Plant Physiol. (1967) 42, 1053–1058

Some Effects of Oxygen Concentration on Levels of Respiratory Intermediates in Buckwheat Seedlings

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Received March 8, 1967.

Summary. Changes in levels of glycolytic and some related respiratory intermediates in young seedlings of buckwheat (*Fagopyrum esculentum*) following transfer A) from air to anoxia, B) from air to 1.5 % O_2, C) from 1.5 % O_2 to anoxia and D) from anoxia to air, are recorded and discussed in relation to other measurements made with these seedlings.

On transfer from air to anoxia the changes are similar to those recorded for pea seeds in which it is inferred that glycolysis is faster in anoxia than in air. The results with buckwheat however can be explained in terms of a decreased rate of glycolysis in anoxia. An alternative hypothesis is developed which states that glycolysis is faster in anoxia than in air and that it is the contribution of the pentose phosphate cycle to the total respiratory catabolism which decreases as the oxygen concentration is reduced towards zero.

The transient changes in concentrations of some glycolytic and related intermediates which occur on transfer from air to anoxia have been described for pea seeds (1) and rhododendron leaves (3). In both of these plant organs the carbon loss in anoxia, measured as CO_2 evolved and ethanol and lactate accumulated, exceeds the carbon loss as CO_2 in air, and the changes observed in concentrations of each glycolytic intermediate exhibit marked similarities in the 2 organs. For the peas, it has been inferred that there is a faster rate of carbohydrate breakdown in anoxia than in air and, based on the observed changes in levels of the respiratory intermediates on transfer from air to anoxia, a hypothesis has been developed to explain an increased rate of glycolysis following the transfer. A central feature of this hypothesis is that the sugar-phosphorylating and other glycolytic enzymes are located in an organized structure which is effectively more permeable to ADP than to ATP (1, 2).

In buckwheat seedlings the rate of carbon loss in anoxia, measured as CO_2 evolved and ethanol and lactate accumulated, is less than the carbon loss as CO_2 in air (4). It was therefore of some interest to compare the changes in levels of glycolytic and related intermediates on transfer from air to anoxia with those observed in peas and rhododendron leaves. In many respects the changes observed in buckwheat proved to be similar to those in peas and rhododendron leaves even though the rate of carbon loss, assessed as described above, decreased in buckwheat on transfer to anoxia but increased in pea and rhododendron.

Consequently, measurements were made of the changes in glycolytic intermediates in the seedlings on transfer from air to the extinction point of fermentation (1.5 % oxygen) and from the extinction point to anoxia. At the extinction point, ethanol and lactate accumulation are suppressed and the respiratory metabolism remains, apparently, wholly aerobic. Carbon loss as CO_2 at the extinction point is nevertheless only 50 to 60 % of the carbon loss in air, and it is approximately equal to the carbon loss as CO_2 and ethanol in anoxia (4). The changes in levels of glycolytic intermediates associated with these changes in rates of carbon loss are discussed in relation to current theories of respiratory control. Measurements of the changes in respiratory intermediates are recorded also for the return to air from anoxia.

Materials and Methods

The preparation of plant material and the determinations of phosphorylated intermediates and glycerol on 30 g fresh weight seedlings by specific enzymatic methods are described in a previous paper (4). Reduced and oxidized pyridine nucleotides were determined on 1 g fresh weight seedlings by the method of Yamamoto (7).

Results and Discussion

The effects of transfer of young buckwheat seedlings from air to anoxia on phosphate ester inter-

[1] The work described in this paper was supported by a Grant-in-aid from the Science Research Council to S. L. Ranson and by a research grant from Facelle Co. Ltd., Canada, to W. R. Effer.

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mediates and glycerol are illustrated in figures 1 and 2. The levels of 3-P-glycerate and P-enolpyruvate decreased very rapidly and then remained steady at much lower levels. Very similar changes occurred in peas (1) and rhododendron leaves (3). Fructose-6-P was present at approximately one-tenth of the concentration of glucose-6-P. Both intermediates showed a rapid small increase followed by a slow decline to values well below those observed in air. Similar slow decreases below the aerobic control level occurred in peas and rhododendron leaves, but the initial rapid increase was absent in peas and very marked in rhododendron leaves. Fructose-1, 6-diP showed a varied behavior in buckwheat; in 2 experiments it increased, in a third it decreased and in a fourth it did not change. In both pea and rhododendron leaves the concentration increased to a very marked degree and in the latter was still much higher than in the air control samples after 24 hours anoxia. In buckwheat dihydroxyacetone-P declined in 1 ex-

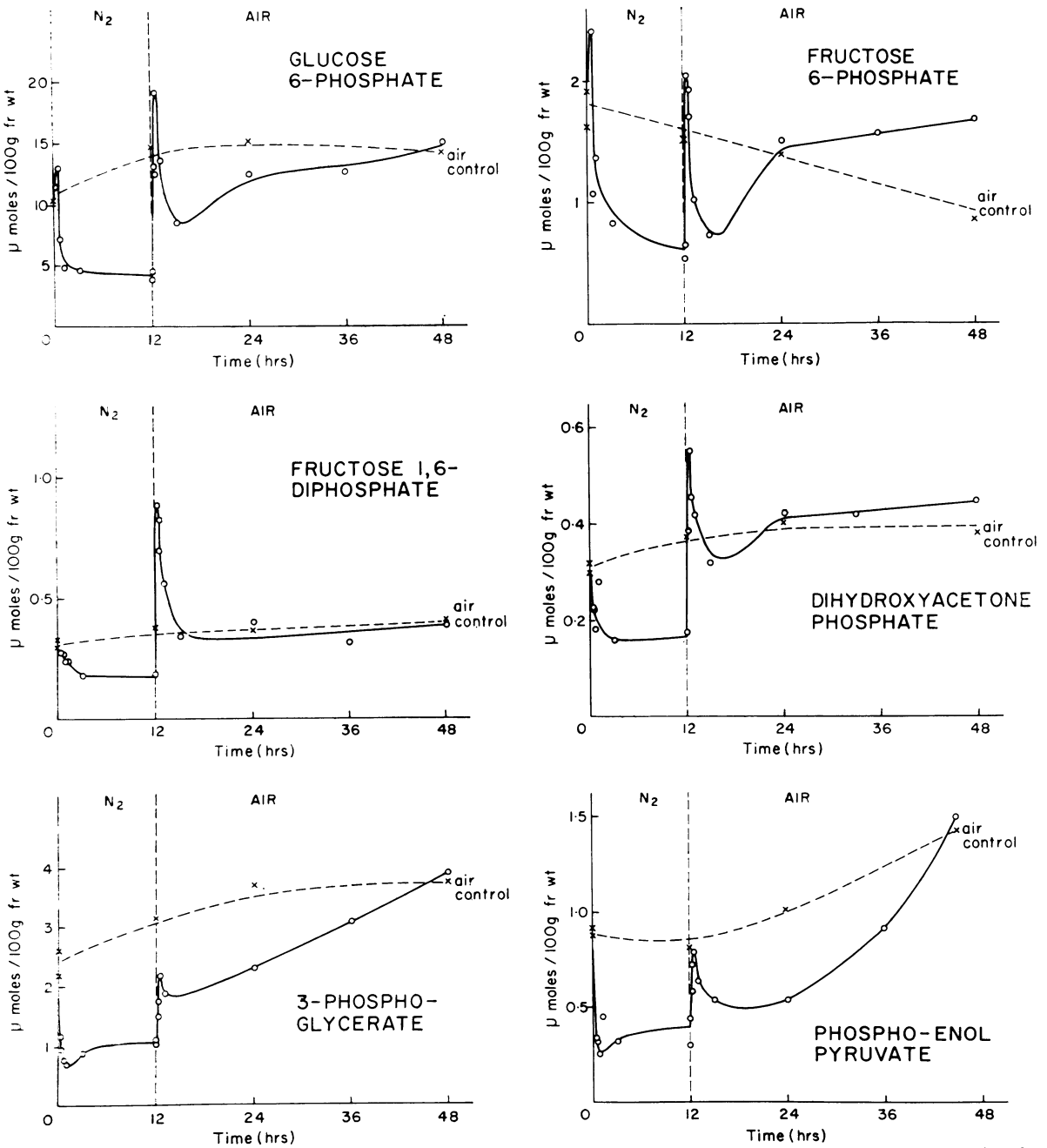

FIG. 1. Concentrations of glycolytic intermediates in air (---), in anoxia (——) and on return to air after 12 hours in anoxia (———).

periment and increased in another whereas in pea the triose-P increased markedly, at least for the first hour of anoxia; no analyses are available for rhododendron leaves. In buckwheat α-glycerol-P and glycerol, presumably produced via dihydroxyacetone-P, accumulated to a marked degree but no data are available for the other 2 plants. A rapid and marked decrease in 6-P-gluconate to a low steady level occurred in buckwheat. Similar results were obtained for rhododendron leaves but no data are available for pea. An obvious limitation of the data obtained is that they represent the average concentrations of each component metabolite in different types of cells and in the various metabolic pools present in each of these cells. It is not possible at present to measure levels in discrete pools in intact organs or tissues. However, the relatively rapid changes observed suggest that the intermediates are in close proximity with pools concerned with respiration or, if present elsewhere, equilibrate readily with such pools. It is therefore provisionally assumed that the data represent changes in pools associated with respiratory catabolism.

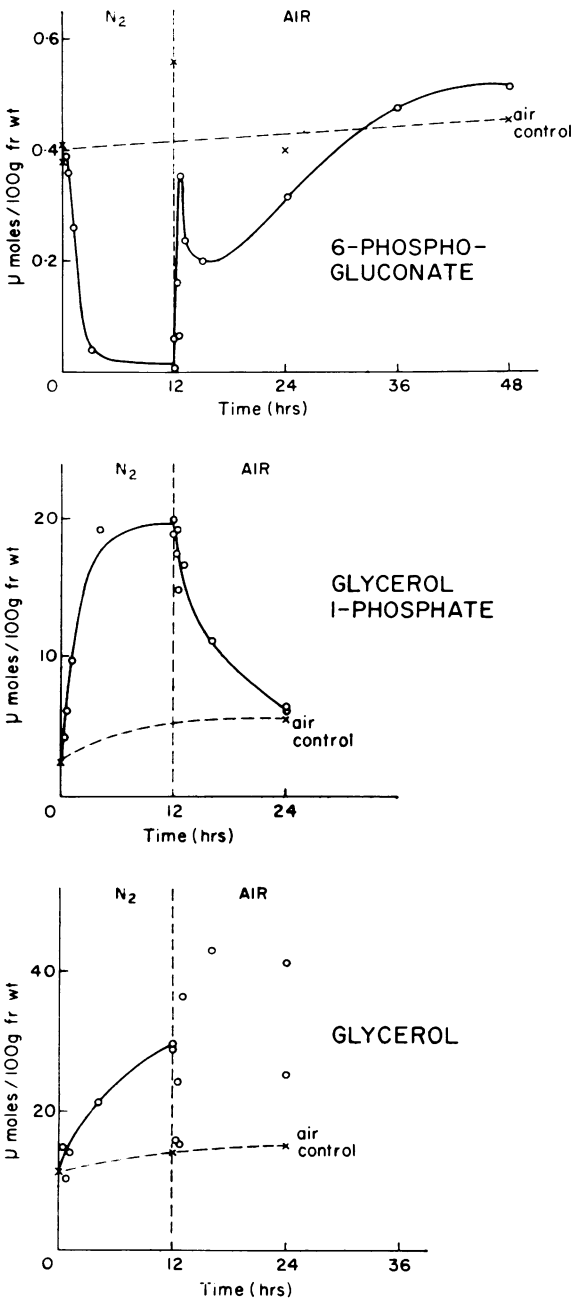

FIG. 2. Concentrations of 6 phosphogluconate, glycerol-1-P and glycerol in air (-----) in anoxia (———) and on return to air after 12 hours in anoxia (———).

In general, the changes in phosphorylated intermediates in buckwheat on transfer from air to anoxia are similar to those reported for peas and rhododendron leaves, although carbon loss in anoxia is less than in air in buckwheat whereas in the other 2 plant materials the carbon loss is greater in anoxia. It is stressed here and elsewhere in this paper that rates of carbon loss refer to values computed from CO_2 output in atmospheres where metabolism is wholly aerobic, and from CO_2 output and accumulated ethanol and lactate when metabolism is wholly or partially anaerobic (4).

If the relative rates of carbon loss reflect relative rates of carbon flux through the glycolytic sequence it would seem that whether transfer to anoxia results in an increase (as in peas and rhododendron) or a decrease in the rate of glycolysis (as in buckwheat) the resulting changes in ester phosphate levels have much in common. The intermediates are produced and consumed in a multi-enzyme sequence where controls may operate at one or more discrete points and so change the overall rate of carbon flux. Changes in levels of a specific intermediate in the sequence may depend on the relative rates of production and consumption of that intermediate and not on the absolute rate of part or the whole of the sequence. However Barker et al. (2) have attached considerable importance to the rapid decrease in levels of 3-P-glycerate and P-enolpyruvate in anoxia. They suggest that these changes reflect an accelerated rate of utilization of these intermediates and, by producing ATP preferentially available for the phosphorylation of glucose and fructose-6-P, lead to an overall increase in the rate of glycolytic flux. If in buckwheat the reduced carbon loss in anoxia is a reflection of a slower rate of glycolysis the similar behavior of 3-P-glycerate and P-enolpyruvate in peas and buckwheat casts some doubt on the general validity of the hypothesis. Barker et al. suggest that the apparently conflicting results may be because the glycolytic enzymes in buckwheat are not present in the type of glycolytic structure which they have postulated for peas. There is some evidence that glycolytic enzymes are structurally orientated (5) but it is still uncertain how such an organization can explain different glycolytic rates.

It is clear that our present uncertainty about the

relative activities of metabolic pathways in respiratory metabolism makes it difficult to be certain that rates of carbon loss can be related directly to rates of glycolytic flux. Further information was sought, therefore, from experiments in which the seedlings were transferred from air to an atmosphere containing 1.5 % O_2 and then from this atmosphere to pure nitrogen. The first transfer results in a 40 to 50 % reduction in rate of carbon loss. The second results in no appreciable change (4). The changes in levels of ester phosphates recorded after each transfer are shown in figure 3. The changes on transfer from 1.5 % O_2 to anoxia were very similar to those on the transfer from air to anoxia. Moreover the initial changes on transfer from air to 1.5 % O_2 followed the same general patterns. These results suggest that the initial change in glycolytic flux, whether it be an increase or decrease, was the same for each of the transfers, air to 1.5 % O_2, 1.5 % O_2 to anoxia and air to anoxia. In 1.5 % O_2 respiratory metabolism is apparently wholly aerobic but the CO_2 output is markedly lower than in air. Seedling growth continues, but at a slow rate in 1.5 % O_2 (6). The reduced CO_2 output and reduced growth, which presumably means reduced consumption of glycolytic and tricarboxylic acid cycle intermediates, are sugges-

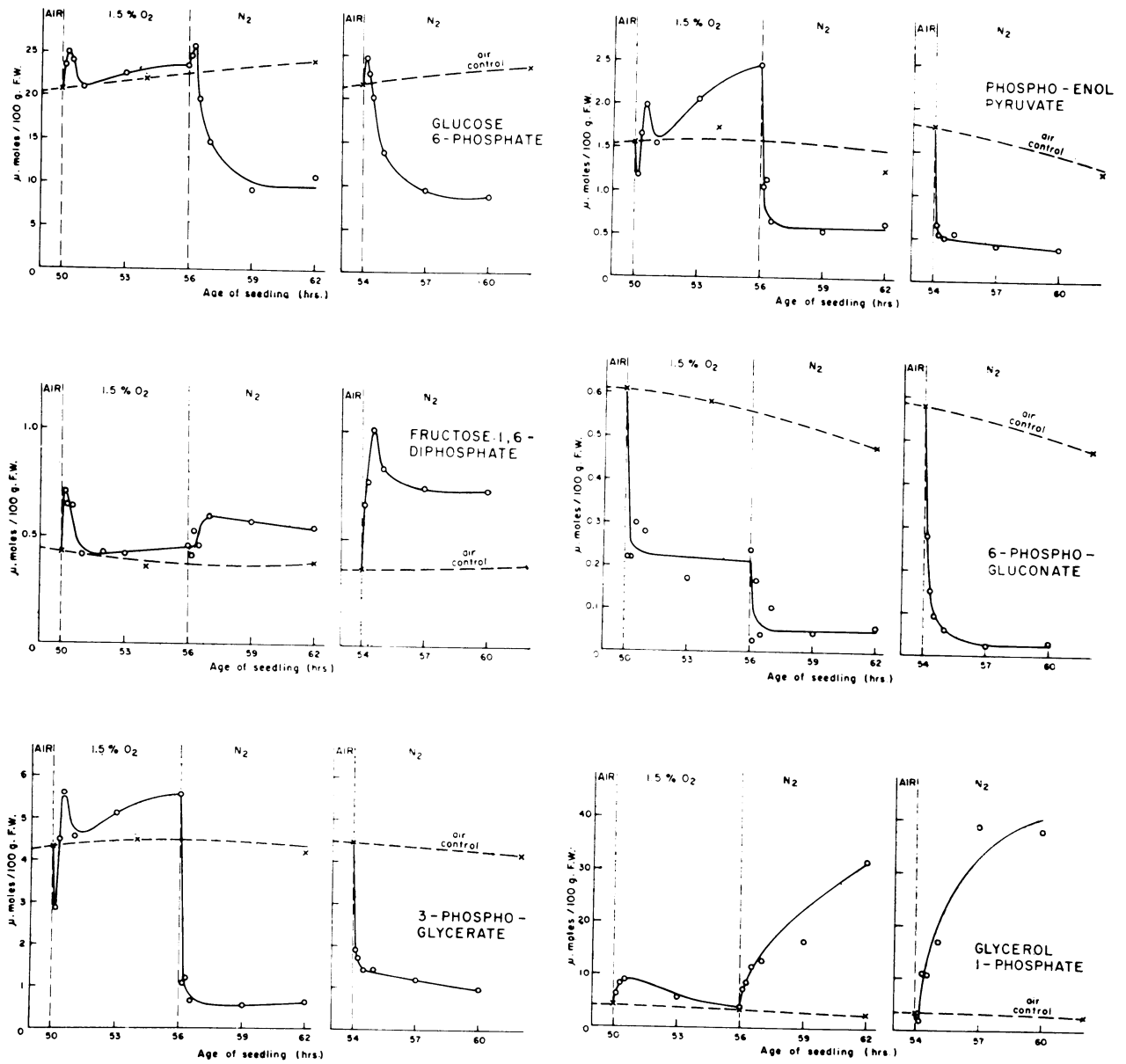

FIG. 3. Concentrations of some phosphorylated intermediates following transfer (a) from air (-----) to 1.5 % oxygen (———), (b) from 1.5 % oxygen to anoxia (———), and (c) from air to anoxia (———).

tive of a reduction in the rate of glycolysis on transfer from air to 1.5 % O_2. The changes in levels of glycolytic intermediates on transfer from air to 1.5 % O_2 (fig 3) would then appear to be associated with a reduction in the rate of glycolysis. As the initial pattern of change in the level of glycolytic intermediates was the same on transfer from air or 1.5 % O_2 to anoxia it might be inferred that glycolysis in buckwheat is slower in anoxia than in air, or 1.5 % O_2.

The observed changes in glycolytic intermediates are explainable in terms of a reduced rate of glycolysis resulting from a reduced level of NAD^+ and, presumably, of ATP in anoxia. The quotient NAD^+/NADH decreased within 5 minutes of transfer to anoxia to a lower steady level (table I). If the reduced availability of NAD^+ and inhibiting effect of accumulating NADH decreased the rate of tri-

Table I. *Levels of NAD^+ and NADH in 2-Day Old Buckwheat Seedlings in Air, in Anoxia and on Return to Air from Anoxia*

Time	NAD^+	NADH	$\frac{NAD^+}{NADH}$
	μmoles/100 g fr wt		
	In anoxia after 2 days growth in air		
0	5.28	0.16	33.0
5 min	4.80	1.07	4.5
15 min	5.04	0.41	12.3
30 min	5.00	1.10	4.5
6 hr	5.12	0.82	6.3
	In air after 6 hr in anoxia		
0	5.12	0.82	6.3
5 min	5.04	0.14	36.2
15 min	4.88	0.25	19.6
30 min	...	0.27	...

ose-P oxidation it would account for the rapid depletion of P-enolpyruvate and 3-P-glycerate, particularly since, in the absence of oxidative phosphorylation, ADP would be in excess. The simultaneous reduction of triose-P to α-glycerol-P and glycerol would tend to counteract the tendency for triose-P to accumulate. This reaction, together with reductions leading to ethanol and lactate production would account for the observation that even though the cells were anaerobic the coenzyme remained mainly in the oxidized form. The initial transient increases in hexose phosphates would be consistent with a hold-up on their consumption resulting from the reduced rate of oxidation at the triose-P level. The subsequent reductions in hexose phosphate concentrations would be explained by a reduced level of sugar phosphorylation because of the reduced level of ATP available in anoxia.

A quite different interpretation of the results may be made however. This is based on a decreasing contribution of the pentose phosphate cycle to the total respiratory metabolism as the O_2 concentration is reduced from that of air to 1.5 % and finally to zero. The postulate is that the decrease in the pentose phosphate component masks an increase in the rate of glycolysis on transfer to anoxia. Some evidence of participation of the pentose phosphate sequence in carbohydrate catabolism in buckwheat seedlings has come from experiments with labeled glucose (4).

After the first hour on transfer of the seedlings from air to 1.5 % O_2 the steady state levels of glucose-6-P, fructose-1,6-diP,3-P-glycerate and P-enolpyruvate had returned to approximately the same levels as in air. This suggests that after the first hour in 1.5 % O_2 the carbon flux through these glycolytic intermediates was similar to that occurring in air. However, since CO_2 output in 1.5 % O_2 was 40 % less than in air it would then follow that the reduction was a result of a lowered CO_2 output from the pentose phosphate sequence, or from some other sequence in which glycolysis does not participate. Reduced activity of the pentose phosphate cycle in 1.5 % O_2 is consistent with the observed marked growth retardation since there is evidence that the cycle is linked with reductive synthetic reactions. Moreover, the steady state level of 6-P-gluconate decreased by 65 % on transfer from air to 1.5 % O_2 and decreased further by another 25 % on transfer from 1.5 % O_2 to anoxia. If these changes reflect similar percentage changes in the CO_2 produced from the pentose phosphate sequence the inference is that carbon flux through glycolysis increased on transfer from 1.5 % O_2 to anoxia, for the rate of carbon loss remains unchanged in this transfer. This interpretation would mean that the changes in levels of glycolytic intermediates, with increase in the rate of glycolysis on transfer to anoxia, follow in essentials the same patterns in buckwheat as those recorded for peas and rhododendron.

Although it is suggested above that the steady state glycolytic rates in air and 1.5 % O_2 were similar, the occurrence of initial changes in the levels of intermediates on transfer of the seedlings from air to 1.5 % O_2, similar to those recorded on transfer to anoxia, would suggest an initial transient increase in glycolysis in 1.5 % O_2. This might simply be a result of a temporarily enhanced availability of hexose phosphate for glycolysis following retardation of pentose phosphate cycle reactions.

Which of the alternative interpretations of the buckwheat data given here is the more accurate must remain in doubt until unambiguous measurements can be made of the rates of carbon flux through the various metabolic sequences which contribute to the total respiratory metabolism of the seedlings in air, 1.5 % oxygen and in anoxia.

Data on the changes in concentrations of respiratory intermediates on return to air from anoxia are shown in figures 1 and 2. The level of each, with the exception of α-glycerol-P increased rapidly, then after decreasing equally rapidly slowly returned to the aerobic control level. The levels of α-glycerol-P and, in 1 experiment glycerol, fell rapidly on return

to air. The CO_2 output rose in this period but substances other than carbohydrates (e.g. ethanol, lactate and succinate) were consumed and pyruvate accumulated (4). The metabolic sequences contributing to CO_2 production were thus probably more complex than those participating in air before the anaerobic period or in the anaerobic period. Interpretation of the changes in respiratory intermediates in the post anaerobic phase is therefore deferred.

The 2 possibilities raised that A) the glycolytic rate remains essentially unchanged on going from air to the extinction point but then increases on going to complete anoxia and B) the activity of the pentose phosphate cycle is reduced on going from air to the extinction point and then further reduced in anoxia, requires further investigation.

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