AUXIN IN ISOLATED ROOTS GROWING IN VITRO[13]

BY J. VAN OVERBEEK AND JAMES BONNER

WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated June 15, 1937

Up to the year 1933 opinion was divided as to whether or not auxin is present in the root. In this year Boysen-Jensen[1] conclusively demonstrated that auxin is present in the normal root. With respect to the isolated root, however, Fiedler[2] has reported that auxin, although initially present, disappears completely and in general within 24 hours, when the root is cultivated *in vitro*. Nagao[3] on the other hand, has shown that auxin may be recovered from isolated roots after 6 days' cultivation *in vitro*. The object of the present work has been primarily to establish, with the aid of improved techniques, whether or not auxin is actually present in isolated roots cultivated *in vitro*. A second but related problem of whether or not auxin is *produced* by such roots will be dealt with in a later communication although experiments of a preliminary nature indicate that it is not produced but is merely carried along in the tip of the isolated root.

Methods.—Pea roots were cultivated *in vitro* under the conditions which have been described elsewhere.[4] Tips ca. 4 mm. long were removed from the roots of germinating pea seeds[5] and cultured under strictly sterile conditions in 10 cm. Petri dishes in a basic nutrient solution containing sucrose, inorganic salts, vitamin B_1 (0.1 mg. per liter) and varying amounts of certain amino acids. After the roots had grown for one week and were ca. 70 mm. long, they were subcultured by removal of a 10 mm. tip to fresh medium. If the roots were to be further subcultured this procedure was repeated at weekly intervals. Since lateral roots are formed only after approximately 10 days' culture *in vitro* under these conditions, these experiments are uncomplicated by the presence of more than one growing point per root.

The technique of the auxin extraction and determination has been described in detail elsewhere.[6] The material to be extracted was placed in highly purified ether, in the cold room (ca. 2°C.) for 15 to 30 hours. The material was neither ground nor acidified[7] since either of these procedures may result in a partial destruction of auxin. The ether extract was then evaporated to dryness and taken up in a known amount of hot agar (1.5%). This agar was in turn analyzed for auxin by means of the *Avena* test. For each determination 24 test plants were used. In order that the auxin content of the roots might be expressed in "indole-acetic acid equivalents," comparable controls with this substance were also run. In general

two concentrations, one of 20 gamma and one of 7 gamma per liter were used for these controls. Each control was also run with 24 test plants. A blank with plain agar blocks was also included in each test. In an earlier paper[8] it has been pointed out that the *Avena* curvature-concentration curve often does not pass through the origin, but that it intersects the abscissa. This means that the curvature is not directly proportional to the concentration. There is a certain "threshold concentration" before a curvature is obtained. This threshold concentration, which is in general about 5 gamma per liter, was determined for most of the tests and was applied to the subsequent calculations. It was, however, found that if this threshold concentration is taken into account, the relationship between auxin concentration and *Avena* curvature can be expressed by a straight line,[9] at least under the conditions of these experiments.

The auxin content of the roots was calculated according to the following formula:

$$\text{auxin concentration} = \frac{(C \times I_1^\circ + O) \times V_{agar}}{W}$$ gamma indole acetic acid equivalents per kg. fresh weight

C = the average curvature obtained in the *Avena* test (extracted material)

I_1° = indole acetic acid concentration giving an increase of 1° in the *Avena* test, in gamma per liter

O = threshold concentration, gamma indole acetic acid per liter

V_{agar} = volume of agar in which the dry extract was taken up, cc.

W = fresh weight of material under investigation, gms.

Auxin Concentration in Roots.—Table 1 shows that under the conditions of our experiments auxin is present in isolated pea roots cultivated *in vitro* even after three weeks, and consequently, after 2 subcultures. There can then be no doubt but that auxin may be present in relatively large amounts, even in the cultured root. Fiedler,[2] as mentioned above, failed to find auxin in isolated roots (*Zea*, *Pisum*, *Vicia*) after more than 48 hours' growth *in vitro*. That Fiedler failed to extract auxin from *Zea* roots of any age, is undoubtedly due to the extraction technique which he employed. It has been shown elsewhere[6] that auxin cannot in general be obtained from *Zea* if acid is employed in the extraction (acid was used by Fiedler). It is shown in table 2 that with the present extraction technique, auxin can be obtained from the roots of sterile, germinating *Zea* seeds. The amount present in these roots is smaller than that present in *Pisum* roots of the same length (table 2). It has been shown earlier[6] that the shoots of *Zea* seedlings, also, contain less auxin than do the shoots of corresponding *Pisum* seedlings. The failure of Fiedler to extract auxin

from isolated pea roots cannot, however, be satisfactorily explained, although this also may have been due to the different techniques employed.

Nature of the Hormone Present.—It was of importance to ascertain whether the auxin of these roots was auxin-a, auxin-b or hetero-auxin (indole acetic acid). This may conveniently be determined by the differential acid-alkali destruction test of Kögl, Haagen-Smit and Erxleben.[10]

TABLE 1

AUXIN CONCENTRATIONS FOUND IN ISOLATED ROOTS GROWING *in vitro*, GAMMA "INDOLE ACETIC ACID EQUIVALENTS" PER KG. FRESH WEIGHT. THE CULTURES WERE ANALYZED ONE WEEK AFTER THEY WERE STARTED. CULTURES OF "ALASKA" ROOTS ARE INDICATED BY A, THOSE OF "PERFECTION" BY P. I, II, III, ETC., ADDED TO A OR P INDICATE RESPECTIVELY THE FIRST CULTURE, SECOND CULTURE (FIRST SUBCULTURE), THIRD CULTURE, ETC. THUS THE P III CULTURE AT THE TIME IT WAS ANALYZED HAD BEEN CONTINUED THREE WEEKS SINCE THE INITIAL ROOT TIP WAS CUT FROM THE EMBRYO

EXPT. NUMBER	CULTURE	γ/KG.	REMARKS
80315	A I	1.69	
80413	A I	2.24	no tip
80418	A II	5.88	
...	...	7.48	
80429	A II	6.40	
80517	roots 4 mm. long from embryo, P 0	22.3	
80315	P I	2.26	
80315	P II	2.28	
80331	P II	2.45	
80418	P III	4.12	

TABLE 2

AUXIN CONCENTRATION AND CONTENT OF 1-CM.-LONG ROOTS OF GERMINATING *Zea*, AND *Pisum* SEEDS. GAMMA "INDOLE ACETIC ACID EQUIVALENTS"

EXPT. NUMBER	PLANTS	C	$(C \times I_1^\circ + O)$	V_{agar}	W	γ/KG.	γ/PER ROOT
80603	*Zea*	3.0	9.4	0.5	2.60	**1.64**	**20.6×10^{-6}**
80523	Alaska	10.6	23.6	10.0	6.60	**36.0**	**300.0×10^{-6}**
80523	Perfection	7.8	18.0	7.5	4.00	**35.3**	**268.0×10^{-6}**
		3.5	9.8	15.0	4.00		

This test is based on the facts (*a*) that indole acetic acid is heat stable to alkali but is destroyed by acid, (*b*) that auxin-a is stable to acid but is destroyed by alkali and (*c*) that auxin-b is unstable to both acid and alkali. Three separate tests of this nature showed that the auxin extracted from pea roots was completely destroyed by alkali, unaffected by acid and must hence have been auxin-a. It might be mentioned in this connection that Heyn[11] has shown by another method that the auxin of the roots of germinating *Vicia* seeds is also auxin-a. It might also be noted, however, that one experiment made with *non-sterile* roots of pea seedlings showed the presence of large amounts of indole acetic acid, presumably formed through

the action of microörganisms. Reserve must therefore be exercised in the interpretation of experiments dealing with the auxin relations of non-sterile roots.

The experimental procedure was as follows: A large amount (45 gms.) of roots which had been one week in culture and most of which were without tips (tips had been removed for the subsequent culture) was extracted with ether in the usual fashion, and the extract divided into 4 equal parts. One part was analyzed immediately for the determination of the amount of auxin originally present. The other three portions were placed in flasks and the ether removed completely by distillation. To one sample was then added 3 cc. of 5% HCl; to the second sample, 3 cc. of 0.5 normal NaOH and to the third sample 3 cc. of distilled water. The samples were then refluxed on a boiling water bath for 15 minutes, cooled, neutralized (slightly acid) and extracted with ether. This ether was then tested in the manner described above. The results of one experiment are summarized

TABLE 3

DETERMINATION OF THE NATURE OF AUXIN IN STERILE, ISOLATED ROOTS GROWING *in Vitro*. EXPT. NUMBER 80509

REFLUXED WITH	C	$(C \times I_1^\circ + O)$	V_{agar}	W	γ/KG.
HCl	2.6	7.7	0.6	$1/4 \times 44.6$	**0.420**
NaOH	+0.9	0	0.6	$1/4 \times 44.6$	**0**
H_2O	2.0	6.9	0.6	$1/4 \times 44.6$	**0.378**
not refluxed	2.3	7.5	1.0	$1/4 \times 44.6$	**0.681**

in table 3. It is of interest to note that the entire analysis was carried out with 30 millionths of a milligram "indole acetic acid equivalents." This corresponds, according to Kögl, Haagen-Smit and Erxleben,[12] to 15 millionths of a mg. of auxin-a.

Distribution of Auxin in the Root.—Table 4 shows that pea roots both at the end of the first culture, i.e., one week *in vitro*, and at the end of the second culture, i.e., two weeks *in vitro*, exhibit a very pronounced gradient of auxin concentration from the tip toward the base. This gradient is much steeper than that found by Thimann[7] for roots of *Avena* seedlings, but is similar to that found by Boysen-Jensen[1] for roots of germinating *Zea* and *Vicia* seeds.

Is Auxin Produced by Isolated Pea Roots in Vitro?—This question will be treated in detail in a later communication. However, the present experiments permit of a comparison of the total amount of auxin per average initial root tip (the 4 mm. tip cut from the germinating seed) with the total amount of auxin per average root after it has been two weeks in culture (and has hence been subcultured once). The average initial root tip (4 mm.) contains 137×10^{-6} gamma indole acetic acid equivalents. The total auxin content of the average root at the end of the second week is

TABLE 4

DISTRIBUTION OF AUXIN-a IN STERILE, ISOLATED ROOTS GROWN *in Vitro*, GAMMA "INDOLE ACETIC ACID EQUIVALENTS" PER KG. FRESH WEIGHT

EXPT. NUMBER	CULTURE	PART	LENGTH (AVERAGE)	C	$(C \times I_1° + O)$	V_{agar}	W	γ/KG.
80524	P I	tip	15 mm.	11.2	25.3	0.5	1.001	**12.7**
		middle	15 mm.	2.1	8.9	0.5	2.003	**2.22**
		base	24 mm.	15.9	34.0	0.5	7.609	**2.23**
80511	P II	tip	15 mm.	2.6	8.7	0.6	0.856	**6.10**
		middle	25 mm.	1.9	7.5	0.4	2.500	**1.20**
		base	25 mm.	1.1	6.0	0.5	8.900	**0.337**
80516	P II	tip	15 mm.	5.3	18.8	0.5	1.020	**9.2**
		middle	15 mm.	4.6	16.4	0.5	2.695	**3.04**
		base	20 mm.	3.2	12.0	0.5	8.490	**0.71**

70×10^{-6} gamma indole acetic acid equivalents. Thus although the root at the end of the second week does *contain* auxin, it appears to contain substantially less of this substance than does the initial tip.

Summary.—(1) Isolated *Pisum* roots cultivated *in vitro* were found to contain auxin for at least three weeks after the original tip was removed from the germinating seeds. (2) This auxin obtained from roots under sterile conditions has been shown to be auxin-a. (3) A steep auxin gradient was found in these isolated roots, the highest concentration being found in the tip. (4) Roots after two weeks' cultivation *in vitro* appear to contain less auxin than did the initial root tips.

[1] Boysen-Jensen, P., *Planta*, **19**, 354 (1933).

[2] Fiedler, H., *Zeit. Bot.*, **30**, 385 (1936).

[3] Nagao, M., *Sci. Rep. Tohoku Imp. Univ.*, **12**, 191 (1937).

[4] Bonner, J., and Addicott, F., *Bot. Gaz.*, **99**, 144 (1937).

[5] Pea seeds of the variety "Perfection," supplied by the Ferry-Morse Seed Co., San Francisco, were used. Seeds of the variety "Alaska" were used in a few experiments with substantially the same results.

[6] van Overbeek, J., *Proc. Nat. Acad. Sci.*, **24**, 42 (1938) and *Bot. Gaz.* (1938) (in press).

[7] Thimann, K. V., *Jour. Gen. Physiol.*, **18**, 23 (1934).

[8] van Overbeek, J., *Ibid.*, **20**, 283 (1936).

[9] Compare: Thimann, K. V., and Schneider, C. L., *Amer. Jour. Bot.*, **25**, 270 (1938).

[10] Kögl, F., Haagen-Smit, A., and Erxleben, H., *Zeit. Physiol. Chem.*, **228**, 104 (1934).

[11] Heyn, A. N. J., *Proc. Kon. Akad. Wetenschap., Amsterdam*, **38**, 1074 (1935).

[12] Kögl, F., Haagen-Smit, A., and Erxleben, H., *Zeit. Physiol. Chem.*, **228**, 90 (1934).

[13] Report of work carried out with the aid of the Works Progress Administration, Official Project Number, 465-03-3-342, Work Project N-9199.