The Influence of Growth Regulating Substances on the Development of Enhanced Metabolic Rates in Thin Slices of Beetroot Storage Tissue

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Summary. Freshly cut disks of beetroot tissue develop high rates of respiration, uptake of phosphate and activity of the enzyme invertase after having been washed for 18 hours in 0.01 M potassium maleate.

Incubation of the disks in solutions of indole-3-acetic acid or kinetin completely prevented the development of the higher activities in all 3 systems assayed, while incubation in gibberellic acid had no inhibitory effect. Using a series of synthetic plant growth regulating compounds it was possible to establish that there was no correlation between the activity of the compound as an auxin and the ability of the compound to prevent the development of the enhanced rates of metabolism.

When bulky storage organs are cut into thin slices there is a general increase in their metabolic activity which develops with time. The increase in the rate of respiration has been the most frequently studied aspect of this phenomenon and has been observed to occur in a wide variety of tissues (6, 9, 18). Considerable evidence exists to show that induced respiration may differ qualitatively from basal respiration (7, 14, 16) and is probably mediated by the operation of different metabolic pathways (1, 15). An increase in the rate of several other metabolic processes has been shown to occur simultaneously with the change in the rate of respiration, these include a dramatic increase in the rate of absorption and esterification of phosphate (8), and a higher rate of protein synthesis (1, 3, 11) which may be associated with the development of much higher levels of the enzymes invertase and ascorbic acid oxidase, as found in slices of Jerusalem artichoke (5). Similar patterns of change in the rate of phosphate absorption and possibly of protein synthesis have been observed in excised segments of etiolated pea epicotyl (13). In this tissue the rate of respiration fails to increase during incubation, in contrast it actually decreases to approximately half the original rate in 24 hours.

The mechanism causing increased metabolic activity in these 2 systems is essentially unknown. However 2 observations have been made which suggest that plant growth substances can modify the pattern of changes. In the first case it has been shown that the incubation of slices of Jerusalem artichoke in 10^{-3} M indoleacetic acid (IAA) prevented the appearance of higher levels of invertase activity (4), and secondly that washing segments of etiolated pea stems in 2×10^{-4} M 2,4-dichlorophenoxyacetic acid (2,4-D) rather than water prevented the increase in the rate of phosphate absorption (12).

The objective of the work described in this paper was to investigate whether other plant growth substances were active in inhibiting the changes in metabolic activity.

Materials and Methods

Fresh beetroots (*Beta vulgaris*) were obtained from a local market and stored at between 1 to 4°. The plant growth substances were obtained from commercial sources. The phenoxyacetic acids were recrystallised several times from ethanol and IAA was recrystallised from ammonium hydroxide before use. These compounds were then dissolved in 0.01 M potassium hydrogen maleate and the pH adjusted to 5.2 for use in all the experiments.

The disks were prepared by removing cores of beet tissue, using a cork borer 0.6 cm in diameter, which were then cut into 1.0 mm slices with a hand microtome. Disks in this condition were referred to as fresh disks. Changes in the metabolic rates were induced by placing 10 fresh disks in 10 ml of solution in a 50 ml Erlenmeyer flask which was then gently shaken on a mechanical shaker at 25° for 18 hours. The incubation medium was not changed during the 18 hour period since trial experiments, involving the addition of penicillin and streptomycin to the medium or changing the solution at frequent intervals did not alter the final levels of metabolic activity. At the end of the incubation period the disks were removed and washed in a large volume of distilled water at 0°. Disks in this condition were referred to as washed disks.

Assays of Metabolic Activities. A) *Respiration Rate.* Respiration was measured using a conventional Warburg manometer. Fifteen disks were suspended in 3.0 ml of water.

B) *Rate of Absorption of Phosphate.* Ten disks were placed in 10 ml of 3×10^{-6} M potassium dihydrogen phosphate (pH 5.2) containing 50 μc of ^{32}P per liter and gently shaken at 25°. After 1 hour the disks were removed and washed for a total of 60 seconds in 3 changes of distilled water at room temperature, further washing at 0° failed to remove any more ^{32}P. The samples were then blotted dry and digested in a sulphuric/nitric acid mixture and the amount of ^{32}P determined using a M6 Geiger-Muller liquid counting tube connected to an appropriate scaler.

C) *Invertase Activity.* Since invertase is bound to the cell wall its activity was assayed in a cell wall preparation obtained by homogenizing 50 disks in 0.5 M KH_2PO_4 at 1° and pH 6.5 and then centrifuging at 20,000 $\times$ *g* for 20 minutes. The resulting cell wall preparation was washed in the phosphate buffer and again centrifuged down at 20,000 $\times$ *g*. To commence the assay the preparation from 50 disks was suspended in 10 ml of 0.025 M sucrose, samples were removed at the appropriate times and the reducing sugar levels were measured using the colorimetric method of Nelson and Somogyi (11, 17) calibrated against glucose as a standard.

Results

Changes in the Rate of Metabolism after Incubation in 0.01 M Potassium Hydrogen Maleate. Figures 1 and 2 show the characteristic pattern of changes in the rates of metabolism which occurred when beetroot disks were washed in a 0.01 M maleate buffer solution, which was used as the control treatment in subsequent experiments described in this paper.

Figure 1 shows that the rate of respiration of the disks more than doubled after they had been washed for 18 hours and also that the sensitivity of O_2 uptake to inhibitors had changed. After the washing period the rate of respiration was no longer stimulated by 2×10^{-5} M dinitrophenol and was much less inhibited by 10^{-3} M sodium azide. In these respects beetroot disks were similar to slices of potato and chicory root tissue (7, 19). The rates of absorption of phosphate and the activity of the enzyme invertase in both fresh and washed disks are given in figure 2. In both systems it is apparent that very large stimulations were brought about by washing. The enzyme invertase exhibited very little activity in fresh disks while in washed disks it was very active. Washed disks showed a high linear rate of uptake of phosphate whilst fresh disks took up phosphate at a much reduced rate which fell off with increasing time. In this respect the uptake

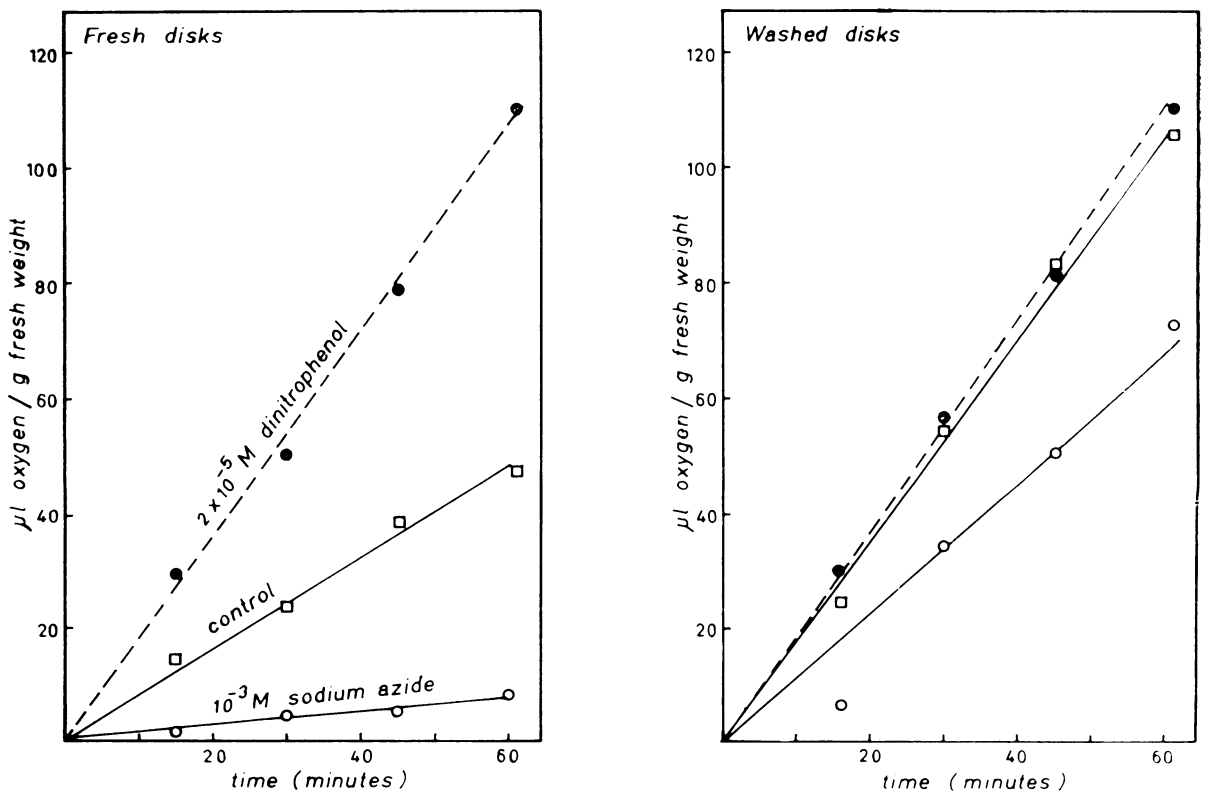

FIG. 1. The rate of respiration of beetroot disks before and after being washed for 18 hours in 0.01 M potassium hydrogen maleate (pH 5.2). The pH of the dinitrophenol was adjusted to 5.2 before use.

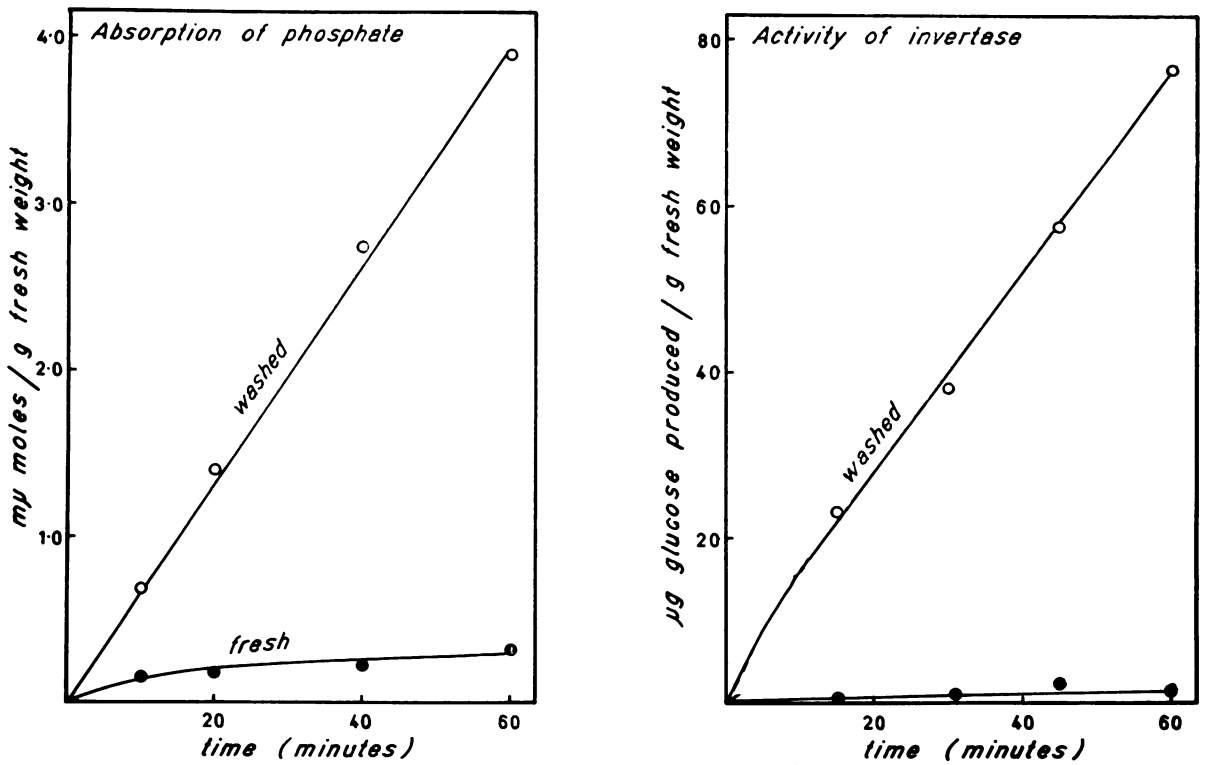

FIG. 2. (left) The rates of absorption of phosphate by beetroot disks from 3×10^{-6} M KH_2PO_4 before and after being washed for 18 hours in 0.01 M potassium hydrogen maleate. (right) The levels of activity of the enzyme invertase in cell wall preparations obtained from beetroot disks before and after being washed for 18 hours in 0.01 M potassium hydrogen maleate.

of phosphate by beetroot disks was similar to that found in segments of pea epicotyls (13).

The Effect of Incubating Disks in Various Plant Growth Substances. The relative levels of the rate of respiration, the rate of phosphate absorption and the activity of invertase found after incubating the disks in various concentrations of IAA for 18 hours are shown in figure 3. The data have been presented as a percentage of the control value found after washing the disks in 0.01 M maleate buffer solution. From these results it is apparent that incubating disks in 10^{-3} M IAA almost completely prevented the development of any change in metabolic activity, and that 5×10^{-5} M IAA brought about a significant reduction in the subsequent rate of respiration and the level of invertase activity but had little effect on the rate of phosphate absorption.

The metabolic activities obtained after treating the disks in either gibberellic acid or kinetin for 18 hours are shown in figure 4. Treatment in gibberellic acid resulted in a considerable increase in the activity of invertase in relation to the control as already observed by Edelman and Hall (14), whilst there was very little effect on the rates of respiration or on the uptake of phosphate. In contrast, treatment in kinetin was highly effective in pre-

Table I. *Rates of Metabolism Obtained after Incubating Disks of Beetroot Tissue in Various Plant Growth Substances for 18 Hours*

The rate of respiration is given in μ liters of oxygen consumed per gram fresh weight per hour, the rate of uptake of phosphate in mμ moles per gram fresh weight per hour and the activity of invertase as the amount of reducing sugar produced measured as μg of glucose per gram fresh weight per hour.

Composition of incubation solution	Rate of respiration	Rate of phosphate uptake	Activity of invertase
Control (potassium maleate only)	92.8	3.70	103.0
10^{-3} M indole-3-acetic acid	13.9	0.62	21.4
10^{-3} M 2,4-dichlorophenoxyacetic acid	9.3	0.55	30.0
10^{-3} M 2,4,6-trichlorophenoxyacetic acid	11.6	0.65	21.7
10^{-3} M 2,3,6-trichlorobenzoic acid	53 4	0.33	36.5

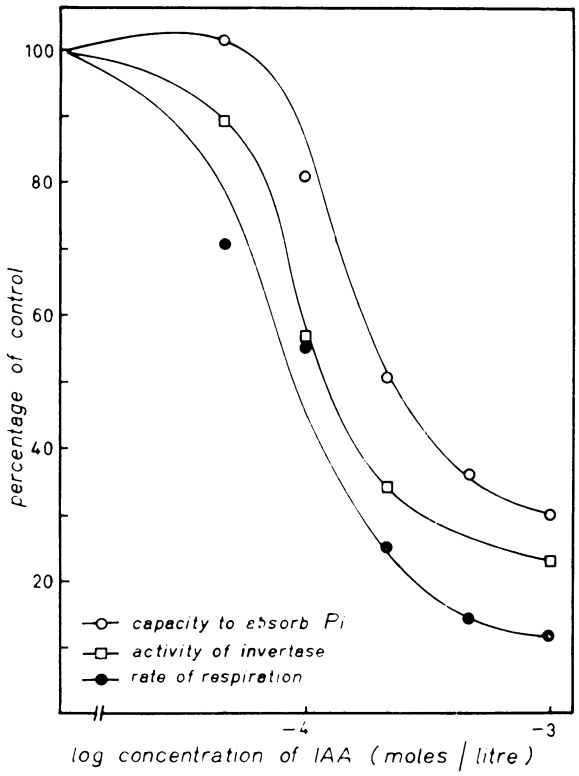

FIG. 3. The rate of metabolism obtained after incubating beetroot disks in indole-3-acetic acid for 18 hours. The levels of the control treatment in 0.01 M potassium, hydrogen maleate is used as 100 % activity.

venting any increase in the rate of each of the systems tested.

The activity of 2,4-D, 2,4,6-trichlorophenoxyacetic acid and 2,3,6-trichlorobenzoic acid were investigated in an effort to relate their activity as auxins to their ability to prevent the changes in the rates of metabolism. Both 2,4-D and 2,3,6-trichlorobenzoic acid exhibit strong auxin activity whilst 2,4,6-trichlorophenoxyacetic acid shows little or no activity as an auxin. The results obtained are shown in figure 5 and indicate that at high concentrations both 2,4-D and 2,4,6-trichlorophenoxyacetic acid were equally able to prevent the changes in the rates of respiration and absorption of phosphate. However, at lower concentrations 2,4-D had much less effect than 2,4,6-trichlorophenoxyacetic acid. Data in table I shows the effect of IAA, 2,4-D, 2,4,6-trichlorophenoxyacetic acid, and 2,3,6-trichlorobenzoic acid on all 3 systems, each of these compounds being active in preventing the occurrence of enhanced rates of metabolism, although the rate of respiration occurring after washing disks in 2,3,6-trichlorobenzoic acid for 18 hours was much greater than in the case of the other growth substances tested.

In the light of these results it would appear that the ability of IAA to modify the changes in metabolism caused by washing the disks was not necessarily connected with its activity as an auxin. This view was further strengthened when it was found (fig 6) that adenine, which bears some overall structural relationship to kinetin and IAA, is also

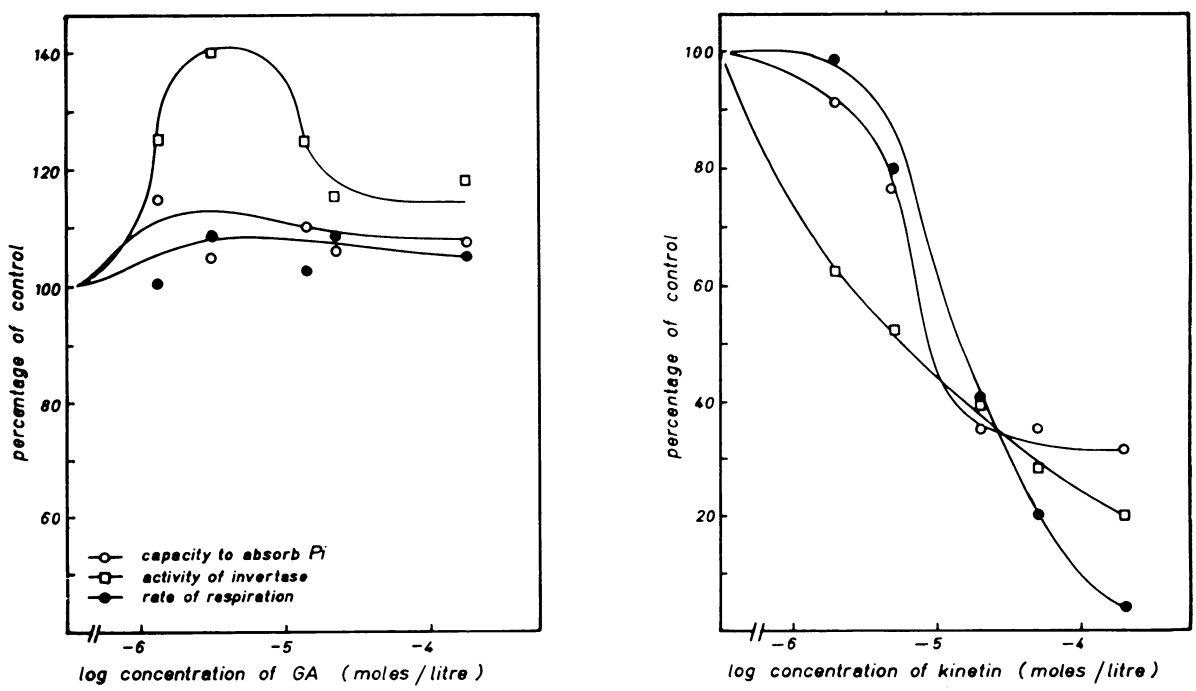

FIG. 4. (left) The rates of metabolism obtained after incubating beetroot disks in gibberellic acid for 18 hours. (right) The rates of metabolism obtained after incubating beetroot disks in kinetin for 18 hours.

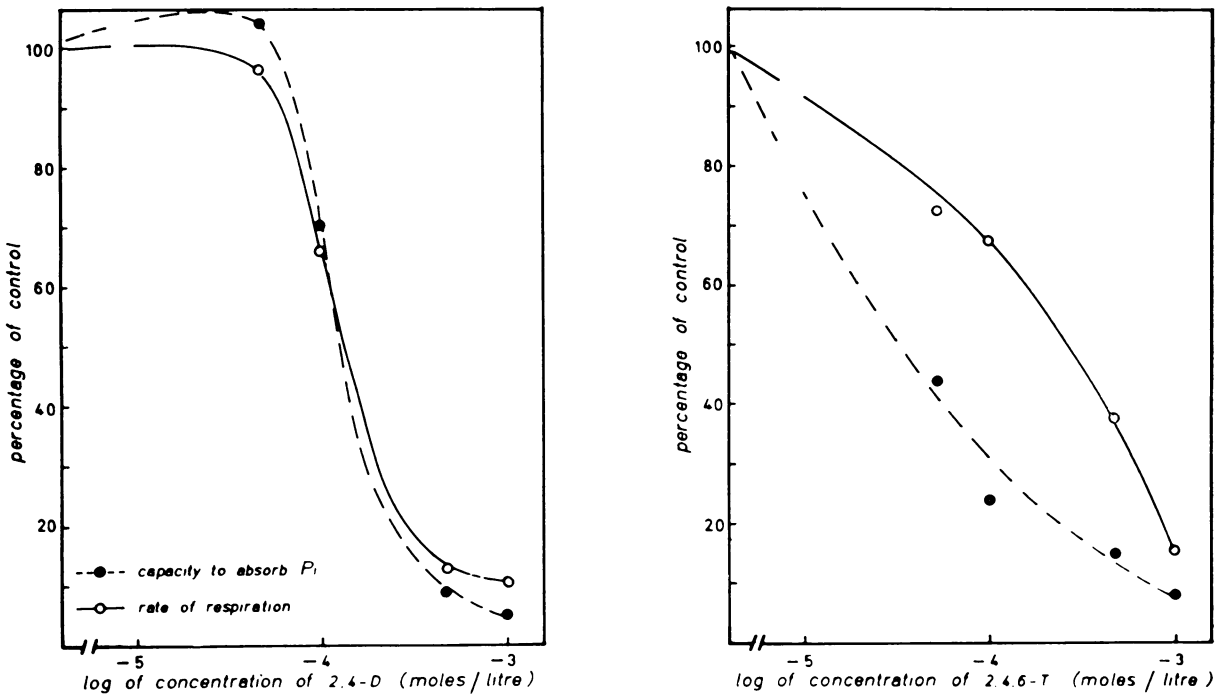

FIG. 5. The rates of metabolism obtained after incubating beetroot disks for 18 hours, in either 2,4-dichlorophenoxyacetic acid or 2,4,6-trichlorophenoxyacetic acid.

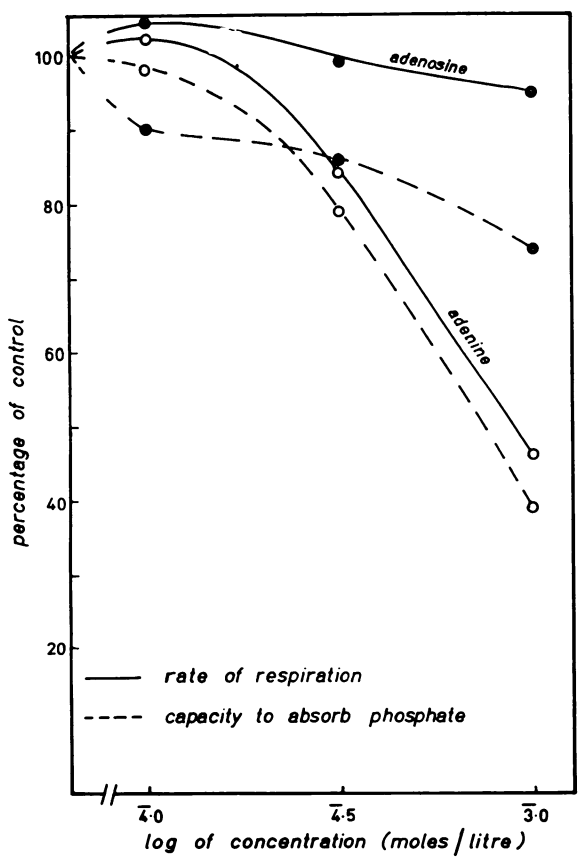

FIG. 6. The rates of metabolism obtained after incubating beetroot disks for 18 hours in either adenine or adenosine.

active in preventing the induction of the high rate of metabolism, on the other hand adenosine was found to be essentially inactive.

Discussion

Although it has been known for over half a century that cutting storage tissue into slices results in enhanced rates of metabolism, especially in the rate of respiration, very little is known concerning the mechanism by which this enhancement occurs. There are at least 2 possible mechanisms which have received experimental support. It has been suggested that cutting and washing the disks might either A) reduce the level of an endogenous inhibitor of respiration, thereby causing the rate of respiration to increase (6), or B) cause a change in the rate or pattern of nucleic acid synthesis which would consequently result in a change in the rate or pattern of protein synthesis (3).

The majority of studies of this phenomenon were carried out using slices of dormant storage tissue which might reasonably be expected to have lower levels of metabolic activity than actively growing tissues. Since there is considerable evidence to support the suggestion that plant growth substances play an important role in maintaining and breaking dormancy it is conceivable that they might also have some effect on metabolic processes in thin slices of tissue. The data presented in this paper show that addition of either IAA or kinetin to the incubation medium prevented the increase of metabolic activity normally found; on the other hand gibberellic acid showed no such inhibitory

activity. No definite correlation could be established between the ability of the compounds to prevent the changes in the rate of metabolism of beetroot disks on washing and their activity as plant growth regulators. 2,4,6-trichlorophenoxyacetic acid and adenine are both relatively active in preventing the appearance of the increased rates of metabolism, yet both of these compounds are very weak plant growth regulators. It is also apparent that in most cases incubation in the presence of the plant growth regulators had an equal effect on the level of all 3 metabolic processes assayed, namely the level of invertase activity, the rate of respiration and the rate of uptake of phosphate. This observation suggests that the changing rates of these 3 systems may result from some change in a single unknown key reaction which controls them and that the plant growth regulators tested may exercise their influence by affecting this key reaction.

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