SIMILAR ACTIVATING EFFECTS OF LIPIDS ON CYTOCHROMES AND ON PLANT HORMONES

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Trace amounts of several fatty acid esters have recently been shown nearly to double the growth-stimulating activity of indoleacetic acid (IAA) and gibberellic acid (GA_3) in a standard plant bioassay (Stowe, 1958). Further study now shows that the specificity of this response of higher plant tissues is very much like that reported for preparations of animal cytoplasmic particulates containing cytochromes.

The materials employed were 10 mm epicotyl sections cut from directly below the apical bud of 7 day old etiolated pea seedlings. For reasons not germane here, the dwarf pea variety Laxton's Progress was used, but the major conclusions of this study have been found to also be valid for the standard Alaska pea. Ten sections were rotated gently in 20 ml of solution in a Petri dish in the dark at 25° and their length measured after 24 hours. Details of the procedure are available elsewhere (Christiansen & Thimann, 1950; Stowe, 1958). Lipids were prepared as a stabilized emulsion (Stowe, 1959).

Results obtained without lipids and with methyl linoleate, vitamin K_1, and vitamin E acetate are shown in Table I. It will be noted that without lipids the two plant hormones together at optimum concentrations only bring section growth up to 67%, while with the lipids the elongation reaches about 100%.

* Now at: J.W. Gibbs Lab., Botany Dept., Yale University.

Not one of the lipids had any influence by itself -- but they all activated the effect of the plant hormones. The effect on

Table I

Lipid Effects on Percentage Growth of Dwarf Pea Sections Induced by Auxin and Gibberellic Acid

Treatment	No lipid	+ methyl linoleate (50 μM)	+ vitamin K_1 (4 μM)	+ vitamin E (10 μM)
Basal medium*	39.1 ± 3.6	38.4 ± 3.2	40.6 ± 3.5	35.7 ±5.9
+ GA_3 0.3μM	41.8 ±15.0	46.9 ± 5.8	51.2 ± 8.2	43.6 ±9.4
+ IAA 1.7μM	55.7 ± 6.2	68.7 ± 6.5	62.9 ± 7.6	65.9 ±4.6
+ GA_3 + IAA	67.4 ± 5.6	99.7 ± 5.0	104.3 ±10.2	94.0 ±6.2

*1.25% sucrose + 50 μM $CoCl_2$ + 5 mM KH_2PO_4 (pH 5.5) + 0.004% Pluronic F-68. Standard deviations are cited.

the action of GA_3 seems to be minimal unless the auxin, IAA, is also present. These observations also hold for other auxins like α-naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid. It thus appears that an auxin is required for lipids to activate growth. It should be noted that the effective lipid concentrations are comparable to those of auxin and are thus within the hormonal range.

Study of the specificity of this lipid synergism has led to the data summarized in Table II, where it is compared with data from three recent papers on animal particulate electron transport systems. Of the 19 substances which have been studied with both experimental materials, only four show activity in one system and not in the other. In each of these cases the activity observed was submaximal. In peas, the hydrocarbon chain appears to be necessary, since the K analogs, K_5 and menadione, were inactive. Mevalonic acid also did not stimulate growth, nor did compounds of length less than C_{12} and a wide variety of lipid metabolism cofactors (Stowe, 1959).

Table III shows that respiration of pea sections is en-

Table II

Comparison of Effectiveness of Lipids on Pea Sections and on Particulate Cytochrome Preparations

Lipid	Relative effectiveness on pea sections*	Percent restoration of particulate cytochrome activity**
Natural lipid extract, vitamins E & K_1, phytol, methyl linoleate and linolenate	+++	100
Triolein	+++	76
Trilinolein	+++	50
Ethyl palmitate	+++	50
Ethyl stearate	+	55
Monopalmitin	+	0
Tristearin	++	0
Tripalmitin	+	0
Menadione	0	50
Vitamin A; lauric, myristic, palmitic, stearic, and oleic acids	0	0

*Data of Stowe, 1959. **Compiled from data of Crawford et al., 1959; Donaldson et al., 1958; and Weber et al., 1958.

Table III

Oxygen Uptake of Dwarf Pea Sections During Log Phase of Growth

Treatment	µl O_2/mg fresh wt. /hour	percent increase	µl O_2/mg fresh wt. /hour	percent increase
Basal medium*	0.342	--	0.310	--
+ GA_3 + IAA**	0.398	16	0.347	12
Basal medium + methyl myristate (40 µM)	0.429	25	- - -	--
Basal medium + vitamin E acetate (10 µM)	- - -	--	0.433	40

*1.5% sucrose + 50 µM $CoCl_2$ + 5 mM KH_2PO_4 (pH 5.5) + 0.002% Pluronic F-68.
** GA_3 0.3 µM, IAA 1.8 µM.

hanced by methyl myristate and vitamin E. Although this effect is not large, it is comparable to that induced by auxin (Christiansen & Thimann, 1950), and hence to the growth increments observed. It would appear then, that the lipids may be activa-

ting the cytochromes of these tissues *in situ*. As these are not disrupted tissues, but merely amputated from the rest of the plant, positive proof of this will not be easy to obtain without loss of the linkage to growth.

Other observations do not match the analogy presented here. Limited tests of Coenzyme Q_{10} (Crane, 1959) have been as yet inconclusive* . Nor has antimycin A inhibition of section growth been reversible -- but irreversibility has been noted in some particulate preparations too (Crawford *et al.*, 1959). Not all plant systems respond, since growth of *Avena* coleoptile sections has not been further promoted by those lipids tested (Stowe, 1958). Also, another plant hormone bioassay -- for wound hormones -- which also responds to lipids appears to have a distinctly different spectrum of specificity (Haagen-Smit & Viglierchio, 1955).

However, Hackett and Schneiderman (1953) proved that auxin action on pea sections is mediated by cytochrome oxidase, and therefore the participation of other members of the cytochrome system is to be expected. Furthermore, the potent growth stimulation of cobalt on peas (Miller, 1954; Thimann, 1956) could also be a respiratory effect, since in yeast cobalt induces respiratory deficiencies (Lindegren *et al.*, 1958). It may be then that both lipids and cobalt shift respiratory pathways to one particularly effective in promoting hormonal action. If this viewpoint is valid, pea sections would appear to be a particularly favorable material to study relationships between respiration and hormone action.

Finally, it should be noted that *section* growth is markedly less than growth of the same zone on an *intact* plant. Presum-

* Coenzyme Q_{10} was generously supplied by Dr. F. L. Crane.

ably the internode normally receives lipoidal synergists of hormone action from the rest of the plant, the limiting nature of these compounds not being apparent until the section is cut out. It thus seems likely that in this case cofactors of the cytochrome system are acting as natural agents of growth regulation.

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