

PROTEIN NITROGEN CONTENT AND PHOSPHORYLATIVE ACTIVITY OF APPLE FRUITS DURING RIPENING AND SENESCENCE

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Summary

Respiratory activity, protein nitrogen content, and rate of esterification of infiltrated ^{32}P have been followed during ripening and senescence at 20°C in detached fruits of five apple varieties.

A peak in protein nitrogen content accompanied the respiratory climacteric, and at least one additional peak occurred during senescence. The senescent increase in protein nitrogen was in most cases much greater than the increase at the climacteric. It is suggested that the diversion of energy to the synthesis of this protein may play a role in hastening the onset of senescent breakdown of the fruit tissue.

While no conclusions could be drawn with respect to quantitative changes in phosphorylative activity, the tissue was shown to retain the capacity to esterify added phosphate for as long as the experiment continued, which was up to 4 months after the occurrence of the climacteric. Democrat apple tissue still showed this capacity after the fruit had been stored at 1°C for 12 months.

I. INTRODUCTION

Although the respiratory climacteric which occurs in many ripening fruits has been studied for over forty years, its mechanism remains unexplained. Biale (1960) has reviewed evidence used in the formulation of hypotheses to explain the phenomenon, which marks the culmination of the processes of development and maturation, and the onset of senescent breakdown. In the present paper the period embracing the preclimacteric minimum and the climacteric peak in the respiration rate of fruits will be referred to as the ripening phase. The rest of the life of the fruit following the climacteric will be termed senescence.

Protein nitrogen increases during ripening in apples (Kidd, West, and Hulme 1939), and in tomatoes and avocados (Rowan, Pratt, and Robertson 1958). The increase observed in apples was considered by Hulme (1961) to be due to the development of a malate decarboxylating system. The latter was found by Hulme and Neal (1957) to be present in the peel and pulp of postclimacteric and senescent apples but not of preclimacteric ones.

The level of high-energy phosphate compounds also increases during the ripening of avocados (Rowan, Pratt, and Robertson 1958; Young, Popper, and Biale 1962), but not of tomatoes (Rowan, Pratt, and Robertson 1958). Tissue slices taken from avocados during ripening have shown an increasing ability to esterify inorganic phosphate (Young, Bielecki, and Biale 1961). Marks, Bernlohr, and Varner (1957) reported that while esterification of ^{32}P -labelled phosphate injected into tomato fruits proceeded at a maximum rate early in maturation and at the climacteric peak, it had fallen to zero by 4 days after the climacteric.

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As part of a larger study of the relation of nitrogen and phosphorus metabolism to physiological storage disorders, the work reported here aimed at determining whether or not the protein increase observed in ripening apples is accompanied by an increase in phosphorylative activity as in the avocado. Moreover an attempt was made to follow phosphorylative activity and protein nitrogen content through the comparatively extended period of senescence to determine whether or not they were marked by the same kind of steady decline which characterizes the respiration rate during this period.

II. MATERIALS AND METHODS

In all, 4–5 bushels of apples per tree were picked at commercial maturity from trees of five varieties which characteristically show marked differences in maturation rate and keeping quality (see tabulation below).

Variety	Days from Full Bloom to Maturity (approx.)	Breakdown	Bitterpit
Cox	140	Very susceptible	Very susceptible
Jonathan	150	Susceptible	Occurs rarely
Granny Smith	170	Not susceptible	Susceptible*
Sturmer	180	Susceptible	Susceptible
Democrat	200	Not susceptible	Occurs rarely

* Applies to light crop fruit only.

Pairs of trees differing in crop size or nitrogen status or both were available in the varieties Granny Smith, Cox, and Jonathan, but only single trees of Democrat and Sturmer were used because of the uniformity of cropping among available trees of these varieties.

Immediately after harvest the apples were divided into about 20 random samples of 20 fruits and placed at 20°C. Two samples were used for measurement of respiration rate during the next 50–125 days, depending on the variety. Over a somewhat longer period the remaining samples were removed from storage successively, every 2 or 3 days at first, and later at increasingly longer intervals. After removal of a sample, disks were first cut from the cortical tissue for the determination of phosphorylative activity. The fruit was then peeled and the remaining cortical tissue was cut up, immediately frozen at –15°C, ground to a powder, and stored at this temperature for subsequent determination of total and protein nitrogen.

Respiration rate at 20°C was estimated by a modification of the weighed-tube method of Eaves (1935), evolved carbon dioxide being absorbed in Carbosorb soda asbestos contained in a U-tube.

Phosphorylative activity at 20°C of disks of cortical tissue was estimated by the method described by Lewis and Workman (1964), except that the chromatogram scanner was equipped with two opposed Geiger–Müller tubes which provided 4 π detection. The period of incubation in the presence of carrier-free [³²P]H₃PO₄ was 6 hr. Activity was expressed as the percentage of inorganic ³²P esterified per hour.

Total nitrogen was determined in the frozen material by digestion with sulphuric acid followed by steam distillation in a Markham-type microKjeldahl distillation apparatus.

Protein nitrogen was considered as that fraction of the total nitrogen which remained after Soxhlet extraction of samples of frozen tissue for 16 hr with 75% ethanol (Turner 1949). Protein nitrogen was expressed as a percentage of total nitrogen according to Hulme (1954). Since total nitrogen content of cortical tissue does not change significantly in storage, expression of protein nitrogen content on this basis reflects absolute values. At the same time it eliminates the complications that would otherwise be introduced by decrease in moisture content of cortical tissue during prolonged storage at 20°C, and by variation between samples in the total nitrogen content.

III. RESULTS

The courses of respiration rate and protein nitrogen content of the fruit from the different trees, and of the rate of esterification of ^{32}P in disks from these fruits, are illustrated in Figures 1-8.

During the first few days after harvest, all samples of fruit showed a climacteric pattern of respiration rate which was characteristic of the variety. The respiration rate of the fruit from both Jonathan trees had passed through the preclimacteric minimum before the first measurements were made.

In disks from Sturmer, Jonathan, and heavy crop Cox fruit, a significant ($P < 0.05$) peak in the rate of ^{32}P esterification was observed at about the time of the respiratory climacteric. During the subsequent senescent decline in respiratory activity, the esterification rate showed an increase which gave rise to a peak which was significant ($P < 0.01$) in every instance, being most prominent in disks from Granny Smith fruit (Figs. 5 and 6).

In accordance with the findings of earlier workers, protein nitrogen also attained a peak at about the time of the climacteric. The peak was significant ($P < 0.05$) in all but the Cox heavy crop fruit. In addition, a marked increase in protein nitrogen during senescence led to the occurrence of a "senescent peak" which was significant ($P < 0.01$) in every instance. With the exception of Jonathan, this peak was considerably higher than that which occurred during ripening. There was evidence for the occurrence of an additional peak during senescence in the Jonathan and light crop Cox fruit. Finally protein nitrogen declined at a rate which varied widely between varieties. Fifty-five days after reaching its maximum value, the protein nitrogen content of the light crop Granny Smith fruit had fallen by one-third. The Sturmer fruit, at the other extreme, had lost only one-tenth of its protein after 105 days. The largest protein loss was observed in Democrat. During 116 days the percentage of the total nitrogen which existed in the form of protein fell from 71.6 to 38.7.

Among the five varieties no consistent relationship could be detected between differences in the course of ^{32}P esterification rate in disks and protein nitrogen content on the one hand, and differences in characteristic varietal maturation rate or disorder susceptibility on the other.

Between-tree differences in the nitrogen status of Jonathan fruits, and in both the nitrogen status and crop size of Cox fruits, exerted no appreciable effect

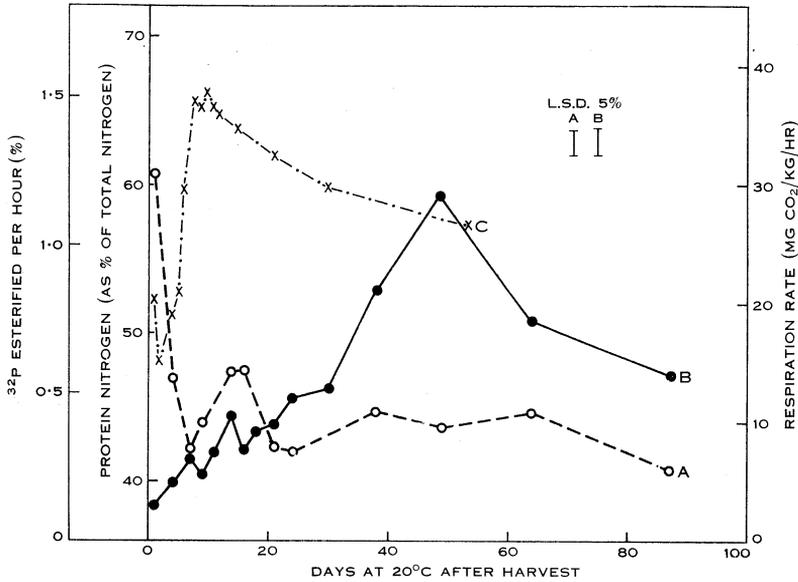


Fig. 1.—Cox variety, heavy crop tree: changes with time in ³²P esterification rate (A), protein nitrogen content (B), and respiration rate (C) of detached fruits held at 20°C.

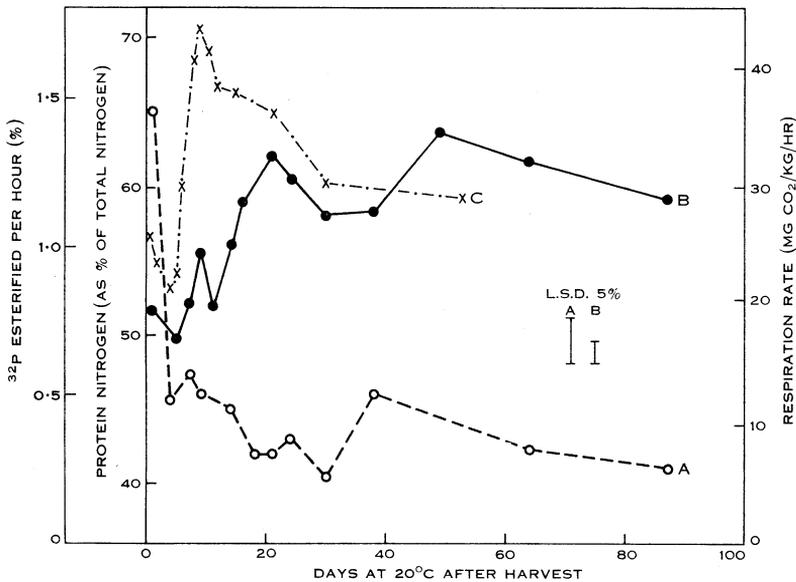


Fig. 2.—Cox variety, light crop tree: changes with time in ³²P esterification rate (A), protein nitrogen content (B), and respiration rate (C) of detached fruits held at 20°C.

on the course of either ³²P esterification rate or protein nitrogen content during senescence. The two Granny Smith trees differed in crop size, the mean weight of the

light crop fruit being 17% greater than that of the heavy crop fruit. The rate of ^{32}P esterification was slightly less in disks from the heavy crop fruit for the first 35 days after harvest, but during the next 90 days it became continually greater relative

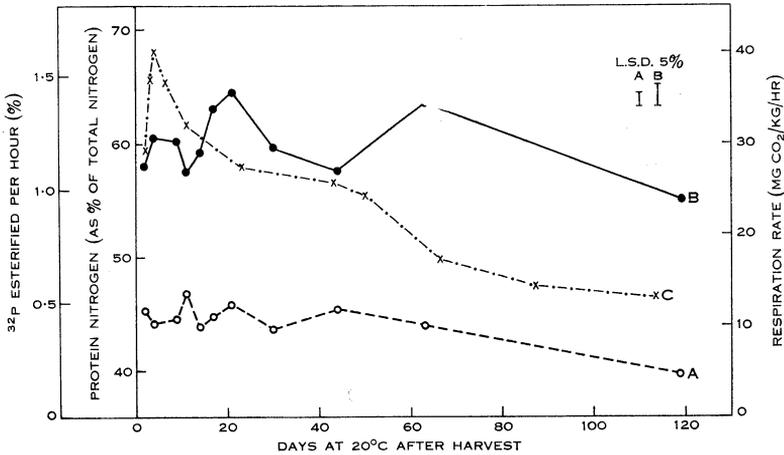


Fig. 3.—Jonathan variety, high nitrogen tree: changes with time in ^{32}P esterification rate (A), protein nitrogen content (B), and respiration rate (C) of detached fruits held at 20°C.

to the rate in disks from the light crop fruit. By 90 days after harvest all the light crop fruit showed severe core browning and some browning in the cortical region.

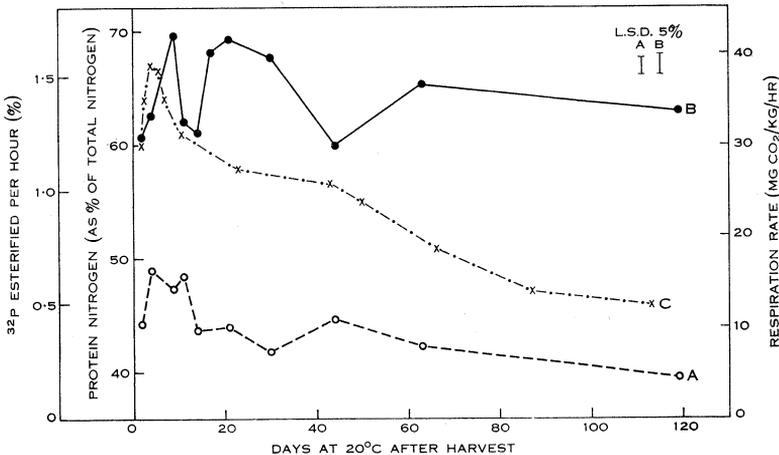


Fig. 4.—Jonathan variety, low nitrogen tree: changes with time in ^{32}P esterification rate (A), protein nitrogen content (B), and respiration rate (C) of detached fruits held at 20°C.

In the heavy crop fruit, on the other hand, no browning of the cortex occurred, and core browning did not appear until 150 days after harvest.

IV. DISCUSSION

The results indicate that throughout the period of observation, which lasted up to 4 months after the occurrence of the respiratory climacteric, the tissue in

all cases retained some capacity to esterify added inorganic ^{32}P . It is of interest to note that Democrat apples stored at 1°C for 12 months still had this capacity. These

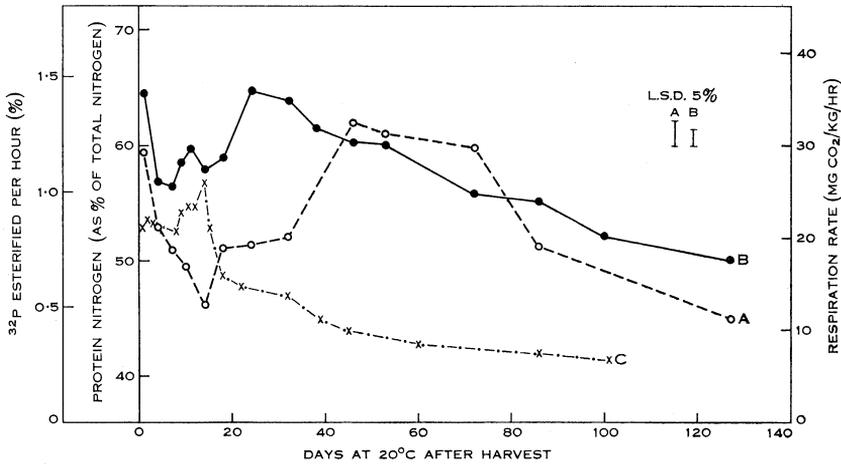


Fig. 5.—Granny Smith variety, heavy crop tree: changes with time in ^{32}P esterification rate (*A*), protein nitrogen content (*B*), and respiration rate (*C*) of detached fruits held at 20°C .

findings contrast with those of Marks, Bernlohr, and Varner (1957) who reported that no esterification of ^{32}P -labelled phosphate occurred when it was injected into intact ripened tomato fruits.

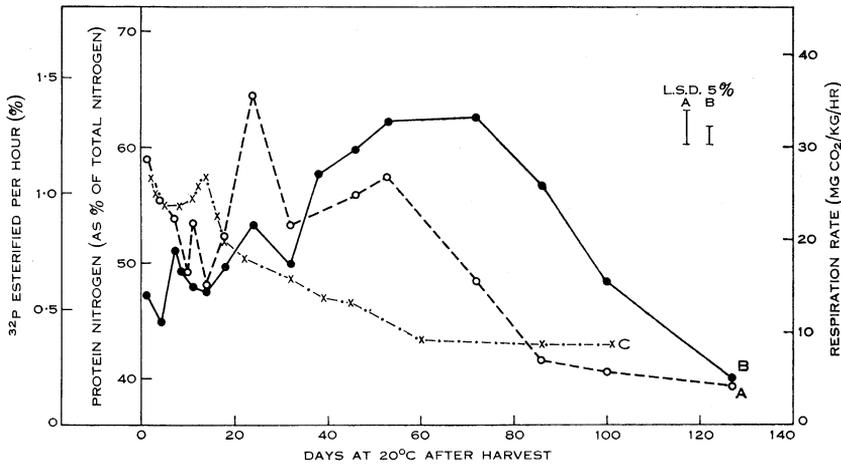


Fig. 6.—Granny Smith variety, light crop tree: changes with time in ^{32}P esterification rate (*A*), protein nitrogen content (*B*), and respiration rate (*C*) of detached fruits held at 20°C .

It is now realized that the results obtained with the technique designed to estimate phosphorylative activity do not necessarily represent a true measure of this activity. The following are among the reasons for this. Cutting the fruit tissue

into disks results in the wounding of an appreciable proportion of the cells and in a considerable change in the environment of undamaged cells. One effect is an increase in the respiratory activity relative to that in the intact fruit (Pearson and

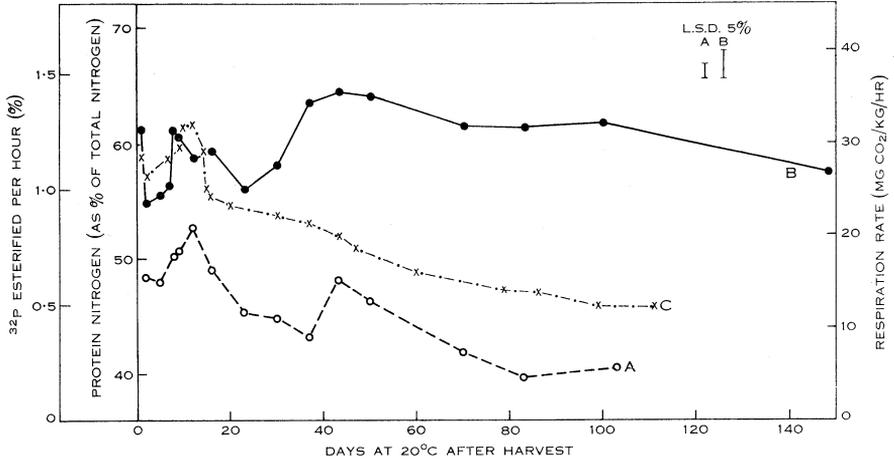


Fig. 7.—Sturmer variety: changes with time in ³²P esterification rate (A), protein nitrogen content (B), and respiration rate (C) of detached fruits held at 20°C.

Robertson 1954). Vacuum infiltration of the disks with ³²P solution further alters the environment of the cells. Where previously a cell was in contact with inter-

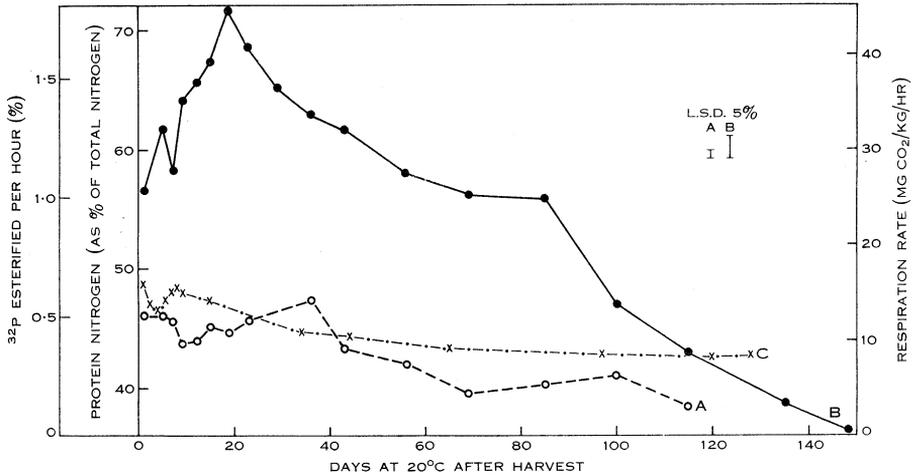


Fig. 8.—Democrat variety: changes with time in ³²P esterification rate (A), protein nitrogen content (B), and respiration rate (C), of detached fruits held at 20°C.

cellular air it is now in contact with an aqueous solution. The concentration of phosphate in the carrier-free ³²P solution is so low that its entry into the cell is not

likely to affect metabolism by altering the phosphate concentration. However, while the concentration of inorganic phosphate in the tissue may be simply determined, the size of the inorganic phosphate metabolic pool which becomes labelled by added ^{32}P is unknown. An increase in the percentage of added ^{32}P which becomes esterified in a given time may indeed be due to an increase in phosphorylative activity, but it may alternatively be due to a decrease in the size of the metabolic pool or to a combination of both these changes. Such an increase in phosphorylative activity could be thought to reduce the size of the inorganic phosphate pool, so that an increase in the percentage of ^{32}P esterified in unit time may in fact indicate that there has been some increase in phosphorylative activity. However, it is apparent that it is unsafe to draw any conclusions from the present results with respect to quantitative changes in phosphorylative activity in apple fruits, even though there is a similarity among the different varieties in the pattern with time.

The above considerations suggest that more care may be needed in drawing conclusions from the results reported by Lewis and Workman (1964) with disks from tomato fruits, and by Marks, Bernlohr, and Varner (1957) who injected ^{32}P into intact tomato fruits at different stages of maturity. Perhaps analysis of the tissue for high-energy phosphate compounds, after the manner of Rowan, Pratt, and Robertson (1958), but using newer and more specific methods, may yield a truer indication of the energy status of the tissue.

The rise in protein nitrogen level which accompanies the climacteric appears to be less important than that which has here been shown to occur during the early part of senescence. It seems likely that the net synthesis which results in an accumulation of protein during senescence is of no use to the cell in maintaining itself. If this is so, then the proportion of the ever-decreasing supply of energy from respiration (which itself is steadily diminishing during senescence) which is available for useful purposes is declining.

If, as a result of a diminution in the energy supply, the normal integrity of intracellular membranes fails to be maintained, an increase in the permeability of these membranes may be expected. Preliminary investigations in this laboratory have indicated that the permeability of apple cells to potassium ions does in fact increase during senescence. Such an increase in permeability could lower the level of adenosine triphosphate in at least two ways, firstly by necessitating an increase in adenosine triphosphate utilization for the maintenance of concentration gradients across membranes, and secondly by bringing about conditions which are favourable to the observed net synthesis of protein. These favourable conditions could result either from the release of amino acids to the active sites or from the loss of acid from the vacuole causing a decrease in cytoplasmic pH and an enhancement of the activity of the appropriate enzyme.

It is postulated that the synthesis of apparently non-functional protein, which commences soon after the respiratory climacteric, may play a part in hastening the onset of senescent breakdown by accelerating the decline of the adenosine triphosphate supply to a level below that required for the maintenance of cellular organization.

It has yet to be determined whether the observed increase in protein nitrogen content during senescence is due to an increase in the level of one or more proteins already present in the tissue, or to the formation of a different protein not initially present.

V. ACKNOWLEDGMENTS

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