LITERATURE CITED


INTERMEDIATES OF THE PENTOSE PHOSPHATE PATHWAY AS RESPIRATORY SUBSTRATES\textsuperscript{1,2}

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In the past few years a considerable amount of evidence has accumulated in favor of the participation of reactions of the pentose phosphate pathway in the carbohydrate metabolism of plants. This evidence, which has been summarized recently by Axelrod and Beevers (1), has come both from work on extracted enzyme systems and from a variety of experiments on intact tissues. Among the latter may be mentioned those which have demonstrated that the interconversion of labeled sugars, the incorporation of fragments of labeled sugars into other compounds, and the respiratory utilization of glucose labeled in specific positions occur in many cases in a fashion which is predictable from the operation of the pentose phosphate cycle.

To facilitate presentation of the results and discussion an outline of the major reactions of the pentose phosphate cycle is shown in figure 1. A detailed consideration of the individual reactions as they occur in plants is given elsewhere (1). The essential features of the cycle, represented in figure 1 as beginning with 6 molecules of glucose-6-phosphate are: (a) oxidative and decarboxylating steps at the aldehyde end in which glucose-6-phosphate becomes converted first to 6-phosphogluconate and then to pentose-5-phosphate; and (b) a sequence of splitting and transfer reactions brought about by transketolase and transaldolase which results in the conversion of the pentose

\textsuperscript{1} Received March 9, 1956.

\textsuperscript{2} This paper is based on work performed under contract No. AT(11-1)-330 with the Atomic Energy Commission.
phosphates into hexose phosphates, with sedoheptulose phosphate as an important intermediate.

The successful demonstration of these reactions in cell free systems from plants and particularly the work of Gibbs and Horecker (7) using specifically labeled pentose phosphate as a substrate for pea root preparations have given good grounds for supposing that the path taken by the individual C-atoms is essentially that shown in figure 1.

The fact that several tissues have given evidence of metabolizing supplied glucose by reactions in which C-1 makes an early contribution to the CO$_2$ (3, 6) and the successful demonstration of the oxidation of 6-phosphogluconate and pentose-5-phosphate by pea extracts (5) encouraged the hope that further evidence of the operation of the pentose phosphate pathway in vivo might be obtained by investigating the utilization of intermediates in the reaction sequence.

In the present report then, experiments are described in which intermediates of the cycle (or more correctly the parent compounds corresponding to some of the phosphate esters shown in figure 1) were provided to respiring plant tissues, and their utilization followed by techniques similar to those used in the glucose experiments (2, 3). C$^{14}$-labeled gluconic acid, the pentoses D-ribose and D-xylose, and sedoheptulose were used in order to find (a) whether they were metabolized in air and under nitrogen and (b) whether anything could be deduced as to the mechanism of their utilization.

**Materials and Methods**

**Plant Material:** Young corn coleoptiles from 2- to 3-day-old seedlings of variety Wf9/38-11 were used in many of the experiments. In addition, tissues of pea seedlings (Alaska) grown on sand and for comparison a variety of other plant materials, mentioned in the text, were employed.

**C$^{14}$-Labeled Compounds:** D-Xylose and D-ribose, labeled specifically in the C-1 position were supplied by the National Bureau of Standards (Dr. H. S. Isbell). The sodium salts of gluconate-1-C$^{14}$, gluconate-2-C$^{14}$ and gluconate-6-C$^{14}$ were prepared from the corresponding labeled glucose (N.B.S.) by the method of Moore and Link (10). Sedoheptulose, uniformly labeled with C$^{14}$ (sedoheptulose-U-C$^{14}$) was

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**Fig. 1.** Diagrammatic representation of the major reactions of the pentose phosphate cycle. The numbers refer to the positions occupied by the carbon atoms in the original hexose phosphate molecules.

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The diagram shown in figure 1 summarizes the various pathways shown above and the relationships in the metabolism of hexose phosphates and pentoses. These have been derived from the work of Horecker and his collaborators and of others (2, 3, 5, 7).

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generously supplied by Dr. N. E. Tolbert, Oak Ridge National Laboratory. It had been separated from Sedum which had received C14O2 (15).

Usually 20 to 50 micromoles of labeled substrate was shaken at 25° with about 1 gm of thinly sliced tissue suspended in 3 to 4 ml of a solution containing 0.5 ml 0.1 M phosphate at pH 5.0. Large (100-ml) Warburg flasks were employed, and the CO2 was collected in CO2-free KOH and converted to BaCO3 at the end of the experimental run. It was then washed, plated and dried and its radioactivity determined in a windowless gas flow counter. The figures in the tables are in counts per minute (cpm) corrected for self absorption and background.

In the anaerobic experiments the substrates were added from the side arms of the flasks after flushing with purified nitrogen for 15 min and a further 15 min equilibration period. When alcohol was collected it was converted to ethanol as described earlier (2). The acetate was then divided into two equal samples, one of which was used for a total oxidation (13) and the other for the determination of radioactivity in the COOH group by the Schmidt reaction (12). The total activity in the CH3 group was then calculated by difference. For experiments in which alcohol produced under aerobic conditions was required, arsenite was added (2). In all cases, the use of large amounts of tissue resulted in yields of CO2 and alcohol which were large enough to render the addition of carriers unnecessary. The high endogenous rates of respiration were not noticeably increased by the addition of any of the substrates used in the experiments.

RESULTS

Pentose Experiments, Aerobic: Table I summarizes the results of a series of experiments in which D-xylose or D-ribose was supplied in air to a variety of tissues and the respiratory CO2 collected. In most cases the experimental period was 4 hrs. It will be seen that all of the tissues are capable of converting C-1 of both of the supplied pentoses to CO2 although some of the rates were quite low. In general, the more actively respiring tissues were more efficient in using the pentoses. Figure 2 shows the effect of increasing the amount of corn coleoptile tissue at fixed concentrations of ribose and xylose. It will be seen that the respiration of each pentose proceeds in an orderly fashion, with ribose the slightly more effective substrate. The response of a given quantity of tissue to increasing substrate concentration is shown in figures 3 and 4. Again there is a clear parallel in the effects of the two substrates. Ribose is again somewhat more rapidly utilized but half maximum rates of C14O2 release are obtained at roughly the same substrate level (15 micromoles in 2 ml).

Table II

Anaerobic Dissimilation of D-XYLOSE-1-C14
(Experiments in which the Distribution of Tracer in the CO2 and Alcohol Accumulating under Nitrogen Was Determined)

<table>
<thead>
<tr>
<th>MATERIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TYPE</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Corn</td>
</tr>
<tr>
<td>Root tips</td>
</tr>
<tr>
<td>Coleoptiles</td>
</tr>
<tr>
<td>Pea</td>
</tr>
<tr>
<td>Root, 5 da</td>
</tr>
<tr>
<td>Coleoptiles</td>
</tr>
<tr>
<td>Plunule, 3 da</td>
</tr>
<tr>
<td>Carrot</td>
</tr>
<tr>
<td>Root discs</td>
</tr>
</tbody>
</table>
Fig. 2. Respiration of labeled pentoses by corn coleoptile tissue. The effect of varying the amount of tissue on the yield of C\textsuperscript{14}O\textsubscript{2} from 45 micromoles of D-ribose-C\textsuperscript{14} (25,000 cpm supplied) and D-xylose-1-C\textsuperscript{14} (32,200 cpm supplied). Experimental period 280 min.

Figs. 3 and 4. The effect of increasing the amount of D-ribose-1-C\textsuperscript{14} (555 cpm/micromole) and D-xylose-1-C\textsuperscript{14} (715 cpm/micromole) on the radioactivity recovered in the respiratory CO\textsubscript{2} from 300 mg tissue. Experimental period 280 min.

Anaerobic: Radioactive CO\textsubscript{2} was also released when certain of the tissues were provided with labeled pentoses under nitrogen (column 6 in Table II and Table III). By far the most active tissue in this regard was the corn coleoptile; this is also the tissue which has the highest and most prolonged rate of endogenous CO\textsubscript{2} release. An important feature of the results was that in every tissue, the alcohol which was produced, as well as the CO\textsubscript{2}, was labeled. When this is taken into account it is seen that in the case of the corn coleoptile up to 40% of the ribose and about 30% of the xylose had appeared in these two fractions at the end of a 5- to 6-hr experimental run. Of greater significance is the fact that the pattern of labeling in the alcohol recovered is the same in each case (Tables II and III). The radioactivity was very largely confined to the CH\textsubscript{3} group and in only one experiment had the CH\textsubscript{2}OH group more than 10% of the total counts in the alcohol.

Moreover, as a comparison of columns 6 and 7 in these tables shows, there appears to be a fairly constant relationship between the total activity recovered in the CH\textsubscript{3} of the alcohol and that in the CO\textsubscript{2}. The figures in the final columns show that this value is interestingly close to 2 in the majority of the experiments.

Aerobic Experiments with Arsenite: Table IV shows the results of experiments in which the aerobic accumulation of alcohol was induced by the addition of 0.005 M arsenite. Since aerobic respiration is only partially inhibited under these conditions (2) the CO\textsubscript{2} has a dual origin, and only part of it corresponds to the alcohol which accumulates. It is clear, however, that the alcohol is labeled, and the activity is again largely confined to the CH\textsubscript{3} group, although a significantly higher amount is found in the CH\textsubscript{2}OH group than was so in the aerobic experiments.

Gluconate Experiments: Table V shows that gluconate-1-C\textsuperscript{14} was utilized effectively by both coleoptile and root tissues, but that in nitrogen, the release of C\textsuperscript{14}O\textsubscript{2} was strikingly reduced. Having established that coleoptile tissue was capable of metabolizing gluconate, a series of experiments was carried out in which equivalent amounts of gluconate-1-C\textsuperscript{14},...
Table III

Anaerobic Dissimilation of D-Ribose-1-C\textsuperscript{14}
(Experiments in Which the Distribution of Tracer in the CO\textsubscript{2} and Alcohol Accumulating under Nitrogen Was Determined)

<table>
<thead>
<tr>
<th>Material</th>
<th>Type</th>
<th>AmT</th>
<th>Duration of Expt</th>
<th>Ribose Added</th>
<th>Activity Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>gm</td>
<td>hours</td>
<td>micromoles</td>
</tr>
<tr>
<td>Corn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root tips</td>
<td>1.3</td>
<td>5</td>
<td>30</td>
<td>10.8</td>
<td>180</td>
</tr>
<tr>
<td>Coleoptiles</td>
<td>0.85</td>
<td>6</td>
<td>30</td>
<td>10.8</td>
<td>1075</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>5.5</td>
<td>30</td>
<td>10.8</td>
<td>1437</td>
</tr>
<tr>
<td>Pea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root, 5 da</td>
<td>2.0</td>
<td>5</td>
<td>50</td>
<td>18</td>
<td>171</td>
</tr>
<tr>
<td>Root, 3 da</td>
<td>2.5</td>
<td>6</td>
<td>50</td>
<td>18</td>
<td>58</td>
</tr>
<tr>
<td>Plumule</td>
<td>2.5</td>
<td>6</td>
<td>50</td>
<td>18</td>
<td>156</td>
</tr>
<tr>
<td>Carrot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root discs</td>
<td>3.0</td>
<td>8</td>
<td>50</td>
<td>18</td>
<td>162</td>
</tr>
</tbody>
</table>

Sedoheptulose Experiments: Results from experiments on the utilization of sedoheptulose-U-C\textsubscript{14} are shown in table VII. It is clear from these that, both in air and nitrogen, this substrate is metabolized, and the yields of C\textsuperscript{14}O\textsubscript{2} under the two conditions are not greatly different. The alcohol produced anaerobically is also labeled, and as shown, the radioactivity is fairly equally divided between the two carbon atoms. An unsuccessful attempt to increase the utilization of sedoheptulose by the addition of (unlabeled) pentose is also shown in table VII.

Discussion

The demonstration that pentoses, gluconate and sedoheptulose can be used as respiratory substrates by plant tissues can not in itself be considered as evidence of the occurrence of the pentose phosphate pathway, but it is clearly consistent with the operation of such a reaction sequence. The particularly active utilization of the pentose by corn coleoptiles indicates that, whatever pathway the breakdown is achieved, the capacity of the system may be quite considerable.

Evidence as to the mechanism of pentose utilization is provided by the data from the anaerobic ex-
Each material (0.9 gm) was incubated for 7 hrs with 50 micromoles gluconate-1-C14.

Experiments with ribose-1-C14 and xylose-1-C14. In these, it will be recalled, the CO2 and the alcohol which were produced were both labeled; the CH3 group of the alcohol accounted for almost all of the activity in the alcohol and contained roughly twice that found in the CO2. Clearly no simple cleavage of a pentose chain singly labeled in a terminal position would give rise to alcohol and CO2 both containing radioactivity, and on this evidence alone some more complex breakdown pattern of the pentose must be suspected. It will now be shown that the results are singularly well explained by supposing that the pentose does in fact undergo the rather complex sequence of reactions shown in figure 1.

It will be seen from this figure that the net result of the reactions subsequent to pentose formation is the production of 4 moles of glucose phosphate and 2 moles of triose phosphate (which are shown in fig 1 as giving rise to a further hexose unit). The positions which the original C-atoms of the pentose would be predicted to take in the resulting hexoses are also indicated in the figure. Of the five hexoses produced, only four contain C-1 of the pentose. In two of these hexose units, C-1 of the pentose (the carbon atoms 2 in fig 1) appears twice, i.e., in the C-1 and C-3 position. These are the hexoses which are produced by the transfer of carbons 1, 2, 3, as a unit, from the sedoheptulose to triose phosphate. Since carbons 1-3 of the sedoheptulose originated from the transfer of C-1 and C-2 from pentose to another pentose unit, both carbons 1 and 3 of the sedoheptulose are derived from C-1 of the pentose. The other two hexoses containing C-1 of the pentose are those produced during the transfer of C-1 and C-2 from the pentose, to a tetrose unit from sedoheptulose. This tetrose unit, like the triose mentioned above, is derived from the lower carbons of the original pentose, and does not contain any C-1. Thus, considering the four hexose units together, all of them would have C-1 derived from C-1 of the pentose and in addition half of them would have C-3 derived from C-1 of the pentose. The average distribution of the original C-1 of the pentose in any pyruvate produced during the action of Embden-Meyerhof-Parnas enzymes on this hexose would then be 2/3 in the CH3 group, and 1/3 in the COOH group, and if the pyruvate were converted to CO2 and alcohol, the CH3 of the alcohol would contain 2/3 of any of the C-1 in the products, and the CO2 would contain the rest.

Thus if pentose which was specifically labeled in the C-1 position followed exclusively the reactions shown in figure 1 and the resulting labeled hexose phosphates were then broken down to pyruvate, and if the conditions were arranged, as they were in our experiments, so that such pyruvate was converted to alcohol and CO2, the theoretical expectation would be alcohol which was labeled only in the CH3 group and CO2 containing half of this activity. The fact that this expectation comes close to being realized in many of the anaerobic pentose experiments reported here is regarded as a highly significant piece of evidence in favor of the operation in vivo of the reaction sequence outlined in figure 1. It will be seen that, to the extent that one is justified in inferring the pattern of labeling in the hexose phosphate from that observed in the alcohol and CO2, the results agree closely with those described from the pea root preparations of Gibbs and Horecker (7). In their in vitro experiments in which hexose phosphate was produced from C-1 labeled pentose phosphate, roughly 70% of the activity recovered in the hexose was in the C-1 position and 30% in C-3.

The work of Neish (11) on utilization of pentose...
in cellulose synthesis also points to the occurrence in vivo of a similar series of reactions and very recently Ginsburg and Hassid (9) have presented some additional results on pentose utilization in wheat which are susceptible of the same interpretation.

In the anaerobic experiments described above, it should be stressed, the O₂-free conditions presumably limited the entry of glucose (including that from the supplied pentose) into the pentose phosphate cycle. In the arsenite experiments, in which the aim was to determine the labeling in the α and β carbons of aerobically produced pyruvate by inducing its diversion to alcohol, it is again clear that the β carbon (CH₃ of alcohol) has by far the greater activity; the appearance of a somewhat greater amount of activity in the CH₂OH group (α carbon of pyruvate) than is observed in the anaerobic experiments might indicate that some recycling and consequent randomization was occurring in air.

In contrast to the pentose results, in which active utilization occurred both in air and N₂, those obtained in the gluconate experiments show that the withdrawal of O₂ has a large depressing effect on the conversion to CO₂. This is in accord with the requirement for O₂ (or an internal system for the sustained regeneration of TPN) if the oxidative decarboxylation reaction shown in figure 1 is to continue unchecked. In trying to ascertain the breakdown pathway of gluconate the results in figure 5 are an important consideration. These show that, both in air and in N₂, C-1 makes a much larger contribution to the respired CO₂ than C-2 or C-6. Such results would be observed if the gluconate were first converted to pentose as in figure 1. The demonstration that carbons 1 and 6 of the gluconate are not equivalent in their contribution to the respired CO₂ establishes that gluconate is not utilized by reduction back to glucose and subsequent breakdown by the E.M.P. sequence.

Although the anaerobic utilization of gluconate is small, the results of the alcohol degradations in table VI are nevertheless in accord with the regeneration of hexose from gluconate in an oxidative cycle which involves (a) decarboxylation at C-1 and (b) recombination of the pentose carbon by the reactions shown in figure 1 into hexose and subsequent breakdown to pyruvate by the E.M.P. enzymes. As table VI shows, the C-1 of the gluconate made a relatively large contribution to the CO₂ and did not appear in the alcohol. On the other hand, C-6, which would be expected from figure 1 to contribute only to C-1 and C-6 of the hexose and thus the CH₃ group of pyruvate, did not appear in any quantity in the CO₂, and was present, significantly enough, only in the CH₃ group of the alcohol. C-2 of the gluconate would be expected on the basis of figure 1 to become C-1 of the pentose and thus to contribute only to C-1 and C-3 of the hexose in a 2/1 ratio. Table VI shows that in the experiments with gluconate-2-C¹⁴ the CO₂ (derived from the COOH of pyruvate and equivalent to the C-3 of the hexose) was in fact radioactive but considerably less so than the CH₃ of the alcohol. The CH₂OH group of the alcohol was again unlabeled. The actual ratios of (C¹⁴ in CH₃ of alcohol/C¹⁴ in CO₂) observed in the gluconate-2-C¹⁴ experiments are 2.9, 2.3 and 3.1. Although these values are considered to be reasonably close to 2 in view of the fact that the yield of C¹⁴ in the products was small, it will be recalled that in some of the pentose experiments values closer to 3 were occasionally observed. This may be taken to indicate that some side reaction not included in figure 1 may occur and result in an additional incorporation of C-1 of pentose into CH₃ of alcohol.

From table V and figure 5 the presumption seems justified that in air the gluconate is metabolized more rapidly but by the same pathway as that taken in N₂.

The fact that pentose utilization occurred readily in our materials in air and N₂ in a way which appeared to include sedoheptulose as an intermediate, made it desirable to investigate the utilization of this compound. Tolbert and Zill (14) had previously observed that in air this material is rapidly utilized and the carbon incorporated into fructose and a number of other cell components in barley, tobacco and sugar beet leaves but that in N₂ the incorporation was drastically reduced. The results with corn coleoptiles in table VII show clearly that, on the basis of its contribution to respiratory end products (which were not under investigation by Tolbert and Zill), sedoheptulose is metabolized in this tissue both in air and nitrogen. The appearance of radioactivity in the CO₂ produced in air, and in both carbons of the alcohol and

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**Fig. 5.** Respiration of gluconate by corn coleoptile tissue. Fifty micromoles gluconate-1-C⁴, gluconate-2-C⁴ and gluconate-6-C⁴, respectively, were provided to samples of 1.3 gm tissue and the respired CO₂ analyzed for radioactivity after 2, 4 and 6 hrs.
in the CO₂ produced anaerobically are understandable if the sedoheptulose follows the sequence shown in figure 1.

At the outset it had appeared possible that absorption and phosphorylation by the tissues might be so slow as to prevent the active utilization of materials such as pentoses which are not normally thought of as respiratory substrates. In the outcome it was clear that the utilization of all three substrates was at least large enough to allow some firm conclusions to be reached about the metabolism of each. The conclusions are that, in at least one tissue, the corn coleoptile, the utilization of the pentoses, gluconate and sedoheptulose occurs in a way which, when the possible interference of side reactions is taken into account, is remarkably well accommodated in the coherent picture represented by the sequence of reactions shown in figure 1. Insofar as other tissues have been examined in the critical case of the pentoses, a similar conclusion about the utility of the pentose phosphate pathway seems justified for them also.

It should be made clear that the burden of this report has been to establish the existence of a functional pentose cycle in vivo rather than to reaffirm a respiratory role for the cycle. In fact it will be noticed that the tissue we have found the most convenient for many of the experiments is one of a group of young tissues in which it is clear that the Embden-Meyerhof-Parnas pathway of glycolysis is by far the most important (2, 3, 6).

The utilization of gluconate and the pentoses indicate that, in spite of this, enzymes are present in the tissue which can carry out the reactions of the cycle at least from gluconate onwards. Furthermore Gibbs and his associates (4, 8) have recently shown that enzymes of the cycle, including glucose-6-phosphate dehydrogenase, can be extracted from several very young tissues. In this case, and in others in which enzymes of both pathways (see Axelrod and Beevers (1)) may be shown to be present and active in vivo, the unresolved question concerns the internal influence which presumably determines the immediate fate of the glucose-6-phosphate.

SUMMARY

It has been established that D-xylose and D-ribose can be utilized as respiratory substrates, by a variety of plant tissues. One of the most active of these was the corn coleoptile, and this material was used in further experiments which showed that sedoheptulose and gluconate are also respired. Each of the substrates gives rise to alcohol and CO₂ under nitrogen, although the utilization of gluconate, in contrast to that of the pentoses and sedoheptulose, is markedly reduced under these conditions.

C¹⁴ appeared both in the alcohol and in the CO₂ produced anaerobically (and aerobically in the presence of arsenite) from ribose-1-C¹⁴ and xylose-1-C¹⁴. In addition, it was observed that the tracer was characteristically distributed in these products; roughly 2/3 appeared in the CH₃ of the alcohol and 1/3 in the CO₂. These findings are interpreted to mean that the pentoses were being metabolized by way of the pentose phosphate cycle.

Experiments with gluconates labeled respectively in the 1-, 2- and 6- positions showed that release of C-1 is an early reaction in its utilization. The pattern of labeling in the alcohol and CO₂ produced anaerobically from the three gluconates is also consistent with its conversion to pentose by loss of C-1 in an oxidative step and subsequent conversion to glucose in the pentose phosphate cycle.

The fact that a plant tissue is capable of respiring gluconate, pentose, and sedoheptulose, which represent intermediates in the breakdown of glucose by the pentose phosphate cycle, is of interest in itself. As evidence for the presence of such a cycle in the tissue a more incisive finding is that the paths taken by individual C-atoms of the labeled substrates, as indicated by the distribution in the products of anaerobic respiration, are essentially those predicted from the present knowledge of the reaction sequence.

The experimental work on which this paper is based was begun while I was a visiting scientist in the Biology Department of Brookhaven National Laboratory in the summer of 1955. I would particularly like to thank Dr. Martin Gibbs of that department for his help and advice during this time as well as for his hospitality.

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LIGHT EFFECTS ON THE GERMINATION OF SEEDS OF HENBIT (LAMIIUM AMPLEXICAULE L.) \(^1\) \(^2\)

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This paper originated from the observation that seeds of henbit failed to germinate when exposed to light from a north window, but they germinated well in the dark. While the writers have found no literature references to light effects on henbit seed germination, the fact that light affects the germination of certain seeds has been known for many years (3). Certain varieties of lettuce (Lactuca sativa L.), especially Grand Rapids, are classic examples of light-sensitive seeds. Flint and McAlister (6, 7) determined that light of wavelengths between 5200 and 7000 Å stimulated the germination of lettuce seed, whereas far-red radiation (7600 Å) sharply inhibited germination of this seed. These observations have been confirmed in more recent work (1, 2) and extended to other species (9).

This study was undertaken to determine the extent to which henbit conforms to the pattern known to exist in other plants. The effects of light intensity, light quality, and the stage of imbibition at which the seeds are irradiated have been investigated.

EXPERIMENTS

Freshly-harvested seeds of henbit (Lamium amplexicaule L.) are dormant and will not germinate for several weeks regardless of light conditions. Efforts to break this dormancy with a wide variety of treatments were unsuccessful. A water extract of crushed dormant seeds markedly inhibited the germination of tomato seeds and non-dormant henbit seeds. Apparently the dormant seeds contained a water-soluble inhibitor which decreased during dry storage of the seeds.

The effect of light intensity on germination of non-dormant seeds was established by irradiating seeds on moist filter paper in Petri dishes at various distances from an unfiltered 150-watt incandescent light source. Eleven lots of 50 seeds each were used, one of which was maintained in continuous darkness. Light treatments were continuous for 72 hrs and were followed by an additional germination period of 48 hrs in the dark. Germination counts were made at the end of this light period and again after periods of 24 and 48 hrs in the dark. Weak diffused daylight was used to make the germination counts after the 24-hr dark period.

A major inhibitory effect was obtained with the lowest light intensity used, i.e., 0.8 ft-c (table 1). Germination was reduced from 70 to 24% at this intensity. At 2.8 ft-c germination was completely inhibited. After 24 hrs in the dark, recovery from this light effect was evident in seeds which were treated with less than 2.8 ft-c of light. Forty-eight hours after the light treatment most of the inhibition was gone regardless of the intensity of the irradiation used.

Recovery from light inhibition was noted in another experiment. In this case seeds were presoaked in water for 15 hrs before treatment for four minutes with light from an incandescent source filtered through red and blue Cellophane. This filter combination gave maximum transmittance between 7300 and 8700 Å, with no radiation below 7000 Å. The incident energy of this far-red radiation was approximately 8.1 \(\times\) \(10^{-3}\) joules/cm\(^2\) \(\times\) sec. In this experiment good germination was obtained 120 hrs after the inhibiting light treatment.

Light sensitive seeds generally have shown greater sensitivity to one or more particular parts of the spectrum (1, 3, 5, 6, 7). Henbit seeds show a similar selectivity in their response to light. In these experiments triplicate lots of 100 seeds each were exposed after 16 hrs of imbibition to six minutes irradiation with spectral bands having peak transmissions at 3600, 4300, 5200, 5800, and 6000 Å, respectively. These light treatments were obtained with glass filters (Corning Color Filters, Corning Glass Works, Corning, New York) which were found to transmit spectral bands about 400 Å units wide with peak intensity in the approximate middle of each band. Far-red radiation (7300 to 8700 Å) used in this and subsequent experiments was obtained with light from an incandescent lamp filtered through two layers of red and two layers of blue Cellophane. The incident energies-