Relationship Between the Growth and Respiration Induced by Lipids in Pea Stem Sections¹, ²

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Introduction

The elongation of sections from etiolated pea stem in optimal concentrations of indoleacetic acid (IAA), gibberellic acid (GA₃), sucrose and cobalt is markedly less 24 hours after excision than the same zone if it is left on the intact plant (28). The growth of the excised sections can be increased by low concentrations of an emulsion of fatty acid esters and related substances as well as by some isoprenoid compounds (26, 30), although the growth of the sections is still less than the same zone on the intact stem.

The mechanism of action of the lipids is not yet understood, but it is known that they stimulate respiration as well as growth. There is an increasingly large body of biochemical knowledge about the control of respiration, while very little is known biochemically about how plant growth regulators control elongation. Thus, if it can be determined how the lipids stimulate respiration, and if there is a causal relationship between the increased growth and respiration, it may then be possible to identify a particular reaction that will control the rate of growth. In this paper the effects of lipids on growth and respiration have been studied under a variety of conditions in order to examine the nature of the relationship between growth and respiration.

Materials and Methods

Pea seeds (Pisum sativum L.) of the tall variety Alaska or the dwarf variety Progress No. 9 were grown, as described in detail in an earlier publication (30), in vermiculite and under a weak red light source (0.1 ergs/cm² per sec between 600 and 800 nm). Sections 10 mm long were cut from 7-day-old plants 1 mm below the plumule from third internodes that were 15 to 30 mm long. For nongrowing tissue, 10-mm sections were cut 1 mm below the top of the first internode of 8-day-old peas.

Emulsions of lipids were prepared using acetonitrile and Phronic F-68 (Wyandotte Chemical Corporation) as described previously (28) except that 1 ml of acetonitrile was warmed to dissolve triolein. The final emulsion was exposed to 5 minutes of 20 kc ultrasound using a Branson Sonifier. As reported previously (28) the small amount of residual acetonitrile in the lipid preparation does not affect the growth of the sections, nor does this residual acetonitrile have an effect on respiration. No O₂ consumption by these lipid preparations has ever been detected in the Warburg vessels in the absence of pea tissue or cell fractions.

Bioassays and respiration experiments were run in a dark room at 25°. Five sections with a fresh weight between 150 to 200 mg were placed in 3 ml of medium in a 15 ml Warburg vessel. The glassware used in these experiments was always washed in a hot concentrated H₂SO₄-HNO₃ acid bath since some detergents are active in the bioassays.

U-C¹¹ methyl oleate (5–7 mc/m mole) was obtained from Applied Science Lab Inc., State College, Pennsylvania. Before use this was further purified by thin layer chromatography on silicic acid with carboxymethylcellulose as a binder and using a 98:10:1 petroleum ether:ethyl ether:acetic acid mixture as the solvent (20). The palmitonitrile obtained from Lachron Chemicals, Inc. was 99.5% pure.

C¹⁴O₂ was absorbed by KOH contained in small vials that fitted inside the center well of the Warburg vessels. When the experiment was completed concentrated H₂SO₄ was added from the side arm to the medium and the vial was later removed. The CO₂ was released from the vial with acid inside a closed vessel at 0 to 4°. The vessel also held a scintillation tube containing phenethylamine which absorbed CO₂ (34). Absorption of CO₂ was shown to be complete within 2 hours after which scintillation fluid was added and the radioactivity counted in a Packard scintillation counter. The method gave a linear counting rate over a 500-fold concentration of C¹⁴O₂ and the phenethylamine did not decrease the counting efficiency.

Statistics were calculated following (25). Results are usually expressed by letters. One letter (a...a) signifies that 2 values are significantly different at the 5% level. Two letters (bb...bb) mean that the difference is significant at the 1% level.

Results

Effect of Triolein on Respiration and Growth. Previous experiments have shown that triolein induced axial growth of pea stem sections in the bioassay. It
has now been shown (fig 1, table IA, B) that concentrations of triolein that stimulate growth also stimulate respiration. The enhancement of respiration is found in Progress No. 9 (dwarf) and Alaska (tall) peas and does not depend on the presence of the hormones. In the experiments shown in figure 1 the measurements of O₂ consumption did not begin until at least 30 minutes after the sections were added to the medium but the results show that the stimulation probably occurs within 30 minutes.

Non-growing Tissue. O₂ consumption was measured in the presence and absence of triolein with sections from the first internode of 8-day-old plants (table IC). This was one of several experiments to determine whether the triolein-induced growth could be the cause of the increased respiration (see Discussion). The results given here demonstrate that even in the absence of growth triolein will still enhance respiration. The slight increase in fresh weight is within experimental error and no increase in the length of the sections could be detected over the 3-hour period of the experiment.

Palmitonitrile. Of the numerous lipids that stimulate the growth of the sections in this bioassay, α-tocopherol, methyl oleate (27, 29) and now triolein have been shown to stimulate respiration. It has recently been discovered in this laboratory (Stowe and C. Kuiper, unpublished) that some nitrile derivatives of fatty acids are active in the bioassay. One of these, palmitonitrile, has also been shown to stimulate O₂ consumption (table ID). The activity per mole of palmitonitrile on respiration is similar to that of triolein when it is considered that triolein has 3 fatty acid chains per molecule. That 4 dissimilar compounds increase both growth and respiration indicates that there is only 1 site of action for the lipids.

Table I. A Comparison of Hormone and Lipid Effects on Respiration, Elongation and Fresh Weight

Ten-mm sections were taken from the first internode of 8-day-old or from the third internode of 7-day-old red light-grown peas. Length and fresh weight measured after 3 hours and respiration measured over 2 hours within the 3-hour period. The basal medium included in all treatments consisted of 1.5% sucrose, 5 mM KH₂PO₄ (pH 5.5), 50 mM CoCl₂ and 0.004% Pluronic F-68. The concentration of IAA was 1.8 µM and that of GA₃ was 0.3 µM.

<table>
<thead>
<tr>
<th>Variety, internode and treatment</th>
<th>O₂ consumption</th>
<th>△ Length</th>
<th>△ fr wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µl</td>
<td>mg/5 sections per 3 hrs</td>
<td>mg/5 sections per 3 hrs</td>
</tr>
<tr>
<td></td>
<td>O₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Progress No. 9 (3rd)</td>
<td>20</td>
<td>45.5/66</td>
<td>7.7/7.9</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>46.5/66</td>
<td>8.7/8.9</td>
</tr>
<tr>
<td>B. Alaska (3rd)</td>
<td>20</td>
<td>5.5/66</td>
<td>5.5/66</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5.5/66</td>
<td>5.5/66</td>
</tr>
<tr>
<td>C. Progress No. 9 (1st)</td>
<td>20</td>
<td>5.5/66</td>
<td>5.5/66</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5.5/66</td>
<td>5.5/66</td>
</tr>
<tr>
<td>D. Progress No. 9 (3rd)</td>
<td>50</td>
<td>20.5/66</td>
<td>20.5/66</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>20.5/66</td>
<td>20.5/66</td>
</tr>
<tr>
<td>E. Progress No. 9 (3rd)</td>
<td>160</td>
<td>20.5/66</td>
<td>20.5/66</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>20.5/66</td>
<td>20.5/66</td>
</tr>
</tbody>
</table>

Fig. 1. Rate of O₂ consumption by 10-mm sections from red light-grown Progress No. 9 peas. Sections placed in medium indicated 30 minutes before start of measurements. Series with 30 replicates: ■ basal medium, × basal medium plus 1.8 µM IAA and 0.3 µM GA₃, [—] basal medium plus triolein, IAA, and GA₃ at the same concentrations.
Supraoptimal Concentrations of Triolein. Previous experiments have shown that there is an optimum concentration for the lipids and that higher concentrations give less growth and may reduce the growth below that of the control (26). For triolein the optimum is 10 to 20 μM. If triolein increases growth and respiration by acting at separate sites then it is unlikely that the 2 sites will show the same optimum. However, when the effect on respiration was measured with higher concentrations of triolein, a stimulation of both growth and respiration was found with concentrations of triolein as high as 160 μM (table II). This is in apparent conflict with earlier results where this concentration inhibited growth. The difference is in part due to the different times that the measurements were made. When bioassays with 160 μM triolein were measured after 20 hours it was invariably found that the triolein had inhibited growth when compared to a control without triolein. When the same bioassays were measured after 3 hours the results were more variable with sometimes an inhibition even at this time. The other difference between the bioassays and the results given in table I is that CO₂ is present during the bioassays and this could affect the results (table II). However, these results on respiration and growth do not suggest separate optima for growth and respiration and therefore give additional evidence of the close correlation between the effects of lipid on growth and on respiration.

Table II. Effect of CO₂ on Elongation and Fresh Weight

<table>
<thead>
<tr>
<th>+KOH</th>
<th>KOH</th>
<th>20 μM triolein</th>
<th>20 μM triolein</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>7.74</td>
<td>20.5a 26.5b</td>
<td>26.5b</td>
</tr>
<tr>
<td>8.1e</td>
<td>8.9d</td>
<td>30.5a 33.5b</td>
<td>33.5b</td>
</tr>
</tbody>
</table>

CO₂ and Growth. Although low concentrations of triolein are used in these experiments it was possible that the increase in respiration resulted from the utilization of the triolein as a respiratory substrate. This would result in a change in the respiratory coefficient so the effect of triolein on respirations was measured in the presence and absence of KOH. However, an effect of CO₂ on increasing the growth of the sections was found (table II), especially in the presence of triolein. Effects of CO₂ on the growth of pea stem sections have not been noted although effects on other plants, particularly Avena, have received more attention and are mentioned in the discussion. Because of this effect of CO₂ on growth it cannot be assumed that CO₂ does not affect O₂ consumption so that a respiratory coefficient cannot be calculated in the normal manner (32).

Evolution of C₁⁴O₂. It is possible with other manometric techniques to measure whether CO₂ is altering the rate of O₂ consumption (32). However, calculations showed that even if the increased respiration resulted from the utilization of triolein as a substrate the change in the respiratory coefficient would be only 3%. Because of the variability between replicates this was considered too small to detect, so a more direct approach of measuring C₁⁴O₂ release from methyl oleate-U-C₁⁴ was used. Methyl oleate also stimulates respiration and was used because it could be obtained with the isotope uniformly distributed. Replicates were run from 1 to 7 hours and 2 vessels were removed at the times indicated in figure 2 and the C₁⁴O₂ counted by the method described in Materials and Methods.

Fig. 2. Formation of C₁⁴O₂ from methyl oleate-U-C₁⁴ by 10 mm Progress No. 9 pea stem sections incubated in basal medium, 1.8 μM IAA, 0.3 μM GA₃ and 50 μM methyl oleate. Each point is from a separate vessel removed at the time indicated after the start of incubation.

The points fall close to a line after an apparent lag period of about half an hour. Slightly less than 1% of the added radioactivity was found in the CO₂ after 3 hours. Since respiration was measured during the first 3 hours, the 3-hour readings were averaged and used to estimate whether utilization of lipid as a substrate could account for the increase in respiration. The results of these calculations are given in table III. The experiment presented in figure 2 is experiment number 2 in table III. Actual is the amount of C₁⁴O₂ that came from methyl oleate and Expected is the amount of CO₂ that would have to come from methyl oleate to account for the increase in respiration. The experiment has been repeated with triolein-1-C₁⁴ and even less C₁⁴O₂ was released (22).
Table III. Use of Lipid as Respiratory Substrate

Release of C\textsuperscript{14}O\textsubscript{2} in 3 hours from methyl oleate-UC\textsuperscript{14}. Actual is the amount of CO\textsubscript{2} released from 50 μM methyl oleate. Expected is the amount of CO\textsubscript{2} that would have to be released to explain the increase in O\textsubscript{2} consumption. Basal medium, IAA and GA\textsubscript{3} as in table I, with 10-mm stem sections from 3rd internode of Progress No. 9 peas.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Actual μl CO\textsubscript{2}</th>
<th>Expected μl CO\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.0</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
<td>40</td>
</tr>
</tbody>
</table>

From this evidence it is concluded that the increase in respiration induced by lipids is not due to the use of the lipids as a respiratory substrate.

Dodecenyl Succinate. Dodecenyl succinate (3 carboxylic tetracenoic acid) and related compounds have marked effects on water uptake by roots (13), opening of stomata (36) and frost resistance of plants (14). Since these compounds may be considered as derivatives of fatty acids, 10\textsuperscript{-3} to 10\textsuperscript{-5} M dodecenyl succinate was tested in the pea bioassay. This substance is usually used at about 10\textsuperscript{-3} M, but strongly inhibited both growth and respiration of the bioassay tissue at this concentration. There was some inhibition of growth even at 10\textsuperscript{-5} M. No stimulations were noted. A triolein control run at the same time gave the usual promotion of growth and respiration.

Discussion

It has been suggested previously that increased respiration caused the increase in growth, but from what is known it was equally likely that the increased respiration resulted from the enhanced growth, e.g. by an accelerated turnover of ATP. It is frequently difficult to distinguish between cause and effect when 2 phenomena are correlated. One of the main aims of the work presented here has been to determine the nature of the seeming correlation between the lipid enhanced growth and respiration.

Difficulties in measurement and considerable variation between sections made it difficult to detect small differences in the growth rate within an hour of adding triolein. It did not seem possible to decide unambiguously whether the increase in respiration preceded the increase in growth, whether they were simultaneous, or whether the increase in growth preceded the effect on respiration. However, an alternative approach has shown 3 cases where triolein stimulates respiration but has no effect on the growth rate. Two of the cases are with Progress No. 9 and Alaska peas in the absence of hormones (table 1A, B). With the Progress No. 9 peas the slight increase in growth was not significant at the 5% level with 24 replicates, whereas the increase in respiration was significant at the 1% level. In these experiments the Alaska peas did not show the lipid promotion of growth in the presence of hormones that has occurred in other experiments, but this does not affect the conclusion that an increase in growth is not responsible for the increase in respiration. The third case is the nongrowing sections in table IC where there is still a triolein-induced increase in respiration. These 3 examples show that the increase in respiration cannot be solely the result of an increase in the growth rate.

Although a correlation has been shown between the effects of lipid on growth and respiration it was still possible that there is no causal connection between the 2 phenomena and that there are 2 sites of action for the lipid. No attempt has been made to study the effects on respiration of all the compounds that stimulate growth, but it has now been shown that 4 dissimilar compounds, α-tocopherol, methyl oleate, triolein and palmitonitrile do increase both growth and respiration. This would not be expected if lipids affect growth and respiration by acting at different sites. Another fatty acid derivative, dodecenyl succinate, had no promotive effect on growth or respiration.

There is evidence that the exogenous lipids are active as such (22) and do not need to be metabolized before they increase growth and respiration. This conclusion is further supported by the fact that palmitonitrile was found to be active in this bioassay. It is unlikely to be metabolized by the tissue since the enzyme nitrilase (15) is lacking in peas. Thus, no hydrolysis of the nitrile to the acid which could be further metabolized is to be expected, and only 2 of the nitriles tested on peas by Fawcett et al. (9) showed evidence of an α-oxidation reaction.

A stimulation of growth of pea sections by CO\textsubscript{2} does not appear to have been reported before except for a recent report that CO\textsubscript{2} reverses ethylene inhibition of growth (6). Other nonphotosynthetic effects of CO\textsubscript{2} include a similar stimulation of growth with Avena coleoptile sections (11,35) and the expansion of potato tuber slices (11).

Root growth is frequently proportional to CO\textsubscript{2} concentration although inhibition may occur at higher concentrations (10). The length, number and dry weight of pea roots grown in water culture was increased at concentrations of CO\textsubscript{2} above atmospheric (10). A stimulation of cell division by CO\textsubscript{2} is found in Chlorella and there are effects on elongation and on cell division in etiolated Avena seedlings (16). Other effects in bacteria, fungi and Hydra are known (1).

The lipid effect examined here is only 1 of numerous effects of lipids on plant growth. Several of these effects have been summarized previously (30). The use of a small drop of olive oil to hasten development and ripening of unripe figs dates to at least the second century A.D. when religious rules for its application were recorded in Hebraic texts (8).

High concentrations of fatty acids inhibit axillary bud development of decapitated tobacco plants (3)
but it is not known whether a promotion would be found at low concentrations. The results of a large number of experiments on various aspects of the floral development of many plants which were sprayed with lipid extracts from flowering or vegetative plants has recently been summarized by Roberts (24).

Synthetic surface active compounds have frequently been found to increase growth and other processes in plants. Some promotion of the growth of tobacco plants or of excised roots has been obtained with Triton X-100 and Tween 20 (17) and a promotion of flowering in radish has been found with commercial preparations of detergents (19). A number of compounds have been tested for their effects on root, shoot and coleoptile growth and on ion uptake (21). Several marked effects were found although the results apparently did not depend on surface activity. The fatty acid ester detergents Tween 20 and Tween 80 are active in the bioassay used here, but their activity is believed due to their fatty acid ester nature since many other surface active compounds do not possess biological activity. A stimulation of the growth of peas was reported with a 0.0001 % solution of a polyvinyl alcohol (23).

Several reports that saponins affect growth are summarized by Heftmann (12) and recently a marked effect of some saponins on the elongation of *Avena* coleoptile segments has been recorded (33). This activity is apparently different from the effect on elongation investigated here since it is found in the absence of auxin, and the lipids that were active on the pea sections were inactive on *Avena* (26).

Giberrellins can be replaced partly by α-tocopherol in increasing the growth of the dwarf-5 mutant of maize (3, 4) and α-tocopherol can also replace vernalization requirements of winter rye (5). Vitamin K₁ markedly enhances the growth of a crown gall tissue culture from *Catharanthus roseus* (2) and increased the elongation of segments from the *Avena* first internode (18).

These brief summaries show that many of the lipids effective in the pea section bioassay also have widespread and various effects on other plants. As yet there is no evidence whether each response is unique, or whether there is a common mechanism of action in the same way that dissimilar molecules that are classed as auxins may influence many physiological functions in the plant. None of the other examples cited here have been analyzed in as much detail as the present phenomenon, either as to the chemical specificity of the active molecules or to the mechanism of action.

The results presented here support the hypothesis that the lipid-induced increase in respiration is closely related to the stimulation of growth, although conclusive evidence is still lacking. Additional experiments that help understand the regulation of respiration in this tissue will be presented in a subsequent paper (22).

**Summary**

Triolein, methyl oleate and palmitonitrile can increase both growth and respiration in pea stem sections. Under other conditions they enhance respiration without affecting growth. Dodecanyl succinate inhibited both growth and respiration in this bioassay. Section growth is also sensitive to CO₂. The exogenous lipid is not a significant substrate for respiration.

It is concluded that the growth effect of lipids is causally related to the effect on respiration.

**Acknowledgments**

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**Literature Cited**


