to both ordinary and partial differential operators, will be discussed in detail in papers now being prepared for publication elsewhere.

[1] Some of the results are briefly described in *Bull. Amer. Math. Soc.*, abstracts 43-3-114, 43-3-209, 43-9-323. There are two errors of statement in the first abstract: on page 171, line 1, the symbols "$\subset$" should be replaced by "$\supseteq$" and on page 171, line 2, "H(W)" should be replaced by "$\tilde{H}$(W)."

[2] M. H. Stone, *Linear Transformations in Hilbert Space*, New York, 1932, 424-530, especially Theorems 10.7 and 10.18. See also paper of I. Halperin, *Ann. Math.*, **38**, 880-919 (1937).

[3] *Opus cit.*

[4] For a discussion of the geometric aspects of the theory of transformations between Hilbert spaces, see F. J. Murray, *Trans. Amer. Math. Soc.*, **37**, 301-338 (1936), especially 301-312; the hypothesis that the transformations there considered have domains in a Hilbert space $\mathfrak{H}_1$ and range in a Hilbert space $\mathfrak{H}_2$ can be altered, in all of the theorems which we use, to allow either $\mathfrak{H}_1$ or $\mathfrak{H}_2$ or both to be unitary.

[5] J. v. Neumann, *Ann. Math.*, **33**, 294-310, especially 299 (1932); Murray, *loc. cit.*, 305-307.

[6] M. H. Stone has recently communicated to the author a characterization of all reduction operators which can be defined on the graph of the adjoint of a closed symmetric transformation H.

[7] J. v. Neumann, *Math. Ann.*, **102**, 49-131 (1929). See also Stone, *opus cit.*, Chap. IX.

A SIMPLIFIED METHOD FOR AUXIN EXTRACTION

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When the auxin content of a plant or part of it has to be determined two ways are open for analysis. (1) The oldest one is the *diffusion method*. The auxin of the plant material is allowed to diffuse into agar blocks. This method is more suited for determining the free moving auxin and the auxin production of tips and buds than for determining the actual auxin content of the plant. (2) The *extraction method* was first used by Thimann (1934). He extracted Avena seedlings by crushing them in chloroform and HCl. This procedure was repeated twice. Laibach and Meyer (1935) extracted maize and Helianthus with alcohol. The extract was concentrated and taken up in lanolin. This auxin paste was smeared on one side of intact Avena test plants. In corn plants up to 30 cm. in height no auxin could be found with exception of the coleoptile tips. Boysen Jensen (1937) reported good results with Thimann's method on Phaseolus. He also used ether and acetic acid and dropped the ether extract on agar

blocks (micromethod). Van Raalte (1937) extracted auxin from Vicia roots with ether and HCl.

When I tried to extract maize seedlings Thimann's chloroform and HCl method was first used. It failed to give results. Ether and acetic acid gave somewhat better results, but Boysen Jensen's dropping method could not be made to work satisfactorily. When acid was omitted from the extraction procedure, excellent results were obtained with ether extraction. Table 1 shows the increased yield from corn seedlings after omission of acid. It also shows that acid does not increase the amount of auxin extracted from Avena seedlings; in one case it even decreased it. The reason for the poor results with acid extraction may be the following. According to Kögl, Erxleben and Haagen Smit (1934) maize contains large amounts of auxin-b. This auxin is destroyed by acid. Another reason for the unsatisfactory preliminary results is the low auxin concentration of corn plants. After a suitable extraction method had been worked out it appeared that the auxin concentration of maize is about one-tenth of that of Avena. This was the more surprising since coleoptiles of corn produce large amounts of auxin in their tips.

The extraction method finally arrived at is described below. Its main features are the total omission of acid, the avoidance of crushing the material (considerable labor saving if large numbers of extractions have to be made), one single extraction for a prolonged time (overnight) and the evaporation of the ether extract to complete dryness.

a. Immediately before an extraction was made the ether was purified. Commercial ether was purified twice, recovered ether (see *c*) once. The ether was shaken with $FeSO_4$, CaO and water. In the beginning also $FeSO_4$, H_2SO_4 and water after Brandt (see Weisberger and Proskauer 1935) was used. Then the ether was distilled off. It was found that ether giving a negative benzidine test, a test which is considered extremely sensitive for detecting peroxides, still destroyed a hetero-auxin solution when shaken with it.

b. The plant material was measured (see *i*), cut into the parts that were to be analyzed and put uncrushed in Erlenmeyer flasks. Twenty-five to fifty cc. of ether was added per gram of plant material (less than 25 cc. was never used). This was left in a refrigerator for about 15 hours (overnight). If for some reason the material could not be tested after it had been in ether for 15 hours it was found advisable to evaporate the extract to dryness (see *c* and *d*) and store it in the refrigerator rather than to leave it in the ether. Material stored this way for three or four days had not lost in activity.

c. The ether was next separated from the plant material and from the water. Then it was distilled off (water bath 100°C.) until a residue of about 1 or $1^1/_2$ cc. was left. On account of the explosiveness of the ether

and for economical reasons, no amount larger than one of two cc. was allowed to evaporate in the open.

d. The residue was taken up in a pipet and dropped carefully on the bottom of a small vial (5 cc.) suspended in a beaker with boiling water. In this way the extract was evaporated to complete dryness and deposited at the bottom of the vial.

e. A known amount of agar (3%) was next pipetted on to the dry residue. Mostly 0.5 cc. was used. To secure a thorough mixing of agar and auxin the mixture was stirred and shaken vigorously (the vial still being in the boiling water). When the vial was removed from the boiling water it was left for a few hours for further uniform distribution of the auxin in the agar.

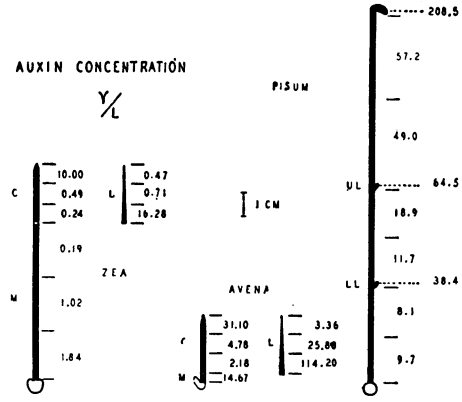

FIGURE 1

Auxin concentrations of etiolated seedlings of corn, oat and pea expressed in gammas hetero-auxin per liter water contained in the plant. It should be noticed that the auxin of the higher plants is of the auxin-a type which has an activity approximately twice that of hetero-auxin. In order to visualize the meaning of the figures it should be remembered that a hetero-auxin concentration of approximately 5 gammas per liter will give a curvature of one degree in the Avena test. Zea plants were 5 days, Avena 3 and Pisum 7 days old, which are stages of development at which these plants are mostly used in the laboratory. C, coleoptile. M, mesocotyl. L, primary leaf. UL, upper lateral bud. LL, lower lateral bud.

f. The agar auxin mixture was melted again in boiling water and poured into a rectangular brass mold (8 x 10.5 x 1.7 mm.). This mold was cooled by ice for a rapid gelification of the agar. Next this agar plate was divided into 12 equal blocks which were ready now to be put on decapitated oat seedlings for the auxin analysis. The extraction proper is thereby finished but in order to determine the auxin concentration in plants the following additional steps were taken:

g. The Avena test method described by Went and Thimann (1937, pp. 27–51) was employed. At the same time with the blocks to be analyzed a control series was run with a known concentration of hetero-auxin. This was necessary in order to calculate the obtained values in absolute amounts of auxin.

h. If weight determinations had to be made, the lengths of the stems, etc., were measured first, then the fresh weight was determined. Next the material was left for 24 hours in a drying oven, whereupon the water content could be determined.

i. The auxin concentration may be calculated from:

$$\frac{C_n \times I_{1^0} \times V_a}{W_n} \text{ gamma hetero-auxin equivalents per liter.}$$

C_n is the curvature of the Avena test for n plants per cm. coleoptile, mesocotyl, etc. I_{1^0} is the concentration of indole-3-acetic acid (hetero-auxin) in γ per liter required to give a curvature of 1° in the Avena test. V_a is the volume of agar in cc. in which the residue is taken up. W_n is the water content in grams per n plants per cm. of coleoptile, mesocotyl, etc.

TABLE 1

EFFECT OF ACID ON EXTRACTION. AUXIN DISTRIBUTION IN OAT SEEDLINGS GROWN IN WATER CULTURE AND IN SAND (70802, 70928)

WAY OF EXTRACTION	PLANT MATERIAL ANALYZED	AMOUNT OF AUXIN EXTRACTED IN DEGREES OF CURVATURE IN THE AVENA TEST	
Crushed and boiled with 50 cc. ether and 1 drop of 1 *N* acetic acid for 1 hour	14 corn seedlings	with acid	5.7
		acid omitted	13.3

Not crushed, left for 15 hours in 50 cc. ether and 0.5 cc. of 0.1 *N* acetic acid	50 oat seedlings	AMOUNT OF AUXIN PER CM.		
		ROOTS IN WATER		ROOTS IN SAND
	Coleoptile (plus leaf)	ACID	NO ACID	NO ACID
	Upper third	7.7	7.7	7.1
	Middle third	2.9	2.7	3.0
	Lower third	8.8	19.9	15.5
	Mesocotyl			
	Upper third			5.4
	Middle third	14.0[1]	11.8[1]	9.3
	Lower third			10.9

[1] Since the entire mesocotyl of water-grown plants was only 4 mm., the actual figures obtained in the assay were respectively 5.6 and 4.7.

In this way the auxin concentration is expressed in terms of hetero-auxin. In higher plants the auxin is of the auxin-a type, the activity of which is about twice that of hetero-auxin (according to Kögl, Haagen Smit and Erxleben).

Since the chemistry of auxin is known and hetero-auxin is readily available, it is my opinion that amounts of auxin extracted from or diffused out of plants should be expressed in absolute units rather than in arbitrary ones. The value of the arbitrary units (*A E*, *p u*, unit/cc., *W A E*) varies with the sensitivity of the test plants. An auxin amount expressed in hetero-auxin equivalents on the other hand is independent of the test method, test plant and sensitivity. However, in many cases where relative

amounts of auxin rather than absolute ones are being considered the auxin amounts may be more conveniently expressed in arbitrary units.

The above-described method has been used for the determination of the distribution of the auxin concentrations in maize, Avena and pea seedlings and also by Yin (unpublished as yet) to study the auxin distribution of Papaya leaves, and has proved to be reliable and to give reproducible results (Avena experiments of table 1). Figure 1 shows the auxin distribution in gammas hetero-auxin equivalents per liter. Note the high auxin concentration in the basal part of the primary leaves of Avena and maize and also the relatively high concentration in the basal parts of those seedlings. The concentration in the lateral buds of the pea seedling is higher than that in the adjacent stem tissue. A detailed paper discussing the relation between growth and the auxin distribution in maize and Avena seedlings and also the relation between auxin concentration and bud inhibition in the pea seedlings will be submitted to *The Botanical Gazette.*

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*LIGHT INTENSITY AND THE NITROGEN HUNGER PERIOD IN THE MANCHU SOYBEAN**

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Leguminous plants grown on a nitrogen-poor substrate and dependent on the fixation of atmospheric nitrogen for their supply of this element frequently exhibit during their development a "period of nitrogen hunger." This period occurs fairly early in the growth of the plant, when the stores of nitrogen in the seed have been exhausted and before the centers of fixation, the nodules, have developed sufficiently to meet the ever-increasing demands of the plant for nitrogen. As would be expected the phenomenon usually occurs under environmental conditions which favor photosynthesis,