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# Oxygen Transport and Root Respiration of Maize Seedlings

## A QUANTITATIVE APPROACH USING THE CORRELATION BETWEEN ATP/ADP AND THE RESPIRATION RATE CONTROLLED BY OXYGEN TENSION

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## ABSTRACT

**Oxygen uptake and ATP/ADP ratio were simultaneously monitored during incubation of excised maize (*Zea mays* L. INRA 508) root tips under varying $O_2$ partial pressure. Both variables were independent of $O_2$ tension until a critical $O_2$ pressure was reached. Below this pressure, ATP/ADP ratio and respiratory rate declined. However, in tissues having a high glycolytic capacity, the correlation between the ATP/ADP ratio and the respiratory rate breaks down as $O_2$ tension decreases, due to the increasing contribution of fermentative processes.**

**In presence of 2 millimolar NaF, the ATP/ADP ratio varied solely as a function of the $O_2$ tension, without interference by fermentative activity, and a close correlation links the ATP/ADP ratio and the respiratory rate of excised maize root tips over the whole range of $O_2$ tensions tested.**

**Using this correlation, a method is proposed for the quantitative determination of the relative cellular respiratory rate permitted by $O_2$ transport from the aerial part of young maize seedlings along the seminal root placed in an anoxic environment.**

**Data are presented which demonstrate the preeminent part played by the cortical air spaces in $O_2$ transport. Their contribution to respiration was high in the first few centimeters nearest the seed and decreased rapidly as the distance from the aerated source increased. It is concluded that $O_2$ transport might contribute to the survival or to adaptive responses of root tissues in flooded soils but that the ventilation of the apical growing zone was inadequate to sustain the growth.**

It is now widely accepted that $O_2$ can move through the gas spaces of plant tissues (for review, see 19). Some water plants, such as yellow water lily (2), have developed anatomical devices very efficient in forcing $O_2$ to the roots and rhizomes buried in the anaerobic sediment several meters below the water surface. In stalked bulrush rhizomes, another marsh plant, $O_2$ provision by diffusion transport is very effective and the internal $O_2$ concentration is hardly affected by the lack of $O_2$ in the rhizome environment (17). The enhancement of the formation of cortical air spaces is another typical adaptive response of mesophytes to anoxic conditions (3, 8). However, the actual physiological significance of this internal transport is still under debate.

For some authors (4, 5), the excretion of $O_2$ into the rhizosphere means that the amount transported exceeds the consumption by the tissues. For these authors, the $O_2$ will meet most of the respiratory requirements of the seminal root of young seedlings over a distance of up to 9 cm. However, according to Crawford (1), it could be questioned that the diffusion coefficient in water being some $10^4$ times lower than in air, $O_2$ would tend to flow through the less resistant pathways, namely the gas spaces, without penetrating significantly into the cells. Its principal favorable effect would not be so much to feed the respiration but to detoxify the surrounding medium by oxidizing the reduced ions. Using the $O_2$-dependent chemiluminescence of plant tissues (18) and inhibitors of respiration (10), Vartapetian *et al.* came to the conclusion that the transported $O_2$, if any $O_2$ transport exists, can support only a very small part of the normal aerobic metabolism of cotton or pumpkin roots.

The debate on the actual significance of $O_2$ transport for root metabolism arises because the methods used do not quantify the amount of $O_2$ actually used in the respiration of tissues along the root *in situ*.

In earlier papers, it has been shown that the value of the ATP/ADP ratio or of the energy charge in lettuce seeds (11, 14) was controlled by the $pO_2$[1]. A method has been proposed, which uses the energy charge value as an indirect assessment of the respiration permitted by the internal $O_2$ transport in tips of intact rice and maize roots placed in an anaerobic environment (13). This method has been used to demonstrate the efficiency of the internal ventilation in bulrush (17). This paper is a refinement of this approach. It shows that in the presence of fluoride a close correlation links the ATP/ADP ratio and the respiratory rate controlled by the $pO_2$ in maize root tips. This correlation is used to demonstrate an $O_2$ transport and to quantify its involvement in the respiratory metabolism along the seminal root of young maize seedlings.

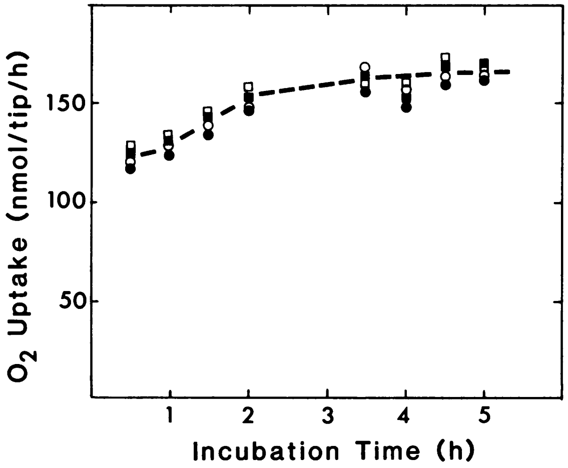

FIG. 1. Rate of $O_2$ uptake of 0.5-cm excised maize root tips in absence (●), or in presence of $10^{-3}$ M NaF (■), 3.3 $10^{-3}$ M NaF (○), $10^{-2}$ M NaF (□). The incubation medium contained 0.1 M glucose. Each point represents the mean of two independent determinations.

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[1] Abbreviation: $pO_2$, oxygen partial pressure.

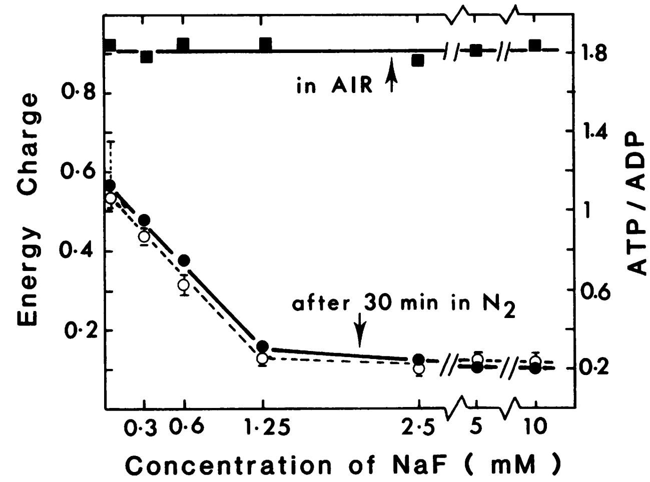

FIG. 2. Effect of various NaF concentrations on adenine nucleotide ratios of 0.5-cm excised maize root tips. Energy charge after 2 h in air (■); ATP/ADP (●) and energy charge (○) after 2 h in air followed by 30 min in $N_2$. Each point is the mean of three determinations on three independent samples, and the bars represent the range of the measurements.

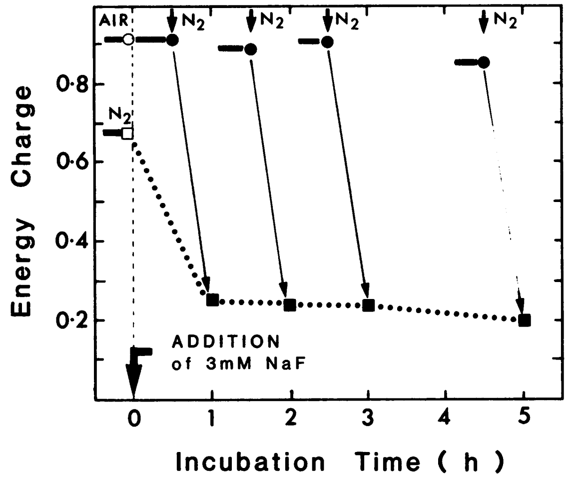

FIG. 3. Energy charge of 0.5-cm excised root tips as a function of incubation time in presence of 3 mM NaF. Energy charge value before addition of NaF in air (○), in $N_2$ (□). The fluoride was added at time zero, and energy charge was determined after various incubation times in air (●) or after the same time in air followed by 30 min in $N_2$ (■). Each point represents the mean of three determinations on three independent samples.

## MATERIALS AND METHODS

Maize seeds (*Zea mays* L. INRA 508) were first infiltrated under vacuum with 2 mM $CaCl_2$ and germinated for 3 d at 25°C between sheets of filter paper soaked with 2 mM $CaCl_2$ and inclined at an angle of 45°.

In some experiments, the seedlings were infiltrated three times under vacuum before the $O_2$ transport studies, in order to fill the gas spaces with the incubation medium. This medium, already described (15), was supplemented with 0.1 M glucose and 2 mM NaF.

**Respiratory Measurements.** The effect of NaF on the rate of $O_2$ uptake was studied by adding it to the medium in a Warburg respirometer as in (15). The effect of $pO_2$ on $O_2$ uptake was studied by polarographic methods. One hundred tips were randomized by shaking for 1 h in an aerated incubation medium (15) containing 2 mM NaF and 0.1 M glucose. After blotting off, they were placed by groups of ten on discs of filter paper imbibed with the medium containing NaF and glucose and introduced into the 3-ml chamber of a polarographic cell maintained at 25°C by a water jacket. The signal recorded by a Clark electrode (Beckman 802 BGA) connected to an $O_2$ analyzer (Beckman Field Lab) was analyzed every 30 s. The rate of $O_2$ uptake was plotted against the $pO_2$.

**Effects of $pO_2$ and NaF on Adenine Nucleotide Ratios.** Series of gas mixtures of $O_2$ and $N_2$ at various partial pressures were prepared. The $O_2$ content was checked with a zirconium $O_2$ analyzer (Ste Mecanalyse, Gaz Purs SARL, France) for $pO_2$ lower than 20 kPa and with a polarographic $O_2$ analyzer (Beckman OM 14) for higher $O_2$ tensions.

Five tips, preincubated for 1 h in the nutrient solution containing NaF as desired and 0.1 M glucose, were blotted off, placed in 50-ml beakers, and flushed for 1 h with a continuous stream of $N_2$ or gas mixtures ($O_2/N_2$) saturated with water as described in Saglio *et al.* (16). The samples were then rapidly frozen by cold diethyl-ether (−100°C) as in (16). After removing the ether by decantation, the tips were extracted and assayed for nucleotides according to Saglio and Pradet (15). Less than 10% of each nucleotide (ATP, ADP, or AMP) is lost during the extraction by TCA, and the values of the ATP/ADP ratios or of the energy charge are not significantly modified by these losses, as compared with standard mixtures.

This procedure which appears to be the most reliable as compared to others (12) has been used to study the effect of increasing NaF concentration on the energy charge of excised root tips. Preincubation in air lasted 2 h followed by 30 min more in air or in $N_2$.

**Study of $O_2$ Transport along the Seminal Root.** The seminal roots (about 8 cm) were inserted in a hole made in the cap of a disposable plastic tube containing the nutrient medium. A gasket of sealing compound permitted a complete separation between the root and the atmosphere of the aerial part. Pure gaseous $N_2$, containing less than 50 μl $O_2$/l, was bubbled through the root medium. The seeds remained either in air or in a compartment flushed with $N_2$.

After 1 h of this treatment, the seedlings were quickly frozen by transferring them to cold diethyl-ether (−100°C). The contact of the roots with air lasted less than 2 s. Then, the frozen roots were cut into 1-cm sections and assayed for adenine nucleotides as in Saglio and Pradet (15). Each sample was constituted by three homologous sections taken from three different roots. Three independent samples were analyzed for each section.

## RESULTS

**Effect of NaF on Respiration and Adenine Nucleotide Ratios.** The effect of various NaF concentrations on $O_2$ uptake by maize root tips is shown on Figure 1. The respiratory rate was not affected by concentrations of up to 10 mM NaF, even after 5 h of incubation. During the same time, the respiratory quotient (RQ = $CO_2$ output/$O_2$ input), measured in presence of 2 mM NaF, remained very stable at values close to 1 as in the control.

In air, the energy charge value was not affected by concentrations of up to 10 mM NaF and remained high as in the control (Fig. 2). In $N_2$, on the contrary, these values were dependent upon NaF concentrations (Fig. 2). The decrease was approximately linear over the concentration range up to 1.25 mM NaF. Higher NaF concentrations did not induce any further significant decrease. The effect of NaF on the value of adenine nucleotide ratios in $N_2$ was maximum within 30 min (Fig. 3) but 4 h of incubation in 3 mM NaF had little effect on the nucleotide ratios of excised root tips in air (Fig. 3). The amounts of ATP, ADP, and AMP actually measured in these experiments were essentially the same as those reported in Saglio and Pradet (15) and Saglio *et al.* (16).

**Rate of $O_2$ Uptake and Values of Adenine Nucleotide Ratios as a Function of $pO_2$.** It appears from the results presented in Figure

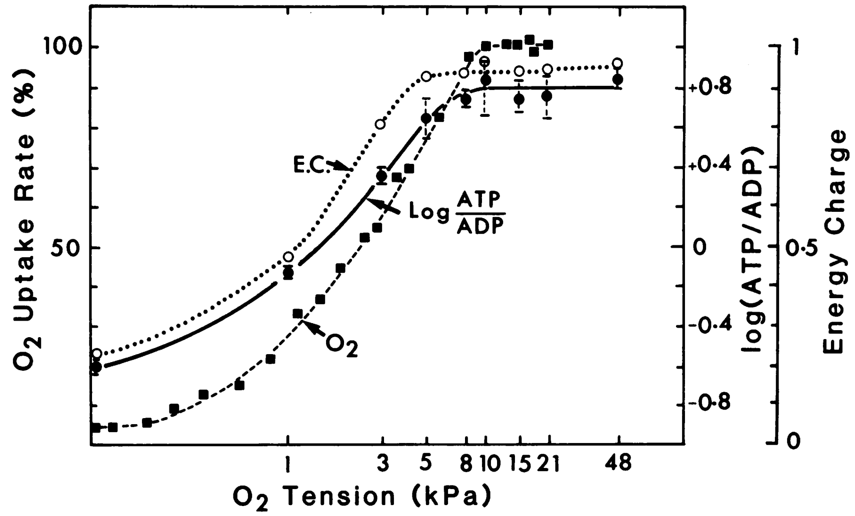

FIG. 4. Rate of $O_2$ uptake and nucleotide ratios of 1-cm maize root tips as a function of $pO_2$ in presence of 3 mM NaF: percent of the maximum rate of $O_2$ uptake (■) which corresponded to 0.24 μmol $O_2$/tip·h; energy charge (○) and log (ATP/ADP) (●). $O_2$ tensions are reported on a logarithmic scale in order to enlarge the scale for low $O_2$ pressures. Apart from $O_2$ uptake rate which has been calculated from the polarographic trace of $O_2$ consumption, each point represents the mean of three determinations on three independent samples. Bars represent the range of the measurements when it was larger than the symbols.

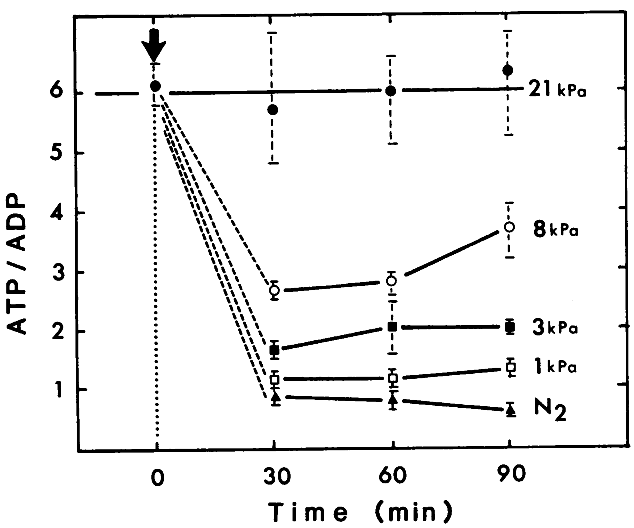

FIG. 5. Stabilization of the ATP/ADP quotient in 1-cm excised maize root tips exposed to various $O_2$ tensions. The arrow corresponds to the beginning of gas flow: (●), 21 kPa; (●) 8 kPa; (■), 3 kPa; (□), 1 kPa; (▲), $N_2$. Each point is the mean of three determinations on three independent samples. Bars represent the range of the measurements.

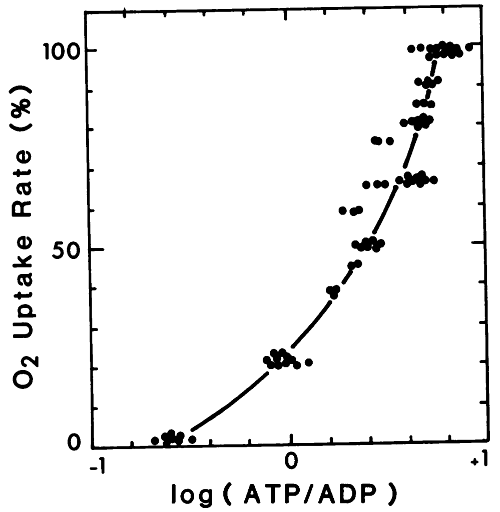

FIG. 6. Correlation between the rate of $O_2$ uptake expressed as a percent of the maximum, and the log (ATP/ADP) in excised maize root tips. Each plot corresponds to the mean of three determinations on one sample. The range was smaller than the symbols.

4 that the ATP/ADP ratio as well as the adenylate energy charge remained roughly stable, as did the respiratory rate for $pO_2$ higher than 10 kPa (about 10% $O_2$ at atmospheric pressure). For lower $O_2$ tensions, both nucleotide ratios and respiratory rate fell. The lower value reached in the absence of $O_2$ but in the presence of 2 mM NaF was 0.25 for ATP/ADP and about 0.2 for energy charge. Under the same conditions, but in the absence of inhibitor, these values were higher than 1 for ATP/ADP and about 0.6 for the energy charge. The values reached by the ATP/ADP ratios at various $pO_2$ remained roughly stable for at least 90 min (Fig. 5).

Simultaneous measurements of ATP/ADP and respiratory rate as a function of $pO_2$ under various conditions were made in a number of experiments not reported here. We always obtained data similar to those reported in Figure 4. The only difference was the value of the $pO_2$, which induced the decrease of both ATP/ADP ratio and $O_2$ uptake rate. This value which depends on the presence of diffusional barriers acting against $O_2$ penetration into the tissues may vary with experimental conditions.

From the data obtained in these experiments, the respiratory rate has been plotted *versus* log (ATP/ADP) in Figure 6, showing the close correlation which linked the two parameters. The log (ATP/ADP) has been used instead of ATP/ADP in order to maintain a similar interval in the deviations recorded in the determinations of the ATP/ADP ratio over the whole range of its values. This deviation tended to enlarge for high values, probably because of the increasing difficulties in assaying very low amounts of ADP. In spite of this relative uncertainty, the correlation was better than with the energy charge, which lacked sensitivity at high $pO_2$.

**Contribution of $O_2$ Transport to Respiration.** The very fast increase of the ATP/ADP ratio value during the transfer of an anaerobic tissue to air (Fig. 7), shows that a contact with air as short as 5 s should introduce some error in the evaluation of the actual values of the ratio in $N_2$. In the following experiments, this

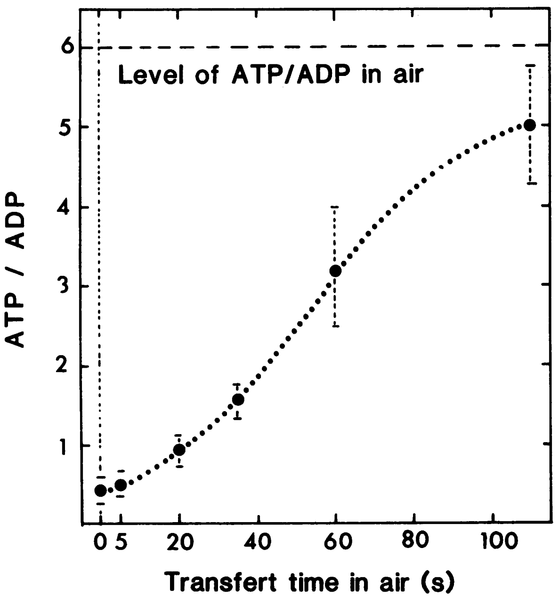

FIG. 7. Rise of ATP/ADP ratio of maize root tips during the transfer from $N_2$ to air. Bars represent the SD of the mean of three independent determinations.

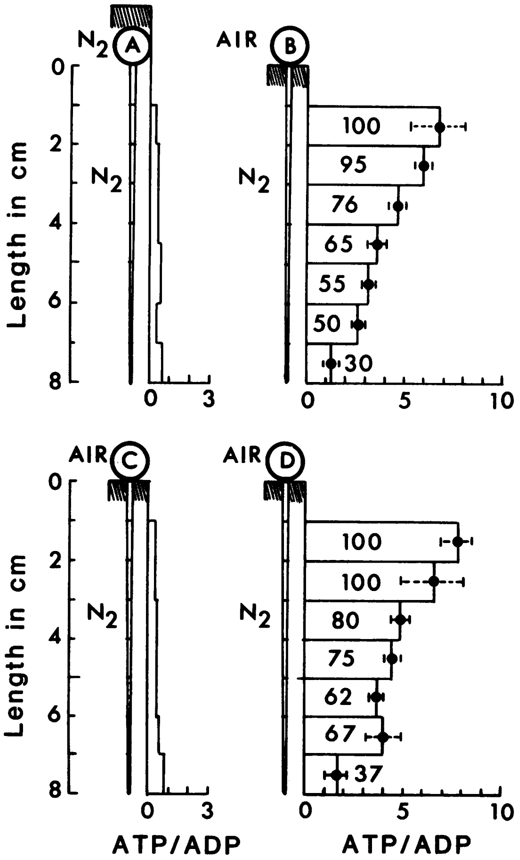

FIG. 8. Effect of $O_2$ transport on the value of ATP/ADP quotients along the root of young maize seedlings maintained in $N_2$. A, B, and D were not infiltrated. C was infiltrated. Bars represent the SD of the mean of three independent determinations. The numbers in the frames are the percent of the maximum respiratory rate deduced from the correlation established between ATP/ADP values and $O_2$ uptake rate (Fig. 6).

transfer time always lasted for less than 2 s and the variation of the ATP/ADP value was negligible.

The respiratory contribution of $O_2$ transport along the seminal root of young maize seedlings maintained in $N_2$ was quantified using the correlation established between the ATP/ADP quotient and the $O_2$ uptake rate. In infiltrated seedlings with the seed in air and the root in $N_2$ (Fig. 8C), the ATP/ADP ratios were very low all along the root as they were in noninfiltrated seedlings whose roots and seeds were both maintained in $N_2$ (Fig. 8A). These low values are typical of anaerobic tissues and indicate that no $O_2$ was transported. In contrast, when the root of noninfiltrated seedlings were placed in $N_2$ and the seed in air, the values of the ATP/ADP ratio varied along the root (Fig. 8, B and D). High values in the first 2 cm nearest the seed, reflect a high contribution of the transported $O_2$ to the respiration, which was close to the maximum. The values of the ATP/ADP ratio then decreased as the distance from the seed increased, indicating a parallel decrease of the respiratory rate, which represented only about 30% to 40% of the maximum rate in the tip situated at 8 cm from the seed. Such a percentage corresponded roughly to the respiratory rate of tips placed at a $pO_2$ of 2 kPa.

## DISCUSSION

Relationships between the value of the ATP/ADP ratios and the respiratory rate have been obtained in various systems *in vitro* (for review, see 7) and *in vivo* (14). They are largely dependent on the experimental system used, being either positive or negative. The use of the $^{31}P$ nuclear magnetic resonance technique for *in vivo* measurements has recently questioned the general concept that the ATP/ADP ratio could be somehow tied to respiratory control (7). However, whether or not this work supports the concept, it does show that there is a correlation between this ratio and the respiration rate under limiting $O_2$ tensions, especially when fluoride is present.

The effect of NaF under anoxia indicates an inhibition of glycolysis. In contrast to uncouplers or to cyanide, which lower the value of nucleotide ratios in air (6), NaF had no effect on these ratios, nor on the rate of $O_2$ uptake. The results indicate that the oxidative phosphorylation pathway was not affected by NaF treatment and that the flux of metabolites in aerobic conditions from glucose to $O_2$ was not slowed down in spite of NaF interaction with glycolysis. Similar results have been reported during the early phases of germination of *Phaseolus mungo* seeds (9).

Whatever the action of NaF, it is clear that it did not modify the characteristics of the tissue with regards to $O_2$. It had the advantage of enlarging the range over which the variations of nucleotide ratios could be observed as a function of $O_2$ tension, by decreasing the influence of the fermentative activity. In tissues having a high fermentative capacity such as maize root tips, it would be difficult, at low $pO_2$ and in the absence of NaF, to discriminate between the part played by the $O_2$-controlled respiration and that played by the fermentation controlled by sugars, in the variation of nucleotide ratios. Such a situation is well illustrated in mammalian kidney cells (20). Under these conditions, there is a good correspondence between the values of the ATP/ADP ratio and the respiratory rate which started to decline at the same $pO_2$ as ATP/ADP or even slightly before. It should be noted, however, that the relative uncertainty of ATP/ADP determination at high values might mask some small adjustment of the ratio.

We have not done enough experiments with other tissues to generalize these results. However, from data obtained with germinating lettuce seeds (14) or animal cells (20), it seems reasonable to assume that this correlation could be quite universal. The value reached by ATP/ADP when the $pO_2$ limited the respiratory rate was not a transient drop, the ratio remained stable at a given $pO_2$ for at least 90 min in maize root tips and up to 24 h in lettuce seeds (14). It corresponds to a new status of the cells' energy balance determined by the rate of ATP utilization which is largely $O_2$ independent and the rate of ATP regeneration which is controlled by the $pO_2$.

By enlarging the variation range of the ATP/ADP ratio as a function of the respiratory rate with NaF, it is now possible to quantify the part played by $O_2$ transport on cellular respiration along roots maintained in an anaerobic environment.

The pattern of ATP/ADP values along the root demonstrates the preeminent part played by gas spaces (aerenchyma) in $O_2$ transport. By filling the gas spaces, infiltration stopped this transport completely. The amount of $O_2$ transported via the phloem stream or through the cells was negligible for respiration. In noninfiltrated tissues, the transported $O_2$ made a large contribution to the respiration in the first few centimeters from the seed. It should be noted, however, that the tip is a highly heterogeneous tissue and the meristematic area, which is very compact and metabolically active, has little or no gas spaces (8). Its ventilation should, therefore, be very poor compared with that of the tissues situated a few millimeters higher. Consequently, the 30% to 40% of the maximum respiratory rate still found in the last centimeters of the root (8 cm from the seed) are average values which certainly overestimate the actual respiratory status of the meristematic region.

In addition, it is likely that in longer roots, unless the plant develops a higher root porosity with age or an adaptive response to low $O_2$ tension (3, 8), only trace amounts of $O_2$ would reach the distal tissues due to the steep respiratory gradient which decreases as the distance from the aerated source increases. These reasons explain why in an earlier paper (13) no $O_2$ transport could be detected in the tips (0.5 cm) of the seminal roots (about 50 cm long) of older maize plantlets (11 d).

As a conclusion of this study, it appears that the relative effectiveness of $O_2$ transport in a mesophyte such as maize should contribute to the survival of the seminal root of young seedlings during an anoxic stress. The high respiratory rate of the upper first centimeters should also stimulate the growth of newly formed roots, adapted to $O_2$ transport usually promoted by anaerobic conditions (3) and the limited rate of respiration above the tip should be able to provide part of the energy necessary for the absorption of ions and transport processes.

However, even in this very young material, the ventilation of the apical growing zone was inadequate to sustain the growth (result not shown).

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