Chemistry and Physiology of Conjugates of Indole-3-Acetic Acid

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Auxins are hormones that promote plant growth. They occur naturally and, in structure, generally possess a planar aromatic ring, an unsubstituted, or electronegatively substituted position ortho to a side chain of, at least, two carbons, and with a carboxyl group on the side chain (1, 2). Examples of auxins are indole-3-acetic acid (3), phenylacetic acid (4), and 4-chloroindole-3-acetic acid (5, 6). Knowledge of the structure of indole-3-acetic acid--IAA--(7) led to the chemical plant growth regulator industry with annual agricultural savings in Michigan alone equal to the cost of all U.S. financed plant hormone research.

Utilization of auxins date to antiquity, as for example, the use of germinating seeds to promote rooting of cuttings (8). The discovery of hormones in roots by the Polish horticulturalist, Teofil Ciesielski (9) and in shoots by the British naturalist, Charles Darwin (10) was made one century ago. They observed that the tip of the root or shoot controlled growth of the tissue some distance from the tip. Thus, an "influence" must have diffused from tip to growing region. Sixty years later, F.W. Went developed the "*Avena* curvature test" for the "influence" (3), and Bonner developed the "straight growth assay" (11). These techniques plus knowledge that tryptophan could be converted to auxin in fungal cultures (12) and that "precursors" in seeds could be converted to auxins by alkaline hydrolysis (13, 14, 15, 16) led to knowledge of the structure of IAA (7).

We stress these assays because, while leading to the discovery of IAA, they imposed structure-activity requirements precluding study of the IAA conjugates--and possibly the discovery of other auxins. For a substance to be active in the assays required that they: 1) permeate membranes in a cut tissue surface, 2) be transported to the growing zone, and 3) promote growth in that zone. Hopefully these three requirements may someday be studied independently.

The biological effects of the auxins are diverse and range from rapid effects, usually growth promotion and occurring within

minutes, to growth differentiation, obvious only in days (17). How IAA can elicit profound changes in the size and form of a plant is totally unknown. At the cellular level it is known that the plant cell walls must be "softened" for growth promotion to occur (cf. 18). Such effects, however, may be concomitants of growth and not the "primary" effects of auxin (19).

We suggest that research directed towards correlating and developing biological and physiochemical assays of the auxins, structural characterization of auxins and auxin conjugates and studies of what permits auxins to move to different parts of the plant and, perhaps selectively into different organelles, will be productive approaches. Thus, I shall confine my remarks to, 1) the chemistry of IAA conjugates, 2) the quantitative assay of IAA and its conjugates, 3) the "turnover" of the indolylic compounds of the plant, 4) an indication of how knowledge of pool size and turnover permitted identification of the seed auxin precursor, 5) a demonstration of the equilibrium between IAA and its conjugates, 6) a demonstration of the perturbability of the equilibrium and, lastly, 7) a working hypothesis concerning how a hormonal homeostatic system can be attuned to the environment.

The Structure and Concentrations of Indoles of *Zea mays*

Figure 1 summarizes the structures and concentrations of the IAA conjugates of the kernels of corn (*Zea mays*), the only plant to have been studied in detail. This work was done by my colleagues Drs. Labarca, Nicholls, Ueda, Piskornik and Ehmann (20-25). We have not detected appreciable amide linked IAA in *Zea* but there are three major classes of esters: the IAA-*myo*-inositols, constituting about 15%; the IAA-*myo*-inositol glycosides, about 25%; and the high molecular weight IAA β1→4 glucan, about 50% of the total IAA. Free IAA, the 2-O, 4-O and 6-O IAA glucose esters and the $(IAA)_n$ inositols comprise the remainder. The vegetative tissue of corn contains 300 μg/kg fresh weight of ester IAA and 30 μg/kg of free IAA (26). A major portion of the esters of the shoot is IAA-*myo*-inositol (cf. 27 and Nowacki, unpublished).

The seeds of oats (*Avena sativa*) have been studied by Dr. Percival and shown to have 85% of their IAA esterified to a glucoprotein (28). The glucan is of the lichenan type having both β1→3 and β1→4 linkages. Recently, Ms. P. Hall in our laboratory (personal communication) has isolated IAA-*myo*-inositol from rice (*Oryza sativa*) thus showing the compound originated early in cereal evolution.

This completes our knowledge of the chemistry of the naturally occurring IAA conjugates. IAA-aspartate is known to be formed following exogenous application of IAA to plants of *Pisum sativum* and was the first IAA conjugate to be structurally characterized (29). There are some data indicating that IAA-aspartate occurs naturally (30). In addition, 1-O glucosyl IAA has been reported to be formed following application of IAA to plants (31).

Definitive characterization of the biosynthesized 1-O glucose ester has not been published although the compound has been synthesized chemically (32).

Quantitative data on the amounts of free IAA, ester IAA and amide linked IAA have been supplied by Ms. A. Schulze (33). Her data, shown in Table I, permit several conclusions: first, all plants examined contain greater amounts of IAA conjugates than free IAA; secondly, the cereals contain mainly ester IAA; and, thirdly, legumes contain mainly amide linked IAA.

The discovery by Ms. Schulze of the ubiquity of IAA-conjugates convinced us that covalently bonded hormone conjugates were of metabolic importance. Especially significant was the occurrence of IAA conjugates in seedlings where growth rate is a function of IAA concentration. We concluded that, if an equilibrium exists between IAA and its conjugates, then anything that shifts that equilibrium would affect growth rate. To further study this system for hormonal homeostasis two additional things were required, one, a more convenient and sensitive assay for IAA and, two, knowledge of pool size and turnover rates of the indolylic components of the plant.

Methods of Assay

Bio-assays, in the hands of careful workers (cf. 3), have provided almost all of our knowledge of plant hormonal metabolism. Such assays, however, measure activities of extracts and are not equatable to amounts of a chemical entity. Thus, one must rely upon both chemical and bioassays. With regard to chemical assays, it is our contention that the planar indole structure and the number of π bonding electrons renders IAA so unstable that recoveries will be both low and variable thus making internal standards obligatory (cf. 33, 34). We first used ^{14}C-IAA-isotope dilution assays in 1961 (35) and refined the assays in 1974 (33). The original assays required kilogram amounts of tissue and a week to obtain a single value. More recently we developed two new assay procedures which permit one assay in a day or two of work and require only 10 to 50 grams of tissue (36, 37). Our assays are laborious but, in this early stage of chemical assays of IAA, such labor may be desirable.

One method involves the use of 4,5,6,7 tetradeutero-IAA, recently synthesized by Dr. V. Magnus (36). There are two advantages to use of this as an internal standard: first, the deuterium in these positions is stable to the alkaline hydrolysis we use to assay the IAA in the conjugates, and secondly, the presence of four deuterium in the standard moves the ions of the standard away from the isotope cluster normally observed in mass spectrometry owing to the naturally occurring heavy isotopes. We add d_4-IAA, plus a trace of ^{14}C-IAA, to the acetone in which we homogenize the plant material. Then, with or without alkaline hydrolysis, depending upon whether we wish to measure free or

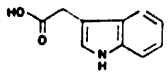
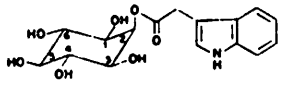
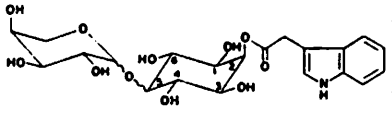
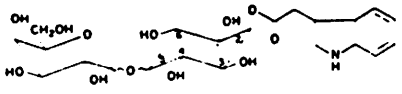
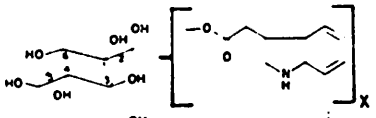
COMPOUND	STRUCTURE	AMOUNT IN DRY SEED MG/KG		PERCENT OF TOTAL
Indole-3-acetic acid		0.5		0.8%
Indoleacetylinositols		10.1		15.2%
2-*O*-(indole-3-acetyl)-*myo*-inositol		7.0	10.5%	
1-DL-(indole-3-acetyl)-*myo*-inositol		3.1	4.7%	
Indoleacetylinositol-arabinosides		15.4		23.2%
5-*O*-β-L-arabinopyranosyl-2-*O*-(indole-3-acetyl)-*myo*-inositol		11.7	17.6%	
5-*O*-β-L-arabinopyranosyl-1-DL-(indole-3-acetyl)-*myo*-inositol		3.7	5.6%	
5-*O*-β-L-galactopyranosyl-2-*O*-(indole-3-acetyl)-*myo*-inositol		5.4		8.1%
Trace compounds		0.2		0.3%
Di-*O*-(indole-3-acetyl)-*myo*-inositol		0.00	-	
Tri-*O*-(indole-3-acetyl)-*myo*-inositol		0.03	-	
2-*O*-(indole-3-acetyl)-D-glucopyranose		x 0.02	-	
4-*O*-(indole-3-acetyl)-D-glucopyranose		0.02	-	
6-*O*-(indole-3-acetyl)-D-glucopyranose		0.05	-	
LOW M.W. COMPOUNDS -- TOTAL		31.2		47.6%
(indole-3-acetyl)-glucan	β 1 4 cellulosic glucan with 7 to 50 glucose units per IAA	35.0		52.5%

Figure 1. The structure and concentration of indolylic compounds in kernels of Zea mays

TABLE I

Concentrations of Free and Bound IAA in Various Plant Tissues

Species	Tissue	IAA content		
		Free IAA[1]	Ester IAA[2]	Peptidyl IAA[3]
		µg/kg		
CEREALS				
Avena sativa	vegetative tissue	16	5	69
Avena sativa	seed	440	7620	n.d.
Hordeum vulgare	seed (milled)	40[5]	329	-
Oryza sativa	seed	1703	2739	-
Panicum miliaceum	seed	366	3198	-
Triticum aestivum	seed	123	511	-
Zea mays	vegetative tissue	24	328	60
Zea mays	seed	500 to 1000	71600 to 78500	-
LEGUMES				
Glycine max	seed	4	50[5]	524
Phaseolus vulgaris	seed	20[5]	30[5]	136
Pisum sativum	vegetative tissue	35	5	43
Pisum sativum	seed	93	n.d..	202
OTHERS				
Cocos nucifera	liquid endosperm	0	905	-
Fagopyrum esculentum	seed	40	127	25
Helianthus annus	seed	30[5]	110[5]	-
Lycopersicum esculentum	fruit	trace	trace	-
Saccharomyces cerevisea	packed cells	290	n.d.	-

[1]No alkaline hydrolysis.
[2]IAA after hydrolysis with 1 N alkali minus the free IAA.
[3]IAA after hydrolysis with 7 N alkali minus the free and ester IAA.
[4]Seedlings and fruits are fresh weight, seeds are air dry and yeast cells contain 30% dry matter.
[5]A visual estimate of IAA on a TLC plate as colorimetry was precluded by contaminants.

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total IAA, we re-isolate the IAA by partitioning, DEAE-sephadex, and high pressure liquid chromatography. The resultant mixture of d_4-IAA and the plant derived IAA is methylated and then the indole nitrogen is acylated with heptafluorobutyric anhydride. The methylheptafluorobutyryl IAA derivative is used for gas chromatography-selected ion mass spectrometry (gc-sim-ms). As shown in Figure 2, we monitor four masses, 385 and 389, the molecular ion for methylheptafluorobutyryl IAA (and its d_4 analog), and 326 and 330, the base peaks for IAA and the d_4 standard. The agreement between the ratios of d_4-IAA to IAA at the molecular ion and at base peak give assurance of the validity of the assay. Is this finally an absolute assay, giving data such that error is impossible? We think it is close in that for an error to occur a compound would have to cofractionate with IAA on a DEAE and HPLC column and coemerge from the gc column and then yield the same percentages of ions at m‡ and at base peak as IAA. Still, we do occasionally observe anomalous results and can only warn other workers that dealing with nanogram amounts of indoles is difficult.

A second method of assay of IAA has been developed by Mr. J. Cohen and involves a "double internal standard" usually ^{14}C-IAA and ^{14}C-indole-3-butyric acid (37). I will not discuss this method of assay except to indicate that it is possible to develop assays not involving mass spectrometry but with comparable sensitivity and good selectivity.

Metabolic "Turnover" of Plant Indoles

Ms. Pat Hall and Drs. J. Nowacki and E. Epstein have provided our knowledge of the amounts and rate of metabolic turnover of the indolylic components of the kernels of *Zea mays* (cf. 27, 28; Nowacki, unpublished; Epstein, unpublished). This knowledge has enabled us, 1) to identify the "seed auxin precursor"--that is the compound which is transported from the seed to the growing shoot (39), and 2) has provided a portion of the proof that IAA-*myo*-inositol and IAA are in reversible equilibrium in the shoot tissue. Proving that IAA and IAA esters are in reversible equilibrium in the tissue is essential if we wish to postulate hormonal homeostasis.

These experiments required labeled IAA and tryptophan, which are available commercially, and ^{14}C-labeled IAA-*myo*-inositol. This compound was synthesized by Dr. Nowacki by reacting ^{14}C-IAA-imidazole with *myo*-inositol (40). Application of these labeled compounds to corn kernels, followed immediately by homogenization of the tissue in acetone permitted us to determine the amounts of each constiuent in the kernel by the isotope dilution method of Rittenberg and Foster (41). An extension of this method, whereby the kernels are incubated for varying periods of time after application of the isotopically labeled compound permits determination of the "turnover" of the pool. Such data are shown in

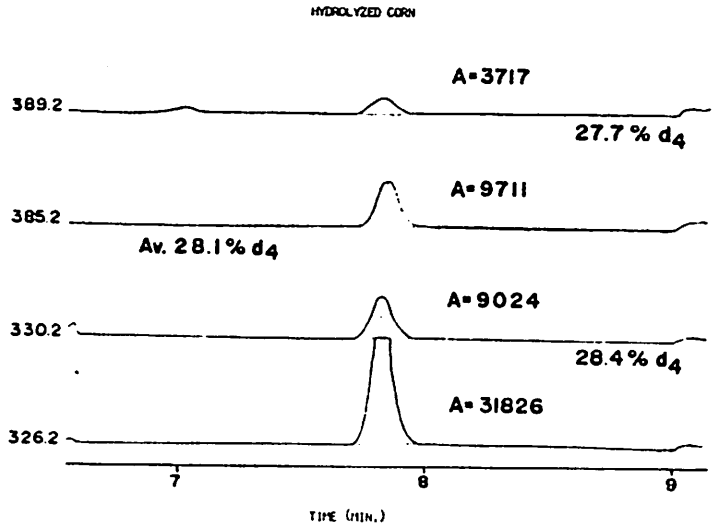

Figure 2. Selected ion chromatogram of a mixture of the methyl esters of tetrafluorobutyryl IAA and d_4-IAA. The IAA was from an extract of corn seedlings and the d_4-IAA added during homogenization. Retention time is in minutes and the masses monitored are 326.2 and 385.2 for IAA and 330.2 and 309.2 for d_4-IAA. The percent d_4-IAA has been computed by the area of the peaks at 330.2/326.2 + 330.2 (base peak) and 389.2/385.2 + 389.2 (molecular ion).

Table II. They showed that tryptophan, IAA and IAA-*myo*-inositol are turning over--that is made and then destroyed, or used, at rates such that $t_{\frac{1}{2}}$ was 5, 3.2 and 12 hrs, respectively. Such data permitted several important conclusions concerning the metabolism of these compounds, for example, that it is the IAA esters, and not tryptophan, which serve as a source of IAA for the germinating seed and secondly that the IAA-inositols are turning over at such a rapid rate that they must be in equilibrium with the IAA-*myo*-inositol glycoside pool perhaps acting as glycosylation reagents (Epstein, unpublished).

TABLE II

Concentration and Metabolic Turnover of Some Indolylic Compounds in *Zea* Kernels

Compound	Incubation Time	Recovered Sp. Act.	k	$t_{\frac{1}{2}}$
	hrs	dpm/µg	hrs^{-1}	hrs
IAA	0	31,000		
	4	8,900	0.22	3.2
	8	5,400		
Tryptophan	0	15,200		
	8	5,000	0.14	5.0
IAA-*myo*-inositol	0	935		
	8	590	0.06	12.0

The "Seed Auxin Precursor"

Of great importance, for these studies, was knowledge of both pool size and turnover. This knowledge permitted calculation of the specific activity of the applied isotopically labeled compound at any desired time. Thus, radioactivity, from a labeled compound applied to the endosperm and appearing in the shoot could be translated in amounts of compound moved from seed to shoot.

For these experiments, minute amounts of labeled IAA, tryptophan, or IAA-*myo*-inositol were applied to an incision in the semi-liquid endosperm of 4 day germinated *Zea* seedlings. After 8 hrs of incubation, the shoots were harvested and the IAA, tryptophan or IAA-*myo*-inositol isolated using rigorous purification techniques. Now, knowing the specific activity of the applied compound at the mid-point of the experiment we could calculate the

average rate of transport of that compound from endosperm to shoot. A summary of a portion of these data (cf. 27; Nowacki, unpublished; Epstein, unpublished) is shown in Table III. As can be seen IAA-*myo*-inositol moves from endosperm to shoot at a rate of 6 pmols·shoot^{-1}·hr^{-1}. We had previously estimated that about 9 pmols·shoot^{-1}·hr^{-1} of IAA compound must be moving from endosperm to shoot to sustain indole concentrations in the shoot (38) and Gillespie and Thimann (42)measured 5 pmols·shoot^{-1}·hr^{-1} of IAA diffusing down from excised *Zea* tips. On the assumption that what goes down must come up, at least 5 pmols·shoot^{-1}·hr^{-1} must be going up. By contrast free IAA moves from endosperm to shoot at a rate of 0.015 pmol·shoot^{-1}·hr^{-1} and tryptophan in the endosperm appears as IAA in the shoot at less than 0.2 pmol·shoot^{-1}·hr^{-1}. This last figure is high and could, in fact, be zero since non-enzymatic conversion of tryptophan to IAA occurs so readily. Thus these data establish that IAA-*myo*-inositol is the "seed auxin precursor" for *Zea mays* (cf. 27 and Nowacki, unpublished).

TABLE III

Rate of Transport of Indolylic Components From Endosperm to Shoot

Compound applied to endosperm	Compound isolated from shoot	Rate pmols·shoot^{-1}·hr^{-1}
^{3}H-IAA	IAA + ester IAA	0.015
^{3}H-tryptophan	IAA + ester IAA	0.15
^{14}C-IAA-*myo*-inositol	IAA + ester IAA	6.2

The Equilibrium Between IAA and IAA-*myo*-inositol *in vivo*

Dr. Hamilton earlier observed that ether extraction of tissue induced autolysis, liberating active esterases and glycosidases, and thus leading to more free IAA than extraction of tissue by polar, and thus, enzyme-denaturing solvents (35). Thus, we knew then that there were enzymes in the tissue capable of hydrolyzing IAA esters. Much later, Kopcewicz demonstrated the presence of an enzyme system which could synthesize IAA-*myo*-inositol from IAA, ATP, Mg^{++} and CoASH (43). More recently, Mr. Lech Michalczuk (unpublished) has shown that IAA-CoA will acylate inositol only in the presence of other nucleotides. Thus, the reaction is complex, but there is no doubt that enzymes to make and hydrolyze the IAA esters are present in corn.

Now, are the enzymes active *in vivo*? The data of Ms. Schulze and Hall and Nowacki, Epstein and Cohen (27, 38; Nowacki, unpublished; Epstein, unpublished) demonstrate that they are. We

showed in 1972 that there is 93% esterified IAA and 7% free IAA in the shoots of *Zea* seedlings (26). The desired experiment then was to apply labeled IAA-*myo*-inositol or labeled IAA to the endosperm of *Zea* kernels and determine whether we approach the same value--93% ester, 7% free--starting either from ester or free labeled IAA. Hydrolysis or synthesis of ester in the endosperm becomes unimportant since the pools are so large that we would not see appreciable radioactivity in the shoot if hydrolysis or esterification occurred in the endosperm. Thus, we can determine whether equilibrium is attained after the labeled compound enters the shoot. Mr. Nowacki applied labeled IAA-*myo*-inositol to the endosperm and found 94% ester and 6% free IAA in the shoot (unpublished). Ms. Hall applied labeled IAA to the endosperm and found 70% ester and 30% free IAA in the shoot (38). These values approximate those found for natural *in vivo* concentrations by Ms. Schulze. The conversion of free IAA to ester, as studied by Ms. Hall, are low but these were early experiments done before we were aware of the ease of hydrolysis of the esters. The results demonstrate that one approaches the same equilibrium amounts of ester and free IAA starting from either compound. We use the word equilibrium to denote that ester IAA can be hydrolyzed to free IAA and free IAA can be converted to ester IAA. We do not imply that this is a reversible reaction catalyzed by a single enzyme (43). This constitutes the first demonstration in biology of an *in vivo* equilibrium between a hormone and its covalently linked conjugates.

Is the Equilibrium Between IAA and its Conjugates Perturable by an Environmental Input

An attempt to answer the question of whether the environment controls plant growth by perturbing the equilibrium between free and covalently conjugated hormone is the major effort of our laboratory. To date only one environmental input has been tested and that is photoinhibition of growth. It has been known for many years that a brief flash of light will inhibit the extension growth of an etiolated seedling plant (cf. 44). The question then becomes, when photoinhibition of growth occurs, will there be a concomitant decrease of free IAA and a commensurate increase in ester IAA? The results of this experiment are shown in Table IV. A 20 second light flash resulted in a 43% inhibition of growth as measured 90 minutes after the light flash. The free IAA decreased by 35% and ester IAA increased by a commensurate amount (45). Thus, our working hypothesis that growth is controlled by the relative amounts of free and conjugated hormone and that it is this ratio which reflects the environment is, in this case, confirmed.

TABLE IV

Photo-Induced Change in Growth and in Free and Free Plus Ester IAA

	Dark	Light	Δ	%
		mm/90 min		
Growth	3.6	2.6	-1.1	-34
		µg/kg		
Free IAA	23	13	-10	-42
Free plus ester IAA	68	77	+9	+11

An Hypothesis Concerning Hormonal Homeostasis

The "take home lesson" I wish to leave with you is that the hormones, IAA, the gibberellins, cytokinins, and abscisic acid, all occur in free and conjugated form (46, 47, 48, 49) and that anything that affects the relative amounts of free and conjugated hormone will control growth. A similar hypothesis, concerning mainly the gibberellins has been made (50). In the special case of IAA we have demonstrated that IAA is in equilibrium with its conjugates and that this equilibrium can be shifted by light. Thus, from these limited data, we propose, as a working hypothesis, that the environment affects the rate of plant growth by causing changes in the relative amounts of free and conjugated hormone. This concept is illustrated diagramatically in Figure 3.

We envisage, Figure 3, that environmental stimuli, as for example light, heat, gravity, water stress, etc., impact upon one or more sensory apparatuses. In the case of light this would be a pigment, whereas other stimuli would impact upon the plant counterpart of a "solion" (51). A "solion" senses changes in heat, sound, pressure, gravity, etc., using a reversible redox system and a minute applied potential. Since plant cells have a suitable bio-electric potential (52) and redox systems in their cytoplasm, they can be, in a very real sense, "solions". The sensor then transfers its signal, perhaps a hydride ion from a flavin, to one of the transducer enzymes. Chemically this could mean using the hydride ion to reduce a disulfide bond in an enzyme that synthesizes or hydrolyzes hormone conjugates--thus changing the activity of the enzyme. If the hydrolyzing transducer is activated then more active hormone results. If the synthesizing transducer is activated then there is less free hormone and less hormone effect occurs. We do not know what hormones do to control growth nor how many processes must occur for

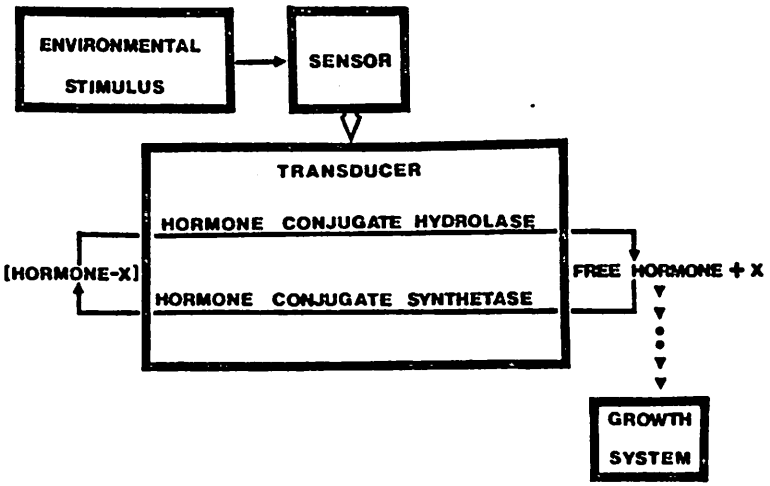

Figure 3. Diagram of a system for control of plant growth by varying the relative amounts of free and conjugated hormone

growth to result so we show a dotted arrow leading to growth. My personal feeling is that what the hormone does cannot be discovered until *in vitro* hormone-responsive systems are available (19). Here we encounter the Heisenberg uncertainty principle as applied to biology.

Conclusion

I wish to close now upon an optimistic but cautious note. Ultra-micro scale chemical assays of labile plant hormones will never be easy and routine. Nonetheless, use of internal standards and the detectors now available for gc and hplc, or the sensitive and selective mass spectrometer, permits assays of the hormones in 1 to 10 gm amounts of tissue thus permitting physiological experiments. We must someday return to bio-assays, using a definitive chemical assay and a physiologically sensitive bioassay simultaneously. This is true because the tissues contain both hormones and high amounts of, usually inhibitory, phenylpropanes, as will be discussed later in this symposium. Also, as Professor J. van Overbeek has indicated (personal communication), we will not understand the physiology of the organism until we simultaneously know what happens to all the hormones and their conjugates during complex developmental phenomenon. Difficult as this sounds, it will be possible, provided we confine ourselves to a few plants and seriously try to understand the hormonal system.

Lastly, I believe our working hypothesis of hormones and their conjugates as homeostatic hormone systems will lead to new and answerable questions. I emphasize, however, our data apply to one hormone, and to one environmental input and only to seedling *Zea* plants. Will there be a generality to our working hypothesis that the environment controls the ratio of free to conjugated growth hormone and thus controls growth? We feel that the answer to this question has great implications for the control of food and fiber production by applied growth regulators. I think we should work hard to answer this question.

Abstract

Most of the indole-3-acetic acid (IAA) in plants occurs as ester or amide-linked conjugates. This preponderance and apparent ubiquity led to a study of the functions of the conjugates. Evidence for four physiological roles has been found: 1) Conjugation is reversible and provides the plant with a way to regulate its IAA levels, and thus its growth rate, in accordance with the environment; 2) one of the conjugates (IAA-*myo*-inositol) is the chemical form in which IAA is transported from the seed of corn to the shoot, suggesting that conjugation provides the plant with information concerning where the hormone-precursor should be delivered; 3) IAA conjugates serve as a source of IAA for the seed and seedling; and 4) conjugation of IAA protects it against peroxidative attack. To our knowledge, this is the first case in biology where hormone levels are controlled by the formation and hydrolysis of a covalent bond.

Acknowledgements

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Literature Cited

1. Wain, R.L.; Fawcett, C.H. *In* F.C. Steward, Ed. "Plant Physiology VA"; Academic Press: New York, 1966, pp. 231-296.
2. Katekar, G.F. Phytochem., 1979, 18, 223.
3. Went, F.W.; Thimann, K.V. "Phytohormones"; Macmillan: New York, 1937.
4. Haagen-Smit, A.J.; Went, F.W. Konikl. Ned. Akad. Wetenschap., Proc., 1935, 38, 852.
5. Gandar, J.C.; Nitsch, J.P. *In* J.P. Nitsch, Ed. "Regulateurs Naturels de la Croissance Végétale"; Centre Nat. de la Rec. Sci. Quai-Anatole: Paris, 1964, pp. 169-178.
6. Marumo, S.; Hattori, H.; Abe, H.; Manakata, K. Nature, 1968, 219, 959.
7. Haagen-Smit, A.J.; Leech, W.D.; Bergen, W.R. Amer. J. Bot., 1942, 29, 500.
8. Weaver, R.J. "Plant Growth Substances in Agriculture"; Freeman: San Francisco, 1972, p. 120.
9. Ciesielski, T. Beitr. Bio. Pflanzen, 1872, 1, 1.
10. Darwin, C.; Darwin, F. "The Power of Movement in Plants"; D. Appleton: London, 1880.
11. Bonner, J. J. Gen. Physiol., 1933, 17, 63.

12. Thimann, K.V. Ann. Rev. Biochem., 1935, 4, 545.

13. Avery, G.S.; Creighton, H.B.; Shalucha, B. Amer. J. Bot., 1940, 27, 289.

14. Avery, G.S.; Berger, J.; Shalucha, B. Amer. J. Bot., 1941, 28, 596.

15. Haagen-Smit, A.J.; Leech, W.D.; Bergren, W.R. Science, 1941, 93, 624.

16. van Overbeek, J. Amer. J. Bot., 1941, 27, 1.

17. Evans, M.L. Ann. Rev. Plant Physiol., 1974, 25, 195.

18. Cleland, R. Ann. Rev. Plant Physiol., 1971, 22, 197.

19. Bandurski, R.S.; Piskornik, Z. *In* F. Loewus, Ed. "Biogenesis of Plant Cell Wall Polysaccharides"; Academic Press: New York, 1973, pp. 297-314

20. Labarca, C.; Nicholls, P.B.; Bandurski, R.S. Biochem. Biophys. Res. Commun., 1966, 20, 641.

21. Ueda, M.; Bandurski, R.S. Phytochemistry, 1974, 13, 243.

22. Piskornik, Z.; Bandurski, R.S. Plant Physiol., 1972, 50, 176.

23. Ueda, M.; Bandurski, R.S. Plant Physiol., 1969, 44, 1175.

24. Ehmann, A. Carbohy. Res., 1974, 34, 99.

25. Ehmann, A.; Bandurski, R.S. Carbohy. Res., 1974, 36, 1.

26. Bandurski, R.S.; Schulze, A. Plant Physiol., 1974, 54, 257.

27. Bandurski, R.S. *In* W.W. Wells and F. Eisenberg, Eds. "Cyclitols and Phosphoinositides"; Academic Press: New York, 1978, pp. 35-54.

28. Percival, F.; Bandurski, R.S. Plant Physiol., 1970, 58, 60.

29. Andreae, W.A.; Good, N.E. Plant Physiol., 1955, 30, 380.

30. Tillberg, E. Physiol. Plant, 1974, 31, 271.

31. Zenk, M.H. Nature, 1961, 191, 493.

32. Keglevic, D. Carbohy. Res., 1971, 20, 293.

33. Bandurski, R.S.; Schulze, A. Plant Physiol., 1977, 60, 211.

34. Little, C.H.H.; Heald, J.K.; Browning, G. Planta, 1978, 139, 133.

35. Hamilton, R.H.; Bandurski, R.S.; Grigsby, B.H. Plant Physiol., 1961, 36, 354.

36. Magnus, V.; Bandurski, R.S. Plant Physiol., 1978, 61(S), 63.

37. Cohen, J.D.; Schulze, A.; Bandurski, R.S. Plant Physiol., 1978, 61(S), 63.

38. Hall, P.L.; Bandurski, R.S. Plant Physiol., 1978, 61, 425.

39. Skoog, F. J. Gen. Physiol., 1937, 20, 311.

40. Nowacki, J.; Cohen, J.D.; Bandurski, R.S. J. Labelled Comp., 1978, 15, 325.

41. Rittenberg, D.; Foster, G.L. J. Biol. Chem., 1940, 133, 737.

42. Gillespie, B.; Thimann, K.V. Plant Physiol., 1963, 38, 214.

43. Kopcewicz, J.; Ehmann, A.; Bandurski, R.S. Plant Physiol., 1974, 54, 346.

44. Elliott, W.M.; Shen-Miller, J. Photochem. and Photobiol., 1976, 23, 195.

45. Bandurski, R.S.; Schulze, A.; Cohen, J.D. Biochem. Biophys. Res. Commun. 1977, 79, 1219.

46. Yogota, T.; Hiraga, K.; Hisakazu, Y. Phytochem., 1975, 14, 1569.

47. Milborrow, B.V. Ann. Rev. Plant Physiol., 1974, 25, 259.

48. Peterson, J.B.; Miller, C.O. Plant Physiol., 1977, 59, 1026.

49. Morris, R.O. Plant Physiol., 1977, 59, 1029.

50. Sembdner, G. *In* K. Schreiber, H.R. Schütte, and G. Sembdner, Eds. "Biochemistry and Chemistry of Plant Growth Regulators"; Inst. of Plant Biochem.: Halle, GDR, 1974, pp. 283-302.

51. Hurd, R.M.; Lane, R.N. J. Electrochem. Soc., 1957, 104, 727.

52. Lund, E.J. "Bioelectric Fields and Growth"; The University of Texas Press: Austin, 1947.

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