ENZYMATIC LIBERATION OF AUXIN FROM PLANT TISSUES

RECENTLY it has become increasingly clear that the quantitative extraction of auxin from green tissues by the use of solvents is attended with considerable difficulty. In a study of this problem,[1] using *Lemna minor,* grown under controlled conditions, as source material, and the standard *Avena* method for auxin assay, we have found that complete removal of the auxin present is obtained only after repeated extractions spread over a period of several months. This is true even when ether, which was found to be the most suitable solvent, was used. It is true also for a number of different plant tissues.

The results which we have obtained by several methods demonstrate that the slowness of the extraction is due to the very gradual liberation of free auxin from some bound form. From the behavior of the material in regard to boiling and drying, it was deduced that this liberation is in the main enzymatic and probably hydrolytic in nature. In order to hasten the extraction, the effect of adding some proteolytic enzymes, available in pure form, has been investigated. It was found that crystalline trypsin brings about a small increase in yield, while chymotrypsin was highly effective under the conditions used. When duplicate samples of ground and dried *Lemna* were allowed to stand 24 hours at pH 8 and room temperature, and then acidified and extracted with ether, the yield of auxin was increased from 20 units in the control to 93 units in the enzyme-treated sample. Incubation with the enzyme at 37° C was still more effective.

In the table are given the results of one such experiment. Fresh *Lemna* was rapidly dried, kept at 70° C overnight, ground and divided into samples weighing 0.365 gms, corresponding to 3.0 gms fresh weight each. Water was added to all samples and some were boiled for 15 minutes. Another sample was cytolysed with ether, ground thoroughly with water, and the extract divided into two 3 cc portions. All samples were brought to pH 8 with Na_2CO_3, enzyme added where necessary, and the final volumes of the solutions brought to 2 cc. After 24 hours' incubation at 37°, the samples were acidified and extracted with ether (first extraction). They were then kept under ether for two further periods of 7 and 8 days, respectively, and the extracts so obtained, together with the rinsings, also tested.

Table I shows that both in the boiled and unboiled material, the enzyme increases the yield from three to four times. In another experiment increases as great as six times have been obtained with as little as 0.1 mg enzyme. Controls on the chymotrypsin solution alone, either fresh or carried through the procedure above, gave in 3 out of 4 experiments no auxin at all; on one occasion 12 units per 5 mg were obtained. The greatly increased auxin yield could therefore not be the result of either active impurities or decomposition products of the enzyme itself.

[1] Details of the work will be published elsewhere.

TABLE I

EFFECT OF CHYMOTRYPSIN ON THE YIELD OF AUXIN FROM LEMNA MINOR*

	First extraction	Second + third extraction	Total
		Unboiled material	
Control	250	310	560
Chymotrypsin 1 mg	1144	884	2028
Chymotrypsin 5 mg	638	982	1620
		Boiled material	
Control A	138	157	295
Control B	203	89	292
Chymotrypsin 1 mg	469	582	1051
Chymotrypsin 5 mg	313	505	818
		Water extract	
Control	101	114	215
Chymotrypsin 5 mg	280	118	398

* All data in units per cc per 3 gms fresh weight.

The results with the water extract are suggestive rather than significant, but they are included because they set an upper limit to the amount of activity which could possibly have been contributed by the enzyme.

It may be concluded that the auxin in *Lemna* is bound to a protein, from which it is liberated on hydrolysis. It is probable that this conclusion applies to a variety of plant tissues.

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BOOKS RECEIVED

ARNOLD, JOHN G. and TIMOTHY L. DUGGAN. *Laboratory Manual of General Biology.* Fourth edition. Pp. 275, loose leaf. Mosby.

BELL, D. J. *Introduction to Carbohydrate Biochemistry.* Pp. viii + 112. University Tutorial Press, London.

BIRKHOFF, GARRETT. *Lattice Theory.* Vol. XXV of Colloquium Publications. Pp. v + 155. American Mathematical Society, New York.

Contributions to American Anthropology and History. Vol. VI, Nos. 30–34. Pp. 299. Carnegie Institution of Washington. $3.00.

COTT, HUGH B. *Adaptive Coloration in Animals.* Pp. xxxii + 508. 84 figures. 48 plates. Oxford University Press. $8.50.

DIAMOND, MOSES and JOSEPH P. WEINMANN. *The Enamel of Human Teeth.* Pp. 105. 50 figures. Columbia University Press. $1.50.

Heller Committee for Research in Social Economics. *Quantity and Cost Budgets for Four Income Levels.* Pp. 107. University of California Press. $0.75.

MAYER, JOSEPH E. and MARIA G. MAYER. *Statistical Mechanics.* Pp. xi + 495. Wiley. $5.50.

Municipal Court of Philadelphia; Twenty-sixth Annual Report, 1939. Compiled by the Statistical Department, FRANK S. DROWN, Statistician. Pp. lii + 419. The Court.

SCHILLETTER, JULIAN C. and HARRY W. RICHEY. *Text-Book of General Horticulture.* Pp. ix + 367. 136 figures. McGraw-Hill. $5.00.