a hydrostatic pressure sufficiently high to eliminate tension forces in the tissue which might initiate cavity formation (*3*).

It is now clear that a focused beam of ultrasound may be used to produce discrete lesions in the central nervous system without destruction of blood vessels or surrounding tissues. By careful control of the dosage, it is possible to destroy large cells only and leave small cells, fibers, blood vessels, and glia intact. Work is now in progress on the production of such selective lesions in order to determine the anatomical and physiological consequences.

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A Microbiological Assay Method for Microgram Quantities of Manganese in Biological Material

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Bentley, Snell, and Phillips (*1*) and MacLeod and Snell (*2*) have described microbiological assay methods for manganese involving acidimetric and turbidimetric measurement of growth response. The observation that filter paper disks, saturated with solutions of manganous ions, exhibit zones of apparent growth stimulation on agar plates seeded with various bacilli (*3*) suggested to us the possibility of a simple diffusion plate assay for this element. This growth stimulation is especially suitable as the basis for an assay, inasmuch as it appears to be a unique property of manganese (*3*). Moreover, the diameter of the stimulated zone was found to bear a linear relationship to the logarithm of the manganese concentration (Fig. 1).

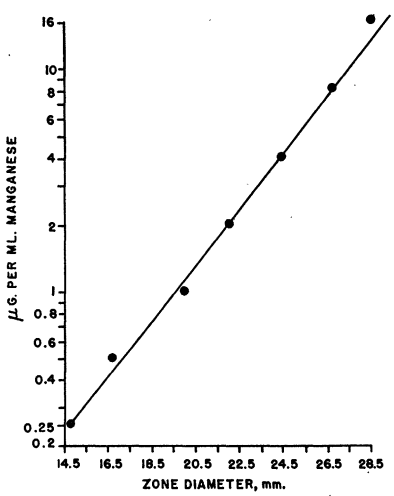

FIG. 1. Relationship between concentration of manganese and diameter of zone of stimulation. Diameter of paper disks was 13.5 mm.

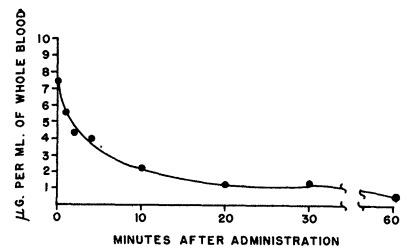

FIG. 2. Blood levels of manganese determined by diffusion plate assay following intravenous administration of 20 mg Mn as $MnCl_2$ to a 2-kg rabbit.

Using the Vincent and Vincent (*4*) assay method for penicillin as a model, the procedure finally developed was a filter paper disk assay employing a modified high-low technique, the high and low levels of manganese being 8 μg/ml and 2 μg/ml, respectively, in a 0.2% solution of Rochelle salt. The use of the latter as a complexing agent for manganese was adopted to avoid the possibility of binding or precipitation of the element during the assay. The standard solutions were conveniently obtained daily from a stock solution containing 80 μg Mn^{++}/ml, stored in a polyethylene container.

Three Petri dishes of nutrient soy agar, seeded with a suitable suspension of *B. subtilis* ATCC 6633, are used for each sample. On each of the plates is placed a disk saturated with the high standard, low standard, and duplicate test solutions, respectively, all solutions containing 0.2% Rochelle salt. Incubation of the plates and reading of the zone diameters are carried out as for any diffusion plate assay.

The mean of the three high and low standards are plotted on semilog paper with the zone diameter in millimeters as the abscissa, and the concentration of manganese in μg/ml as the ordinate; a straight line then is drawn through the two points. The mean value of the 6 test replicates is then referred to the curve to obtain the concentration of manganese in the test sample. This value, multiplied by the dilution factor used in preparation of the sample, yields the final result.

In order to define the applicability and limitations of the method, the determination of the blood levels of manganese following intravenous administration of manganese was attempted. As a preliminary test, it was found that whole rabbit blood contained no manganese detectable by this method, and, further, that added manganese could be completely recovered. Twenty mg of manganese as $MnCl_2$ was injected into the ear vein of a 2-kg rabbit. One-ml samples of blood were removed from the opposite ear vein at intervals and added to 9 ml of 0.2% Rochelle salt. After lysis of the red cells had ensued (approximately 1 hr at room temperature), the samples were assayed directly. The results obtained are shown graphically in Fig. 2.

The method described suffers from one severe limi-

tation. It is not suitable for the determination of quantities of manganese less than 0.5 μg/ml and therefore is incapable of detecting levels of manganese naturally present in most biological material unless preliminary concentration is carried out. Where microgram quantities of added manganese are to be determined in biological material, however, the method is both specific and convenient.

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Acceleration and Retardation of Abscission by Indoleacetic Acid[1]

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IAA (indoleacetic acid) and the related growth regulators, notably 2,4-D (2,4-dichlorophenoxyacetic acid) and NAA (naphthaleneacetic acid), are considered potent retardants of abscission in higher plants. Their widespread horticultural use to retard abscission of leaves and fruits has been described in a recent review of the physiology of abscission (*1*). The present paper is primarily concerned with the retardation that followed the application of growth regulators to the distal side of abscission zones, and the acceleration that followed the application of IAA to the proximal side of abscission zones.

In 1936 La Rue (*2*) observed that IAA retarded the abscission of the debladed petioles of *Coleus*. To obtain more precise information regarding this retardation, La Rue's experiment was repeated in modified form, using Black Valentine beans. This experiment was conducted in the greenhouse on fully expanded first trifoliolate leaves of plants that were 26 days old. Four groups of leaves were used: intact leaves, debladed leaves untreated, debladed leaves treated with lanolin, and debladed leaves treated with IAA-lanolin. The leaves were debladed by a cut between the leaflet blade and the leaflet pulvinus, exposing a surface at the distal end of the pulvinus. Lanolin and IAA-lanolin were applied to this surface. The IAA-lanolin contained 1 g IAA/l lanolin. The leaves were examined daily for abscission of the leaflet pulvinus. The data obtained during the 33 days of the experiment are summarized in Fig. 1. The two groups of debladed leaves, one with and one without lanolin, showed almost identical records: Each showed no abscission for the first 2 days, about 50% (with lanolin, 43%; without lanolin, 55%) on the 3rd day, 100% on the 4th day. The group of intact leaves showed no abscission for 21 days, 12% abscission on the 22nd day, 65% on the 23rd day, 90% on the 25th day, 100% on the 33rd day. The group of debladed leaves with IAA-lanolin showed no abscission for 3 days, 10% on the 4th day, 59% on the 10th day, 94% on the 17th day, 100% on the 32nd day.

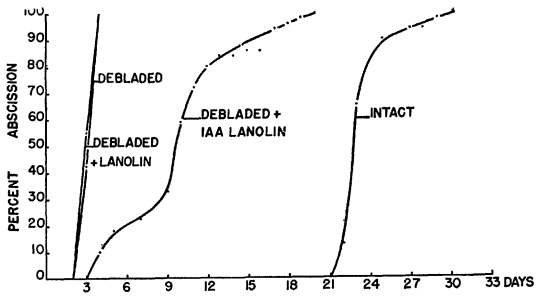

FIG. 1. Effect of indoleacetic acid on the time of abscission of debladed leaflet pulvini of beans.

The results of this experiment not only confirm La Rue's results—they show an even greater degree of retardation. The failure of the IAA-lanolin to replace the blade completely in retarding abscission is not surprising in view of the importance of other factors, especially carbohydrate, in retarding abscission (*1*); it is likely that debladding greatly reduces the amount of carbohydrate available to the abscission zone.

These results, as well as La Rue's, were further confirmed in experiments with excised abscission zones (explants) from greenhouse Black Valentine beans. An explant consisted of a leaflet pulvinus and 10 mm of the subtending leafstalk, with the included abscission zone lying between pulvinus and stalk. Explants were mounted on glass pins in Petri dishes and kept at 25° C. The method is described in detail in a previous publication (*3*). By means of a hypodermic syringe, 0.005-ml droplets of the solutions were applied daily to the explants.

When an application of IAA was made to the pulvinus of the explant, distal to the abscission zone, the results varied from slight retardation to complete inhibition of abscission, in direct proportion to the concentration of IAA applied. The range of concentrations employed was 10–1,000 mg/l; concentrations of 500 mg/l or higher produced complete inhibition. Similar results were obtained with 2,4-D and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). Moreover, like concentrations of all three growth regulators produced like effects, in contrast to the diverse effects commonly found in the study of other reactions (*4*).

The retardation and inhibition of abscission in the laboratory experiments described above were anticipated in view of the similar results consistently obtained in greenhouse and field experiments. What was not anticipated was the effect of the application of IAA to the stalk of the explant, proximal to the abscission zone. When the application of IAA was

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