Final Report

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Respiration of roots. Response to low O_2 stress.

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In the two years covered by this agreement the following papers have been published:

1. Alpi, A. and H. Beevers. 1983. Effects of O_2 concentration on rice seedlings. Plant Physiology 71: 30-34.
2. Shimomura, S. and H. Beevers. 1983. Alcohol dehydrogenase and an inactivator from rice seedlings. Plant Physiology 71: 736-741.
3. Shimomura, S. and H. Beevers. 1983. Alcohol dehydrogenase from rice seedlings: properties and intracellular location. Plant Physiology 71: 742-746.

These papers describe the progress on the original proposal submitted to DOE. A brief summary follows.

The unusual ability of rice seedlings to germinate in the complete absence of O_2 has been known for many years. However, the growth is limited; no roots are produced and only the coleoptile elongates. We showed that although the coleoptile produced in pure N_2 is longer than in air, it is deficient in several respects. Its dry weight, protein content, sugar and

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cellulose content were all markedly less than those in air. In seeking for an explanation of even this limited growth in anaerobic conditions we showed that it is not due to a higher tolerance for the accumulating ethanol than that shown by other seedlings which cannot grow at all in N_2 . It was also shown that leakage of ethanol from the tissue must be taken into account when assessing the metabolism of the seedlings in N_2 , and that this leakage occurs by a strictly passive process of diffusion. We discussed the suggestions that have previously been made to account for the unusual ability of rice to grow in N_2 and conclude that none is adequate. A particular recent proposal that the energy charge is maintained at a high level is an inadequate explanation, since it does not take into account the actual rate of ATP synthesis.

In the papers with Dr. S. Shimomura we describe our investigations on alcohol dehydrogenase in rice seedlings. This enzyme plays an important role when tissues are deprived of O_2 and one of the suggestions that has been made previously is that in seedlings that are resistant to low O_2 the alcohol dehydrogenase activity remains low, whereas in seedlings that are killed by low O_2 this enzyme activity increases in anoxia. We show that in rice seedlings the alcohol dehydrogenase activity increases strikingly in N_2 and thus that the ability of rice to withstand low O_2 cannot be ascribed to the lower production of ethanol due to a lower alcohol dehydrogenase activity. An important aspect of this work was the finding of an inactivator, which rapidly attacks the alcohol dehydrogenase as soon as the cells are broken during extraction.. Failure to recognize this inactivator (which may be quite general) can lead to erroneous estimates of the alcohol dehydrogenase and, unless assays are done immediately after extraction, can lead to the conclusion that no enzyme is present.

Since the alcohol dehydrogenase is such a key enzyme in anaerobic conditions, much work has been done previously on this enzyme. Our work shows that the action of the inactivator can be completely prevented by dithiothreitol, an S-S reducing agent, and accurate assays of enzyme activity are thereby obtained. The inactivator is not present in dry seeds. It increases in seedlings grown in air, but not in those grown in N_2 . It is not clear yet what the function of this inactivation is in vivo, and from the fact that active enzyme can be obtained from tissues with high levels of inactivator (sufficient to inactivate the enzyme in minutes once the cells are broken) we conclude that the inactivator and enzyme must be in separate compartments in vivo.

In the second paper with Shimomura we show that the inactivator is associated with an organelle fraction sedimenting in sucrose gradients close to the endoplasmic reticulum, whereas the alcohol dehydrogenase is all free in the cytosol. The inactivator acts on alcohol dehydrogenase from several plant sources. We show that the inactivator, which appears to be a protein, does not act proteolytically. Instead there was a loss of SH groups from alcohol dehydrogenase which paralleled the loss of activity when the inactivator was added. This loss of activity could be prevented by dithiothreitol and by withholding O_2 from the incubation medium. The work on alcohol toxicity and on rice cells growing in culture is being continued with funds from other sources.

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