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LITERATURE CITED


EFFECT OF TISSUE AGE ON HEXOSE METABOLISM. I. AN ENZYME STUDY WITH PEA ROOT 1, 2

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Through the use of a method involving the measurement of the rate of glucose 6-C14 and glucose 1-C14 oxidation (C6/C1 ratio) by plant tissues, Gibbs and Beevers (5) found that while immature tissue respired glucose either exclusively or to a large extent via the classical EMP4 glycolytic pathway, the participation of the HMP pathway was increasingly pronounced as the tissue aged and differentiated. It is clear that the relative concentrations of the enzymes of the various pathways might determine the extent to which each participates in the metabolism of a given tissue. The purpose of this investigation was to determine whether the concentration of some enzymes of the two pathways varied with the developmental age of the plant tissue. Fructose 1,6-diphosphate aldolase, fructose 1,6-diphosphatase and 6-phosphogluconate dehydrogenase were selected as enzymes representing the two pathways. Also assayed were the enzymes catalyzing the anaerobic disappearance of ribose 5-P. The root of the pea plant was selected as the test tissue for the following reasons: 1) large numbers of this plant can be conveniently grown in hydroponic tanks; 2) root tissue in comparison to other plant organs showed a sharper decreasing C6/C1 ratio with successive tissue segments; and 3) previous publications from this laboratory (4, 6, 7) have demonstrated that these enzymes could be quickly and easily determined in cell-free extracts of this plant.

In this paper, attention is directed to the entire root. In a subsequent report, enzyme activity in parts of the root will be treated. A preliminary report of this investigation has appeared (2).

MATERIALS AND METHODS

Growth of Plants: Pea seeds (Pisum sativum var. Alaska, Rogers Brothers Seed Company, Idaho Falls, Idaho) were immersed overnight in distilled water at 4° C. The following day which corresponds to day 1 in the table, they were washed for 15 minutes with a 3.5 % calcium hypochlorite solution, followed
by a distilled water soaking. They were placed in stainless steel trays between moist towels. On the 5th day, the seedlings were of sufficient size to be transferred to the hydroponic tank containing the nutrient solution of Hoagland and Snyder (10). The temperature in the greenhouse was kept at 18 to 22° C. On occasion, the lack of sunlight became a growth factor (experimental period was February 13 to March 15). The few plants which became etiolated were discarded.

By day 7, when the 1st sample was taken, a short somewhat thick tap root had developed. Two sets of compound leaves and rudimentary secondary roots had appeared by the 12th day. On day 16, when the 4th sample was taken, there were 5 compound leaves. Flower primordia, 8 compound leaves and a mat of secondary roots had appeared by the 20th day. When the last samples were taken, 12 compound leaves had been formed.

**ENZYME PREPARATIONS:** Pea root acetone powder was prepared by homogenizing for 1 minute the thoroughly washed roots with 400 ml of acetone at -10° C in Waring blenders and filtering with suction. The dry powder was reblended with another 200 ml of acetone. When dry, the powder was screened through a plastic no. 20 sieve to remove course fibers. The powder was stored at -18° C in a desiccator containing P₂O₅. The enzyme extract was made the following day by grinding 200 mg of acetone powder with 15 ml of 0.1 M TRIS buffer, pH 8.5, at 4° C for 2 hours with a mechanical mortar. After removal of most of the insoluble material by centrifugation, the supernatant fluid was filtered through a plug of glass-wool. The volume was approximately 10 ml. Pea seed acetone powder was prepared by homogenizing 50 g of seeds which had been immersed in water for 16 hours at 4° C with 400 ml of acetone at -10° C in a Waring blendor and then treated in the same manner as the roots.

**REAGENTS AND ASSAYS:** Unless otherwise specified, the methods and materials employed for protein assay, aldolase, 6-PG dehydrogenase, R-5-P and F-1,6-diP phosphatase were as previously described (4, 6). Further reduction of TPN by the products of 6-PG metabolism is not significant since R-5-P does not bring about the formation of reduced TPN under the same conditions.

**RESULTS AND DISCUSSION**

Some of the pertinent data concerning the sampling and growth of the plants are recorded in Table I. They indicate that in spite of the weather conditions prevailing in the area during the winter months, growth and development of this particular crop of peas was fairly normal.

In discussing the data of figure 1, the following assumptions have been made: 1) aldolase activity represents the metabolism of G-6-P via the EMP pathway while 6-PG dehydrogenase activity reflects the entry of G-6-P into the HMP pathway; 2) the curve

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**Table I**

**Characteristics of Plants**

<table>
<thead>
<tr>
<th>Day of Sampling</th>
<th>No. of Plants per Plant</th>
<th>FW *</th>
<th>FW Root**</th>
<th>Length Top†</th>
<th>Length Root†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>G</td>
<td>MM</td>
<td>MM</td>
</tr>
<tr>
<td>7</td>
<td>150</td>
<td>0.58</td>
<td>0.06</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>0.82</td>
<td>0.13</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>13</td>
<td>61</td>
<td>1.25</td>
<td>0.34</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>16</td>
<td>36</td>
<td>2.90</td>
<td>0.54</td>
<td>150</td>
<td>170</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>2.50</td>
<td>0.80</td>
<td>230</td>
<td>220</td>
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<tr>
<td>24</td>
<td>31</td>
<td>3.40</td>
<td>0.95</td>
<td>370</td>
<td>300</td>
</tr>
<tr>
<td>29</td>
<td>31</td>
<td>4.40</td>
<td>1.02</td>
<td>500</td>
<td>300</td>
</tr>
</tbody>
</table>

* FW = fresh weight
** The root is defined here as the distance from the cotyledon to the root tip.
† Distance from cotyledon to highest point of plant.
designated R-5-P metabolism represents the rate at which pentose phosphate is converted anaerobically to hexose monophosphates and triose phosphates via the transketolase-transaldolase sequence of reactions; 3) the curve designated fructose-1,6-diphosphatase represents the rate of conversion of F-1,6-diP to fructose 6-phosphate; 4) a change in enzyme activity is due to a change in enzyme concentration and is not due to inhibitors or accelerators; and 5) the enzyme isolation procedures are representative for each sampling period.

The highest ratio between aldolase and the other 3 systems tested was found in the seed sample (fig 1). This would suggest that the most active mechanism for carbohydrate metabolism in this tissue is probably the EMP type. That this is the case has been demonstrated convincingly by the recent observations of Hatch and Turner (9). In their pea seed extracts, no evidence could be found for an anaerobic pathway operating alternatively to the EMP pathway. This finding of Hatch and Turner is interesting in light of our observation of an active G-6-P dehydrogenase and a somewhat more active 6-PG dehydrogenase in the seed extracts. This would suggest that the mechanism regulating the entry of G-6-P into the HMP pathway in seed extracts is not the absence of the alternative pathway enzymes. The insignificant rate of R-5-P metabolism by the extracts of seeds may be another indicator of a non-functional HMP pathway.

Attempts to bring about in plant extracts a partial reversal of glycolysis, i.e., the conversion of pyruvate to hexose monophosphate, have generally been unsuccessful. The rate of the forward reaction is far greater than the reverse reaction. The cause of this difference has usually been thought to be a lack of adenosine triphosphate and reduced pyridine nucleotide. Another controlling mechanism may be the amount of fructose 1,6-diphosphatase which is only 10% as active as F-1,6-diP aldolase (fig 1).

By the 7th day, when the 1st root samples were assayed, the activity of the 4 enzyme systems had changed considerably. The system catalyzing the disappearance of R-5-P had multiplied the most. Between the 7th and 13th day, aldolase activity decreased slowly followed by a sharper drop between the 13th and 16th day. In comparison, R-5-P metabolism and fructose 1,6-diphosphate activity showed little change while 6-PG dehydrogenase decreased until day 13, then remained stable.

The 2 most striking changes illustrated in this figure are: 1) the appearance of the system catalyzing the metabolism of R-5-P between the seed sample and the first root sample which may reflect the formation of a functional HMP pathway; and 2) the sharp drop in aldolase activity between the 16th and 20th day without a concomitant decrease in 6-PG dehydrogenase and R-5-P metabolism. It is interesting to speculate that flower initiation which occurred in this period may be linked to this decrease.

In tissues which possess enzymes of both pathways the unresolved question concerns the internal influence(s) which apparently determines the fate of the G-6-P. In the embryonic tissue which appears to respire hexose mainly via the EMP sequence, an internal influence may be a high TPNH/TPN ratio. Clearly the possibility exists that as root embryonic areas differentiate during growth, a low TPNH/TPN ratio combined with a high DPNH/DPN ratio could shunt G-6-P into the HMP pathway since the one oxidizing step of the EMP pathway in pea roots is DPN-specific (3). A control mechanism of this type has been fully documented in bacterial and animal extracts (1, 8). If the C6/C1 ratio is an indicator of carbohydrate dissimilation, then the data presented in this paper are consistent with the concept that while the EMP pathway plays a dominant role in hexose metabolism in embryonic tissue, the HMP pathway makes an increasing contribution as aging occurs. This would further imply that enzyme concentration does, indeed influence the carbohydrate metabolism of at least part of the maturing plant. Turner and Turner (11) made a similar suggestion after demonstrating a linear relationship between the rate of starch formation and starch phosphorylase activity in newly developing pea seeds.

**Summary**

The purpose of this investigation was to determine whether the developmental age of the root of the pea plant had any effect on the enzyme concentration of fructose 1,6-diphosphate aldolase, 6-phosphogluconate dehydrogenase, fructose 1,6-diphosphatase and the enzymes catalyzing the anaerobic metabolism of ribose 5-phosphate. Extracts prepared from acetone powders of 7, 10, 13, 16, 20, 24, and 29 day old roots and from seed which had been immersed overnight in water were assayed. In the extracts of the seed tissue aldolase activity was found to be approximately 2.5 times higher than that of 6-phosphogluconate dehydrogenase while the enzymes catalyzing ribose 5-phosphate disappearance were essentially absent. When the first root sample was taken on the 7th day, the activities of aldolase, 6-phosphogluconate, ribose 5-phosphate metabolism and fructose 1,6-diphosphatase had approximately increased by factors of 5, 8, 50 and 8, respectively. In subsequent samples, both a decrease in aldolase activity and 6-phosphogluconate activity was found. However, the aldolase activity decreased significantly more so that by the 20th day, the dehydrogenase activity was approximately 50% higher. The ribose 5-phosphate metabolism rate remained about half that of the dehydrogenase. The phosphatase rate was the lowest of the systems investigated being approximately one third that of the dehydrogenase. It was concluded that the concentration of enzymes of Embden-Meyerhof-Parnas glycolytic sequence and of the hexose monophosphate pathway may be an internal influence on determining the immediate fate of glucose 6-phosphate.
The authors would particularly like to thank Mr. Frank German and Mr. Robert Gaffga of the Biology Department of the Brookhaven National Laboratory for their assistance in the growing of the plants.

LITERATURE CITED

AN AIRFLOW PLANIMETER FOR MEASURING THE AREA OF DETACHED LEAVES1

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Competition for light within crops and pastures has received considerable attention in the last few years. The area of leaf is the principal factor governing the ability of the plant to intercept light energy and thus the measurement of leaf area has been of direct importance in growth and competition studies. But measurement of leaf area has been a tedious process, particularly in mixed pastures where leaves of many species have to be measured. A rapid and accurate method of measuring leaf area despite differences in shape and texture is required. The apparatus described in this paper is presented as meeting these needs.

The more widely used methods of measuring leaf area were reviewed by Frear (2) and more recently by a series of papers presented at the Third Easter School in Agricultural Science, University of Nottingham (4). Briefly these methods are:

BLUE-PRINT AND PLANIMETERING: The leaf is held flat by glass plates on light sensitive paper; on exposure and printing a record of the shape of leaf is obtained; the area is then measured by a planimeter. This method is presumably accurate, but it is very slow and tedious; it has the unique advantage in that a permanent record of individual leaf sizes and shapes is obtained.

COUNTING SQUARES ON GRAPH PAPER OR GLASS GRIDS: This method has been reported for the measurement of turnip leaves; with smaller leaves of pasture species and particularly with long narrow grass leaves this method would be impracticable.

LIGHT INTERCEPTION: Leaves are placed between a light source and a photronic cell; the intercepted light (measured by the photronic cell) is proportional to the area of the leaves. The accuracy of this method depends on leaves being placed flat and at right angles to the column of light. Many detached pasture plant leaves will not lie flat unless held by glass plates in which case the method becomes slow and tedious.

MEASURED PARAMETERS: Where the shape of the leaf is closely related to a geometric pattern the area of leaf is calculated in the same way as the particular geometric pattern; this method is not applicable to most pasture plant species. In a similar manner methods have been developed whereby the area of leaf has been empirically related to a linear measurement of some part of the leaf (1). These methods require considerable preliminary work for each species to be measured and are impracticable for most pasture samples.

MATERIALS AND METHODS

Basically the apparatus (fig. 1) consists of 2 identically perforated plates mounted in the top of an airtight drum, which is connected to a constant speed

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1 Received January 19, 1959.