The results obtained do not support the view that, for the species investigated, intact plants grown from seed can benefit from additions of vitamin B_1 to an otherwise favorable nutrient medium. Such fluctuations in dry weight as were obtained are probably within limits of experimental error. It appears that under the conditions of these experiments the rate of vitamin B_1 synthesis was not limiting growth for any of the species investigated or, stated in other terms, such differences as do exist in the rate of vitamin B_1 synthesis among these plants are apparently compatible with their different needs.

Although these findings are regarded as favoring the contention that green plants when propagated under favorable conditions from seed are capable of synthesizing adequate amounts of vitamin B_1, they do not bear directly on the question of the effect of vitamin B_1 on the rooting of cuttings.[11] An important distinction should be made in this connection between sexual and vegetative propagation. The seed is a storage organ for vitamin B_1 (and is valuable for that among other reasons in animal nutrition) and other growth and food substances essential for the initial growth of the seedling, which soon embarks on photosynthesis, inorganic nutrient absorption and the other metabolic activities of the growing plant. A cutting, on the other hand, is a vegetative organ, not characterized by the storage of nutritive and growth substances to the extent observed in the seed. Cuttings of different species, or for that matter cuttings of the same species, taken under varying conditions may differ in vitamin B_1 content which in some cases may conceivably become a limiting factor in root development. Thus the beneficial effect of vitamin B_1 on the rooting of certain cuttings is not inconsistent with the finding that, when propagated from seed, green plants are capable of synthesizing their own vitamin B_1 requirements. In the light of the observation that in higher plants vitamin B_1 is synthesized in the green leaves under the influence of light[5] it is interesting to note that the presence of leaves on cuttings usually promotes root formation.[12]

This discussion is based on work done with several species of plants grown under favorable nutrient and climatic conditions. Whether under conditions adverse to the synthesis of vitamin B_1, these and other plants, even when grown from seed, may respond favorably to additions of vitamin B_1 from without, is a question of potential agricultural interest which can not be answered definitely at this time. Evidence was obtained, however, to show that several widely differing species, when grown from seed under favorable conditions, are not limited in their growth by an inherently low rate of vitamin B_1 synthesis.

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[11] F. W. Went, J. Bonner and G. C. Warner, SCIENCE, 87: 170, 1938.

[12] Review by H. L. Pearse, Imp. Bur. Hort. and Plantation Crops, Tech. Com. 12, 1939.

SOME DIFFICULTIES ENCOUNTERED IN THE EXTRACTION OF GROWTH HORMONES FROM PLANT TISSUES[1]

ALTHOUGH there are nearly as many methods of extracting plant growth hormones (auxin) as there are investigators, there is no satisfactory quantitative method. For over two years the author has been engaged in studying growth hormones in a number of plants, and during that time he has tried out many of the existing methods. Van Overbeek's[2] method has been used as the standard against which the others have been compared. It has proven to be as satisfactory as any, but it does not extract the auxin quantitatively from the material under investigation. The trouble has been that only after weeks or months of extraction was a point reached where no more auxin was obtained from the material.

The auxin assay has been carried out in the usual way with *Avena* coleoptiles under standard conditions of humidity and temperature and calculations made according to the equation formulated by Van Overbeek. In his publication of the method, Van Overbeek stated that he obtained complete auxin extraction in a period of 24 hours, and that succeeding periods gave no further active material. The writer has not found this to be true with his material, even when the extraction was done on an agitator, as shown in Table I.

TABLE I

AUXIN CONTENT IN OVARIES OF THE SUNFLOWER. THE AUXIN CONCENTRATION IS DENOTED IN TERMS OF INDOLE ACETIC ACID EQUIVALENTS, AND THE FIGURES ARE GAMMAS PER KG OF FRESH MATERIAL

Extraction	Time of extraction in hours	Amount of auxin obtained H_1	Amount of auxin obtained H_6
1	37	0.6	2.22
2	25	1.74	1.46
3	26	0.81	0.97
4	27	0.70	0.77
5	37	1.04	1.81
6	45	1.21	1.63
7	27	1.17	0.75
8	24	1.35	0.44
9	20	0.74	0.99
10	22	0.85	0.98
11	23	0.41	1.22

Note: During the next two weeks several more extractions were made and each showed the presence of auxin.

Table II gives the data from an experiment with young tomato plants.

These two experiments are typical of the many that have been performed during the past two years. It is obvious that not all the auxin, which is finally extracted

[1] This investigation has been financially aided by the Horace H. Rackham Trust Fund of the University of Michigan.

[2] J. Van Overbeek, *Proc. Nat. Acad. Sci.*, 24: 42–46. 1938.

TABLE II

AUXIN CONTENT IN TOMATO SEEDLINGS. THE AUXIN CONCENTRATION IS DENOTED IN TERMS OF INDOLE ACETIC ACID EQUIVALENTS, AND THE FIGURES ARE GAMMAS PER KG FRESH MATERIAL

Time of extraction	Conc. of auxin	Time of extraction	Conc. of auxin
1939–			
4–10	3.98	8–27	1.76
	*1.06	9–18	9.55
	*1.82	25	1.07
15	1.87	10– 3	0.60
22	2.27	7	1.09
29	0.97	14	0.96
5– 4	1.00	24	0.76
11	0.91	11– 4	1.30
21	1.07	18	1.95
25	0.78	21	1.91
30	1.30	28	0.5
6– 5	1.31	12– 8	1.54
7	1.12	15	0.74
15	1.03	1940–	
7–13	0.68	1– 5	0.93
15	1.36	16	1.47
19	1.21	27	1.9
21	1.20	2–10	1.33
24	1.50	20	1.24
27	1.50	27	1.76
31	0.96	3– 9	2.39
8– 4	1.17	23	2.50
9	2.29	4– 8	2.50
14	†1.62	5– 6	3.66
17	1.58		

* Refluxed 4¼ and 3 hours, respectively.
† Material frozen and ground finely.

from a lot of plants, was there in an active form at the time the plants were collected.

DuBuy's[3] method of extracting the frozen and ground plant material with cold water has not been found to be satisfactory. Avery's[4] recently published method of extracting with alcohol, while satisfactory with dried corn, does not work with fresh plants. Thimann[5] used chloroform as a solvent, and in an experiment with *Malvaviscus* sp. it has been compared with ether. The total auxin obtained in 14 extractions was nearly twice as great with ether as with chloroform.

Table III shows the importance of proper dilution.

TABLE III

INFLUENCE OF DILUTION UPON THE APPARENT AMOUNT OF AUXIN OBTAINED. THE AUXIN CONCENTRATION IS DENOTED IN TERMS OF INDOLE ACETIC ACID EQUIVALENTS, AND THE FIGURES ARE GAMMAS PER KG OF FRESH MATERIAL. THE AMOUNT OF MATERIAL WAS THE SAME IN ALL DETERMINATIONS

Amount of agar in cc added to the extract				
.6	.7	1.0	1.5	2.0
2.00		3.64	5.06	
3.20		3.84	7.65	
	3.38	4.10	6.32	6.28
2.49		3.72	6.07	5.94

Of the solvents used ether seems to be the best, but it does not extract all the auxin except after many extractions. Care must be taken to have proper dilutions. This has previously been discussed by Went and Thimann with respect to indole acetic acid but not previously stressed with extracted material.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE METHOD OF OBTAINING PURE CULTURES OF EMBRYONIC HEART MUSCLE

A BRIEF statement concerning the cultivation of embryonic chick heart muscle *in vitro* may serve a twofold purpose, adding to the data available on the tolerance of tissue cells from warm-blooded animals to subnormal temperatures[1] and suggesting a possible method of obtaining pure cultures of embryonic heart muscle.

Fragments of 8-day embryonic chick heart muscle were explanted to a medium consisting of 1 drop of fowl plasma (dil. 2:1 with Tyrode solution) plus 2 drops of freshly prepared 8-day embryonic juice (dil. 1:2 with Tyrode solution). One culture of a limited series remained in the incubator continuously for a period of 18 days without renewal of the nutrient medium. The temperature during incubation varied between 33°–35° C.

Microscopic examination of the living culture on the eighteenth day of cultivation revealed a network of refractile cells which were contracting vigorously. The nuclei of the cells appeared unusually clear with particularly prominent nucleoli. Connective tissue cells had entirely disappeared from the culture.

Microscopic examination of the fixed and stained preparation (Champy-Kull process followed by Heidenhain's iron alum haematoxylin method) showed that histodifferentiation had not taken place. No striated myofibrils were present in the network of cells. There was an accumulation of uniformly fine fat droplets in a relatively small number of cells, while a still smaller number contained large vacuoles. The nuclei of the vacuolated cells showed evidence of degenerative changes. No mitotic figures were present.

The fact that the muscle cells remained functional while all types of connective tissue cells degenerated suggests a simple method of obtaining pure cultures of embryonic chick heart muscle. The more usual method of subdividing cultures at the time of transplantation is worthless in the case of heart muscle,

[3] H. G. DuBuy, *Jour. Agr. Res.*, 56: 155–158. 1938.
[4] G. S. Avery, *Am. Jour. Bot.*, 26: 679–682. 1939.
[1] A. Fischer, *Arch. f. exper. Zellforsch.*, Bd. 2, S. 303–305, 1925–26; L. Buccianti, *ibid.*, Bd. 11, S. 397–423, 1931; E. F. Stilwell, *ibid.*, Bd. 21, S. 447–476, 1938; D. C. Hetherington and J. C. Craig, *Jour. of Cell. and Comp. Phys.*, 14: 197–203, 1939.
[5] K. V. Thimann, *Jour. Gen. Physiol.*, 18: 23–34. 1934.