# Metabolic Processes in Cytoplasmic Particles of the Avocado Fruit VII. Oxidative and Phosphorylative Activities throughout the Climacteric Cycle[1, 2, 3]

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Many fruits including the avocado exhibit a marked increase in respiration during the ripening process which is referred to as the climacteric rise (2). Explanations of this phenomenon have been put forward by a number of investigators (1, 10, 15, 18, 23) but in general the mechanisms that initiate the respiration climacteric remain obscure. There have also been a number of recent reports in which the isolation of subcellular particles either from ripe fruit alone (20, 21, 27) or from various stages during the ripening process (6, 10) have been carried out. It was considered that a detailed study of the evolution in biochemical behavior of particles from avocado fruit between maturity and senescence might shed some light on the energy relationships and their importance in the ripening process. By somewhat modifying a method (27) for the production of particles from ripe avocado fruit, it has been possible to obtain mitochondria which appear to be in a good physiological state of preservation from fruit at any stage of ripeness up to the postclimacteric (overripe) condition. A close comparative study of the metabolic properties of these particles has been made especially on the trends in their respiratory activities. The ability to show the phenomenon of respiratory control (5) [preferably called acceptor control by Lehninger (13)] with various substrates, the variation in the ADP phosphorylated to $O_2$ consumed ratios, and responses to the action of various metabolic inhibitors of mitochondria isolated from fruit at distinct stages during the ripening process have also been examined.

## Materials and Methods

*Preparation of Mitochondria.* The method of Wiskich et al. (27) for the isolation of active particles from ripe avocados was used as a basis for developing a procedure which could be used with fruit at any stage of ripeness. The modified method has been described in detail (9) and only a summary will be given here.

Thoroughly chilled fruit tissue (80 g) was slowly passed through a stainless steel grater with 2-mm holes and dispersed in 240 ml of 0.4 M sucrose containing 4 mM cysteine, 1 mM $MgCl_2$, 10 mM KCl, 50 mM Tris-10 mM EDTA buffer pH 8.1, and bovine serum albumin at a standard amount of 0.75 mg per ml. The pH was kept close to 7.6 by the dropwise addition of 1 N KOH. The brei was squeezed in a muslin bag and the extract, diluted with 160 ml of 0.4 M sucrose containing 10 mM KCl and albumin, was subjected to the following centrifugations: A) 1500 × $g$ for 15 minutes, precipitate discarded; B) 12,000 × $g$ for 20 minutes, supernatant discarded; C) 2500 × $g$ for 10 minutes, precipitate discarded; D) 10,000 × $g$ for 15 minutes, supernatant discarded. The precipitate at stage B was suspended in 320 ml of 0.4 M sucrose containing 10 mM KCl, 10 mM Tris-10 mM $KH_2PO_4$ buffer pH 7.2 and albumin. The final suspension was made in about 1 ml of this medium. $O_2$ consumption was measured polarographically at room temperature using the apparatus, method and reaction medium similar to that described by Wiskich et al. (27). The medium contained 0.25 M sucrose, 10 mM potassium phosphate buffer pH 7.2, 10 mM Tris-HCl buffer pH 7.2, 5 mM $MgCl_2$, 0.5 mM EDTA and albumin (0.75 mg per ml) in a final volume of 3 ml.

*Mitochondrial Nitrogen.* This was determined by the method of Thompson and Morrison (24) modified according to Biale et al. (4). Due allowance was made for the presence of Tris and bovine serum albumin in the medium in which the mitochondria were suspended.

*Materials.* The variety of avocado fruit (*Persea gratissima*, Gaertn.) used in this work was Fuerte but the variety Hass was shown on a number of occasions to be equally suitable. Fruit were grown in southern California and when mature were picked

and immediately transferred to a dark store at 8°. For the first week after harvest, fruit kept at this temperature remained in an early preclimacteric state (see 9), but after about 10 days developed late preclimacteric characteristics. For more advanced stages of ripening, individual fruit were transferred to respirometer jars at 20° and their respiration followed continuously by means of a paramagnetic $O_2$ analyzer (28). Fruit were removed for extraction of mitochondria when the respiration rate corresponded to a selected point on the climacteric curve (2). Postclimacteric fruit were obtained by allowing climacteric peak fruit to remain at 20° for at least 2 days.

All the organic acids and bovine serum albumin (fatty acid poor, B grade) were obtained from California Corporation for Biochemical Research, ADP and ATP from Pabst Laboratories and oligomycin from the Wisconsin Alumni Research Foundation.

## Results

*Particle Preparation.* It may be noted that the concentration of magnesium salt included in the dispersing medium was only one-sixth of that recommended by Wiskich et al. (27). In our experience a higher concentration of magnesium than this caused the aggregation of large quantities of mitochondria which precipitated during the low speed centrifugations.

Unripe fruit tissue needed to be grated very slowly and finely to insure a satisfactory yield of particles. Excessive grinding in a mortar and pestle or the addition of abrasives such as sand or alumina were found to be detrimental to the retention of respiratory control. The use of blending machines also caused a loss of control and in summary it can be said that the less violent the methods used for breaking up the tissue the better appears to be the integrity of the mitochondria from a functional viewpoint.

Unless avocado fruit mitochondria are isolated in a medium affording some osmotic protection as well as containing some high purity bovine serum albumin (BSA), the measure of respiratory control will suffer. An illustration of this is shown in figure 1, in which a uniform batch of avocado tissue was extracted by 2 types of medium, one with and the other without the addition of BSA. Following the final suspension of the 2 pellets of mitochondria, each was assayed in 2 media, to one of which BSA had been added. In the complete absence of BSA, no respiratory control was shown (fig 1A), but this property was partially restored by BSA addition to the assay medium (fig 1B). The presence of BSA throughout preparation and assay gave the best results (fig 1C) but these were adversely affected when BSA was omitted from the assay medium (fig 1D). Thus, albumin is a desirable constituent of media to which avocado mitochondria are exposed if unnecessary physiological damage is to be avoided.

Albumin was shown to be superior to other macromolecular substances such as ficoll, dextran, thiogel and polyvinylpyrrolidone in its protective action on mitochondria. The mechanism through which BSA shows its effect is not known at present but it may be connected with the fatty acids present in avocado fruit; further studies on this question are being carried out. It was observed that, if during the preparation, a small amount of seed tissue of the avocado was accidentally grated with the flesh, no respiratory control was given by the particles. This would suggest that the high polyphenolic content of the seed had affected the mitochondria. The addition of substances such as nicotine and caffeine which are able to counteract the action of certain polyphenolic compounds never appeared to improve the respiratory control in ordinary preparations.

The pH of the extraction and assay media was not of critical importance for retaining respiratory control, but in order to promote uniformity of mitochondrial preparations from day to day the extraction medium was kept at pH 7.6 and subsequent media at pH 7.2. The presence of a small amount of potassium salts consistently improved the uniform appearance of the mitochondrial pellet and possibly increased respiratory control values. EDTA may be of benefit to the mitochondria in certain circumstances but higher concentrations in the media are detrimental to the metabolic activities of the particles.

For the extraction procedure used in this study the number of times the mitochondria were washed was less than that recommended by Wiskich et al. (27), but the particles nevertheless showed good respiratory control. The amount of washing, inevitably, is a compromise between a progressive reduction in the endogenous concentration of phosphate acceptors and substrates on the one hand, and a deterioration due to aging as well as losses due to aggregation on the other. In a comparative study, a single wash was found to be satisfactory and was adopted for the general method.

*Oxidation of Succinate, Malate and α-Ketoglutarate.* An account of the observations that formed the basis for the suggested splitting of the preclimacteric stage of ripeness into early and late divisions has already been given (9), and in summary is based on the differences in the effectiveness of thiamine pyrophosphate (TPP) on the oxidation of α-ketoglutarate. A consideration of the stages of ripeness of avocado fruit between which clear-cut differences occur either in mitochondrial behavior or in respiratory activity by the intact fruit has led to a division of the ripening process into 4 phases, early preclimacteric, late preclimacteric, climacteric rise to climacteric peak, and postclimacteric.

Using particles from fruit in each of these 4 distinct conditions and succinate as substrate, a picture of the evolution in oxidative ability for this substrate was built up. Figures 2A and 2B illustrate the behavior of particles from the first and last of these stages. The rate of oxidation following the addition of substrate is referred to as the basic rate (see table I), which was increased sharply by the addition of
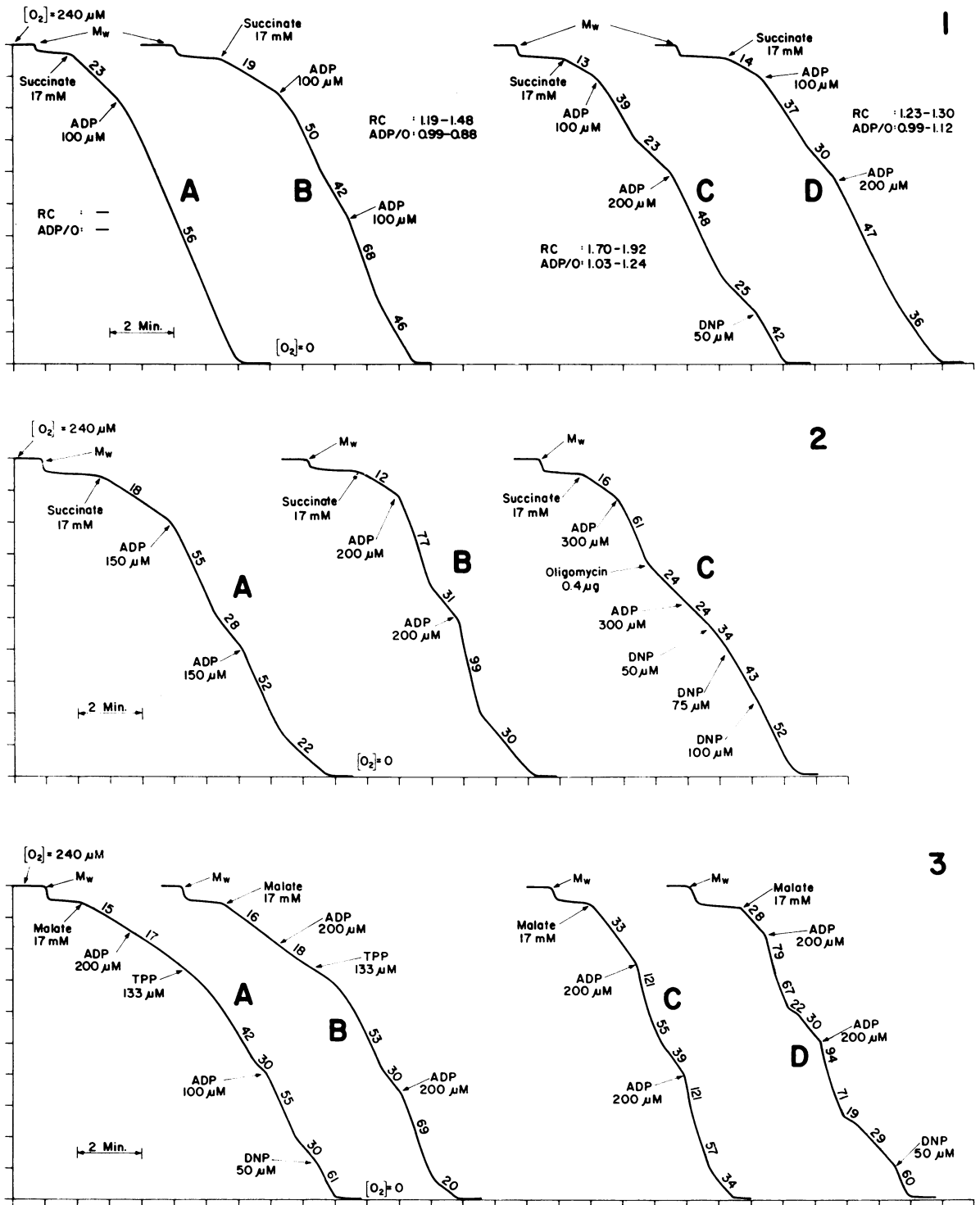
FIG. 1. Polarograph traces showing the influence of the inclusion of bovine serum albumin (BSA) in the extraction and assay media on the activity of mitochondria from early preclimacteric avocado fruit. Trace A, no BSA; trace B, BSA in the assay medium only. Trace C, BSA throughout; trace D, BSA in the extraction medium only. Mw indicates the addition of washed mitochondria, the substrate used was succinate. The mitochondrial nitrogen, expressed as $\mu g/ml$, was 54 $\mu g$ N for traces A and B, 35 $\mu g$ N for traces C and D. The numbers on the traces represent the decrease in $m\mu moles$ $O_2/minute$ per ml. Additions are shown as final concentrations on all the traces.

Table I. *Rates of Oxidation of Succinate, Malate and $\alpha$-Ketoglutarate by Avocado Fruit Mitochondria*

The rates of oxidation, $Qo_2(N)$, are expressed as $\mu l$ $O_2$/hour per mg mitochondrial nitrogen. Basic respiration rate refers to the rate immediately following the addition of substrate (in the absence of ADP), to distinguish it from state 4 oxidation rate immediately following a state 3 rate. Values for state 3 and state 4 rates are computed from the average of the rates following the first and second additions of ADP. The particular conditions for each substrate are given on the relevant figure.

| Substrate | | Respiration rate | Early preclimacteric | Late preclimacteric | Climacteric rise to climacteric peak | Post climacteric |
|---|---|---|---|---|---|---|
| | | | | Stage of ripeness | | |
| Succinate | $Qo_2(N)$ | Basic | 420 | 280 | 430 | 420 |
| | | State 3 | 1440 | 1270 | 1590 | 1430 |
| | | State 4 | 780 | 490 | 580 | 510 |
| Malate | $Qo_2(N)$ | Basic | 210 | 210 | 390 | 310 |
| | | State 3 beginning | 240 | 270 | 1170 | 1110 |
| | | end | ... | ... | 660 | 910 |
| | | State 3 + TPP | 620 | 600 | ... | ... |
| | | State 4 | 430 | 330 | 230 | 210 |
| $\alpha$-Ketoglutarate without malonate | $Qo_2(N)$ | Basic | 55 | 74 | 89 | 108 |
| | | State 3 | 200 | 700 | 640 | 700 |
| | | State 3 + TPP | 660 | ... | ... | ... |
| | | State 4 | 310 | 280 | 280 | 210 |
| With malonate | $Qo_2(N)$ | Basic | 46 | 55 | 49 | 73 |
| | | State 3 | 110 | 470 | 450 | 400 |
| | | State 3 + TPP | 480 | ... | ... | ... |
| | | State 4 | 200 | 120 | 120 | 110 |
| No. of expts | | | 5 | 4 | 7 | 5 |

ADP [state 3 rate of oxidation according to Chance and Williams (5)]. After a short interval depending on the amount of ADP added there was a marked reduction in the rate of oxidation caused by the utilization of all the phosphate acceptor (state 4 rate of oxidation), thus demonstrating the phenomenon of respiratory control. In this study a distinction was made between a state 4 rate of oxidation immediately following substrate addition (basic rate), and the state 4 following a state 3, which was invariably the higher of the two. A general observation on all the traces was that subsequent additions of ADP after a display of respiratory control always gave control ratios which were higher than the first figure. It is suggested that the active phosphorylation which follows the initial introduction of ADP results in an improvement in the structure of the mitochondrial membrane leading to tighter coupling of oxidation and phosphorylation. As an illustration of this strong coupling, oligomycin, an inhibitor of

oxidative phosphorylation, drastically reduced the oxidation rate of mitochondria in state 3 (fig 2C), and the particles remained insensitive to a further addition of ADP. The state 3 rate was, however, regained by additions of 2:4-dinitrophenol (DNP).

Values for the $Qo_2$ (N) with succinate as substrate at each of the 4 ripening stages are given in table I. The basic and the state 3 rates indicate little change as the fruit ripened, but the decrease in the state 4 rate may be accounted for by an improvement in the respiratory control ratio as shown in table II. It can also be seen from this table that the ADP/O ratio improves from stage to stage, continuing even into the postclimacteric condition.

The oxidation of malate by mitochondria from fruit at certain stages of ripening presents a rather more complex picture. The basic respiration rate rises considerably between the preclimacteric and the climacteric conditions. On ADP addition to preclimacteric mitochondria oxidation increased only

FIG. 2. Respiratory control with succinate as substrate. Trace A illustrates oxidation by mitochondria from early preclimacteric avocados (40 $\mu g$ N), and traces B and C by mitochondria from postclimacteric fruit (73 $\mu g$ N in each case). Trace C shows the effect of oligomycin (0.4 $\mu g$/ml) and DNP. Other conditions are given in figure 1.

FIG. 3. The oxidation of malate by avocado mitochondria. Trace A is typical for particles from early preclimacteric fruit (65 $\mu g$ N), trace B from late preclimacteric fruit (117 $\mu g$ N), trace C from fruit near the beginning of the climacteric rise in respiration (127 $\mu g$ N) and trace D from fruit near the climacteric peak (100 $\mu g$ N). Other conditions are given in figure 1.

slightly to give a slow linear rate which persisted until the $O_2$ supply became exhausted. If, however, TPP was added it caused a gradual acceleration in oxidation ending in an exhibition of respiratory control (table I, fig 3A and 3B). The addition of DNP had the expected effect of uncoupling oxidation from phosphorylation, causing a return to the state 3 rate.

Traces C and D in figure 3 illustrate the extremes in behavior of mitochondria during the climacteric period, one at the beginning and the other at the end of this stage. The rate of oxidation of malate on ADP addition increased sharply, showing that the complete dependence on the presence of TPP for this reaction, which is characteristic for preclimacteric fruit, has been relieved. The oxidation rate, however, gradually fell away with time until breaks in the curves indicated that respiratory control had taken place. In the case of particles from fruit near the beginning of the climacteric rise (fig 3C), the state 4 rate was close to that for basic respiration, while those from fruit near the climacteric peak (fig 3D) showed a transient state 4 rate, much lower than the basic rate, which after a short while reverted to the basic value. This was the only instance in which significant differences in behavior were found between fruit at the beginning and end of the climacteric rise.

An example of the characteristics of postclimacteric mitochondria is given in figure 4A, showing a gradually decreasing oxidation rate following ADP addition as with climacteric peak mitochondria. The length of the plateau immediately following respiratory control appeared to be proportional to the amount of ADP added, but in the presence of TPP (fig 4B)
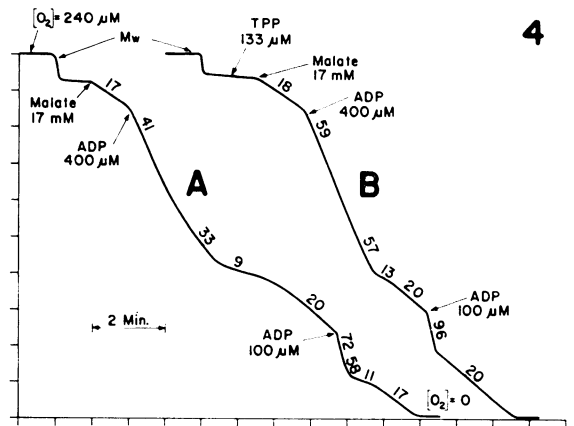


FIG. 4. The oxidation of malate by mitochondria from postclimacteric avocados. Trace A shows a typical respiratory pattern while trace B illustrates the influence of the addition of TPP to particles from the same preparation (144 $\mu$g N in each case). Other conditions are given in figure 1.

the rate of oxidation during the state 3 phase became almost linear, with only a very small plateau following respiratory control. In fact, on the second addition of ADP (100 $\mu$M), a linear state 4 was immediately assumed, which leads to the suggestion that TPP aids in the removal of some substance that inhibits malate oxidation. Quantitative data on the oxidation of malate are shown in the relevant parts of tables I and II, and demonstrate that whereas the rates of oxidation differed markedly between preclimacteric particles and those from riper stages,

Table II.   *Oxidative and Phosphorylative Properties of Avocado Fruit Mitochondria*

The effectiveness of ADP in promoting the state 3 oxidation rate is expressed as the percent increase over the basic respiratory rate brought about by the first addition of ADP. Respiratory control (RC = state 3 rate/state 4 rate) and ADP/O ratios ($\mu$moles ADP phosphorylated/$\mu$atom $O_2$ absorbed) are computed from the average results from the first and second additions of ADP. When TPP significantly affects the oxidation rate, RC values are calculated using the higher state 3 rate. The particular conditions for each substrate are given on the relevant figure.

| | | | Stage of ripeness | | |
| | | | | Climacteric rise | |
| Substrate | | Early preclimacteric | Late preclimacteric | to climacteric peak | Post climacteric |
|---|---|---|---|---|---|
| Succinate | ADP stimulation | 240 | 370 | 260 | 280 |
| | RC ratio | 1.86 | 2.61 | 2.79 | 2.72 |
| | ADP/O ratio | 1.08 | 1.38 | 1.40 | 1.49 |
| Malate | ADP stimulation | 32 | 30 | 150 | 270 |
| | RC ratio | 1.58 | 1.81 | 3.31 | 4.34 |
| | ADP/O ratio | 1.04 | 1.49 | 1.70 | 1.93 |
| $\alpha$-Ketoglutarate without malonate | ADP stimulation | 200 | 930 | 620 | 730 |
| | RC ratio | 2.22 | 2.93 | 2.93 | 3.01 |
| | ADP/O ratio | 1.30 | 1.92 | 2.05 | 2.32 |
| With malonate | ADP stimulation | 230 | 830 | 780 | 880 |
| | RC ratio | 2.40 | 4.09 | 3.85 | 4.75 |
| | ADP/O ratio | 1.62 | 1.97 | 2.26 | 2.72 |
| No. of expts | | 5 | 4 | 7 | 5 |
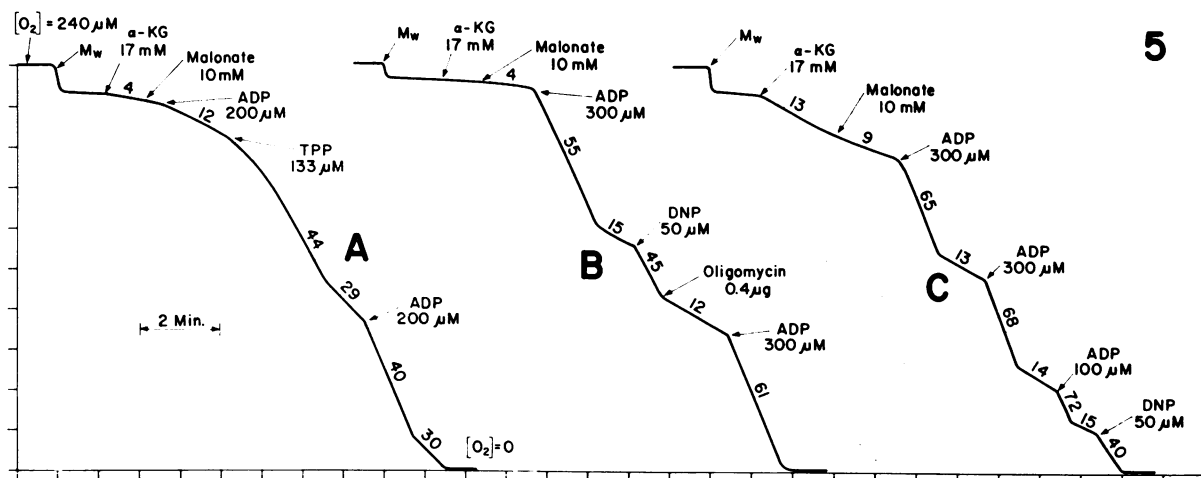
FIG. 5. The oxidation of $\alpha$-ketoglutarate by avocado mitochondria. Trace A shows oxidation by particles from early preclimacteric fruit (170 $\mu$g N) and trace B those from late preclimacteric fruit (175 $\mu$g N) in the presence of oligomycin (0.4 $\mu$g/ml) and DNP. Trace C illustrates oxidation by particles from climacteric peak fruit (165 $\mu$g N). Other conditions are given in figure 1.

when the cofactors of ADP and TPP were present the rates of oxidation became much more similar. The progressive decrease in the state 4 rate was the result of a continuous improvement in the respiratory control. The ADP/O ratios also rose throughout ripening to the postclimacteric condition.

Experiments using $\alpha$-ketoglutarate as substrate were carried out both in the absence and in the presence of malonate in order to limit the oxidation to a one-step reaction by inhibiting succinic dehydrogenase. The tables show that there was, in the presence of malonate, a general reduction by some 30 to 40 % in the rates of oxidation and concomitant improvements in respiratory control and ADP/O ratios. With early preclimacteric mitochondria ADP caused some increase in oxidation which was further enhanced by TPP addition (fig 5A), ending in a demonstration of respiratory control. Late preclimacteric fruit showed no such dependence on TPP (fig 5B) and this disparity between the behavior of early and late fruit provided a biochemical criterion for their separation (9). The action of TPP on the rate of oxidation of $\alpha$-ketoglutarate by early preclimacteric mitochondria suggests that these particles are probably deficient in this required cofactor but in subsequent stages an endogenous supply is probably available. Respiratory control ratios improved from stage to stage (fig 5C and table II), and in general the basic respiration rate also increased but the state 3 rate in the presence of the required cofactor remained almost constant.

In furtherance of the work of Wiskich et al. (27) on the balance between the respiratory chain and substrate level phosphorylations during the oxidation of $\alpha$-ketoglutarate, the action of DNP and oligomycin on late preclimacteric mitochondria was investigated (fig 5B). The addition of DNP immediately al-

lowed the oxidation rate to rise, but this effect was reversed by oligomycin. The interpretation of these results (27) is that $\alpha$-ketoglutarate oxidation is limited by the substrate-level phosphorylation step and that the stimulation by DNP is not primarily due to an uncoupling of oxidative phosphorylation, since this condition would not be sensitive to oligomycin, but rather is it thought to be due to a DNP-stimulated oligomycin-inhibited adenosine triphosphatase releasing additional amounts of ADP. This was confirmed by a stimulation of oxidation by direct addition of ADP. Particles at the preclimacteric stage thus behaved in an essentially similar manner to those at the climacteric stage.

## Discussion

It is now clear that by the use of the method described in this study it is possible to produce ADP controlled mitochondria from unripe as well as ripe avocado fruit and from a number of other tissues as well (9), provided that the pH is kept within close limits, the cells are disintegrated in a carefully controlled way, the tonicity is within tolerance, a small quantity of BSA is present at all stages of isolation and assay of the mitochondria, and phenolic substances are not allowed to damage the particles (14). There is no evidence in our present work to suggest that preclimacteric avocados possess a more active adenosine triphosphatase than mitochondria from climacteric peak fruit, an explanation (3) that has been used to account for the difficulties in the isolation of preclimacteric fruit particles showing respiratory control. It is also unlikely that differential damage to the mitochondria from fruit at the various stages of ripeness could account for the distinct characteristics found. The usefulness of following mitochondrial

activities during ripening has been pointed out by Pierpoint (16) while Tager (22) stressed the dangers of drawing firm conclusions from apparent differences between ripening stages. The point is well taken, and with the increasing number of reports of particles from fruit at selected stages of ripeness (6, 10, 17) much care must be taken in avoiding confusion of biochemical differences and physiological damage to the particles.

Until recently the consensus of opinion was that the process of oxidative phosphorylation would be amongst the first of the systems to suffer from the effects of senescence as fruit became overripe. An increasing amount of evidence (6, 7, 10, 17, 18) as well as that in the present study strongly suggests that active phosphorylation, and the synthesis of specific enzymes, persist well into the senescent phase. As the ADP/O ratios of particles continued to rise up to the postclimacteric condition with all the substrates used in this study, there is every indication that other biological systems suffer disruption and disintegration (19) some time before mitochondrial breakdown begins.

In the presence of the necessary cofactors, taking the state 3 rate as the most reliable criterion, no major changes in the oxidative abilities of mitochondria from avocados during the ripening process have been found. This is, of course, in direct contrast to observations with intact fruit where the climacteric rise at 20° brings about a 3- to 5-fold increase in the respiratory rate (2). There is no evidence that the climacteric rise may be accounted for by an increase in the number of mitochondria during this period. The strong influence of TPP on the oxidative ability of preclimacteric mitochondria perhaps shifts the emphasis away from the particles themselves towards their cofactor requirements for the expression of maximum activity. The subtlety of the changes that allow a release from a dependence on added TPP for the effective oxidation of $\alpha$-ketoglutarate merely by allowing the fruit to remain at 8° for about a week is worthy of further investigation.

Some aspects of the metabolism of malate by mitochondria from avocados in various conditions need to be examined in the light of the conclusions from previous observations (26, 27). The very much higher rates of oxidation of malate by climacteric or postclimacteric mitochondria compared with preclimacteric particles, a situation that is partially relieved by TPP, is evidence that the cofactor is acting by catalyzing the removal of an inhibitor of malate oxidation. The length of time in the presence of ADP and TPP that is required by preclimacteric particles to reach the maximum rate of oxidation could be a function of the rate of penetration of TPP into the mitochondria. In addition, a scheme for the removal of some inhibitor of malate oxidation involving TPP is likely to implicate more than one step in the reaction, possibly contributing to the delay in reaching maximum velocity.

In the case of climacteric mitochondria, the high initial rate of malate oxidation brought about by ADP decreases progressively, presumably because of some inhibition by a product of the reaction (11, 27). The inhibitor is presumed to be metabolized slowly and a high rate of malate oxidation causes its accumulation. This conclusion is substantiated by 3 additional pieces of evidence. First, the initial part of the state 4 rate immediately following control is markedly lower than the basic respiration rate (fig 3D, 4A). Second, as has already been mentioned, the length of this plateau between state 3 and a true state 4 rate is in direct proportion to the amount of ADP added and hence to the amount of inhibitor produced. Third, when TPP is added to the system (fig 4B), the rate of oxidation becomes almost linear and the plateau much less obvious or nonexistent; TPP appears to be active in relieving the metabolic restraint. The break in the state 4 trace introduces a difficulty in computing the respiratory control ratio, since the initial rate of oxidation following state 3 is a temporarily inhibited state 4.

These results lead to the following suggestion, at least as a working hypothesis, that oxaloacetate could be the inhibitor concerned with the oxidation of malate. It is the direct product of the oxidation of this substrate and is known to inhibit malic dehydrogenase (11). Wiskich et al. (27) have suggested that in avocado mitochondria this product could inhibit malate oxidation and they have presented evidence that a transamination reaction with glutamate is able to relieve this inhibition. The action of TPP could be thought of as acting in an equivalent way. However, it is unlikely (25) that TPP is directly involved in the decarboxylation of oxaloacetate to yield pyruvate, but much more probably it takes part in the oxidative decarboxylation of endogenous pyruvate, the product condensing with oxaloacetate to form citrate, thus relieving the inhibition.

An investigation into the sensitivity of $\alpha$-ketoglutarate oxidation towards DNP and oligomycin showed that mitochondria contained an active adenosine triphosphatase (27). Under the same experimental conditions it might be expected that the oxidations of succinate and malate would be similarly affected, but with these substrates phosphorylation is through the electron transport chain. Furthermore, even if adenosine triphosphatase were stimulated by DNP to the same extent as with $\alpha$-ketoglutarate, the rates of oxidation of succinate and malate are so much greater that the contribution due to the triphosphatase would be of minor importance.

## Summary

By a modification of a previous method, particles showing respiratory control have been isolated from avocado fruit at 4 selected stages of ripening, early and late preclimacteric, climacteric and postclimacteric. The particles displayed a basic ability to oxidize succinate, malate and $\alpha$-ketoglutarate. Succin-

ate was oxidized by particles from fruit at all ripening stages at about the same rate. With malate there appeared to be a block in the oxidation at the preclimacteric stage and a progressive inhibition of the oxidation rates at the climacteric and postclimacteric stages. Both these points of inhibition were overcome by thiamine pyrophosphate addition, suggesting an involvement of oxaloacetate in the mechanism. Oxidation of $\alpha$-ketoglutarate required the addition of thiamine pyrophosphate only with particles from preclimacteric fruit. In general, respiratory control ratios increased with ripening, as did the ADP to oxygen uptake values. Indications are that a cofactor requirement could form a regulatory mechanism and act as a contributory factor in the climacteric rise.

## Acknowledgment

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