GROWTH PROMOTION IN PEA STEM SECTIONS. I. STIMULATION OF AUXIN AND GIBBERELLIN ACTION BY ALKYL LIPIDS.[1,2]

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A section of pea stem after removal from the plant elongates markedly less than the same zone when it is left on the intact plant. No growth factor or combination of known factors restores more than half of this missing growth. This is particularly evident in studies with the gibberellins. The intact pea plants respond to gibberellin with large growth increments whereas sections cut from replicate plants are only slightly stimulated (6,11,22).

Our previous paper (20) showed that several types of fatty acid esters can partly restore the missing growth of the excised sections. The increased growth was found to be due to a stimulation of auxin and gibberellin action by the fatty acid esters, since the latter substances were inactive by themselves. These unexpected findings indicate that lipid substances have a hitherto unappreciated action in plant growth. Their role in normal plant growth regulation may be exerted through a synergistic effect on hormones rather than as hormones in themselves. The present study has been directed at defining the chemical structure required for this synergism.

MATERIALS AND METHODS

Stem sections of *Pisum sativum* L. were used. These were cut 10 mm long from directly below the apex of the third internode at the time the apical bud and leaves were perpendicular to the stem (about seven days of age). The technique has been described elsewhere (8, 20). The standard variety Alaska was employed for comparison with dwarf peas. A survey of dwarf pea varieties revealed that Laxton's Progress was the most suitable for use under our conditions. Some lots of this variety have not been fungus-free. Therefore Phygon (active ingredient, 2,3-dichloronaphthoquinone) has been satisfactorily used as a fungicide during the initial soaking period. Control experiments have shown that the use of Phygon does not affect the growth response of the sections, and Alaska peas which have never needed Phygon treatment respond as well to these fatty growth factors.

All plants were exposed to continuous weak red light from the 3rd day onward (cf. 20). Plants so exposed are more uniform and have shorter internodes than when grown in the dark. The response to lipids is not markedly influenced by the amount of red light received, either before or during the test period. Ten sections were placed in Petri dishes containing 20 ml of solution and were kept on a slow speed rotary shaker for 24 hours in the dark. Measurements of the length of the sections were then made.

A major technical difficulty in this study has been the need to apply lipid substances to the plant sections via an aqueous emulsion. Simple lipid dispersions result in unstable emulsions, leading to uncertainty as to the amounts of lipid actually available to the plant material. It was found that the most efficient way to disperse a lipid in water is to dissolve it in a relatively small volume of any fat solvent which is also highly soluble in water, and to inject this solution into the aqueous medium. The following solvents have been found suitable: acetonitrile, acetone, dimethylformamide, dimethyl sulfoxide, and tetrahydrofuran. Dimethylformamide and dimethyl sulfoxide, although they have no effect on pea section growth up to 0.1 M, cannot easily be removed from the system. Acetone, tetrahydrofuran, and acetonitrile, having high vapor pressures, can be partly removed from the aqueous medium by simple aeration. Tests of these volatile solvents revealed that acetone frequently and erratically had an inhibitory effect on pea sections. Tetrahydrofuran, while it was the best solvent for many lipid compounds, inhibited the growth of pea sections at initial concentrations above 0.01 M and had to be frequently repurified. Acetonitrile was the most satisfactory solvent and was usable even at initial concentrations of 0.3 M (20). This concentration, however, must have been substantially reduced by volatilization during the 24 hour rotation of the solutions on the shaker. As normally employed, the lipid stock emulsions were aerated for 5 to 15 minutes prior to use, so that the actual concentrations must have been even lower. Wächter (27) and Denny (9) have reported that acetonitrile causes leaf epinasty, but our acetonitrile (Matheson) has never shown any indication of a growth effect on peas under our conditions. It is unlikely that ambient vapor pressures in our experiments ever approached the

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levels that probably prevailed in Wächter's or Denny's bell jar experiments.

A satisfactory lipid emulsion must also remain dispersed. The Tweens could not be used as they proved to be growth stimulants in themselves (20), and since many other alkyl compounds had this property, other commercial emulsion stabilizers were investigated. The best so far encountered are those of the Pluronic and Tetronic types manufactured by the Wyandotte Chemical Corp. These are based on lipid-soluble polyoxypropylene to which water-soluble polyoxyethylene chains are attached. The resulting compounds have molecular weights and properties which depend on the relative ratios of the two components of the molecule. Pluronics L62, L64, P75, P84, and P85 showed toxicity in our system even at 0.01 %, while Pluronics F77, F88, and Tetronic 707 had moderate inhibitory effect at 0.1 % levels. Pluronics F38, P66, F68, and Tetronic 908, however, were only slightly inhibitory at 0.1 % and could be used at somewhat lower concentrations without any apparent effect on the plant material. For uniformity Pluronic F68 was adopted as the standard compound, but the other substances of this last group would probably also have been suitable.

We are indebted to Professor R. P. Geyer of the Harvard School of Public Health for his advice on these matters, and the technique which follows is based on his procedures. A typical emulsion was prepared in this way: 5.4 mg of methyl palmitate and 4 mg of Pluronic F68 were dissolved in 0.25 ml of acetonitrile and injected with a 1 ml syringe fitted with a No. 18 needle into 10 ml of water in which 4 mg of Pluronic F68 had previously been dissolved. The emulsion was then aerated for 5 to 15 minutes to remove much of the acetonitrile. The final stock emulsion is 0.5 mM in methyl palmitate and 0.08 % Pluronic F68.

This emulsion remains stable for days, even in the cold room. For the tests reported here, however, all emulsions were routinely made up immediately prior to the bioassays. For less soluble substances such as the higher alkyl compounds, and in particular their alcohols, warming of both the acetonitrile and the aqueous solution before injection is necessary. Although twice the lipid weight of Pluronic F68 is generally sufficient to ensure a stable emulsion, higher quantities are occasionally required. The amount of acetonitrile can also be increased, or tetrahydrofuran may be used if the lipid material is insufficiently soluble in acetonitrile. In most of the tests the final concentration of Pluronic F68 was set at 0.004 %.

As many of these lipid substances resist normal washing procedures, all glassware was routinely cleaned in a hot acid bath.

Results

Comparison of Section Growth in situ and in vitro: It was first necessary to establish by what margin excised sections fail to match growth of the intact plant. For this purpose 10 mm zones were marked on ten intact pea plants with India ink at the same position from which sections were cut on the replicate plants. The sections were floated in solutions of sucrose, pH 5.5 phosphate buffer, cobalt chloride, indoleacetic acid (IAA), and gibberellic acid (GA_3) at the concentrations which had been found to provide the largest growth increments. Tests have shown these optimum concentrations are not appreciably altered by the presence of the lipid factors. The intact dwarf plants were small enough to be placed flat within 150 mm Petri dishes in 60 ml of solution and were rotated on the shaker in precisely the same manner as the sections. This procedure resulted in a slight geotropic curvature of the third internode as the cotyledons somewhat restricted the plant motion during this period. Apparently this procedure, even though auxin was in contact with the roots, had

Table I

Percentage Growth of 10 mm Zone on Intact Pea Plants Compared to 10 mm Section

Additive (μM)	Alaska (standard)	Laxton's Progress (dwarf-2 expts)	
	Sections		
Controls	52.7 ± 6.7	44.9 ± 5.6	39.9 ± 3.5
GA_3 (0.3)	....	54.6 ± 5.3	39.9 ± 7.9
IAA (1.7)	....	67.5 ± 7.0	65.1 ± 10.5
IAA + GA_3	94.3 ± 16.1	84.3 ± 6.4	70.1 ± 12.0
Ditto + methyl linoleate (68)	....	113.8 ± 7.3	107.4 ± 9.7
(same at 41)	123.3 ± 15.1	....	....
	Intact plants		
Controls	204.9 ± 24.2	132.4 ± 15.3	125.0 ± 16.8
GA_3 (5 % Paste)	262.2 ± 10.8	....	....
IAA + GA_3	....	201.1 ± 14.6	234.9 ± 24.1

All sections and the intact dwarf plants were grown in 1.25 % sucrose + 50 μM $CoCl_2$ + 5 mM KH_2PO_4 (pH 5.5) and additives at concentrations indicated in parentheses. Solutions initially 0.2 M in acetonitrile. Percentage increase in length in 24 hours and standard deviations are indicated.

no deleterious effects since, as in the experiments of Brian and Hemming (5), the growth of dwarf peas was brought up to that of a standard variety. Since the larger intact Alaska pea plants could not be handled conveniently in the same manner a set was left in the growing trays (8) in the darkroom at the same temperature as the sections and treated with 5 % GA_3 in lanolin paste, applied at the base of the third internode. Another set was left untreated as controls. Gibberellic acid applied to the root solution gave only about 20 % of the growth increment caused by the lanolin treatment. This probably was due to the time required for translocation of the gibberellin. The work of many other investigators (cf. 1, 16) has established that externally applied auxin has only a small growth promoting effect on intact pea plants. Our results are listed in table I.

These experiments confirm that the growth response of both Alaska and dwarf pea stem sections to IAA plus GA_3 is similar, but the sections from dwarfs consistently show slightly less growth. The concentration of IAA selected here (1.7 μM) was that which is usually optimal for the sections from dwarfs; in some tests the Alaska sections reached their optimum at a higher (5.7 μM) concentration. Adding methyl linoleate to the bioassay medium causes a statistically significant increase in growth of both section types. None of these treatments, however, brings the growth of either type of section up to the level it would have reached on the intact plant. The discrepancy is shown most strikingly by the data for the GA_3 treated intact plants (their growth is twice that of the maximum elongation of excised sections). Even with the addition of any of the lipid factors studied here the growth of sections remains submaximal, probably requiring another factor or factors normally provided by the rest of the plant. It is possible that the action of GA_3 depends on these additional factors more crucially than does that of IAA, since the sections in this experiment and in others responded more markedly to the auxin (see also table I in both 20 and 21).

Comparing Effectiveness in Growth Promotion of Different Lipids: The growth promotion by lipids has two noteworthy characteristics. It appears at low lipid concentrations (even 10 μM), and it is exerted by more than one class of long chain alkyl compound (20). It seemed desirable to delineate further this apparent lack of specificity, as well as the relative activities of different compounds. For this purpose an extensive series of alkyl compounds has been tested.

The procedure is illustrated in table II for the oleic acid derivatives. A series of dilutions for each compound over its presumed range of activity was run in two separate experiments (a and b). In this manner both the optimum concentration and the maximal section growth obtained with each compound could be estimated. Both criteria led to the following order of effectiveness: triolein > methyl oleate > monolein >> oleic acid. Triolein was equated with three times greater molarities of the other compounds as it has three oleyl residues per molecule. A comparison of its concentration with that of IAA shows that only 6 to 18 times more oleyl groups than auxin molecules are required for maximal growth. Triolein was one of the most active compounds tested.

Since only a few compounds could be tested together in a single experiment one of the most active compounds, methyl linoleate, always was included as a standard. In the course of this work, however, a paper appeared (2) which indicated that emulsions of ethyl linoleate are rapidly autoxidized in the presence of air at room temperature. Therefore, another, equally active but more stable compound, namely methyl myristate, was substituted as standard in subsequent experiments. The relative activities of different compounds, compared to these standards, are shown in table III.

These data clearly demonstrate that the most active class of compounds are the methyl and ethyl esters of the fatty acids, followed by the triglycerides. But not one of these compounds increased growth of the

Table II

Comparison of Growth Stimulating Effect of Oleic Acid Derivatives

	μM	Oleic acid		Methyl oleate		Monoolein		μM	Triolein	
Expt.		*a*	*b*	*a*	*b*	*a*	*b*		*a*	*b*
	1	...	...	...	...	...	...	0.3	...	76.1
	4	...	...	...	...	...	...	1.3	...	90.2
	10	71.2	75.8	70.1	...	68.2	61.6	3.3	101.5	99.9
	20	70.7	...	78.1	69.0	69.2	...	6.7	107.9	92.0
	30	69.3	...	107.6	95.6	88.2	...	10	110.7	...
	40	67.2	84.2	105.3	95.3	74.1	97.1	13	95.4	94.0
	50	...	...	...	92.0	98.2	...	17	98.3	...
	100	...	68.7	...	...	...	77.8	33	...	...
	300	...	75.6	...	...	...	67.7	100	...	...
						a	*b*			
				Controls		40.6	34.2			
				IAA + GA_3		74.4	70.5			

Percentage increase in length of 10 mm sections of Laxton's Progress pea epicotyls after 24 hours in 1.25 % sucrose + 50 μM $CoCl_2$ + 5 mM KH_2PO_4 (pH 5.5) + 0.003 % Pluronic F-68, and additives as listed. All but controls also 1.7 μM IAA and 0.3 μM GA_3.

sections above the values reported for methyl linoleate in table I. Since all these fatty acids are common constituents of plant fats, it can be assumed that most natural fats would be active. Accordingly, no further study has been made of the natural glyceride fraction isolated earlier (20), particularly since the behavior of this fraction in the bioassay was not different from that of the chemically better defined substances studied here.

Remarkably, the free acids have shown no consistently significant activity in any test series (the lone datum for oleic acid at 40 μM could not be repeated in other tests). But two monoglycerides, the higher alcohols, and selachyl alcohol accentuated section growth. In addition to the substances in table III, methyl or ethyl esters of the C_{10}, C_8, C_7, C_6 acids, monacetin, monopropionin, and monobutyrin, as well as acetate, triacetin, and glycerol have also been tested at various concentrations. In no case has any of these, or any other compound containing a chain of less than 12 carbon atoms, been found to exhibit action on the section growth, except to inhibit it at relatively high concentrations.

Studies of Mechanism of Action of Alkyl Lipids: A number of experiments have been run in an attempt to clarify the mechanism of action of these substances. As briefly mentioned previously (20), the activity of Tween detergents and the unsaturated fatty acid esters, as well as the inactivity of free acids, are reminiscent of the growth response of both a Lactobacillus and a Venturia mutant. But in other respects, this analogy fails. Esters of saturated fatty acids are active in the peas but not with the mutants. Conversely, biotin is active in the latter, but is inactive (0.1-50 μM) on the peas. The pH is important for the response of the mutants (but tests of the isolated pea glycerides (20) and of methyl linoleate showed that varying pH between 4.5 and 7 did not much modify the growth of the sections). It thus seems unlikely that the pea response is really analogous to that of the two microorganisms.

The smut fungus, *Ustilago zeae,* produces in culture quantities of ustilagic acid (a mixture of diglucosyl dihydroxy hexadecanoic acids) (4). The malformations characteristic of smut infection are believed to be at least partially due to its production of auxin (29). In the thought that ustilagic acid might also be involved, a sample (kindly supplied by Dr. R. H. Haskins) was tested on the pea sections. No growth promotion was noted, but inhibition was found at low levels (10 μM), with toxic effects appearing in the tissues at two to three times this concentration.

The activity of the Tween 20, 80, and 81 detergents (20) made it possible that some type of surface action is responsible for the growth promotion. However, other non-ionic surfactants, such as the Igepals CO-610, CO-630, and CO-710 (nonylphenoxypolyoxyethylenes—General Aniline and Film Corp.), and the Pluronics and Tetronics mentioned earlier, were inactive over a wide range of concentrations. An anionic wetting agent, Alrowet D-65 (sodium dioctylsulfosuccinate—Geigy Chemical Corp.), at 3 and 30 mg/l failed to stimulate growth. Some cationic detergents, Sarkosyls NL-30, O, and S (lauroyl, oleoyl, and stearoyl sarcosine—Geigy Chemical Corp.), at 3 to 300 μM were impotent or inhibitory. Carboxymethyl cellulose (CMC-70 premium low viscosity—Hercules Powder Co.), used commercially as a colloidal emulsion stabilizer, was inhibitory at concentrations greater than 0.01 % and without any

Table III

Relative Activities of Alkyl Lipids on Dwarf Pea Epicotyl Section Elongation

Alkyl group		Free acid	Ester *	Mono-glyceride **	Triglyceride	Alkyl-glyceryl ether	Alcohol
$C_{12}H_{24}$	Lauric	0	+(Me)	...	...	...	...
$C_{14}H_{28}$	Myristic	0	+++(Me)	0	...	...	0
$C_{16}H_{32}$	Palmitic	0	+++(Me, Et)	+	+	...	0
$C_{18}H_{36}$	Stearic	0	+(Et)	0	++	0 (Batyl)	+
$C_{18}H_{34}$	Oleic	0	+++(Me)	++	+++	+++(Selachyl)	...
$C_{18}H_{32}$	Linoleic	0	+++(Me)	...	+++	...	...
$C_{18}H_{30}$	Linolenic	...	++(Me)	...	...	...	...
$C_{20}H_{40}$	Arachidic	...	...	0	...	...	++
$C_{20}H_{32}$	Arachidonic	...	+++(Me)	...	...	...	...
$C_{22}H_{44}$	Behenic	...	...	0	...	...	++
$C_{22}H_{42}$	Erucic	...	...	...	...	...	++
	Brassidic	0	...	...	...	...	...
$C_{18}H_{32}$	Chaulmoogric	...	++(Et)	...	...	...	...

Basal medium as in table II, including IAA and GA_3. Relative activities are compared to maximum growth promotion over controls obtained with methyl myristate or methyl linoleate on the same day: 0 = less than 20 % of maximum promotion; + = from 20 to 50 % of maximum promotion; ++ = from 50 to 80 % of maximum promotion; +++ = same as (± 20 %) maximum promotion.

* Me = methyl ester and Et = ethyl ester of corresponding acid.

** Kindly supplied by Distillation Product Industries.

action at lower percentages. Furthermore, the large number of active compounds in table III do not seem to have any obvious common physical property which would not be shared by some of the inactive substances. Surface action thus appears to be an untenable explanation unless it is exerted inside the cell after some metabolic modification of the molecules.

The next most likely explanation of the growth promoting effect of the lipids was considered to be an action on the cell membrane. The site of auxin action is believed to be on the cell wall or membrane, and furthermore, auxins are known to increase cell permeability (25). As phospholipids are constituents of membranes, it is possible that the lipids are entering into their metabolism and in some way promoting auxin action. Bennet-Clark has proposed an hypothetical auxin mechanism involving phospholipids (3). The metabolic sequences thought to be involved in phospholipid synthesis have been summarized by Kennedy (15). Of the various cofactors he cites, choline (0.4, 4, 40 μM), phosphoryl choline (10, 30, 100, 300, 10^4 μM), and cytidine (5, 50 μM) were all found to be ineffective on the pea sections, as were uridine (5, 50 μM) and uracil (5, 50, 100 μM). A characteristic requirement of phospholipid synthesis is that both saturated and unsaturated fatty acids are needed. When mixtures of methyl palmitate and methyl linoleate were tested on our system, however, their maximal effect on section extension was no greater than that of either compound alone. At submaximal concentrations simple additivity of their action was indicated. A phospholipid, lecithin (0.0003-0.003 %), was also inactive.

If the cell membrane is involved it might also be that the entry of auxin into the cell is facilitated. Some crude tests with C^{14}-carboxyl-labeled IAA have been carried out, but did not show any convincing increase in the disappearance of IAA from the external solution in the presence of lipid factors as compared to non-lipid-treated controls. Thus, no evidence implicating an action of the growth-promoting lipids on the membrane has yet been found.

Another group of long carbon chain compounds found in plants is the phytosphingolipids containing phytosphingosine. Their role in plant metabolism is obscure, but Carter et al (7) believe that they also contain inositol, hexuronic acids (probably galacturonic acid), glucosamine, arabinose, galactose, and mannose. These compounds at 0.01 and 0.1 % were accordingly tested both in the presence and absence of methyl myristate, but no further promotion of section growth was noted. Phytosphingosine (10-50 μM), generously supplied by Professor Carter, was similarly without effect.

Finally, the possibility that these lipids might be acting via the conventional degradative pathway for fatty acids was examined in several ways. The action of cobalt and sucrose in promoting section growth (18, 24) is not understood, and since pea sections are rich in stored materials (8), it seemed possible that sucrose was acting as a remote source of lipid materials via fatty acid synthesis. Figure 1 shows a test of this hypothesis. It demonstrates that the lipid response is almost completely dependent on added sucrose and hence the action of sucrose cannot primarily be as a substitute for alkyl lipids. Also, as noted above, acetate and glycerol were inactive. So was sodium pyruvate over a concentration range of 0.03 to 3 mM. Marrè and Arrigoni (17) have reported slight promoting effects of reduced glutathione on pea sections under different conditions than ours. In our bioassay, cysteine (3 and 10 mg/l), reduced glutathione (0.1, 0.3, 1 mM) and thioctic acid (5, 20, 60 μM) failed to accelerate section growth either in the presence or absence of lipid. Equally ineffective were coenzyme A (5, 20, 60 μM) and pantothenic acid (1, 10, 100 μM).

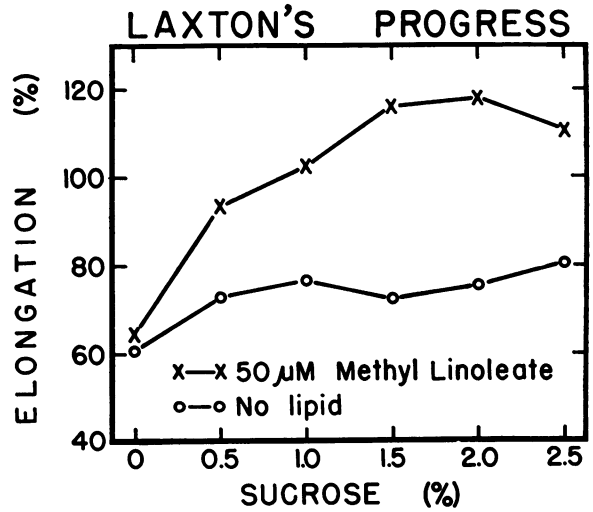

FIG. 1. The effect of sucrose on the growth of dwarf pea epicotyl sections and its promotion of the growth stimulation caused by a lipid. All sections treated with 1.7 μM IAA and 0.3 μM GA_3 and basal medium as in table I.

The action of many lipid-soluble substances on pea sections seems at first sight similar to the wound hormone factors reinvestigated recently by Haagen-Smit and Viglierchio (12). When a detailed comparison was made, it was the differences between our system and the Wehnelt bean test they used which became really striking. First, traumatic acid (10) was inactive on peas (4-130 μM). Next, these workers found the most active substances to be free fatty acids, including lauric, myristic, and linoleic acids. In our tests these substances were not active at all. Finally, the wound response of the bean pods to linolenic acid was greatly promoted by cytochrome c and ascorbic acid. In our tests horse heart cytochrome c (0.03-3 μM) and ascorbic acid (3 μM) added to the pea sections both singly and together, as well as in the presence of methyl myristate (30 μM),

were ineffective. Cytochrome c did cause moderate inhibition at the higher concentrations. None the less, the fact that results of Haagen-Smith and Viglierchio also show an apparently hormonal response to long-chain alkyl substances at concentrations lower than 200 μM suggests that bean plaques and pea sections share some common hormonal mechanism.

Discussion

While this work was in progress Vlitos and Crosby (26) isolated a higher alkyl alcohol from tobacco and showed it to be active in the Avena first internode auxin assay, as were several other alkyl compounds. Struckmeyer and Roberts (23) have also found a higher alcohol in plant extracts and believe it interacts with auxin. These discoveries, when compared with the data of Haagen-Smit and Viglierchio (12), the earlier work on wound hormones (10) and that of this paper, make it clear that trace amounts of lipid substances do play some role in the hormonal physiology of plants. These substances cannot be said to be limiting in all auxin bioassay material, however, as our tests have so far failed to show any effects on *Avena* coleoptiles (20).

Further support for our conclusion that lipids are involved in plant growth regulation can be found in studies of the use of auxin sprays for horticultural and herbicidal purposes. As shown by Harley et al (13), M. N. Westwood (private communication), and Hitchcock and Zimmerman (14), the efficiency of such sprays can be increased by adding Tween 20. This phenomenon has been interpreted as due to increased wetting or penetration of the spray. Harley et al (13), however, noted that Tween 20 was a weak thinning agent in itself. And recent work by L. L. Jansen (private communication) indicates that a number of commercial detergents can by themselves increase the growth of several intact plants. It would be interesting to compare the effectiveness of alkyl with non-alkyl detergents in tests such as these.

A link between lipid substances and auxin action has also been indicated by the work of Christiansen and Thimann (8) who showed that the ether-soluble fraction of pea stem sections dropped during growth. More significantly, this drop was reduced by the same inhibitors that reduced cell elongation. These workers, in summarizing previous work as well as their own, concluded that some interaction between fat and reducing sugar levels was indicated. The present work shows that sugars are needed for lipids to enhance growth. The added fats cannot be a quantitatively important source of sugars here since nearly all the alkyl lipids tested are most effective in the range of 10 to 50 μM. This is only 6 to 30 times the concentration of IAA which was employed in the tests; it suggests that a hormonal rather than a nutritive requirement is involved. Inasmuch as it was necessary to employ emulsions of these lipids the actual molar ratios could even be lower and approach mole to mole values.

The data of Christiansen and Thimann show further that during growth only a third of the neutral ether-solubles are utilized. The amount still left in the section is five times more than would be required to produce the effects discussed in this paper. Therefore, since nearly all normal fat constituents have activity in the bioassay, it appears that not all lipids present in the tissue are available to take part in the synergism. However, sections were not grown by Christiansen and Thimann in the presence of added sucrose so that their analyses are not strictly comparable. It will be necessary to analyze lipid metabolism in pea sections and in the intact plant in detail before any conclusions can be made concerning the natural importance of the synergism of lipids with plant hormones.

Neither can any firm conclusions yet be drawn as to the role these lipids are playing in plant metabolism. The tests described in this paper, although limited to the bioassay technique, have given no support to the idea that well known biochemical pathways of lipids are involved. The most promising explanation developed so far has come out of further work which indicates that these factors may be activating the cytochrome system; the preliminary evidence for this has been outlined elsewhere (21).

This work also makes it clear that some unexpected factor other than lipids is involved in section growth. Dwarf peas were employed because it was anticipated that they would be more sensitive to gibberellin (5). Yet in every instance their sections gave a response remarkably similar to that of Alaska pea sections (see table I in this paper and figure 1 in 20). This amplifies the results of von Abrams (1) who could not find appreciable differences between a dwarf and a standard pea when several aspects of auxin relations were considered. Hence it appears that the striking effect of gibberellic acid on dwarf peas discovered by Brian and Hemming (5) is still in some way restricted to intact plants. The data of Lockhart (16) and Galston and Warburg (11) also suggest that the ineffectiveness of gibberellic acid on sections may be due to the lack of a factor produced by the rest of the plant, as a greater response was shown by decapitated sections left attached to the base of the plant. Our attempts to extract the factor indicated by such experiments have not as yet been successful. Comparing these papers with the older publication of Went (28) shows that the factor gibberellin action requires may well be the "caulocaline" which Went showed to be produced by roots and which only passed through living tissue. The appearance of the caulocaline effect required pea tip auxin, but as Lockhart (16) has shown, pea tips also produce gibberellin.

But a completely different explanation based on the results of Phinney and Neely (19) cannot yet be excluded. They found that dwarf mutants of maize vary in their response to different gibberellins, and also found that one mutant (dwarf-1) was much less responsive than the others to a natural gibberellin, bean factor II (GA_5), isolated from a higher plant.

They have advanced the hypothesis that this indicates a biochemical pathway of gibberellin synthesis. Therefore, it may be that GA_3 (and the GA_1 in the mixture employed by Lockhart) are not active on growth of peas in themselves, but must undergo some alteration before they participate in the growth process. Thus the relative inactivity of GA_3 on pea sections may merely mean that the sections do not possess the enzymes necessary to convert it to an active form and that these enzymes are elsewhere in the plant. In any case, further analysis of the puzzling difference between the growth of intact plants and excised sections seems certain to cast more light on the factors involved in natural growth regulation.

Summary

A technique is described for preparing stable aqueous emulsions of lipids, allowing their routine use in bioassays.

Excised sections of both dwarf and Alaska peas, even under optimal conditions, fail to attain half the growth they would have reached in situ on an intact plant.

Part of this missing growth can be restored by many lipid substances, especially by several types of fatty acid esters. The specificity of this response is delineated. The most effective compounds found so far are: methyl myristate, methyl palmitate, methyl oleate, methyl linoleate, methyl arachidonate, triolein, trilinolein, and selachyl alcohol. At their optima, these materials can bring the growth of sections up to just half that upon the intact plant.

To observe the growth promoting activity of these lipids, it is necessary that sugar and auxin be supplied in the medium. Their action is enhanced further by gibberellic acid.

Tests of various cofactors of lipid metabolism have failed to establish a connection with the growth effect shown by the alkyl lipid compounds. This effect seems most likely to be hormonal in nature since the amounts of lipid required are comparable to that of the auxin used.

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BIOSYNTHESIS OF SUCROSE AND SUCROSE-PHOSPHATE BY SUGAR BEET LEAF EXTRACTS[1]

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Cardini and coworkers (6) reported as one part of a plant materials survey that "negative or non-reproducible results" were obtained in sucrose synthesis studies with sugar beet leaves and roots. They stated that these results "may be attributed to the presence of interfering enzymes".

Burma and Mortimer (5), using isotopic tracer techniques, reported the synthesis of sucrose by sugar beet leaf homogenates when fructose-6-phosphate and uridine diphosphoglucose (UDPG) were present. They demonstrated that sucrose-phosphate was formed and assumed that it was subsequently dephosphorylated by phosphatase to sucrose. No sucrose synthesis occurred when fructose or fructose-1,6-diphosphate and UDPG were added to sugar beet leaf homogenates. Burma and Mortimer stated that their experiments with sugar beet leaves eliminated Leloir and Cardini's (11) first enzyme mechanism demonstrated with wheat germ, which was the interaction of UDPG and fructose for the direct formation of sucrose plus pyrophosphate.

This paper presents data showing that the assumption of Burma and Mortimer that only sucrose-phosphate is synthesized directly does not seem to be correct. Rather, it shows that sugar beet leaf tissue contains enzyme systems for the direct synthesis of sucrose as well as sucrose-phosphate through UDPG and fructose and UDPG and fructose-6-phosphate, respectively.

Any sucrose-phosphate which is formed is ultimately dephosphorylated by enzymes in sugar beet tissue at some stage prior to storage in the root. Thus the synthesis of both sucrose and sucrose-phosphate also has some bearing on the important unresolved question of whether sucrose or sucrose-phosphate is the principal sugar transported to the root for storage (4, 8, 9, 10, 18).

MATERIALS AND METHODS

Preliminary experimentation resulted in adopting the following procedures for preparing beet leaf enzyme fractions active in the synthesis of sucrose and sucrose-phosphate. All operations were carried out at 0 to 5° C.

Six hundred grams of fresh leaves of 6 to 7 week old sugar beet seedlings (*Beta vulgaris* var GW 304) were used for preparing the enzyme fractions. A 100 g aliquot of seedling leaves was blended with 100 ml of 0.05 M, pH 7.2 phosphate buffer in a high speed blender. This homogenate was then squeezed in a fine nylon cloth. The liquid obtained was used for blending the next 100 g aliquot of leaves. This process was repeated until all 600 g of leaves had been homogenized, using only the original 100 ml of phosphate buffer diluent. The pH was periodically adjusted to 6.8 to 7.0 with NaOH during the blending procedure. Approximately 600 ml of filtered homogenate was obtained. This was centrifuged at 13,000 × G, and the precipitate obtained was discarded. The supernatant was gradually taken to pH 4.9 with acetic acid and immediately centrifuged at 13,000 × G for 15 minutes. The precipitate was discarded and the clear yellowish supernatant was left overnight at

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