# THE RELATIONSHIP OF HIGH-ENERGY PHOSPHATE CONTENT, PROTEIN SYNTHESIS, AND THE CLIMACTERIC RISE IN THE RESPIRATION OF RIPENING AVOCADO AND TOMATO FRUITS

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*Summary*

The relationships between respiration rates and the content of protein nitrogen and high-energy phosphate were investigated in ripening avocado and tomato fruits. The climacteric rise in respiration rate was accompanied by a rise in total high-energy phosphate in avocados ripened after picking; in tomatoes ripening on the plant the change in total high-energy phosphate was not significant. The ratio of protein nitrogen to total nitrogen increased during the climacteric rise in both fruits. It is concluded that, while the rates of respiration and synthetic processes may be controlled by the phosphate transfer system, the evidence suggests that uncoupling of respiration and phosphorylation does not explain the respiratory climacteric.

## I. Introduction

Recent speculations on the control of respiration rate in ripening fruits have associated the mechanism with the generation of high-energy phosphate (~P). Millerd, Bonner, and Biale (1953) and Pearson and Robertson (1952, 1954) showed that the respiration of tissue cut from pre-climacteric fruits was markedly stimulated by 2,4-dinitrophenol, but tissue cut from fruit at the climacteric showed little stimulation. In both papers, it was suggested that the concentration of phosphate acceptor, adenosine diphosphate (ADP), limited the rate of respiration of immature fruit and that this limitation was removed as the fruit matured. Millerd *et al.* considered that, as the fruit (avocado) ripened, a compound was formed which uncoupled phosphorylation and respiration, thus preventing re-synthesis of adenosine triphosphate (ATP). With the consequently rising concentration of ADP, the limitation previously imposed on the respiration rate would be removed. Pearson and Robertson (1954) suggested that the increasing demands for the maintenance of protein and other unstable compounds in the cell might reduce the concentration of ATP and increase that of ADP to bring about the same effect on respiration rate. These workers showed that net protein synthesis would, in fact, continue after the rise in respiration. Hulme (1954) showed that the ratio protein/total nitrogen ran

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parallel to the rate of respiration of apple fruit. Marks, Bernlohr, and Varner (1957) have shown that the percentage esterification of $^{32}P$ reached a maximum in pre-climacteric tomato fruit and did not decrease until after the onset of pink colour; 2,4-dinitrophenol applied to green mature fruits inhibited the esterification, and prevented normal ripening.

The objects of the experiments reported here were to measure the concentration of ~P, protein nitrogen/total nitrogen ratios, and respiration rates at different stages of ripeness in avocado and tomato fruits. This work is preliminary to programmes on fruit ripening now being carried out independently at Davis, California, and at Melbourne, Australia.

## II. Materials and Methods

The avocados (var. Anaheim) used in these experiments were obtained from Murwillumbah, N.S.W. Fruits were harvested early in the morning and sent to Sydney by air freight. On the evening of the same day, they were examined at the laboratory and placed in a room at 25°C. Single fruits were placed immediately in the respiration chambers of a Pettenkofer apparatus, through which a current of $CO_2$-free air was passed continuously; measurements of the respiration rates began the next morning. Fruits were sampled immediately and after various intervals of time depending on the probable state of ripeness as deduced from the individual respiration curves and the rate of softening of the fruit. As fruits were taken for analysis, they were replaced in the chambers by other individuals which had been kept at the same temperature. The time of sampling individual fruits was chosen so that the various samples would represent different stages of ripeness and different points on an idealized climacteric curve of respiration. On removal from the respiration chambers, some of the tissue was used for the extraction of acid-soluble phosphates and some was dried for subsequent dry weight and nitrogen determinations. This procedure was followed with two different lots of fruit, providing data from a total of 13 individuals; these data were combined for discussion as one experiment.

Tomatoes (var. King of All) were harvested early in the morning from unheated glass-houses near Sydney and taken to the laboratory. The fruits were sorted into lots of eight different stages of ripeness, judged by external appearance as follows: (1) slightly immature, entirely green with little or no cork formation at the stem scar; (2) mature green; (3) first trace of colour; (4) about half-coloured; (5) orange; (6) light red; (7) full red; (8) soft ripe. Within each colour grade fruits were matched into paired samples of 12 fruits each. One of these samples was placed in a respiration chamber at 25°C, and the other was used for analysis. Respiration measurements were taken by the Pettenkofer method on the next 2 days after harvest, and these readings were averaged to represent the probable respiration rate of the paired sample; the latter was analyzed immediately after harvest for acid-soluble phosphates, dry weight, and nitrogen.

In order to minimize analytical variation due to tissue differences within the fruit, these procedures were followed: Each avocado was cut into 16 approximately

equal radial longitudinal slices, the first cut being made on the axis of bilateral symmetry. Alternate sixteenths were taken, one for drying and the other for phosphate extraction. With tomatoes, each fruit in the sample was also cut into 16 radial longitudinal slices. Where seeds were found they were removed. Every fourth slice was used for extraction, so that the total extraction sample consisted of four-sixteenths of each of 12 fruits. One-sixteenth of each fruit was taken for the drying sample.

The extraction of the acid-soluble phosphate was carried out at 0°C. The chopped fruit tissue was placed in a Waring Blendor with a measured volume of 1·2N $HClO_4$ (1·5 ml/g fresh tissue), and blended at full speed for 3 min; the sample was then centrifuged at approximately 1500 *g* for 15 min, the supernatant was poured off and filtered, and the filtrate was adjusted to pH 7·6 with KOH, the volume of filtrate taken being recorded before and after neutralization. After an interval of frozen storage, the $KClO_4$ precipitate was centrifuged off and the phosphate fractions were subsequently determined on the supernatant. High-energy phosphate was measured by the enzymic method of Slater (1953) as modified by Rowan (1958). Uridine triphosphate (UTP), which is included in the analysis of ~P, is present in both fruit, but chromatographic examination has shown that ATP is the predominant nucleoside triphosphate (Rowan, unpublished data). For nitrogen and dry weight determinations, the tissue was dried in an air-draught oven at 70°C to constant weight (12–16 hr); this was followed by drying in a vacuum oven for 6 hr, and the dried sample so obtained was ground in a Waring Blendor. Nitrogen determinations were by the Parnas-Wagner method (Turner 1949); the protein nitrogen was taken as that fraction insoluble in 70 per cent. ethanol.

## III. Results

### (*a*) *Avocados*

Drifts in respiration of different individual fruits appeared to follow a similar pattern, the rate decreasing to a pre-climacteric minimum, increasing to a climacteric maximum 24–36 hr later, and then decreasing again slowly. The time required to reach the pre-climacteric minimum varied between individual fruits. In presenting the data (Fig. 1(*a*)) the placing of the curve for each fruit on the time-axis is arbitrary, but is based on the comparative ripeness of the individual fruits at the time of the final sampling and an estimation of the probable time of occurrence of the rise from minimum to maximum. Figure 1(*b*) shows all estimations of the total ~P concentrations determined in the same fruits when they were taken from the respiration chambers.

These results show that the concentration of ~P tends to follow the drift of respiration rate from the time of the pre-climacteric minimum onwards, with a maximum ~P concentration at the peak of the climacteric. The concentration of protein nitrogen in the fruit was closely correlated with the concentration of ~P. However, the ratio of protein nitrogen to total nitrogen (Fig. 1(*c*)) rose steadily during ripening suggesting that the levels of ~P were adequate for this synthesis.

### (*b*) *Tomatoes*

The results for the tomatoes are shown in Figure 2. The respiration rates and analytical values are plotted against the eight stages of ripeness at which the fruits

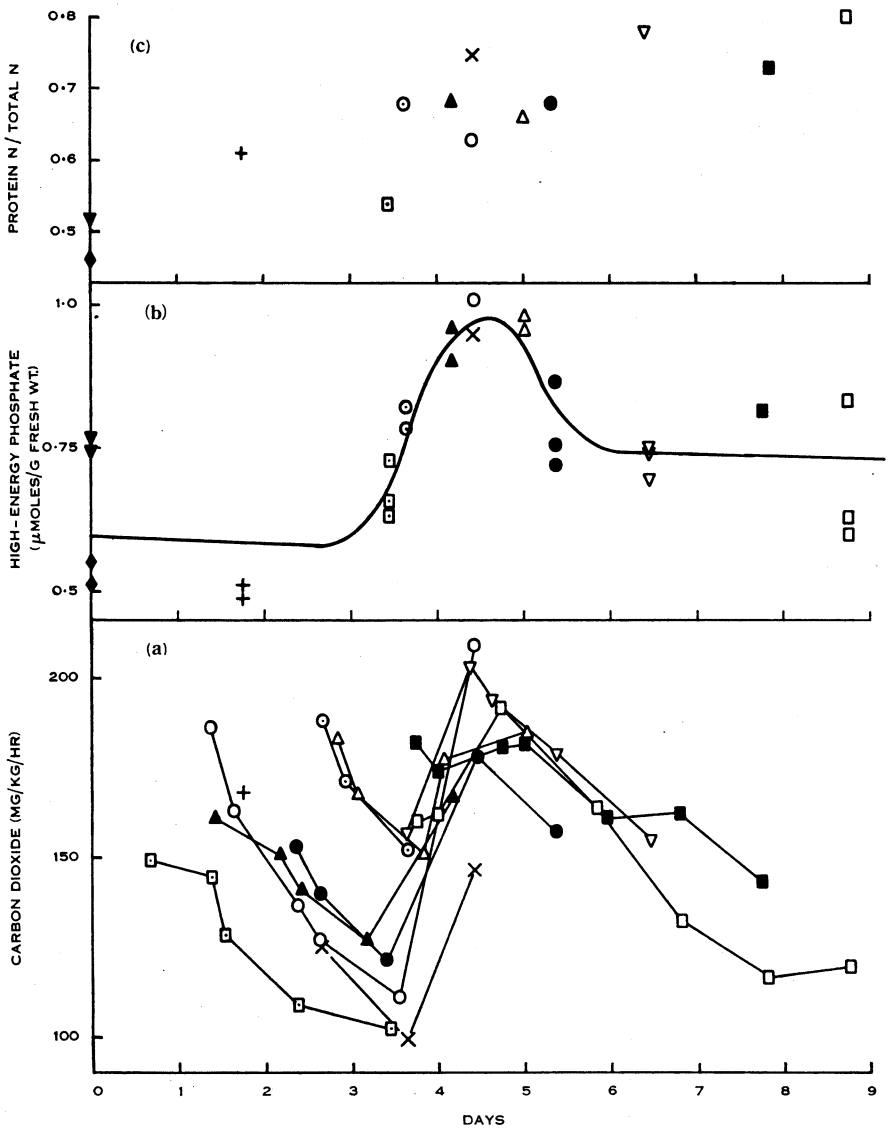


Fig. 1.—Changes in avocado fruit with time. (*a*) Rate of respiration per gram fresh weight; (*b*) concentration of high-energy phosphate per gram fresh weight; (*c*) ratio of protein nitrogen to total nitrogen.

were sampled. Using these stages of ripeness as an arbitrary time scale, the respiratory pattern shows the expected climacteric rise (Fig. 2(*a*)). It has been demonstrated with tomatoes that such a curve can be established by this means or

by following the respiration rate during ripening through the same colour stages of a single sample of mature green fruits (Beadle 1937; review in Workman, Pratt, and Morris 1957). A rapid rise in respiration is noted between stages 2 and 4. During this period there was no significant change in the concentration of ~P (Fig. 2(*b*)), though the rise in the mean between stage 2 and stage 4 is only just short of

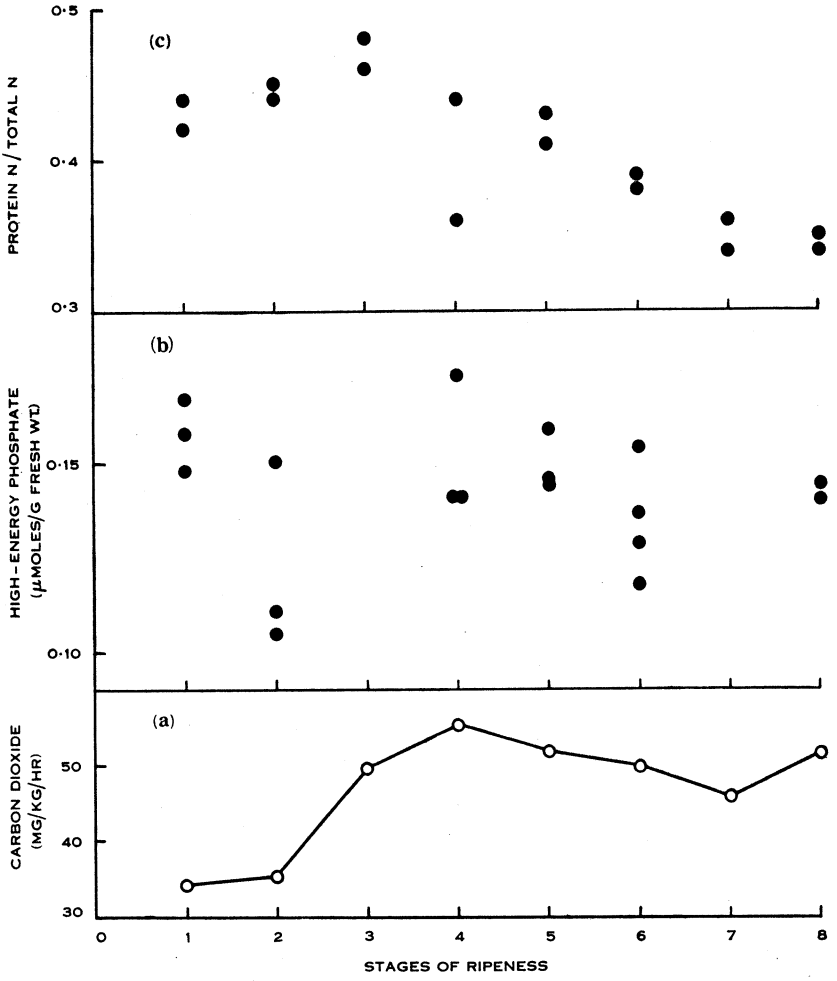


Fig. 2.—Changes in tomato fruit with stage of ripeness. (*a*) Rate of respiration per gram fresh weight; (*b*) concentration of high-energy phosphate per gram fresh weight; (*c*) ratio of protein nitrogen to total nitrogen.

being significant. After the peak in respiration, no significant change in concentration of ~P was observed. The ratio protein nitrogen/total nitrogen (Fig. 2(*c*)) increased in the first three stages and then decreased steadily in the later stages.

These results present some contrast to the results for the avocado. There is no spectacular increase in the concentration of ~P with the large increase in the

rate of respiration, and the ratio protein nitrogen/total nitrogen falls, although the rate of respiration remains high and the concentration of ~P does not change.

## IV. Discussion

The results with the two fruits, avocado and tomato, suggest that the relationships of energy-rich phosphate, respiration, and protein levels are not uniform. In avocado, ripening after picking, ~P increased with respiration, and the ratio protein nitrogen/total nitrogen increased throughout the experiment. In tomato, ripening on the plant, ~P did not rise significantly at the climacteric. The ratio protein nitrogen/total nitrogen increased to a maximum at one stage of ripeness before the maximum rate of respiration and decreased progressively in the later stages of ripeness. Marks *et al.* (1957) reported a marked decrease in the rate of esterification of $^{32}P$ after the climacteric. This is consistent with the steady state concentration of ~P shown in the present study only if the ratio ADP/ATP increases as the fruit matures.

The measurements of ~P reported here, and the work of Marks *et al.* suggest that the rise in respiration is probably not to be interpreted in the manner preferred by Millerd *et al.* (1953) because if uncoupling were taking place the concentration of ~P would be expected to fall as the respiration increased. The increase in ~P observed is consistent with the failure of dinitrophenol, in the work of Millerd *et al.*, to stimulate the respiration of tissue slices at the time of the climacteric, because the relative effect of dinitrophenol would be less when more ATP was being synthesized.

Pearson and Robertson (1954) suggested that the climacteric in apple fruit might be due to an increase in ADP. In the experiments reported here, ADP was not measured, but as ~P increased at the climacteric in avocado, it is probable that the concentration of ADP increased also. Such an increase in ADP would account for the increased respiration. Howard, Yamaguchi, Pratt, and Rowan (unpublished data) have recently observed an increase of ADP during the climacteric in ripening tomato fruit. During the period of increasing respiration, the ratio protein nitrogen/total nitrogen also increased, so increase in ADP might be due to, in part, increased rate of protein synthesis. After the climacteric, the avocado differed from the tomato because the ratio protein nitrogen/total nitrogen remained high, though the respiration rate fell markedly and ~P fell slightly.

While certain consistent changes have been noted, the initial cause of the increases accompanying the climacteric remained unexplained. It is likely that the rise in the respiration which has been called the climacteric and is associated with ripening has different causes in different fruits. The complexities may become obscured by discussions about "the cause" when causes are what should be sought. Some of the possibilities and the limitations of the present evidence have been reviewed by Laties (1957).

The increase in synthetic activity and the respiration rate observed in our experiments reflect a change in the pattern of metabolism which is under the control of some factor yet unknown. When the rate of synthesis of protein rises, the concentration of a number of enzymes might rise, including those concerned in

respiration, and further changes associated with ripening may be induced. Tager and Biale (1957), for instance, have shown an increase in activity of carboxylase and aldolase in banana fruit passing through the climacteric. It is perhaps relevant that Turner and Turner (1957) have shown that a rapid synthesis of starch in the developing pea seed is associated with an increase in concentration of starch phosphorylase.

## V. Acknowledgments

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