

Potentiating Effect of Pure Oxygen on the Enhancement of Respiration by Ethylene in Plant Storage Organs: A Comparative Study¹

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ABSTRACT

A number of fruits and bulky storage organs were studied with respect to the effect of pure O₂ on the extent and time-course of the respiratory rise induced by ethylene. In one group, of which potato (*Solanum tuberosum* var. Russet) and carrot (*Daucus carota*) are examples, the response to ethylene in O₂ is much greater than in air. In a second group, of which avocado (*Persea americana* Mill. var. Hass) and banana (*Musa cavendishii* Lambert var. Valery) are examples, air and O₂ are equally effective. When O₂-responsive organs are peeled, air and O₂ synergize the ethylene response to the same extent in parsnip (*Pastinaca sativa*), whereas O₂ is more stimulatory than air in carrots. In the latter instance, carrot flesh is considered to contribute significantly to diffusion resistance. The release of CO₂, an ethylene antagonist, is recognized as another element in the response to peeling.

The potentiating effect of O₂ is considered to be primarily on ethylene action in the development of the respiratory rise rather than on the respiration process *per se*. On the assumption that diffusion controls O₂ movement into bulky organs and the peel represents the major diffusion barrier, simple calculations indicate that the O₂ concentration in untreated organs in air readily sustains respiration. Furthermore, in ethylene-treated organs in pure O₂, the internal O₂ concentration is more than enough to maintain the high respiration rates. Skin conductivity to O₂ is the fundamental parameter differentiating O₂-responsive from O₂-nonresponsive fruits and bulky storage organs. The large preceding the earliest response to ethylene, as well as the magnitude of the ethylene-induced respiratory rise, is also controlled by permeability characteristics of the peel.

induced elevated respiration rates, or does O₂ play a role in the very process of respiratory augmentation? Furthermore, can the synergistic effect of O₂ be achieved by the simple expedient of diminishing the barrier to O₂ diffusion? In what follows, we examine a variety of fruits and storage organs with respect to the influence of O₂ on their response to ethylene and the effectiveness of peeling in air as a substitute for pure O₂.

MATERIALS AND METHODS

Plant Material. Potato tubers (*Solanum tuberosum* var. Russet) were grown by the Department of Vegetable Crops, University of California, Davis, and kindly provided by Professor Herman Timm. Tubers were stored at 7°C and preconditioned at room temperature for 7 d before use (8, 12). Avocado fruits (*Persea americana* Mill. var. Hass) were collected from a private orchard in Los Angeles. Untreated banana fruits (*Musa cavendishii* Lambert var. Valery) were shipped from Central America and obtained immediately upon unloading. The following storage organs were purchased from local markets and used immediately: sweet potato (*Iopomoea batatas*); parsnip (*Pastinaca sativa*); carrot (*Daucus carota*); rutabaga (*Brassica napus*); turnip (*Brassica rapa*); Jerusalem artichoke (*Helianthus tuberosus*); red beet (*Beta vulgaris*); jicama (*Pachyrhizus erosus*); Daikon radish (*Rhaphanus sativus*); and horseradish (*Armoracia rusticana*). Sausage fruit (*Kigelia pinnata*) was collected from trees on the University of California, Los Angeles, campus.

Intact storage organs were treated with 10 μl/l ethylene in the presence of air or pure O₂ at room temperature, as previously described (15). CO₂ output was monitored with a Beckman IR CO₂ analyzer.

RESULTS

O₂ Responsiveness of Ethylene-Treated Fruits and Storage Organs.

Relative Effect of Air and O₂. The prototypical synergistic action of ethylene and O₂ is demonstrated in potato in Figure 1. As previously reported (8, 12), potato tubers respond to ethylene in air, but the response is exaggerated in the presence of pure O₂. Other bulky organs fall into two groups. One group behaves much as potato, wherein O₂ is more effective than air in evoking the ethylene response (Fig. 2). The lag period preceding overt response is frequently shorter than in potato. In the second group, foremost in which are avocado and banana, air and O₂ are equally effective (Fig. 3). The indifference of avocado to elevated O₂ concentrations in connection with ethylene responsiveness was shown earlier by Biale (2).

Effect of Peeling on O₂ Responsiveness. The primary resistance to gas penetration into bulky organs is most often the skin (4, 6,

The quintessential fruit ripening hormone, ethylene, not only induces the respiratory climacteric in fruits (2, 13, 15) but also elicits a similar respiratory burgeoning in bulky storage organs (11, 13, 16). A second consequence of ethylene action in potato is that the respiration of fresh slices and mitochondria from ethylene-treated tubers is CN-resistant (8, 12), in marked contrast to the CN-sensitivity of slices and mitochondria from untreated tubers. With the provocative discovery by Rychter *et al.* (12) that the effect of ethylene is enhanced by pure O₂, questions arise with respect to the ubiquity of the phenomenon and the role of O₂ therein. Do increased O₂ levels serve simply to sustain ethylene-

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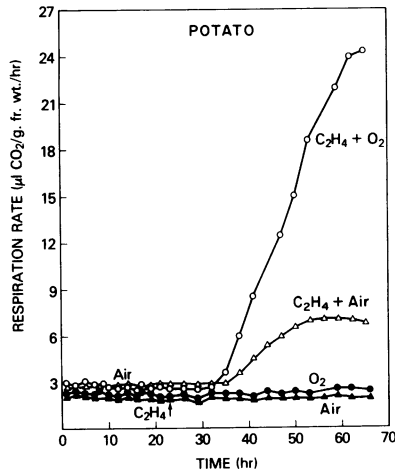


FIG. 1. Effect of ethylene in the presence of air or O_2 on potato tuber respiration. Tubers were kept in air for 24 h and subsequently treated with $10 \mu\text{l/l}$ ethylene in air or pure O_2 , as described in "Materials and Methods." CO_2 evolution was monitored continuously.

7). Accordingly, the effect of peeling was investigated on three O_2 -responsive organs, viz. parsnip, carrot, and red sweet potato. The effect of peeling on parsnip and carrot is depicted in Figure 4, while the effect on sweet potato is noted in Figure 5. The ethylene-induced respiration rise in air in peeled parsnips is greater than that in unpeeled roots in O_2 , in spite of the fact that the internal O_2 concentration is almost surely higher in the latter instance (see "Discussion"). Pure O_2 shows little advantage over air in the peeled organ. Accordingly, the effect of peeling that cannot be explained solely by O_2 enhancement may well be due to the escape of CO_2 , since CO_2 has been shown to be an antagonist of ethylene action (5). The modest rise with time in the peeled organ in the absence of ethylene, both in air and in O_2 , is attributable to the so-called wound-induced respiration at the peeled surface, much as that observed in thin slices (8). When the wound-induced respiration is considerable in slices, O_2 is known to lead to more highly developed rates than in air (9). In carrot, peeling alone does not evoke a maximal respiratory response to ethylene; pure O_2 is required as well (Fig. 4). The basis of this seeming anomaly is taken up in the "Discussion."

Sweet potato responds to peeling much as does parsnip, air and O_2 being equally effective in peeled roots (Fig. 5). Removal of the peel also shortens the time until the first evidence of ethylene responsiveness and increases the rate of responsiveness thereafter. The ultimate maximum is much the same, however, in unpeeled roots in O_2 and in peeled roots in air or O_2 . Where air and O_2 are equally effective, as in avocado (Fig. 3), peeling has been shown to be without effect (1).

Effect of Ethylene Concentration on the Kinetics of the Induction Process. The virtual abolition by peeling of the lag period in the initiation of the ethylene-induced rise in sweet potato (Fig. 5) suggested that the penetration of ethylene may be yet another factor controlled by the major diffusion barrier, the skin. Accordingly, the rate of ethylene penetration in unpeeled sweet potatoes was increased by raising the external ethylene concentration. As seen in Figure 6, the attainment of the maximal induced rate is hastened by increasing the diffusion gradient; however, the maximal rate is ultimately the same in $10 \mu\text{l}$ ethylene per L and $1,000 \mu\text{l}$ ethylene per L. Thus, as might be expected, the skin is a barrier to O_2 and ethylene diffusion alike (as well as to the escape of CO_2).

DISCUSSION

Soon after it was demonstrated that ethylene evokes a burgeoning of respiration in potatoes (11, 16) and other bulky storage

organs (13, 17) akin to the respiratory climacteric in fruits, it was shown that the stimulation of respiratory activity by ethylene in potatoes is markedly enhanced by pure O_2 (12). When small potato tubers the size of a pea developed in culture are treated with ethylene, however, air and O_2 are equally effective (H. W. Janes, personal communication). Under ordinary conditions, air is adequate to sustain respiration fully, since elevation of the external O_2 concentration has no effect on organ respiration rate (10). In this paper, we have shown that the synergistic effect of ethylene and O_2 is widespread, and, in this connection, we have addressed two main questions. Is pure O_2 effective because an adequate O_2 supply to sustain the high respiration rates in ethylene-stimulated organs is limited by diffusion? Does the ethylene-stimulated response to pure O_2 reflect the operation of a low-affinity (high $K_m^{O_2}$) O_2 system involved in ethylene-induced respiratory development? Whatever the case, the ethylene-induced O_2 demand can be met either by providing oxygen in lieu of air (Fig. 2) or by peeling (Figs. 4 and 5).

In most fruits (4) and bulky storage organs (6, 7), the peel or periderm is the major barrier to gas diffusion, gas exchange taking place through gas-filled pores, or lenticels (4). Peel permeability reflects the number and size of the latter. When the resistance of the peel is high, O_2 (Fig. 2) or peeling (Fig. 4, 5) increases the respiration of ethylene-treated organs. When the peel is relatively porous (as in avocado and banana) air and O_2 are equally effective (Fig. 3).

The bulk of fruit and storage organ respiration is seemingly sustained by an oxidase with high O_2 affinity, viz. Cyt oxidase (2, 10, 19). In potato, there is a 24% drop in respiration as the internal pO_2 drops from about 18% to 4% (10). Throughout this modest decline in respiration rate, there is no change in R.Q. A precipitous drop in respiration rate occurs as the internal pO_2 drops below 1% (10). In banana, there is no accumulation of glycolytic products at external O_2 concentrations as low as 3% (3). Thus, although a low-affinity oxidase may contribute to fruit or tuber respiration (10), whether in the form of the alternative path (14) or the so-called residual respiration (18, 20), there is seemingly adequate O_2 to sustain the energy generating Cyt path (19). In fact, when the respiratory climacteric is elicited by CN and respiration is accordingly mediated by the alternative path with a $K_m^{O_2}$ some 10 times greater than that of Cyt oxidase (13), there is still no sign of O_2 deficiency as evidenced by respiration rates as high as those evoked by ethylene (15). Accordingly, one is led to the view that the response to elevated O_2 or to peeling is due to the participation of a high $K_m^{O_2}$ system in the development of the ethylene-induced respiration (5). In fact, when potato tubers are treated with ethylene- O_2 for 24 h, and the gas phase is then changed to ethylene-air, the ethylene-induced respiration remains at its maximal level (R. E. Christoffersen, G. G. Laties, unpublished). The foregoing observations provide the basis for controlled atmosphere fruit storage practices that involve low O_2 environments.

A simple but convincing calculation establishes that the O_2 concentration in bulky storage organs in air must be high enough to saturate Cyt oxidase, which seemingly is fully active at an internal oxygen concentration of 1% or less (10, 19). Somewhat higher saturating concentrations in slices are the result of H_2O injection of the air spaces of the superficial cell layers (19, 21). Let us assume that oxygen enters a potato tuber, in accordance with Fick's law (4), and that the resistance to penetration (R) resides in the skin (4, 6, 7). Burton has established that the drop in O_2 concentration from beneath the skin to the center of a potato tuber is inconsequential (6). It is axiomatic that, in the steady state, the rate of O_2 penetration equals the rate of its utilization.

⁴ Abbreviations: pO_2 , partial pressure oxygen; R.Q., respiratory quotient.

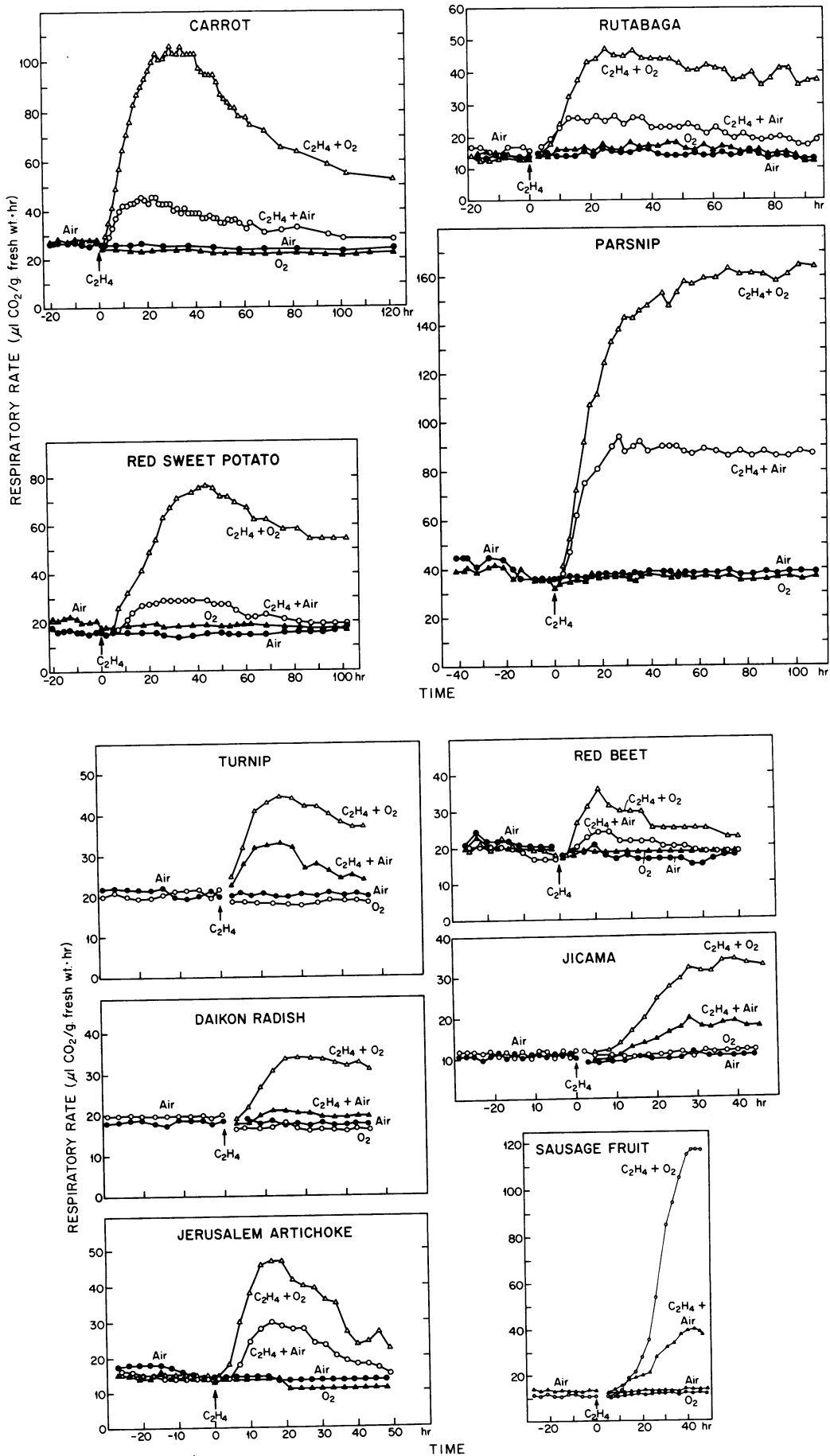


FIG. 2. Effect of ethylene in the presence of air or O_2 on the respiration of O_2 -reponsive bulky storage organs. Sausage fruit is in the preclimacteric stage.

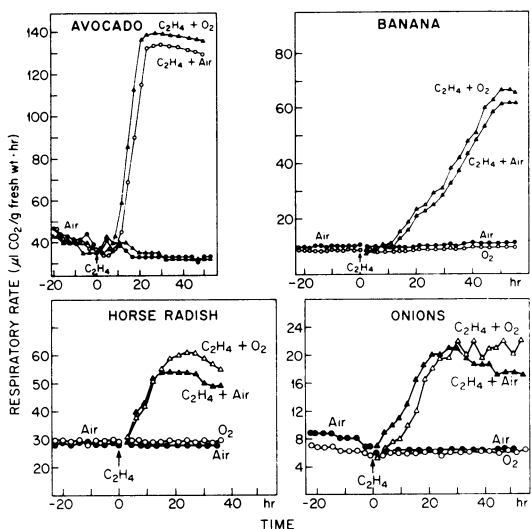


FIG. 3. Effect of ethylene in the presence of air or O₂ on the respiration of O₂ nonresponsive fruits and storage organs.

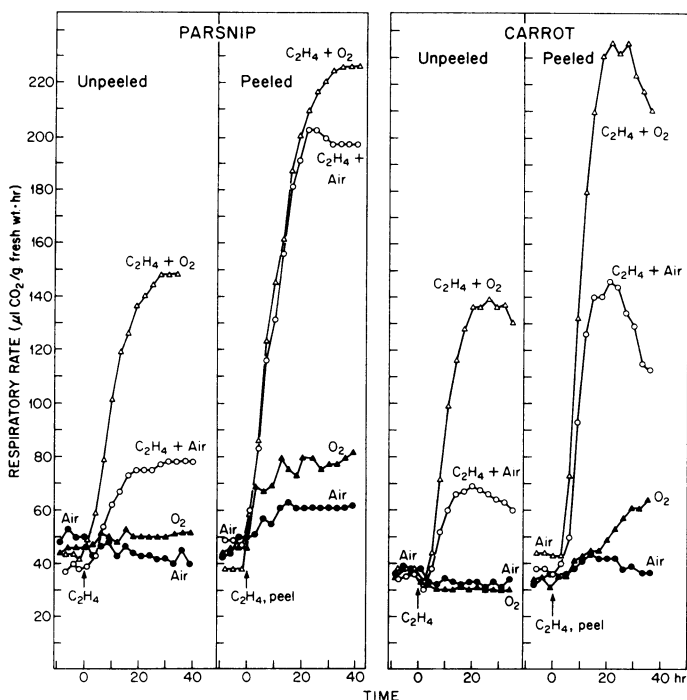


FIG. 4. Effect of ethylene in the presence of air or O₂ on the respiration of unpeeled and peeled parsnip and carrot roots. Roots were peeled, and ethylene was added where indicated by arrows. Experimental details are as for Figure 1.

Thus, for the entire tuber:

$$\text{Respiration rate} \cong \frac{A}{R} (p_{O_2}^o - p_{O_2}^i) \quad \text{eqn 1}$$

where the respiration rate defines the amount of O₂ (e.g. cm³) used per unit time, *t*; *A* represents the area of the tuber surface (cm²); *R* has the dimensions cm⁻¹ *t*; and *p*_{O₂}^o and *p*_{O₂}ⁱ represent the external and internal O₂ concentrations, respectively, expressed as a fraction, the partial pressure, or cm³/cm³_{total gas}. The reciprocal of the resistance can be taken as the conductivity (*C*), and, with the surface area fixed, we have:

$$\text{Respiration rate} \cong C(p_{O_2}^o - p_{O_2}^i) = C\Delta p_{O_2} \quad \text{eqn 2}$$

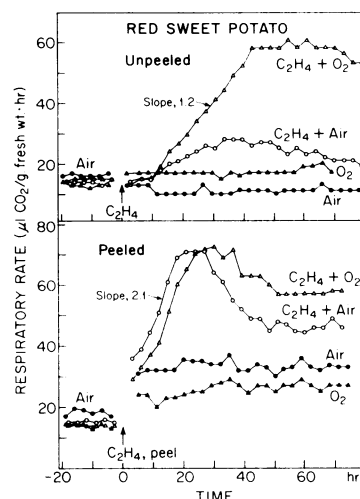


FIG. 5. Effect of ethylene in the presence of air or O₂ on the respiration of unpeeled and peeled red sweet potato roots. Experimental details are as for Figure 1.

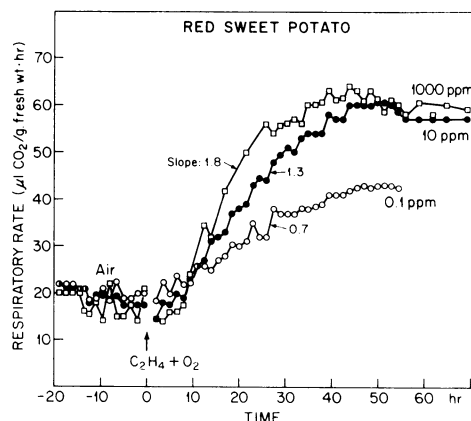


FIG. 6. Effect of ethylene concentration in the presence of pure O₂ on the respiration of intact red sweet potato roots. Roots were kept under a stream of humidified air for 24 h, and, at the time indicated by the arrow, ethylene at various concentrations in O₂ was introduced.

and

$$\Delta p_{O_2} \cong \frac{\text{Respiration rate}}{C} \quad \text{eqn 3}$$

Taking a tentative value of 10% (0.1 atm) for *p*_{O₂}ⁱ of an untreated tuber in air, Δp_{O_2} would be 0.11 atm (21%–10%). Let the respiration rate in response to ethylene in air rise some 3-fold over that in air alone (Fig. 1), and let it be assumed that *C* does not change. To sustain a 3-fold rate increase with constant *C*, Δp_{O_2} must increase three times (eqn 3), from 0.11 atm to 0.33 atm. Since Δp_{O_2} in air can be 0.21 atm at best, it is obvious that the internal O₂ concentration must have been greater than 10% at the outset. For example, if the initial internal O₂ concentration were 15% ($\Delta p_{O_2} = 0.06$ atm), it would drop to 3% ($\Delta p_{O_2} = 0.18$ atm) following a 3-fold increase in the respiration.

When respiration rates in response to ethylene and O₂ are high and considerably exceed the rates developed in ethylene and air (Fig. 2), O₂ may serve to sustain the high rates as well as to implement ethylene-induced respiratory development. For reasons noted above, however, the dominant cause for the effectiveness of high concentrations of O₂ in synergizing ethylene action is the involvement of O₂ in a high *K_m*^{O₂} process other than respiration

(5). We have established that oxygen effectiveness in implementing the ethylene response in potato increases with concentration with apparent Michaelis-Menten kinetics yielding a $K_m^{O_2}$ of 160 μM (14% O₂) (R. E. Christoffersen, G. G. Laties, unpublished). Since increasing O₂ continues to be effective at concentrations above those needed to saturate respiration, as estimated by simple calculations of the type made above, a second role of O₂ is indicated. Whereas the so-called residual, or ground, respiration rises with pO_2 above levels that saturate Cyt oxidase and the alternative path (20), the equivalence of O₂ and air in sustaining high respiration rates in fruits with porous skins (*viz.* avocado and banana, Fig. 3) raises doubts about the residual respiration as the explanation of the high respiration rates induced by ethylene/O₂ (*cf.* Ref. 20).

Inasmuch as respiration rates developed in O₂ in response to ethylene in unpeeled organs are matched or exceeded in air in peeled organs (*viz.* parsnip, Fig. 4; sweet potato, Fig. 5), peeling seemingly acts to remove the major diffusion barrier to O₂, and it may serve to lower the internal CO₂ concentration, as well. This presumption is further strengthened by the quickening of respiratory development by peeling (Fig. 5). An apparent paradox lies in the observation that the developed respiration of peeled carrots in ethylene-O₂ is higher than that of peeled carrots held in ethylene-air, the latter equaling the rate of unpeeled carrots held in O₂. On the basis of our simple presumptions, the internal O₂ concentration in unpeeled carrots in ethylene-O₂ should be higher than that in peeled carrots in ethylene-air. Accordingly, either the rate in the former should be higher or there should be no additional stimulation by O₂ in peeled ethylene-treated roots. The answer may lie in the possibility that, in carrot, the flesh contributes significantly to diffusion resistance (4). By our measurements, carrots have about 8% intercellular gas space, whereas sweet potatoes have 18% and parsnip, 35%. In addition, however, there is the possibility that an inhibitor, specifically the ethylene antagonist, CO₂ (5), is lost on peeling or that respiration is otherwise stimulated by peeling for reasons other than O₂ enhancement. In this connection, it is to be noted that the immediate respiration rate following slicing of potato tubers is not attributable to an increase in tissue O₂ (21).

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