

THE PHYSIOLOGY OF GROWTH IN APPLE FRUITS

VI. THE CONTROL OF RESPIRATION RATE AND SYNTHESIS

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Summary

Experiments are described which test the hypothesis that the rate of respiration is controlled by the phosphate carrier system in apple tissue. The effects of 2,4-dinitrophenol and adenosine triphosphate on the respiration of cut tissue are consistent with the hypothesis that a more rapid utilization of energy-rich phosphate at a critical stage could result in the climacteric rise. The interrelations of starch, organic acid and nitrogen metabolism, and respiration are discussed. An increase in the activity of extracted respiratory enzymes at the time of the climacteric rise has been demonstrated.

I. INTRODUCTION

In earlier papers in this series (Robertson and Turner 1951; Pearson and Robertson 1952, 1953), Maskell's hypothesis was used to explain the rise in respiration rate, the climacteric rise, which occurs in Granny Smith apples developing on the tree. Maskell (1948) has pointed out that the removal of a controlling effect due to a low concentration of phosphate acceptors would result in an increase in respiration rate. Following our work on increase in protein content during the enlargement of the cells of the fruit, we suggested that there would be an increasing rate of turnover of phosphate carriers to meet the greater demand for energy for the protein maintenance. The more rapid turnover of phosphate carriers makes a higher concentration of acceptors available to take the energy-rich phosphates from the respiration process, which consequently increases in rate.

If the rate of respiration in pre-climacteric fruit were controlled by the concentration of phosphate acceptors, it would be expected that either an increase in phosphate acceptors supplied or an uncoupling of the oxidation system from the linked phosphate carriers would increase the rate. In climacteric fruit where the respiration had already risen, a marked increase in rate would not be expected. 2,4-Dinitrophenol is known to uncouple oxidation and phosphorylation and to stimulate oxygen uptake in tissues where the respiration rate is limited by the phosphate acceptor concentration.

Early in 1952, experiments were begun to test this hypothesis of the control of respiration. Recently Millerd, Bonner, and Biale (1953) have reported work along similar lines with the avocado in which the respiration rate is governed

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by a phosphorylative coupling mechanism. The respiratory rate of slices of pre-climacteric fruit but not that of climacteric fruit was increased by 2,4-dinitrophenol.

The purpose of this paper is to report results with apple tissue and with isolated respiratory particles, which support the hypothesis outlined above.

II. METHODS

(a) *Material and Sampling*.—Experiments were carried out on Granny Smith apples from trees at Bathurst during the two seasons 1951-52 and 1952-53. The sampling data are given in Table 1. The fruits were sent to the laboratory within 4 hr of picking. Except when the fruits were very small each fruit was weighed and six were put into glass shells for measurement of respiration rate. The remaining fruits were held at low temperature for 48 hr.

(b) *Respiration*.—The respiration rate of the whole fruit was measured at 25°C by the Pettenkofer method during the 70 hr after picking.

(c) *Preparation of Cut Tissue*.—After measurement on the whole fruits, four of the six were used in the Warburg apparatus for experiments on the respiration of freshly cut tissue. Cylindrical blocks of tissue were removed with a cork borer and disks 1 mm thick were cut from the cortex. One gram was used in each vessel. After weighing 1 g to the nearest whole disk, the disks were placed in a small piece of muslin and rinsed quickly in an isotonic solution of glucose (0.45M) and potassium dihydrogen phosphate (0.01M). They were spread, with as little handling as possible, on filter paper to dry the excess solution and then placed in the Warburg vessel in the isotonic solution (see Hackney 1946).

(d) *Measurements of the Effects of Adding 2,4-Dinitrophenol and Adenosine Triphosphate*.—The solutions in contact with the cut tissue were glucose (0.45M) and potassium dihydrogen phosphate (0.01M). 2,4-Dinitrophenol (DNP) and adenosine triphosphate (ATP) were added from the side-arm to give the desired concentration in contact with the tissue.

(e) *Preparation of Isolated Particles*.—The apples which had been kept at low temperature for about 48 hr after picking, were peeled and the flesh diced. The diced flesh was placed in a Waring Blendor with an equal volume of water or 0.45M sucrose solution and sufficient 5 per cent. potassium hydroxide was added during blending to maintain a neutral reaction with brom-thymol blue. Apple tissue has a pH of about 3 and it was found necessary to neutralize with potassium hydroxide during blending to obtain good activity. Water only was used as the blending medium in 1952. In the second season both water and 0.45M sucrose were used.

The tissue was blended for 30 sec and the homogenate squeezed through three layers of muslin. The muslin-squeezed homogenate was placed in 250 ml centrifuge tubes and spun at 1150g for 20 min. With the exception of the first four samples of the 1952-53 season, each tube contained the muslin-squeezed

homogenate from 100 g fresh tissue. To obtain a washed residue, the residue in one tube was resuspended in 50 ml water and centrifuged at 1150g for another 20 min.

TABLE 1
SAMPLING DATA

Days from Full Blossom	1951-52 Full Blossom 7.x.1951		1952-53 Full Blossom 5.x.1952	
	Number of Fruits	Number of Trees	Number of Fruits	Number of Trees
16			55	1
29			76	1
43			20	1
64			10	1
92			12	12
99			12	12
106			12	12
114			12	12
120			12	12
127			12	12
134	10	1	12	12
141	10	1	12	12
148	10	1	12	12
155	10	1	12	12
162	10	1	12	12
169	10	1	12	12
176	10	1	—	—
177	—	—	12	12
183	10	1	—	—
184	—	—	12	12
191	10	1	12	12
197	10	1	—	—
199	—	—	12	12
204	10	1	—	—
205	—	—	12	4
211	10	1	—	—
212	—	—	12	4
218	10	1	—	—
219	—	—	12	4
225	10	1	—	—
226*	—	—	12	4
232	10	1	—	—
239	10	1	—	—
253	10	1	—	—
260	10	1	—	—
267	10	1	—	—

* Unfortunately no samples were obtained after this during 1952-53 as all the fruits were stolen from the trees.

(f) *Measurement of Particle Activity.*—Each residue in 1952 was resuspended in 3 ml water. In 1952-53 the residue was taken up in 3-7 ml of solution according to the total amount required in each experiment. In most experiments 5 ml were added and all the others were recalculated to correspond to the same dilution. In all experiments 1 ml of suspension was tested for oxygen uptake in a Warburg apparatus with sodium succinate, sodium citrate, or sodium malate as substrate at 27°C (1952) or 25°C (1953).

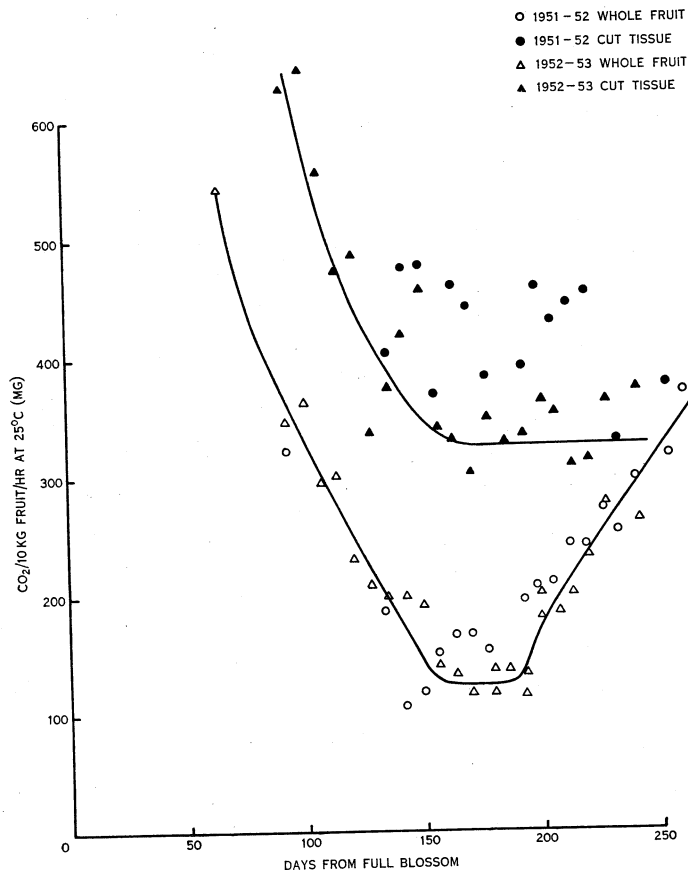


Fig. 1.—Changes in respiration rate (CO_2 output) per unit weight with time, comparing whole-fruit rates with cut-tissue rates in both seasons.

III. RESULTS

(a) *Respiration Rates of Whole Fruits*

The results of the respiration rate measurements for whole fruits are given in Figure 1. Respiration per unit weight fell from a very high rate in the young fruit to a low rate at about 150 days from blossom. At 190 days it rose sharply and continued to rise till the end of the season. The same type of curve has been obtained in all other seasons.

(b) *Cut-tissue Experiments*

(i) *Cut-tissue Respiration*.—The cut-tissue respiration was measured as μ l oxygen uptake per gram per hour at 25°C. On each sample the mean of three readings from each of four fruits was taken, with the exception of the first sample and of the last two samples of the 1951-52 season, when only two fruits were used.

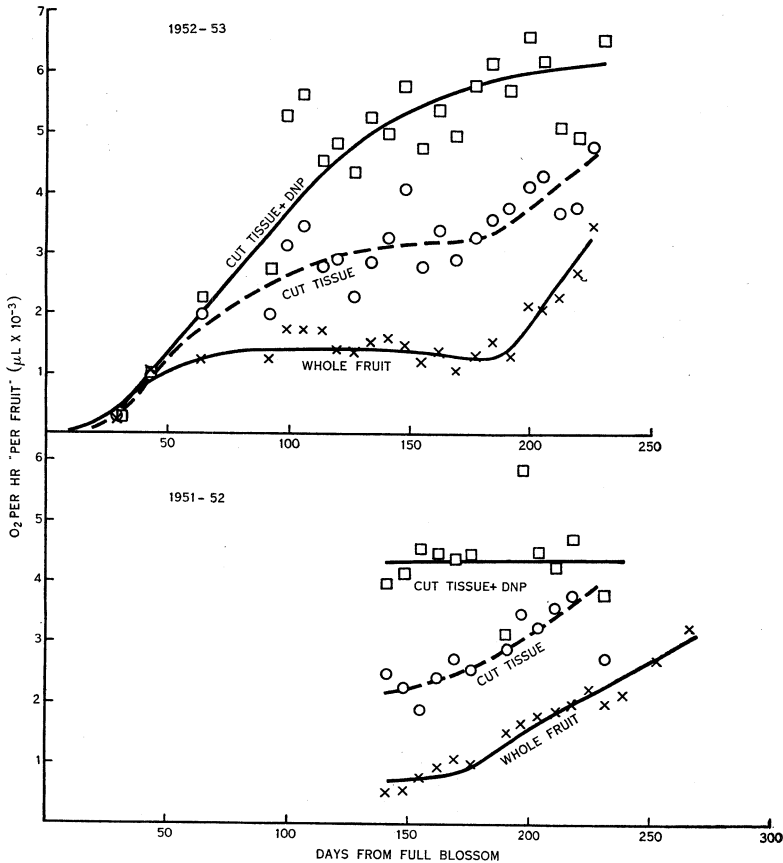


Fig. 2.—Comparison of the oxygen uptake of whole fruit (assuming R.Q. = 1) with the oxygen uptake of cut tissue with and without 2,4-dinitrophenol (DNP). The cut-tissue respiration has been corrected by multiplying by the weight to express it on a "per fruit" basis. The form of the curves would be that expected on a per cell basis since cell number does not change.

In Figure 1, the cut-tissue respiration is shown calculated as mg CO_2 per 10 kg per hour, assuming that R.Q. = 1. The cut-tissue respiration was very high in the young fruits but fell rapidly till 130 days, where it remained with no definite trend till the end of the season. The rate in 1951-52 was higher than in 1952-53.

The cut-tissue respiration may be compared with the whole-fruit rates when allowance is made for the changing weight during the growth of the fruit.

Earlier studies have shown that cell division is complete within 30 days of blossom. The change in respiration rate per fruit should therefore follow the same course as the change in respiration rate per cell. Respiration per fruit ($\mu\text{l O}_2$ uptake per fruit per hour, calculated assuming $R.Q. = 1$) is shown in Figure 2. On a comparable basis, the respiration per g of cut tissue must be multiplied by fruit weight and this curve is also shown in Figure 2. In the early samples the respiration per cell of cut tissue and of whole fruit are similar but by 100 days the cut-tissue respiration per cell is about double that per cell of the intact fruit. After about 190 days when the whole-fruit respiration showed a climacteric rise, the cut-tissue respiration also increased in both seasons.

(ii) *Action of 2,4-Dinitrophenol.*—In a preliminary experiment, 2,4-dinitrophenol was added from the side-arms in the following concentrations: 10, 5, 3, 1, 0.5, and 0.1 mg/100 ml. The respiration was inhibited by concentrations of 10 and 5 mg/100 ml, while 0.1 mg/100 ml gave no effect. Some stimulation was caused by 3 and 1 mg/100 ml, but the greatest stimulation in every trial was given by 0.5 mg/100 ml. This concentration, which corresponds to 0.5 mg/l in contact with the disks, was used throughout the experiments.

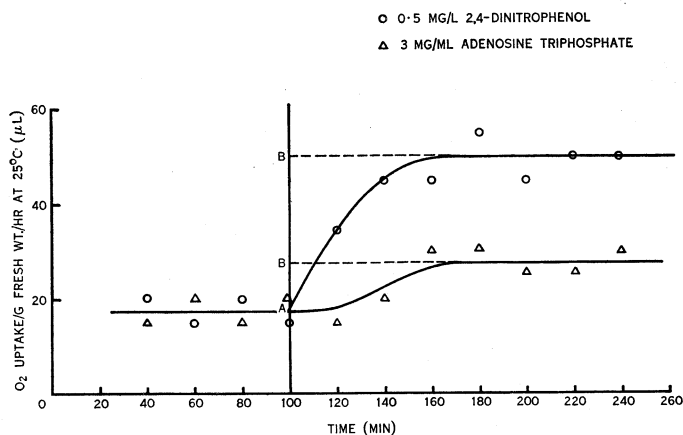


Fig. 3.—Results of a typical experiment to measure cut-tissue respiration rate, indicating the method of obtaining the results.

The contents of the side-arm were added to the disks which had been respiring for about 2 hr in the Warburg vessels. Figure 3 shows results of a typical experiment. These experiments were used for calculating the points shown in Figure 4*. The respiration rate at the time of adding ATP or DNP (point A, Fig. 3) was subtracted from the rate at 80 min after adding the substance to be tested (point B) and the difference calculated as a percentage increase in respiration rate produced after adding the contents of the side-arm.

* The scatter of points is due to high variability between fruits, which influences all respiration results. Samples large enough to overcome this are impracticable to handle.

In the very early samples of the 1952-53 season up to 60 days from full blossom, the effect of adding DNP was small. By 130 days, the DNP had caused about 75 per cent. increase in respiration rate. The increased respiration rate then decreased slowly to 226 days. The percentage increase in respiration rate due to DNP was high at 130 days from blossom in the 1951-52 season also, and low at the end of the season when the whole-fruit respiration was rising. In both seasons stimulation of respiration was marked at about 150 days and its extent decreased as the fruit approached the climacteric rise.

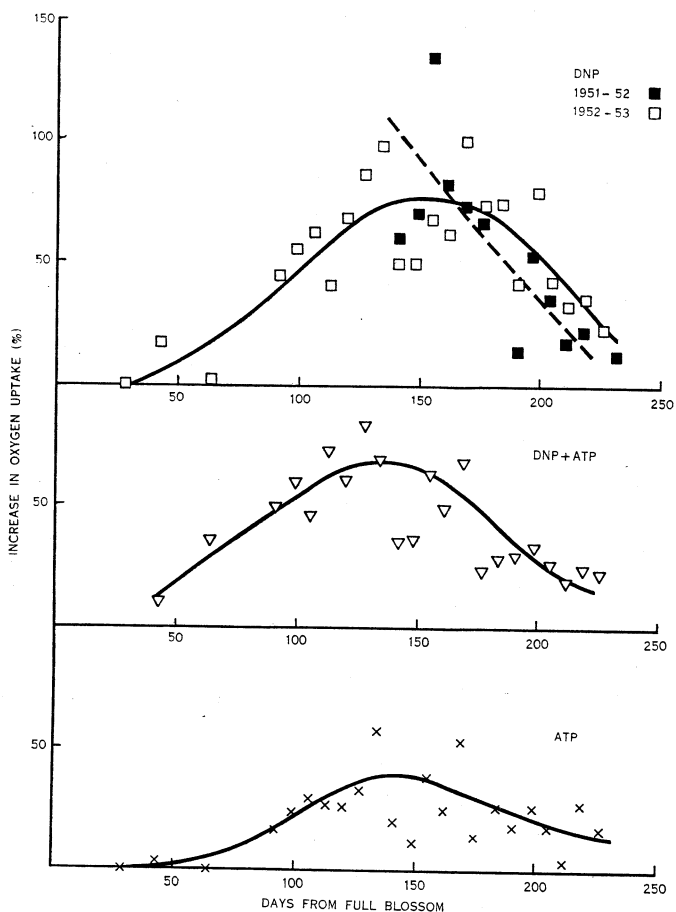


Fig. 4.—Percentage increase in respiration rate of cut tissue after adding 2,4-dinitrophenol, adenosine triphosphate, and both together (DNP, 0.5 mg/l; ATP, 3 mg/ml) at different stages of development.

(iii) *Action of Adenosine Triphosphate.*—In the 1952-53 season adenosine triphosphate (ATP) was added to give 3 mg/ml in contact with the disks (Fig. 4). ATP had no action in the early stages of development but increased the respiratory level by about 35 per cent. at about 150 days. The effect of ATP decreased at the end of the season.

(iv) *Action of ATP and DNP Added Together.*—In the 1952-53 season, ATP (3 mg/ml) and DNP (0.5 mg/l) were added to the disks at the same time. The results of this treatment are given in Figure 4. The curve is similar to that for DNP.

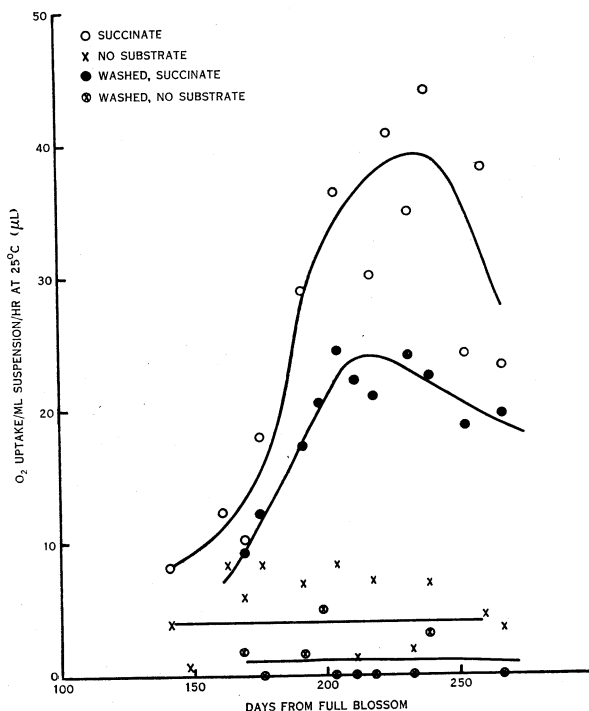


Fig. 5.—Oxygen uptake of cell-free particles prepared in water before and after washing, with and without substrates. Complete system in Warburg vessel: 1 ml particle suspension, 0.05M sodium succinate, and approx. 1×10^{-4} M cytochrome *c*. Temp. 27°C.

(c) Succinoxidase Activity of Isolated Particles

The oxidative activity (unwashed residue prepared in water) in the presence of 0.05M sodium succinate and in the absence of substrate is shown for the 1951-52 season in Figure 5. The activity, given as O_2 uptake per ml suspension per hour, is plotted against days from blossom. The activity increased sharply and then decreased towards the end of the season. The washed residue in 1951-52 (Fig. 5) showed lower activity both in the presence and absence of substrate but the trend was the same with an increase followed by a slight decrease towards the end of the season. The unwashed residue gave greater oxygen uptake both with and without substrate than the washed residue and was also more variable, probably because of the different amounts of substrate carried over.

The oxidative activity of unwashed residue prepared in sucrose in the 1952-53 season was determined. The results in the presence of 0.05M sodium

succinate and in the absence of substrate are shown in Figure 6. The results were very similar to those for the water preparation in the same season. Unfortunately no samples were prepared in sucrose before 64 days from blossom.

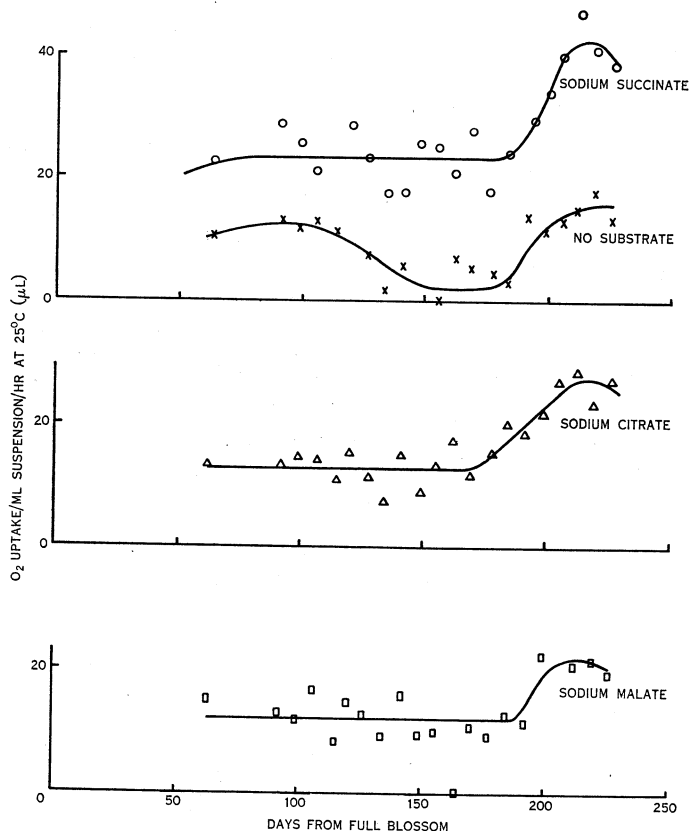


Fig. 6.—Oxygen uptake of cell-free particles (prepared in sucrose) with sodium succinate, sodium citrate, sodium malate, and no substrate. Complete system in Warburg vessel: 1 ml particle suspension, 0.05M sodium succinate, 0.02M sodium citrate or 0.02M sodium malate, 0.001M MgSO_4 , 0.01M phosphate buffer pH 7, 0.001M adenosine triphosphate, and 0.3M sucrose. Temp. 25°C.

Samples prepared in water during this period gave a much higher activity per unit weight of original tissue, indicative of the greater concentration of active material in the small unvacuolated cells. From 70 to 190 days the activity was at the same level as the water preparation but the fluctuations were less in sucrose. At 190 days the respiration rate rose to a higher value at about 215 days. The activity in the absence of substrate was mostly higher in sucrose than in water. In sucrose the curve for oxygen uptake without substrate was very similar to that with substrate and a considerable increase in activity occurred at 190 days.

The marked increase in extractable succinoxidase activity occurring in all these experiments takes place about the same time as the respiration of the whole fruit begins to rise in the climacteric.

(d) *Citrate Oxidation by Isolated Particles*

The oxidative activity of isolated particles prepared in sucrose was measured in the presence of 0.02M sodium citrate (Fig. 6). The rate is about half the average rate with succinate up to about 170 days. The increase in activity occurred earlier and by 210 days the rate had doubled. The activity of the water preparation was also measured with 0.02M sodium citrate but by 150 days the activity was less than in the sucrose preparation and the experiments on the water preparations were not continued.

(e) *Malate Oxidation by Isolated Particles*

The oxidative activity of isolated particles prepared in sucrose was measured in the presence of 0.02M sodium malate. The results (Fig. 6) show a level of activity slightly less than in citrate with a sharp rise occurring at 197 days, which doubled the rate.

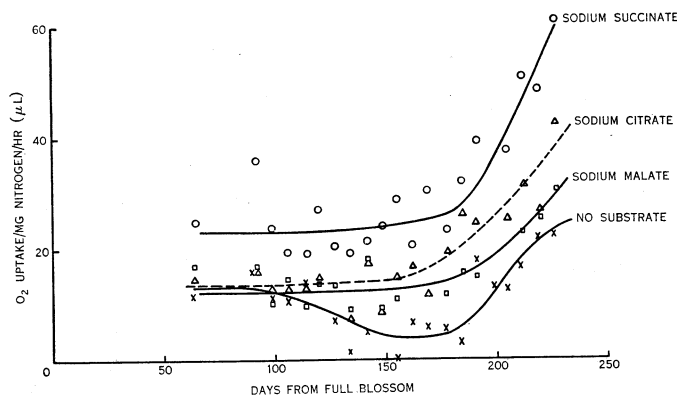


Fig. 7.—Oxygen uptake of cell-free particles prepared in sucrose and expressed per mg nitrogen in the preparation.

(f) *Activity of Isolated Particles*

The total nitrogen of the residue was estimated in each sample and the activity of the residue with succinate, citrate, and malate was expressed per mg nitrogen (Fig. 7). The curves seem to show little change until the sharp rise at 190 days which continued to 226 days when the last sample was taken. The oxygen uptake per mg nitrogen in the absence of substrate seemed to decrease towards 150 days but showed the sharp increase at about 190 days. The decrease in activity in the absence of added substrate between 100 and 150 days may be a reflection of the decrease in concentration of organic acids in the tissue, which would be the substrates carried over during the preparation.

Millerd, Bonner, and Biale (1953) extracted particles from the avocado at higher speeds (two successive spins of 16,000g for 15 min) after a preliminary spin to separate the larger particles such as nuclei. An apple-particle preparation made in the same way gave an activity per mg N which did not differ significantly from that of our standard method; the activity was only about one-tenth that reported by Millerd, Bonner, and Biale for the avocado particles.

The definite utilization of succinic acid by the particles suggests the activity of a cytochrome-cytochrome oxidase system. The utilization of succinate, citrate, and malate by these particles provides evidence for the possible participation of a tricarboxylic acid cycle.

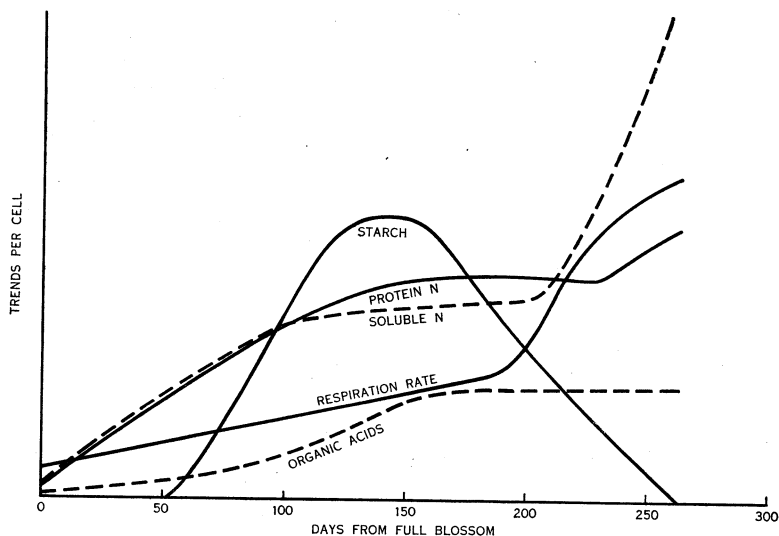
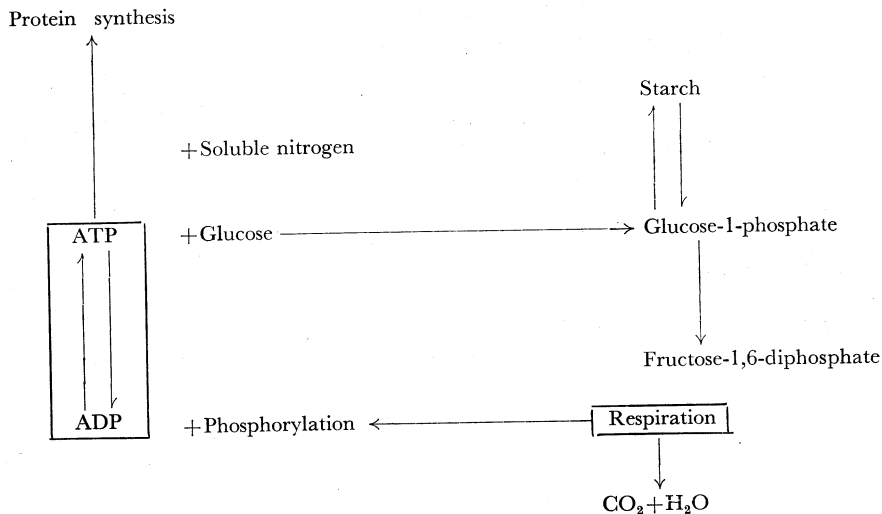


Fig. 8.—Generalized curves based on earlier work on apples (Pearson and Robertson 1953) to show the relations in time between the changes in starch, protein and soluble nitrogen, organic acids, and respiration rate.

IV. DISCUSSION

It has been shown in earlier work on fruit from some of the same trees (Robertson and Turner 1951; Pearson and Robertson 1953) that a number of changes took place in the cells prior to and during the climacteric rise (Fig. 8). Protein, soluble nitrogen compounds, and starch seem to be involved. Starch content approached its maximum at about 120 days from full blossom and decreased from about 165 days onwards. At about the same time, protein and soluble nitrogen, which had been increasing in the cells, stopped increasing for some weeks and then decreased slightly. At about 200 days, just after the onset of the climacteric rise, the soluble nitrogen increased very rapidly in the cells and, after some delay, protein nitrogen began to increase again. These processes as they occurred in 1949, 1950, and 1951, are represented diagrammatically in Figure 8.

The interrelations of starch, protein and soluble nitrogen, and respiration rate can be shown as follows:



The respiration rate increased in the climacteric rise at about 190 days from full blossom and continued to rise while the fruit remained on the tree (Fig. 1). The respiration rate per unit weight of cut tissue fell rapidly till 130 days and then remained at about the same rate till the end of the season. When, however, cut-tissue and whole-fruit respiration are compared on a per-fruit basis (equivalent to a per-cell basis), they are very similar in young fruits (Fig. 2). The cut-tissue rate increases more rapidly than the whole-fruit rate, which remains steady from about 100 to 190 days. At 190 days both whole-fruit respiration and cut-tissue respiration begin to rise and this continues until the end of the observations. Similar trends were seen in both seasons. Millerd, Bonner, and Biale (1953) noted that the climacteric rise was not as marked in slices of avocado as in the intact fruit and that the difference in rates was more marked in pre-climacteric fruit than in climacteric fruit. The figures tabulated by these authors show no climacteric rise in respiration of cut tissue.

(a) Control of Respiration Rate

The rate of respiration in these experiments has been increased in four separate ways: (1) by the climacteric rise, (2) by cutting, (3) by the addition of DNP, and (4) by the addition of ATP. Diagrammatic respiration curves taken from Figures 2 and 4 are compared in Figure 9.

According to the hypothesis outlined in the introduction, one of the most important factors controlling the respiration rate is the phosphate carrier system. If it can be assumed that, as in other tissues, this is adenosine polyphosphate, then the concentration of ADP will govern the rate. This is inversely related to the ATP/ADP ratio at a constant concentration of adenosine polyphosphate. The respiration rate will be low when this ratio is high because the ADP con-

centration will then be low, and will increase, with increase in the ADP to accept phosphate, when this ratio falls. It has been suggested (Pearson and Robertson 1953) that the sharp increase in respiration rate (Fig. 8) at about 190 days from blossom is due to the increase in ADP relative to ATP when the maintenance of protein and other unstable compounds already synthesized makes high demands on the energy-carrying system.

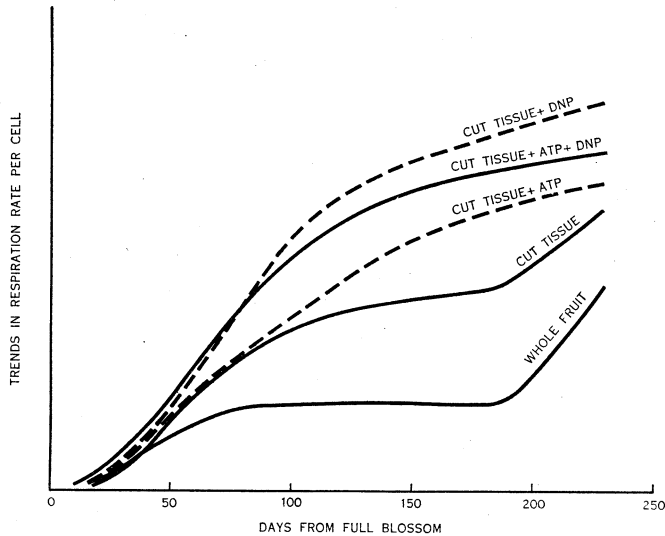


Fig. 9.—Generalized curves for respiration rate per cell based on the changes in 1952-53 season (see Fig. 2).

The increase in respiration rate on cutting (Figs. 1, 2), which occurs between 50 days and about 230 days from blossom, is not inconsistent with the hypothesis. Many workers have observed that mechanical stimulus and wounding both increase the respiration of plant tissue. The greater rate observed in cut tissue may be stimulated by the shearing and squeezing effects on cells not actually cut. At present it is impossible to say by what means this stimulus increases the rate, since it might affect a number of factors. From the hypothesis, however, any effect of cutting likely to lower the ATP/ADP ratio would raise the respiration rate. Such factors would be the increased demands of synthesis as the cells adapted themselves after cutting or increase in the activity of an ATP-splitting enzyme which had been liberated. Millerd, Bonner, and Biale (1953) have suggested that the ATP-ase of mitochondria might take part in the ripening of the avocado.

Whatever the cause of the increased respiration in the cut tissue, it seems that the respiration rate is still controlled to some extent by the ATP/ADP ratio. Up to 65 days from blossom, addition of DNP to cut tissue had very little effect (Figs. 2, 4); thereafter, prior to the climacteric, there was a marked increase due to DNP in the oxygen uptake. The percentage stimulation due to DNP gradually decreased after about 160 days and was at a minimum after

the rise in the whole fruit respiration and in the control cut tissue (Fig. 4). Examination of Figure 9 shows that this decrease in percentage stimulation is due to the increase in cut-tissue respiration, which accompanies the rise in the whole-fruit respiration. These results we interpret as being due to the controlling effect of the ATP/ADP ratio in cut tissue from about 65 days until the climacteric rise. After 65 days, the ratio in cut tissue might have been lower than that in whole-fruit tissue, thus explaining the increase in respiration rate on cutting, which, however, was not as effective in increasing oxygen uptake as the uncoupling effect of the DNP. As the fruits went through the climacteric rise, the oxygen uptake per cell of cut tissue increased too, but the oxygen uptake per cell in the presence of DNP increased only slightly with increase in cell size, i.e. showed no climacteric rise. This suggests that the ATP/ADP ratio remains in control of the respiration at all stages but a change in the ratio allows an increase in both whole-fruit and cut-tissue respiration after 190 days.

The hypothesis that the ADP concentration limits the rate of respiration in fruit from about 65 to 190 days is further supported by the results with added ATP. The hypothesis that the ATP/ADP ratio controls the respiration rate is based on the assumption that the amount of adenosine polyphosphate which occurs naturally in the cells does not alter rapidly. If, however, the absolute amount is increased by the absorption of ATP supplied to the external medium, the amount of ADP will be increased even though the ratio remains the same. This will result in increased respiration rate. No stimulation by ATP addition was obtained until after 65 days. ATP then influenced the oxygen uptake with a trend similar to that shown by the addition of DNP (Fig. 4). As the fruit approached the climacteric rise, the percentage stimulation due to added ATP decreased. As seen in Figure 9, this decrease in percentage is associated with the increase in cut-tissue respiration.

The simultaneous addition of ATP and DNP gave a curve similar to that obtained with DNP alone. The fact that the stimulation due to DNP added simultaneously with ATP was not greater than that due to DNP alone indicated that the uncoupling of respiration from phosphorylation by DNP was complete, since addition of more phosphate carrier did not increase oxygen uptake. No suggestion is offered to explain why the respiration with DNP and ATP was less than that with DNP alone.

The respiration rate is thus interpreted as being controlled by the ATP/ADP ratio, a conclusion similar to that reached by Millerd, Bonner, and Biale (1953) for the avocado. Our hypothesis given earlier (Pearson and Robertson 1953) differs from theirs in attributing the change in respiration rate, at the time of the climacteric rise, to the increased demands of synthetic processes to maintain the protein content of the cells. Millerd, Bonner, and Biale attribute the change to an increase in a natural uncoupling agent which behaves like DNP. This might be ATP-ase. There is at present insufficient evidence to decide between these two hypotheses and it is not necessarily true that the change in ATP/ADP ratio would be due to the same cause in a fruit like an apple developing slowly on a tree and a fruit like an avocado with a sharp ripening phase. The evidence for our hypothesis for the apple is discussed further in the next section.

(b) Control of Synthesis

Using the hypothesis to examine the results of several seasons, we can suggest how synthesis is related to respiration rate during development (see Fig. 8). In the early stages of development up to about 65 days, when the DNP and ATP have very little effect on the respiration, the ATP/ADP ratio can be interpreted as being low. The respiration rate per unit weight at this time is high, though the respiration per cell is low because of the small cell size. The active synthesis of protein and other compounds necessary for the growth of the cell uses the ATP formed in this period. After about 60 days from blossom, starch begins to appear and increases steadily till about 120 days and then decreases from about 165 days. During the period of starch increase and maintenance, the ATP/ADP ratio is probably high and the phosphorylations, in excess of those required for growth, result in the formation of the glucose-1-phosphate, which is condensed to starch. This corresponds to the period during which the respiration is controlled and the stimulation with both DNP and ATP rises to its maximum.

Protein nitrogen increases steadily through the early part of the season but the rate of increase falls off at about the time of the starch maximum. It is possible that the protein increase is checked by the decreased rate of import of soluble nitrogen which occurs at about the 100th day. It is significant, however, that McKee and Urbach (1953) have shown that a marked decrease in the content of glutamine occurs at about this time and that no glutamine could be detected at 138 days in spite of no marked change in glutamic acid. This they point out may be due to insufficient ATP for this reaction. ATP is known to be required for glutamine synthesis *in vitro*. At this time, starch and protein content, both dependent on ATP, are being maintained but by the 165th day the synthesis of starch does not exceed its rate of utilization and this can be taken as further indication that the ATP/ADP ratio is beginning to decrease. This may be due to the protein maintenance, which has increased with increase in cell size, competing with the other processes requiring phosphorylation; the transfer of energy is sufficient only for the resynthesis of the unstable protein and of other compounds in the cells by that time. By 190 days, the starch content has decreased and the decreasing ATP/ADP ratio is shown by the increase in respiration rate. The adjustment of the respiration rate to the ATP/ADP ratio is not continuous but is marked by a sudden rise; kinetic considerations of the hypothesis show that the respiration rate is not an inverse linear function of the ratio, but that the lower the ratio the more rapidly does the respiration increase for a given change of ratio. Even after the respiration rate has increased no net gain of starch is possible and there is no change in the protein nitrogen concentration until about 230 days, despite the fact that the soluble nitrogen has begun its rapid increase at 200 days. When the protein nitrogen does increase again towards the end of the life of the fruit on the tree, only a relatively small fraction of the soluble nitrogen is changed. This increase in protein nitrogen, however, indicates that there is some ATP available for net gain at the higher respiration rate, in addition to the ATP required for maintenance. The fact that the respiration is not completely uncoupled from phos-

phorylations at this stage is also suggested by the stimulatory effect of DNP which, though small, is still apparent at the end of the observations. When the DNP effect has reached its minimum, probably corresponding to the lowest ATP/ADP ratio since the early stages of development (Fig. 4), the spectacular increase in respiration occurs. This increased respiration, after a time, results in the gain in total protein late in the life of the fruit, which uses only a small fraction of the increased soluble nitrogen.

During the period from 60 to 190 days, very little change in the activity of the particles with substrates of succinate, citrate, or malate was observed. When the respiration of whole tissue increased in the climacteric rise, the activity of the particles also increased (Figs. 5, 6). Increased activity per mg of nitrogen in the particles (Fig. 7) implies either that the oxygen uptake by the particles had been controlled by some factor such as the ATP/ADP ratio in the particles, which changed at the time of the climacteric, or that the enzyme concentrations had increased relative to the other nitrogen constituents. The second possibility would seem to be less likely than the first since increase in the concentrations of the oxidative enzymes would be expected to cause a sharp rise in the DNP respiration of cut tissue, but this did not occur (Fig. 4). Control of oxidation rate by ATP/ADP in the extracted particles is consistent with the general hypothesis of control in the intact tissue. In this respect, the apple differs from the avocado investigated by Millerd, Bonner, and Biale, who found no evidence of a change in the activity of isolated particles in pre-climacteric and post-climacteric fruits. This might be a consequence of the differences between a developing fruit and a detached fruit in which the climacteric takes place rapidly.

While it has been postulated that in apples between 65 and 190 days the respiration rate is limited by the ATP/ADP ratio, once the climacteric rise starts at least two factors will contribute to the rise continuing. The breakdown of starch which is taking place concurrently in these fruits will contribute a phosphorylated hexose to enter the respiration system and hence act as a source of substrate in the cytoplasm, so that substrate supply is less likely to become limiting. In this connection, it is important to note that the loss of starch between 185 and 225 days (see Pearson and Robertson 1953) is about 8×10^{-8} g per cell or 0.2×10^{-8} g per cell per day. If all this starch were respired as phosphorylated hexose it would produce 1.4×10^{-7} mg CO_2 /cell/hr, and as the respiration rate at 225 days is 1.2×10^{-7} mg CO_2 /hr, the hexose from starch would account approximately for the increase in respiration rate.

The other factor is the increase in respiratory enzyme activity which suggests that the enzyme systems can keep pace with the increased rate of respiration. In the earlier paper, it was observed that the total organic acid content did not change (Fig. 8) at the time of the climacteric rise. It has now been shown that the activity of the enzymes concerned with the acid cycle had doubled within 36 days of the onset of the climacteric (Fig. 7). The absence of change in the organic acid content at this time indicates either that the gross concentration does not relate very directly to the respiratory activity or that there is an increased rate of turnover in acid without any change in net content.

The occurrence of the appropriate enzymes in the particles is further evidence for the participation of the tricarboxylic acid cycle in the respiration but the cause of the accumulation of organic acids, chiefly malic, which may be in the vacuole, requires more investigation.

Much has been written on the climacteric rise in apples and some of the problems have been discussed at length by Kidd and West (1945). The hypothesis outlined in this paper may serve as a starting point for re-examination of the theoretical implications of results given by Kidd and West in their various publications. Such factors as the influence of ethylene and the effect of time of picking on the subsequent climacteric require explanation. While we do not wish to attempt this in the present paper, it seems likely that the hypothesis we discuss will explain the "auto-regulatory mechanism" controlling respiration during growth suggested by Kidd and West.

(c) Conclusion

Thus it may be concluded that the hypothesis connecting respiration rate with starch, organic acid, and protein metabolism through the ATP/ADP ratio is consistent with our present knowledge. The climacteric rise in respiration is due primarily to increase in ADP concentration with the change in this ratio and to the tissue's capacity for increasing the activity of respiratory enzymes. It is hoped that our hypothesis can be further examined when it becomes possible to estimate the ATP/ADP ratio directly.

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