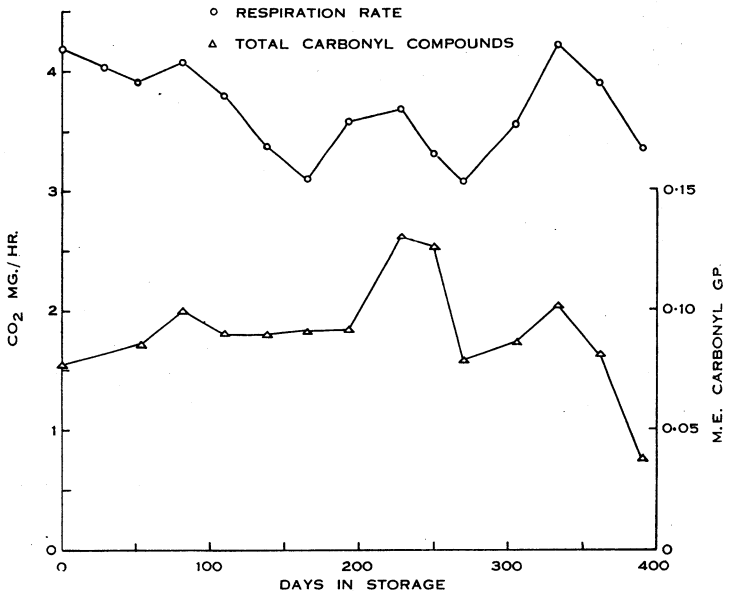

Fig. 1.—Changes in respiration rate and total carbonyl compounds during storage at 0°C. The concentration of the carbonyl compounds is *arbitrarily* expressed in terms of milli-equivalents of carbonyl group: absorption was measured at 5200 Å.

and the rate thereafter drops rapidly until the experiment is concluded. The trend, if any, within each series of readings was in good general agreement with that prevailing at the time in the graph of the mean values (Fig. 1).

The reasons for a mature resting organ, such as the apple, not subjected to external stimuli and having an abundant substrate supply, exhibiting sudden

changes in respiratory activity are obscure. Sugars, which are presumably the initial source of respiratory substrate, are at a high concentration throughout storage (even after 361 days the total sugar concentration is over 72 per cent. of the actual dry weight) and do not fluctuate significantly with the respiration rate. The respiratory rises may be caused by an increase in the activity of one or more of the enzymes involved in carbohydrate metabolism and CO_2 production or by "decrease in organization resistance" (Blackman and Parija 1928).

In most investigations with apples only one respiratory peak has been found (Kidd and West 1930; Onslow, Kidd, and West 1931) and the climacteric has been shown to be associated with the production of ethylene by the fruit (Kidd and West 1932; Gane 1935). If ethylene is the only cause of rises in respiration rate, it must be assumed that in these apples the concentration increases and decreases several times during storage, which would be unusual. The experiments of Kidd and West (1945) showed that ethylene is produced in greatly increased quantities both during and after the climacteric phase so that the normal climacteric is irreversible.

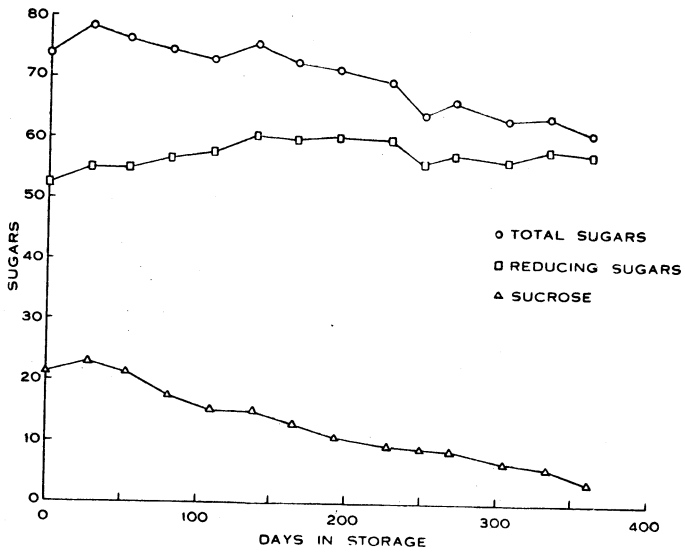

Fig. 2.—Changes in total and reducing sugars and sucrose during storage at 0°C.

(*b*) *Carbohydrates*

The changes in total and reducing sugars and "sucrose" during storage are shown in Figure 2. Total sugars, after reaching a peak of 78.4 per cent. on day 28, show a general fall to 61.3 per cent. at day 361. Reducing sugars rise from an initial value of 52.5 per cent. to 60.5 per cent. at day 138 and thereafter fall only slightly to a final value of 57.7 per cent. Sucrose rises initially to 23.1 per cent. at day 38 and then decreases steadily throughout storage: at the conclusion of the experiment it had practically disappeared.

At the commencement of storage 0.49 per cent. of starch was present: this had completely disappeared by day 28 and its hydrolysis may partially account for the initial rise in total sugars. As shown in Table 1, there is a fall of 1.2 per cent. in the alcohol-insoluble residue during this period so that components of this fraction, other than starch, may contribute to sugar formation. This decrease in alcohol-insoluble residue cannot account for all the observed rise in sugar and this is in agreement with the results of Krotkov and Helson (1946) who conclude that the initial increase in sugar when MacIntosh apples are placed in storage must, to some extent, come from some alcohol-soluble substance.

TABLE 1

CHANGES IN ALCOHOL-INSOLUBLE RESIDUE DURING STORAGE

Storage (days)	Alcohol-insoluble Residue (% dry wt.)	Storage (days)	Alcohol-insoluble Residue (% dry wt.)
0	14.2	228	12.8
28	13.0	250	12.5
53	13.3	270	11.7
81	13.1	305	12.2
109	12.9	333	11.7
138	12.3	361	11.9
165	12.6	390	11.8
193	12.5		

At low temperatures Granny Smith apples have a respiratory quotient of 1, indicating that carbohydrates are the principal substrates for respiration. This is confirmed by the analytical data. The total estimated loss of utilizable substrates in the flesh during the period from day 28 to day 361 is 28,240 mg. CO_2 per 100 g. dry wt., and of this amount, losses due to sugars (25,100 mg. CO_2) and alcohol-insoluble residue composed largely of substances which could give sugars on degradation (1800 mg. CO_2), account for 26,900 mg. CO_2, or 95.4 per cent. of the total estimated losses.

It will be seen from Table 1 that the alcohol-insoluble residue decreases steadily during storage. The main components of this fraction are probably celluloses and pectic components. Carré (1925) has made a detailed study of the changes in the pectic constituents of Bramley's Seedling apples stored at 1°C. and observed a decrease in the total pectic constituents, pectose, and especially the pectic constituents of the middle lamella. Carré assumes that the neutral pectin breaks down to pectinic acids and ultimately to pectic acid. Further breakdown to simpler acids is probable, in which case galactose, arabinose, and methyl pentose would be set free and might subsequently be utilized in respiration. From the data of Carré, it would appear that the decrease in alcohol-insoluble residue observed in the present experiment could be fully accounted for by losses in the pectic constituents. If this material is completely respired, it is a source of substrate next in importance to the sugars, forming 6.4 per cent. of the total CO_2 production calculated from decreases in the compounds estimated.

The concentration of carbohydrate material in the apple is particularly high, as the sum of total sugar plus alcohol-insoluble residue in some samples accounts for over 91 per cent. of the actual dry weight. It seems improbable that respiration is limited at any stage by the level of carbohydrate. This is in agreement with observations on other tissues such as potato (Barker 1936) and leaves (Wood 1942; Wood and Petrie 1942) where it was found that at high sugar concentrations, the respiration rate remains constant when sugar level is varied, i.e. is independent of the carbohydrate content.

Sucrose decreases at a relatively constant rate during storage and may form the preferred initial substrate for respiration or, alternatively, may be hydrolysed to reducing sugars which may enter a glycolytic cycle. It is apparent that up to day 138 at least part of the sucrose disappearing is hydrolysed to hexoses. Towards the end of the storage life sucrose had reached a very low level (3.6 per cent.) and this may be a factor causing the decline of the fruit. Results of this experiment indicate that practically all of the decrease in sucrose over the whole period is needed to account for the observed CO_2 production.

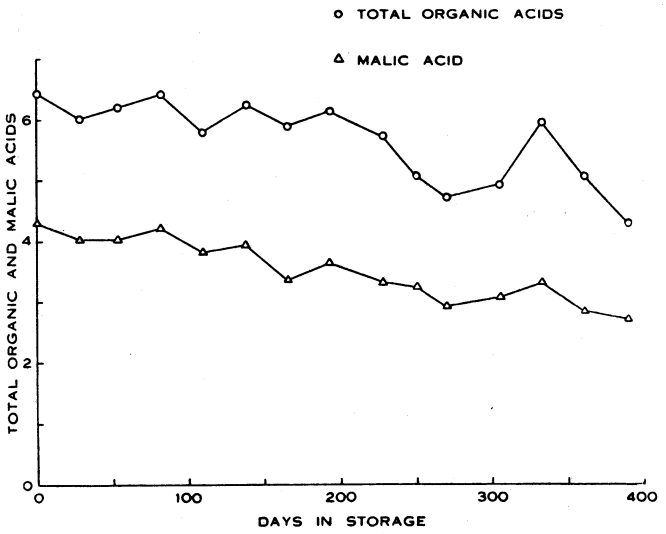

Fig. 3.—Changes in total organic acids (expressed as malic acid) and malic acid during storage at 0°C.

(c) *Organic Acids*

(i) *Total Organic, Malic, and Citric Acids*

Changes in total organic acids (expressed as malic acid) and malic acid are shown in Figure 3, and those of citric acid in Figure 4. Malic acid, in general, accounts for 60-70 per cent. of the total acid and the undetermined residue probably consists of smaller amounts of such acids as succinic, fumaric, and *iso*citric, together with traces of tartaric and α-ketoglutaric. Total organic acids show a tendency to decrease during storage, superimposed on this being fluctuations in the same direction as for respiration rate (Fig. 1), and correlated with it to the extent of a coefficient of 0.71, statistically significant at the 1 per

cent. level. Malic acid (much like total organic acids) exhibits a steady tendency to decrease; again the fluctuations superimposed on this tendency are in the same direction as for respiration rate and correlated with it with a coefficient of 0.73, significant at the 1 per cent. level.

Similarly for citric acid, which tends to increase from approximately the 160th day (Fig. 4), there are superimposed fluctuations which are correlated with a coefficient of 0.73 (significant at the 1 per cent. level) with the corresponding fluctuations in respiration rate.

There is a considerable body of circumstantial evidence (e.g. Vickery *et al.* 1939; Wood, Cruickshank, and Kuchel 1943) that the organic acid cycle of Krebs (cf. Krebs 1943) may play a significant part in plant metabolism. It appears that the results obtained in this investigation are consistent with the existence of the tricarboxylic acid cycle and may provide additional evidence for its operation in certain plant tissues.

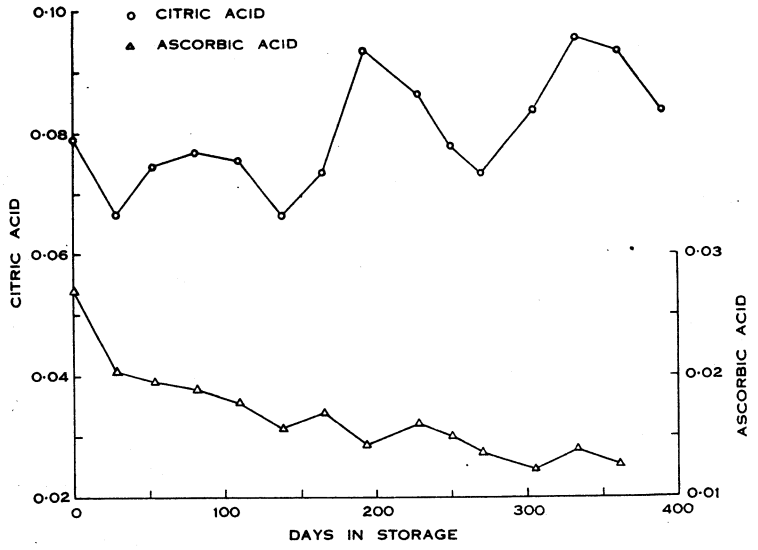

Fig. 4.—Changes in citric and ascorbic acids during storage at 0°C.

Malic acid decreases by 37 per cent. during the course of the experiment and, as no other acid product accumulates, the malic acid lost is probably completely respired to CO_2. This would not significantly change the overall R.Q. (the calculated change being from 1.00 to 1.01). Malic acid is an intermediate of the Krebs cycle and it will be seen (Fig. 3) that at the peaks of respiratory activity this acid actually increases in concentration, presumably because of additional supplies from the acid cycle.

The fluctuations in citric acid content (Fig. 4) when considered with the curve for respiration rate (Fig. 1) indicate that this acid is intimately concerned with carbohydrate oxidation. In the later modifications of the Krebs cycle (Wood 1946), pyruvate (derived from carbohydrates) is degraded to a C_2 compound which condenses with oxalacetate to form *cis*-aconitate. This acid, under the

action of aconitase, is hydrated to both citrate and *iso*citrate, the latter being available for dehydrogenation by *iso*citric dehydrogenase and further metabolism in the Krebs cycle. In the presence of aconitase, the equilibrium between *cis*-aconitate, citrate, and *iso*citrate lies strongly in the direction of citrate formation (Johnson 1939; Krebs and Eggleston 1944) so that citric acid concentration may be used as an indication of the amounts of the other C_6 acids present, i.e. as an indicator of the amounts of these acids passing through the cycle. Thus citric acid is not an actual intermediate in the Krebs cycle but is a side reaction of aconitic acid; the ready interconversion of the three C_6 acids renders this distinction somewhat unimportant.

From the data in Figures 1 and 4, it appears that most of the total citric acid present in the tissue is actively engaged in the metabolic flux. This contrasts with the position of malic acid, which is present in much greater concentration and which also functions as a reserve substrate supply, so that the quantities actually engaged in metabolism at any one time are impossible to estimate. The correlations obtained between the organic acids and respiration rate are probably due to carbohydrate being the main substrate for respiration in the apple and to the absence of the complicating effects of protein, amino acid, and amide degradation such as arise in the study of most other plant tissues.

It can be concluded that the data of this experiment are compatible with the participation of a Krebs cycle in carbohydrate oxidation in the apple, but do not prove that such a cycle operates.

(ii) *Ascorbic Acid*

Ascorbic acid (Fig. 4) shows a pronounced fall during storage life; from an initial value of 0.027 per cent. dry weight the concentration reaches 0.013 per cent., or less than half of the original figure. This acid shows no correlation with respiratory activity and its fate is unknown.

(iii) *Oxalic Acid*

Oxalic acid is a minor acid constituent and remains very constant at 0.19 per cent. dry weight throughout storage. This is in agreement with the data of Vickery *et al.* (1939) with rhubarb leaves in culture and with narcissus plants (1946). These authors found that little change occurred in oxalic acid and that conditions of culture or starvation did not affect it in any way. The impression given is that oxalic acid is a sluggish metabolite under any conditions and it does not appear to play any dynamic role in the metabolism of the apple during storage.

(*d*) *Carbonyl Compounds*

The method of Friedemann and Haugen (1943) for the estimation of total carbonyl compounds involves extraction with trichloracetic acid, incubation with 2, 4-dinitrophenylhydrazine and the addition of sodium hydroxide. Light absorption is then measured at 5200 Å and several other wavelengths. Compounds which react in this method include aldehydes, ketones, and keto-acids of relatively low molecular weight.

It has been found that phenolic substances present in the trichloracetic acid extract of apple flesh interfere in this method. Addition of ferric chloride to the

extract gives a green colouration and addition of sodium hydroxide causes this green colour to become red owing to the formation of a complex iron salt: these reactions are characteristic for *o*-dihydroxy benzene derivatives. The red compound formed on the addition of sodium hydroxide to the ferric chloride-apple extract has a slightly different absorption spectrum from that given by catechol itself. It appears that, although the substance present in the trichloracetic extract has the basic structure of catechol (*o*-dihydroxy benzene), there are additional groups attached to the benzene nucleus. This catechol-like substance may be identical with the chlorogenic acid isolated by Rudkin and Nelson (1947) from sweet potatoes. The phenol present is readily oxidized to an *o*-benzoquinone derivative having a reddish-yellow colour when the solution is made alkaline during the estimation of the total carbonyl compounds. The quinone so formed can react with phenylhydrazine (quinones show reactions of the carbonyl group) and can also oxidize this reagent; these products are coloured red or yellow and it is essential to apply an appropriate correction (using catechol as reference substance) before calculating the amount of carbonyl compound present. Provided the concentration of carbonyl compounds is relatively large this procedure gives satisfactory results especially if, as in the present case, the concentration of the phenol remains constant during the experiment.

The lower curve of Figure 1 shows the fluctuations in total carbonyl compounds during storage, the concentration being *arbitrarily* expressed as milli-equivalents of carbonyl groups per 100 g. dry weight. The carbonyl compounds show several interesting variations: there is a small rise at day 81, followed by maxima at day 228 and day 333 and a pronounced fall at day 390. There is a slight tendency to increase in the first 193 days, and a steady decrease from then on, superimposed on these trends being fluctuations in the same direction as for respiration rate, and correlated with it to the extent of a coefficient = 0.64, which is significant at the 5 per cent. level.

The carbonyl compounds thus appear to play some part in metabolic activity prior to the liberation of carbon dioxide and seem to be closely associated with the respiration rate rises. With each of the peaks there is a simultaneous carbonyl compound maximum, suggesting that the carbonyl compound may be an intermediate in carbohydrate breakdown as carbohydrates are responsible for practically all the CO_2 production in apple tissue. The concentration of the carbonyl compound is small – of the order of 0.1 milli-equivalents per 100 g. dry weight – but would not be unusual for an intermediate of a reactive nature. The magnitude of the role played by the carbonyl compound cannot be determined on the available evidence, but it may be important especially during the periods of increased respiratory activity.

Contrary to expectations based on the reported occurrence of acetaldehyde in apple tissue (e.g. Thomas 1925; Hulme 1933; Fidler 1936), this substance does not appear to be present in significant amounts in Australian Granny Smith apples stored at low temperatures. The reasons for this discrepancy and evidence concerning the identity of the carbonyl present in the apple flesh will be presented

in a subsequent communication. The substance is not an α-keto acid as the extraction method of Friedemann and Haugen (1943), which is relatively specific for the naturally occurring α-keto acids, did not give comparable results.

(*e*) *Nitrogen Metabolism*

Changes in total and protein nitrogen during storage are shown in Figure 5. Total nitrogen remains approximately constant during storage although there is a slight rising trend in the later stages. This, as shown below, may be due to transport of soluble nitrogen components from the skin, but it is probable that the earlier fluctuations in total nitrogen are largely due to sampling errors, as it has been found that a series of individual apples picked from the same tree may exhibit substantial variations in total nitrogen concentration; conversely, protein nitrogen shows remarkably little variation in such a series. During storage, protein nitrogen (see lower curves in Figure 5) shows a significant increase from 0.101 g. per cent. at day 53 to a maximum of 0.137 g. per cent. at day 390.

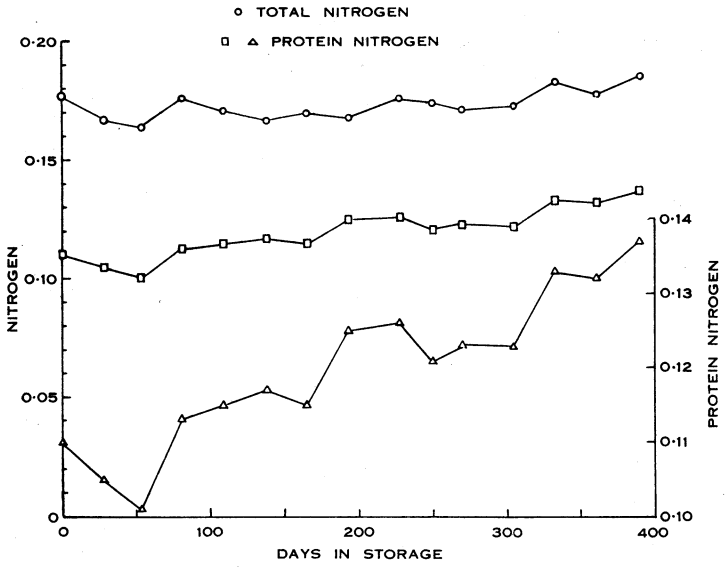

Fig. 5.—Changes in total and protein nitrogen during storage at 0°C. The lower graph for protein nitrogen is on an enlarged scale (shown at right).

Protein synthesis as a percentage of total nitrogen is shown in Figure 6, the graph for non-protein nitrogen (total nitrogen *minus* protein nitrogen) being included. Protein nitrogen, expressed as a percentage of total nitrogen, increases from 61.8 per cent. to a maximum of 75 per cent. at day 361, and, as there is no loss in total nitrogen, protein synthesis has definitely occurred.

During prolonged storage, the green colour of the skin of the Granny Smith apple changes to yellow, indicating chlorophyll breakdown. Yemm (1937) and others have noted that the onset of protein digestion in starving leaves is simultaneous with the beginning of obvious chlorophyll degeneration. It seems

probable that certain of the proteins in the skin tissue are hydrolysed and the products of proteolysis (amino acids etc.) may be transported into the cortex region and so give a higher total nitrogen figure for the flesh during the later stages of storage. It is apparent, however, from the data in Figure 6, that the rise in protein nitrogen during storage is in excess of any increase in total nitrogen in the flesh.

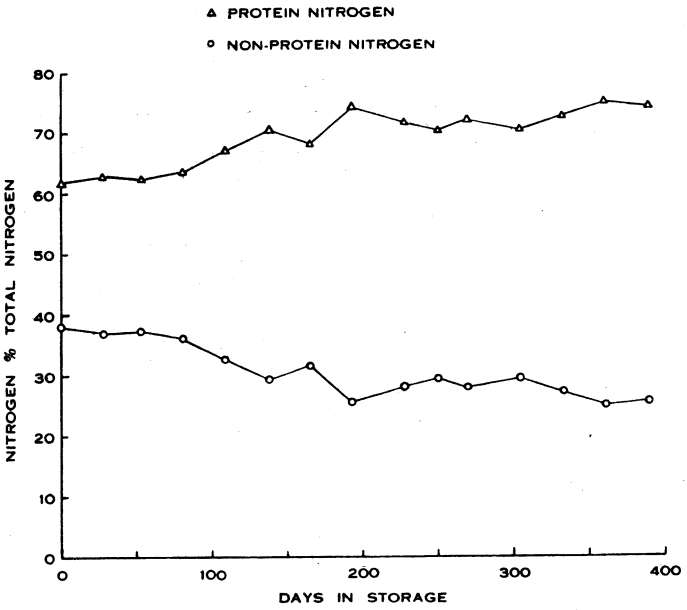

Fig. 6.—Changes in protein and non-protein nitrogen as percentages of total nitrogen during storage at 0°C.

Hulme (1932) has observed a rise in protein nitrogen in Bramley's Seedling apples in storage at 1°C. and the apple appears to be one of the few starving plant tissues which synthesize protein while in an adult resting state. When a leaf is detached from a plant, digestion of protein, with increase in soluble nitrogen, soon becomes apparent (e.g. Vickery *et al.* 1939); an exception to this is found in an experiment of Wood, Cruickshank, and Kuchel (1943) where proteolysis was considerably delayed owing to a high concentration of sucrose in the particular sample. Protein degradation did not occur until this reserve was considerably decreased. A similar observation with leaves having large stores of starch and other carbohydrates was made by Deleano (1912) who found no loss of protein until after 100 hours in culture. The apples used in this experiment provide a similar example in that carbohydrate reserves are very large, total sugars always being greater than 60 per cent. of the original dry weight. A possible explanation of this "protein sparing effect" is that when carbohydrate content is high, carbohydrate degradation products, which are the starting point for amino acid synthesis, are formed in high concentration and are converted into amino acids at a rate greater than the oxidation of the latter. In the present investigation, the three main rises in protein nitrogen (Fig. 5),

which account for most of the total observed increase, occur at days 53, 165, and 305 and coincide with periods of markedly increasing respiratory activity (Fig. 1) so that protein nitrogen level and respiration rate appear to be related. The problem of protein metabolism in the apple will be considered in greater detail in a subsequent publication.

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