AUXIN IN MARINE ALGAE

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(WITH ONE FIGURE)

Introduction

Auxin has been studied almost exclusively in the higher land plants. Therefore, it seemed of interest to obtain some information regarding auxin in very different groups such as the lower water plants. For this purpose Macrocystis was selected. *Macrocystis pyrifera* (L.) Ag. is a large brown alga growing on rocks, usually in 10 to 30 meters of water. A description can be found in SETCHELL and GARDNER (**11**), or OLTMANNS (**10**). The stipe ("stem") becomes 30 to 50 meters long, bearing at its tip a terminal, bladderless, falcate blade and lower down the young blades and along the greater part of its length at regular intervals the mature, lateral blades, each with a pyriform bladder at its base. The mature blades are from 3 to 5 dm. long and 5 to 9 cm. wide. The upper 2 to 10 meters of the thallus usually floats on the surface of the water. The alga is perennial, which offers the advantage that material can be collected throughout the year. Since it was not considered practical to investigate the entire plant, only a young apical part about 50 cm. in length was used. Such a part consists of a terminal blade and a stipe to which are attached on the average 15 blades and bladders of an increasing size toward the base. Before the physiological rôle of auxin of a plant can be determined it is desirable to: (a) demonstrate the presence of auxin; (b), to determine its distribution throughout the plant; and (c) to discover the type of the auxin involved. These are the three points which will be discussed in this paper.

VAN DER WEIJ (**12**) in 1933, for the first time demonstrated the presence of auxin in marine algae. In the cell sap of young *Valonia macrophysa* plants he found between 0.00012 and 0.00021 mg. of auxin per liter, and in the cell walls of the same material about twenty times that amount. DU BUY and OLSEN (**3**) extracted auxin from Fucus. During the summer of 1938 I determined the auxin content of the green alga Bryopsis which had been collected and extracted (**14, 15**) by MR. M. L. DARSIE at Pacific Grove, California. About 80 gamma equivalents of indole acetic acid per kg. fresh weight were found. This is an auxin concentration of the same order as occurs in pea seedlings, but considerably smaller than found in Avena and corn plants (**13, 14, 15**). Another water plant in which such relatively large auxin concentrations were found is Elodea, in which I found auxin concentrations as high as 50 gamma equivalents of indole acetic acid per kg. fresh weight. In Macrocystis, on the other hand, auxin concentrations

of only about one gamma per liter were found, which is close to VAN DER WEIJ'S values for Valonia.

Experimental methods and results

Young undamaged plants free from overgrowths, as far as could be determined macroscopically, were collected in a pail of sea water. The plants were collected by boat at the kelp beds near the breakwaters of Newport Bay, or off the coast. Material that had become detached or was washed ashore was never used. Within one half hour after collection, the material was thoroughly washed with fresh water in order to remove microorganisms that might be attached, and then placed in highly purified ethyl ether. It was left in the ether for 2 to 3 days, and after that time the assay for auxin was made in a previously described manner (**14, 15**). However, one further simplification was introduced (suggested by DR. W. S. STEWART). Instead of evaporating the residue to complete dryness and adding agar to it, the extract was evaporated down to about 2 ml. which were then dropped immediately into the hot agar. In this way the auxin was instantaneously mixed with the agar and the 2-hour period of standing which was previously necessary to insure this mixing was eliminated. Each test included a control curve with known amounts of indole acetic acid which made it possible to bring the threshold value into calculation (**16**).

In order to check whether or not all of the auxin was removed, the extraction method described above was compared with the Soxhlet method, which is one of the most exhaustive methods known. Table I shows that practically the same amount was obtained by both methods. Hence, one can be reasonably sure that the data presented here truly represent the auxin content of the material.

TABLE I

AMOUNTS OF AUXIN* OBTAINED FROM MACROCYSTIS BLADES BY MEANS OF EXTRACTION IN ETHER FOR 2 DAYS AND BY SOXHLET EXTRACTION DURING THE SAME TIME (81101)

PART OF PLANT	SOXHLET	STANDING
Terminal blade	0.61	0.73
Young lateral blades	1.13	1.00

* Gamma equivalents of indole acetic acid per kilogram fresh weight.

Distribution

As was stated in the introduction, only the apical 50 cm. of the thallus was investigated. In table II a typical distribution experiment is represented which shows all of the individual factors necessary for the determination of the auxin concentration in the plant. In the first column is indicated the part of the thallus and the number used for extraction. The column marked W indicates the total fresh weight in grams of the parts

TABLE II

A COMPLETE ANALYSIS OF THE AUXIN DISTRIBUTION IN THE APICAL PART OF A MACROCYSTIS THALLUS. EXPLANATION OF SYMBOLS IN TEXT (81026)

	W	V_a	C	$(C \times I_{1^\circ} + O)$	GAMMA / KG.	GAMMA / PART
	gm.	*ml.*	*°*			
10 terminal blades	17.5	0.5	6.5	19.0	0.54	87×10^{-5}
			5.3	16.0	0.46	
10 stipes	42.0	0.3	7.2	20.0	0.14	59×10^{-5}
100 bladders	59.0	0.5	6.8	19.5	0.16	9×10^{-5}
			7.0			
15 lateral blades	62.0	0.5	7.0	20.0	0.16	78×10^{-5}
			10.5	27.5	0.22	
Controls						
26.5 gamma indole acetic acid per liter gave C = − 10.0°						
13.2 gamma indole acetic acid per liter gave C = − 4.1°						
0.0 gamma indole acetic acid per liter gave C = + 1.8°						

under investigation. The column V_a indicates the volume of agar in ml. in which the extract was taken up, C gives the degree of curvature in the Avena test. The factor $(C \times I_{1^\circ} + 0)$ translates degrees of curvature into concentration of indole acetic acid. Its value can be directly determined from the concentration curve with known amounts of indole acetic acid. I_{1° is the indole acetic acid concentration required to give an increase of curvature in the Avena test of 1°. 0 is the threshold value in gammas indole acetic acid per liter. The auxin concentration in gamma equivalents of indole acetic acid per liter (kg. fresh weight) is calculated from:

$$\frac{(C \times I_{1^\circ} + 0) \times V_a}{W}$$

which is given in the sixth column. The last column finally gives the auxin content of an average terminal blade, stipe, bladder, or young lateral blade.

As table II indicates, the auxin concentration is highest in the terminal blade and lowest in the stipe. This relation was generally found, but the terminal blade does not always have a higher auxin concentration than the upper lateral ones, as follows from table I. In table III a summary of all available data on the auxin distribution is given. There appears to be a definite decrease in auxin concentration in the basal direction although this auxin gradient is not as pronounced as in seedlings of higher plants (**14, 15**). The low auxin concentration in the rapidly growing stipe may be compared with a similar situation in young corn seedlings (**15**). Here it was found that the upper part of the first internode, which is the fastest

TABLE III

DISTRIBUTION OF AUXIN* IN THE UPPER PART OF THE THALLUS OF MACROCYSTIS

TERMINAL BLADE	YOUNG BLADES	OLDER BLADES	BLADDERS	STIPE	EXPERIMENT NUMBER	DATE
1.75	1.00	0.53	.....	.....	80926	(Sept. 26, 1938)
0.84	0.19	.....	.....	0.21	81019	(Oct. 19, 1938)
0.51	.....	.....	.....	.....		
0.54	0.16	.....	.16	0.14	81026	(Oct. 26, 1938)
0.46	0.22	.....	.....	.....		
0.61	1.13	.....	.....	.....	81101	(Nov. 1, 1938)
0.73	1.00	.....	.....	.....		

* Gamma equivalents of indole acetic acid per kilogram of fresh weight.

growing region of the entire corn seedling, has the lowest auxin content. It may be that in these cases the rapidly growing tissue consumes large amounts of auxin as discussed later.

TYPE OF AUXIN

In order to determine the nature of the auxin present in Macrocystis three types of investigation are open.

(a). The most satisfactory is to obtain it in crystalline form and determine its structure in a manner similar to that followed by KÖGL and HAAGEN-SMIT for auxin-a,-b, and indole acetic acid. A glance at tables I, II, and III shows that only about one-half part of auxin per billion (10^9) parts of Macrocystis is present. Hence it would require tons of material to isolate a fraction of a gram of auxin, which makes direct determinations impracticable in this case.

(b) KÖGL, HAAGEN-SMIT, and ERXLEBEN (7) have described a convenient differential acid-alkali destruction test which makes it possible to distinguish between indole acetic acid which is destroyed by acid but stable in alkali, auxin-a which is stable in acid but destroyed by alkali, and auxin-b which is unstable both in acid and alkali. When this method was tried on extracts of Macrocystis blades, invariably the auxin present in it was destroyed by acid, but not by alkali, indicating that indole acetic acid was present. Table IV gives the results of a few experiments of this type. It is noteworthy that in several instances refluxing with sodium hydroxide increased the auxin activity of the extract over that which had been boiled with distilled water. It may be that by boiling with dilute NaOH active auxin is liberated from a precursor, but it is also possible that some inhibitory substance is inactivated. Since HAAGEN-SMIT (private communication) does not consider the differential destruction test reliable for impure ex-

TABLE IV

AUXIN CONTENT* OF MACROCYSTIS EXTRACTS

REFLUXING WITH 5 PER CENT. HCl	REFLUXING WITH 1 N NaOH	REFLUXING WITH DISTILLED WATER	CONTROL NOT REFLUXED	TIME	EXPERIMENT NUMBER
deg.	*deg.*	*deg.*	*deg.*	*min.*	
+0.1	11.3	5.5	.....	45	81025
+1.5	10.0	6.3	.....	.....	
2.3	14.1	12.3	12.8	30	81019
2.9	13.5	10.7	11.6	.....	

* Degrees of curvature in the Avena test.

tracts, the third method which is available for obtaining information about the nature of the auxin was also tried.

(c) This method is based upon the determination of the diffusion coefficient and was first successfully applied by WENT (**17**), who four years before the auxin-a was chemically isolated, had determined its molecular weight. The experiment was carried out as follows: the auxin present in an extract of Macrocystis was taken up in a small agar plate ($10.8 \times 8.1 \times 0.8$ mm.). This plate was then carefully placed on top of a stack of three similar plates containing no auxin. After 40 minutes the 4 plates were separated and the auxin content in each of them determined by the Avena test method. From the relative distribution of the *actual amount* of auxin (rather than Avena curvature) in each of the four plates the diffusion coefficient can be found from diffusion tables after SHEFFER and KAWALKI (**1**). Once the diffusion coefficient (D) is known, the molecular weight can be calculated from $\sqrt{M} = \frac{7 \times 1.07}{D_{22^\circ}}$. Since it is extremely important to know the relative distribution of the actual auxin content in the four agar plates, one is not justified in directly using the degrees of curvature obtained in the Avena test for the determination of the relative auxin distribution in the tables, unless one is sure that there is a direct proportionality between the Avena curvature and the actual auxin concentration in the agar plates. Such a direct relationship does not exist in a great many cases. The curve did not start at the origin, but somewhere on the concentration axis, indicating that there was a threshold value. In order to avoid this error a diffusion test was run with indole acetic acid ($M = 175$) parallel to the diffusion test with the unknown auxin. In this control run the expected relative distribution of the indole acetic acid in the 4 agar plates may be found from the tables. The Avena curvature for each of these 4 plates is found by means of the Avena test. When these Avena curvatures are

plotted against the concentration of auxin obtained from the tables an accurate concentration curve for the conditions of the experiment is obtained. This curve subsequently was used to translate the Avena curvature of the diffusion test with the unknown auxin into relative amounts of auxin. When this was done diffusion coefficients and molecular weights for the auxin in Macrocystis were found which were close to those of indole acetic acid (table V). The difference between the molecular weight found for

TABLE V

DIFFUSION AND MOLECULAR WEIGHTS FOR AUXIN IN MACROCYSTIS. EXPLANATION IN TEXT

	$D_{22°}$	MOLECULAR WEIGHT	EXPERIMENT NUMBER
Auxin-a	0.414	328	.....
Auxin-b	0.426	310	.....
Indole acetic acid	0.567	175	.....
Auxin from Macrocystis	0.612	149	81117
	0.599	156	81207

Macrocystis-auxin and that of indole acetic acid is probably not significant. It is clearly shown, however, that the auxin of Macrocystis cannot be auxin-a or -b.

In determining the molecular weight by means of the diffusion test it was found inadvisable to work with a donor plate which contained an excessive amount of impurities. Such a block gives too low values in the Avena test. It was found that when such an impure block was placed for one hour on top of a block of plain agar, the latter gave a higher curvative than the former. Auxin had diffused into this plate of agar, but the majority of impurities remained behind in the original block. In the experiments mentioned in table V, use was made of this simple method of purification.

Discussion

It is generally assumed that higher plants contain auxin-a and -b and that lower ones contain indole acetic acid. This generalization is based upon the actual isolation of auxin-a and -b from corn oil and malt (**6**), and by a number of molecular weight determinations for higher plants (**4, 7**) all of which showed that auxin of a molecular weight like that of auxin-a or -b is present in these plants. The lower plants so far investigated have been fungi and bacteria. Indole acetic acid was actually isolated from yeast and Rhizopus (**2, 5, 7**), and more indirect evidence for the presence of indole acetic acid was obtained in Aspergillus and *Bacterium coli* (**9, 10**). The lower plants so far investigated were saprophytes in contrast to the

autotrophic higher plants. It is interesting to see that in the case of Macrocystis, an autotrophic lower plant, indole acetic acid or a substance closely related to it is present.

Although auxin has now been demonstrated in at least 3 marine algae, its mere presence does not make it a growth hormone for these organisms. Further researches have been planned in order to elucidate this particular point. There is evidence, however, that auxin is a growth hormone for Macrocystis: (1) the higher auxin concentration in the blades of the apical growing part of the thallus than in the older mature part. (2) The similarity in the relation between growth and auxin distribution in corn seedlings and in the stipe of Macrocystis. In an earlier paper (**15**) it was shown that in young corn seedlings the region of maximal rate of elongation is located in the upper part of the first internode. It was also shown that out of this particular region the smallest amount of auxin of the entire plant could be extracted. Strange though this may seem at first sight the following consideration will show that it is to be expected. If one assumes that auxin is used up during the process of elongation one can expect that the faster a particular region elongates the more auxin it uses. In the upper region of the corn coleoptile the growth rate is relatively small and is not limited by auxin but by other factors. In the lower part of the coleoptile and the upper part of the first internode elongation is limited by auxin. This has been shown by decapitation experiments (**15**). In this upper region of the first internode the other factors necessary for elongation are present in excess, and auxin will cause it to elongate as soon as it arrives at the proper spot. It is thereby rendered inactive. When active auxin is extracted it will be auxin which was present in the plant under the following conditions: (a) on its way to those regions where it will cause elongation; (b) on its way to more basal regions; and (c) as bound auxin. For a discussion of bound and free-moving auxin see (**18**) and a paper by the writer on "Auxin in roots."[1]

It will be clear from the above consideration that in the upper part of the coleoptile, where the auxin is produced and relatively little is used for elongation, relatively large amounts are found upon extraction. On the other hand, the more basal parts of the corn seedling (the upper part of the first internode) will have a relatively low auxin concentration because: (1) they receive only auxin which has been left over by the more apical regions; and (2) since the other growth factors are present in excess, practically all the auxin will be used for elongation. If a similar relation between elongation and auxin content exists in the stipe of Macrocystis, the relatively low auxin content there may be regarded as an indication that its elongation is auxin controlled.

[1] Bot. Gaz. **101**: 450–456. 1939.

Summary

In the brown alga Macrocystis auxin is present in a concentration of approximately 0.5 gamma equivalents of indole acetic acid per kilogram fresh weight. In the green alga Bryopsis up to 80 gammas per kilogram, and in Elodea 50 gammas per kilogram fresh weight were found. These are auxin concentrations of the same order of magnitude as are present in higher plants such as corn (on the average 0.5 gamma equivalents of indole acetic acid per kg.) and pea seedlings (on the average 50 gammas per keg.). The auxin distribution in the upper 50 cm. of the thallus was investigated. The young blades had the highest auxin concentration and the stipe (stem) the lowest (fig. 1). It has been shown that the auxin of Macrocystis is indole acetic acid, or a substance closely related to it, rather than auxin-a or -b.

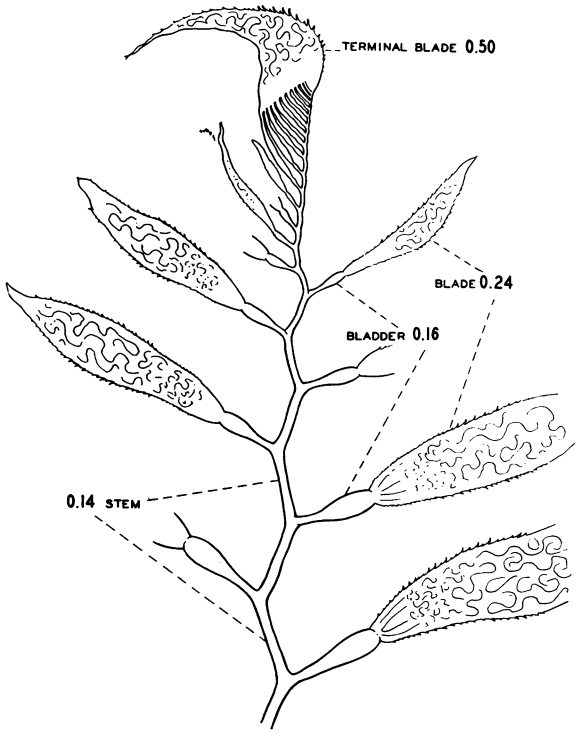

FIG. 1. Apical part of the thallus of *Macrocystis pyrifera* with figures indicating the distribution of auxin in gamma equivalents of indole acetic acid per kilogram fresh weight.

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