York City, and at the American Cyanamid Company. Both substances showed absorption bands similar in frequency and shape at 40 points. The correspondence of these two compounds at so many frequencies would leave little doubt as to their identity.

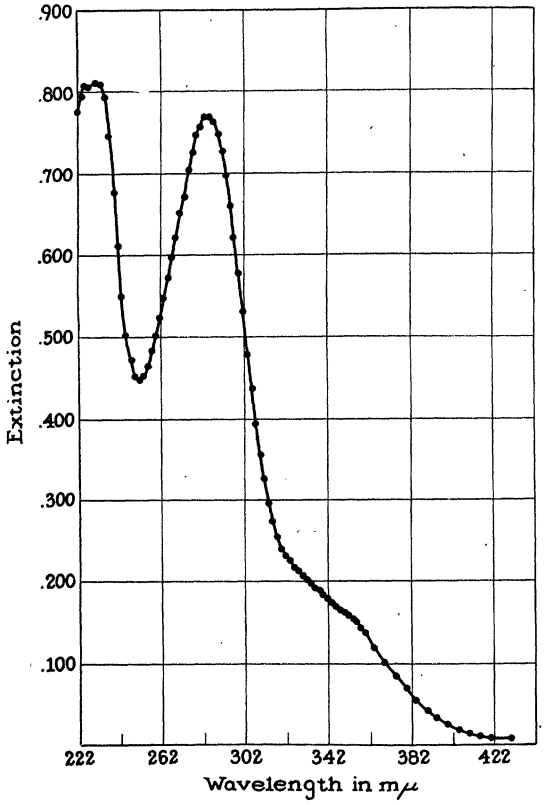


FIG. 2

Further investigations made by X-ray diffraction analysis of the two compounds by J. D. Bernal and I. Fankuchen indicated identical crystal structures. A more detailed account of their studies will be published elsewhere.

The results previously published (*6*) on *in vitro* inhibition of growth of various bacteria by the compound from *Ramalina reticulata* agree in general with those of Stoll and co-workers (*7*). Inhibition of gram-negative organisms is not obtained except at relatively high concentrations, while gram-positive organisms are inhibited by low concentrations. Human and bovine tubercle bacilli are inhibited by low concentrations, but the concentration required for inhibition of the avian strains of tubercle bacilli is higher.

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# Relative Growth Rates of Bean and Oat Plants Containing Known Amounts of a Labeled Plant-Growth Regulator (2-Iodo$^{131}$-3-Nitrobenzoic Acid)


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Succulent dicotyledonous plants are generally more sensitive to growth-regulating substances than are most plants of the monocotyledonous type (*2*), yet very little is known regarding the factors responsible for this difference in sensitivity. In recent experiments with the growth-regulating substance 2-iodo$^{131}$-3-nitrobenzoic acid (INBA) labeled with radioiodine, bean plants were found to absorb and translocate this compound more readily than barley plants (*3*), and a new tool was provided for observing still other differences in the way some dicotyledonous and monocotyledonous plants respond to growth regulators of this type.[1]

The present experiments were undertaken to determine, in part, whether the difference in sensitivity of plants, such as bean and oat, to such a growth regulator as INBA can be accounted for on the basis of a quantitative difference in their ability to absorb and translocate the compound. By applying radioactive INBA in varying amounts to the older leaves of bean and oat plants and then measuring the radioactivity of the young leaves that developed subsequently, it has been possible to compare the rate of growth of the two plant types when the young leaves of each contain equal concentrations of the growth regulator. Additional information which bears directly on this problem has also been obtained by studying the translocation of INBA in a third monocotyledonous plant, namely, corn seedlings.

The compound 2-iodo$^{131}$-3-nitrobenzoic acid was synthesized by diazotizing 3-nitroanthranilic acid and treating the reaction product with radioiodine 131.[2] A water dispersion of the compound was prepared by first dissolving the required amount in a small quantity of a commercial detergent (Tween-20) and then adding sufficient water to make a 4 per cent solution of the detergent in the final mixture. Measured amounts of the dispersion thus obtained were added to measured amounts of a 4 per cent solution of detergent in distilled water in order to make a series of clear, aqueous dispersions containing, respectively, 3.13, 6.25, 12.50, and 25.00 μg. of INBA/0.01 ml.

Bean and oat seedlings were grown from seed in potted soil under greenhouse conditions. Bean seedlings selected for uni-

[1] In the paper referred to it was tentatively concluded on the basis of the indirect evidence then available that INBA was absorbed and translocated as such in the plant. Since this work was published, it has been proved conclusively by the isolation of pure INBA from the stem and bud tissue of bean plants treated with this compound, that INBA is absorbed, translocated, and accumulated, at least in the bean plant, in the form of the intact molecule. While these experiments will be reported elsewhere, this fact is mentioned here to obviate the necessity for considering that a possible degradation product of INBA might be responsible for the results reported in this paper.

[2] Radioiodine was obtained through the Isotopes Branch ,Manhattan District, Oak Ridge, Tennessee.

formity were treated when the second internodes were not more than 1 mm. long but beginning to elongate. Immediately before treatment a thin layer of lanolin was smeared on the upper surface of one primary leaf of each plant in order to cover an area of about 1 cm.[2] above the juncture of the petiole and leaf blade. This was done to block external movement of INBA from the treated leaves to other parts of the plant.

The oat seedlings were treated when they had developed one fully expanded leaf and a second leaf which was still partly enclosed in the leaf sheath. The third and fourth leaves were not outwardly apparent. Before treatment, lanolin was smeared across the upper and lower surfaces of the first leaf of each plant so as to cover a region extending approximately 1 cm. from the upper end of the sheath and toward the tip of the leaf.

Both the bean seedlings and the oat seedlings were divided into 5 groups of 30 plants each. The 30 bean and 30 oat seedlings in the first group of plants were used as controls, and each lanolin-blocked leaf was treated with 0.01 ml. of a 4 per cent solution of the detergent (Tween-20) in water. The solution was applied in each case to the upper surface of the leaf along the midrib in order to cover an area approximately 5 mm. wide and 10 mm. long in about the center of the leaf. The 30 bean and 30 oat seedlings in the other four groups were treated similarly by applying 0.01 ml. of a 4 per cent detergent-water dispersion containing, respectively, 3.13, 6.25, 12.50, and 25.00 $\mu$g. of radioactive INBA to each lanolin-blocked leaf.

For the study of the translocation of INBA in corn, the plants were grown from seeds in potted soil under greenhouse conditions. Fifty-one were selected for uniformity and treated when the first leaf was well expanded and a second leaf was partially expanded. The basal portion of the first leaf of each plant was blocked with lanolin by the technique described above on both the upper and lower surfaces and 0.01 ml. of a 4 per cent detergent-water dispersion containing 25.00 $\mu$g. of radioactive INBA was applied to the upper surface of each lanolin-blocked leaf.

Eight days later the treated leaves of the bean, oat, and corn plants were removed. Stems of the bean plants were severed at the second node, and all parts above the second node were collected. Each part was weighed individually, dried at 80° C. in a well-ventilated oven, reweighed individually, and then the parts were combined by groups and ground to 40-mesh for radioactivity measurements. The oat plants were severed at the uppermost end of the first leaf sheath, and all leaves above this point were weighed and processed as described for the bean plants. The corn plants were severed at the upper end of the first sheath and parts above this level were divided so as to obtain combined samples made up of the second, third, and fourth leaves, respectively. The corn leaf samples were processed as described for the bean plants.

Radioactivity measurements were made on 50-mg. aliquots of the dried, ground plant samples under rigidly standardized conditions using a Geiger-Müller scaling unit and a lead-shielded, Geiger-Müller counter tube having a thin mica window.[3] For measurement, the samples were placed in cylindrical stainless-steel cups having an internal diameter of 32 mm. and a uniform depth of 3 mm. Radioactivity measurements were also made under the same conditions on 50-mg. aliquots of each of a series of reference standards prepared by adding various known amounts of radioactive INBA to samples of tissue from corresponding parts of untreated bean, oat, and corn plants by the procedure previously described (*3*). The amount of INBA present in the experimental plant samples was calculated by relating the measured radioactivity in each case to that of a reference standard of comparable activity. Frequently interspersed measurements of the radioactive INBA standards also obviated correction for decay of the radioiodine (half-life period of $I^{131}$ = 8.0 d.) during the experiments. Proper operation of the counting apparatus was checked routinely throughout the work by measuring the radioactivity of a radium D + E sample.[4] Background measurements (natural radioactivity) were also made at frequent intervals.

TABLE 1

ACCUMULATION OF INBA IN TERMINAL PARTS* OF BEAN AND OAT SEEDLINGS AND ITS EFFECT ON THE SUBSEQUENT GROWTH OF THESE PARTS

| INBA applied per plant ($\mu$g.) | Dry wt. per plant terminal growth avg. (mg.) | Net activity per 50 mg. tissue (counts/minute) | INBA per 50 mg. dry tissue† (mg.) | INBA per plant terminal growth ($\mu$g.) |
|---|---|---|---|---|
| | | Beans | | |
| 0.00 | 185.9 | 10 | 0.00 | 0.00 |
| 3.13 | 83.6 | 3,810 | 0.76 | 1.26 |
| 6.25 | 76.1 | 7,670 | 1.52 | 2.31 |
| 12.50 | 59.4 | 13,724 | 2.72 | 3.23 |
| 25.00 | 58.5 | 21,852 | 4.38 | 5.07 |
| | | Oats | | |
| 0.00 | 38.2 | 152 | 0.00 | 0.00 |
| 3.13 | 41.3 | 2,894 | 0.56 | 0.47 |
| 6.25 | 38.9 | 4,826 | 0.94 | 0.73 |
| 12.50 | 39.0 | 7,194 | 1.40 | 1.10 |
| 25.00 | 40.9 | 9,764 | 1.91 | 1.56 |

* All parts above sheath of first leaf of oat and above second node of bean plants are designated as terminal growth. This portion developed during the period of treatment (8 days).

† Based on standards made from dried leaves of bean and oat plants possessing average net activities of 5,047 and 5,123 counts/minute/$\gamma$ of INBA, respectively.

The concentration of INBA in the most rapidly developing parts of both bean and oat seedlings increased progressively as the amount of INBA applied to the plants was increased (Table 1). In the case of the bean plant the accumulation of the compound in the terminal buds, even at the lowest level of applied INBA, resulted in a marked inhibition in the rate of growth of this tissue (45 per cent of control). At higher levels of application even greater inhibition in growth occurred, with maximum inhibition (approximately 32 per cent of control) attained upon the application of 12 or more $\mu$g. of INBA/plant (Fig. 1). In the case of the oat plant, however, no inhibition in

[3] The apparatus used had the following characteristics: counting circuit scale having 64 units, with a register circuit for operation of an external mechanical register; self-quenching, end-window-type counter tube having a threshold voltage of 1,200 and a mica window 28 mm. in diameter, with a thickness of 3.2 mg./cm.$^2$.

[4] The radium D + E sample used as a standard in these investigations was kindly furnished by L. F. Curtiss, National Bureau of Standards. The sample consisted of radium D plated on thin palladium foil. The absolute beta-ray activity of the sample, due essentially to radium E in equilibrium with radium D, was calculated by the Bureau of Standards to have a va ue of 147 microrutherfords (1 microrutherford = 1 disintegration/second, *1*).

the rate of growth of young leaves occurred, regardless of the amount of INBA applied to, and absorbed by, the plant.

Oat and corn plants absorbed and accumulated appreciably less of the applied INBA than did bean plants. For example, 8 days after the application of 25 μg. of INBA to bean and oat plants, the concentration of the compound per unit of dried oat plant tissue was only half that found in the young leaves and stems of bean seedlings. Similarly, in corn plants, where no inhibition in growth occurred, considerably less INBA was absorbed and accumulated in the young leaves than was

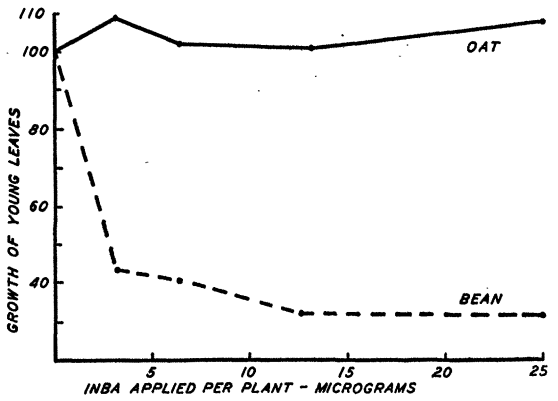


FIG. 1. Growth response of young leaves of bean and oat seedlings created with different amounts of INBA. Values calculated on the basis of tontrols equal to 100 per cent.

absorbed and accumulated in bean plants treated with an equal amount of the compound. Nevertheless, because of the wide range in the amounts of INBA applied to the bean and oat plants, experimental conditions were attained in which the

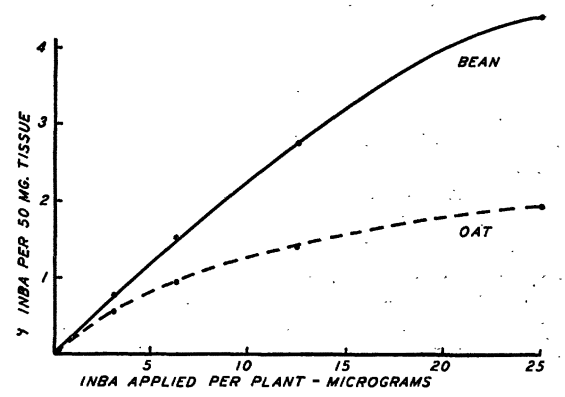


FIG. 2. Effect of applying increasing amounts of INBA to bean and oat seedlings on the concentration of the compound in the young leaves of each plant. (These curves yield essentially straight lines when plotted with logarithm coordinates.)

amounts present in the rapidly growing parts of the two plant types were identical, making it possible to compare the growth-inhibiting effects of this compound in these plants under strictly comparable conditions. This fact is apparent when the INBA accumulation curves plotted in Fig. 2 are considered together with the growth curves given in Fig. 1. Equal concentrations of INBA (1.9 μg./50 mg. of dried tissue) were present in the young leaves and stems of bean plants and in the young leaves of oat plants when the plants were treated with approximately 8.3 and 25 μg. of INBA, respectively. The growth of the young bean leaves and stems containing 1.9 μg. of INBA/50 mg. of dried tissue was reduced to 37 per cent of that of controls, whereas the growth of young oat leaves that contained an equal concentration of INBA was not at all affected.

It can be concluded from these results that the difference in the sensitivity of bean and oat plants to INBA cannot be accounted for on the basis of differences in the ability of the plants to absorb and translocate the compound or on the basis of a difference in the extent to which INBA accumulates in the rapidly growing parts of the plants, since with equal concentrations in the young leaves of each type of plant the growth of bean was greatly reduced but that of oat was not affected. These results lend additional support to one of the two alternative conclusions previously reached (*3*), namely, that the growth-inhibiting effects of INBA in the bean plant and its failure to produce significant inhibition in barley, oat, and corn plants must be due to differences in the manner in which INBA reacts with the plant constituents in each case.

TABLE 2

ACCUMULATION OF INBA IN LEAVES OF CORN SEEDLINGS 8 DAYS FOLLOWING APPLICATION OF 25 μG. OF THE COMPOUND TO THE FIRST LEAF

| Leaf in order of development | Dry weight per plant avg. (mg.) | Net activity per 50 mg. dry leaf tissue (counts/minute) | INBA per 50 mg. dry leaf tissue* (μg.) | INBA per leaf (μg.) |
|---|---|---|---|---|
| Second | 49.6 | 5,298 | 1.27 | 1.26 |
| Third | 63.6 | 4,815 | 1.04 | 1.32 |
| Fourth | 31.8 | 3,621 | 0.78 | 0.50 |

* Based on standards prepared from corn leaves having an average net activity of 4,652 counts/minute/γ of INBA.

Three other implications of the experimental results are noteworthy: (a) Growth of the terminal buds of bean plants was reduced roughly in proportion to the amount of INBA accumulated in them until a concentration of approximately 2.7 μg./50 mg. of dry tissue was reached; at higher concentrations of INBA no greater inhibition in growth occurred. (b) INBA continued to accumulate in terminal buds of bean plants even after maximum inhibition had been attained. (c) In the case of the translocation experiments with corn seedlings, the concentration of INBA was greatest in the oldest leaf assayed and least in the youngest one in which the INBA content was determined. On the basis of these observations and other evidence to be presented at a later time, it is considered probable that INBA, and perhaps other plant-growth regulators of this type, not only accumulates primarily in the plant tissue that is developing most rapidly at the time the compound is applied, but also combines stoichiometrically and, for the most part, irreversibly with certain essential metabolites of these tissues to exert its growth-inhibiting effects.

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