zide was injected intraperitoneally into the pretreated and also into nonpretreated (control) mice, and the number of surviving animals was recorded after 24 hr. The doses protecting 50% of the animals (ED_{50}) were: chloral hydrate, 650 mg/kg; phenobarbital, 61 mg/kg. The therapeutic indices (LD_{50}/ED_{50}) were 1.7 and 5.3, respectively. It is clear that both drugs conferred protection but, because of its favorable therapeutic index, phenobarbital was preferred.

References

1. DUCA, C. J., ROTHLAUF, M. V., and SCUDI, J. V. *Antibiotics and Chemotherapy*, **2**, 16 (1952).
2. GRUNBERG, E., and LEIWANT, B. *Proc. Soc. Exptl. Biol. Med.*, **77**, 47 (1951).
3. ANDERSON, F. E., DUCA, C. J., and SCUDI, J. V. *J. Am. Chem. Soc.*, **73**, 4967 (1951).
4. MEYER, H., and MALLY, J. *Monatsh.*, **33**, 400 (1912).
5. GRUNBERG, E., and SCHNITZER, R. J. *Quart. Bull. Sea View Hosp.*, **13**, 3 (1952).
6. ROBITZEK, E. H., SELIKOFF, I. J., and ORNSTEIN, G. G. *Ibid.*, 27.
7. DEBEER, E. J. *J. Pharmacol. Exptl. Therap.*, **85**, 1 (1945).
8. SCUDI, J. V., KIMURA, E. T., and REINHARD, J. F. *Ibid.*, **102**, 132 (1951).

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Absorption of Nutrients by Stems and Branches of Woody Plants[1]

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Although roots are the principal nutrient absorbing parts or organs of plants, it has been shown that foliage is also capable of absorption (*1–3*). Deficiencies of magnesium have been corrected by foliar applications of Epsom salts (*4, 5*), proper nitrogen levels in fruit trees have been maintained by foliage sprays of urea (*6*), and as much as 7% of the phosphorus requirement of developing fruits of young tomato plants has been supplied through a single application to the foliage (*7*). The question naturally arises as to the absorption of nutrients by stems and branches.

Radioactive isotopes lend themselves well to such studies. K^{42} as potassium carbonate applied in a 6-in. band of cotton gauze around dormant branches of bearing apple trees (*Malus domestica* var. R. I. Greening) in midwinter (February) was detected 24 hr later in both the phloem and the xylem 18 in. above the point of application and 18 in. below, although the air temperature was below freezing during the period and reached a minimum of −3° F. Radiopotassium was detected likewise in branches rising vertically from horizontal branches of apple trees to which it was applied. With the Elberta variety of peach (*Prunus persica*) activity was detected within 48 hr in dormant branches of bearing trees both 6 in. beyond and 6 in. below the point of application.

Potted dormant 2-year-old trees of the McIntosh apple and the Elberta peach were treated with P^{32} *o*-phosphoric acid in a band 2 in. wide on the bark 6 in. above the soil and placed in the greenhouse at 70° F. Radiophosphorus was detected within 28 hr, not only in the stem above the point of application but in the roots as well.

Finally, measurements were made of urea hydrolysis from applications of C^{14} urea made to the branches in full leaf of apple, peach, and cherry (*Prunus cersus* var. Montmorency). Plants were placed in a chamber in the dark, and the atmosphere was circulated at a slow rate in a closed system containing a Geiger-Muller counter and a continuous recorder, as described by Hinsvark, Wittwer, and Tukey (*8*). Applications were made to leaves, to the bark of branches from which leaves had been removed, and to the bark of branches from which leaves had not been removed.

The data are given in Table 1 as enzymatic rate constants (zero order) derived from the initial slopes of the activity time curves. Hydrolysis of urea occurred in all cases following bark applications. The rate of hydrolysis varied with the parts and condition of the plant treated. Most rapid hydrolysis occurred when urea was applied to the bark of branches in full leaf, followed by application to a comparable leaf surface on a newly developed leaf, and the slowest rate of hydrolysis occurred when the urea was applied to the bark of branches from which the leaves had been removed. Activity of the bark and leaves of the apple appears to be approximately three times that of the peach.

TABLE 1

ENZYMATIC RATE CONSTANTS (ZERO ORDER) DERIVED FROM THE INITIAL ACTIVITY-TIME OF THE HYDROLYSIS OF C^{14} UREA, APPLIED TO THE BARK AND LEAVES SEPARATELY OF APPLE, CHERRY, AND PEACH TREES

Point of application	Rate constants (Counts/hr)		
	Apple	Cherry	Peach
Leaves	42	15	15
Bark, leaves removed	30	10	8
Bark, leaves not removed	130	32	20

To determine the concentration of nutrients that could be applied to dormant trees without visible injury, applications were made of calcium chloride, *o*-phosphoric acid, potassium nitrate, and urea in 2, 4, 8, 16, and 32% solutions and slurries to 2-year-old McIntosh apple trees and 1-year-old Montmorency cherry trees. Injury in the form of bud killing and delayed bud start resulted from concentrations greater than 8% calcium chloride, *o*-phosphoric acid, and urea. On the other hand, no injury to dormant trees was observed from potassium nitrate at any of the

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concentrations used. Also, no injury was observed following urea applications to young apple and cherry trees that had just begun growth. This observation is of interest in connection with the data for the hydrolysis of urea given in Table 1 showing greater hydrolysis following bark application to branches with leaves than to branches without leaves.

The surface area of trunk, branches, and twigs of dormant trees and the quantity of nutrients that were retained from bark applications were found to be appreciable. Thus the surface area of a 3-year-old McIntosh tree was found to be 551 cm^2, and that of a 25-year-old McIntosh tree, 86 m^2. The quantity of material in the form of urea, for example, which was retained when applied at 32% concentration was determined at .79 g for a 3-year-old apple tree and 1364.0 g for a 25-year-old apple tree.

It would appear from these data that so-called "foliage feeding" must take into consideration other portions of the plant such as trunk, branches, and shoots as well as foliage. It may be recalled that among the first field applications of commercial nitrogenous fertilizers to fruit trees were sprays to dormant trees (*9*) and that old horticultural practices included coating trunks and branches of fruit trees and vines with various manurial and mineral substances (*10*).

References

1. HAMILTON, J. M., PALMITER, D. H., and ANDERSON, L. C. *Proc. Am. Soc. Hort. Sci.*, **42**, 123 (1943).
2. HAMILTON, J. M., PALMITER, D. H., and WEAVER, L. O. *Phytopathology*, **33**, 5 (1943).
3. WENT, F. W., and CARTER, M. *Am. J. Botany*, **35**, 95 (1948).
4. BOULD, C., and TOLHURST, J. *Ann. Rept. Agr. and Hort. Research Sta., Long Ashton, Bristol*, **1948**, 51 (1949).
5. JONES, R. J., and ROGERS, H. T. *Advances in Agron.*, **1**, 39 (1949).
6. FISHER, E. G., and COOK, J. E. *Proc. Am. Soc. Hort. Sci.*, **55**, 35 (1950).
7. SILBERSTEIN, O., and WITTWER, S. H. *Ibid.*, **58**, 179 (1951).
8. HINSVARK, O. N., WITTWER, S. H., and TUKEY, H. B. *Plant Physiol.* (in press).
9. LEWIS, C. I., and ALLEN, R. W. In *Rept. (1914–15) Hood River (Oregon) Branch Expt. Sta.*, 5 (1916).
10. MILLER, P. *Gardener's Dictionary*. London (1754).

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The Activity of α-Amylase as Determined by Adsorption Indicators

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It has been previously shown that the activity of a proteolytic enzyme may be indicated by the loss of binding capacity of the protein substrate for simple dyestuffs (*1*). This note presents initial results to show that dextrinogenic activity may be determined in a similar manner. The method for determining the activity of hydrolytic enzymes may be considered a general one. In brief, the method consists of selecting a dye which undergoes a spectral change when it is adsorbed by the substrate. As the substrate is hydrolyzed, its affinity for the bound dye is altered. Usually the fragments of hydrolysis have a smaller binding capacity for the dye, and thus the system reverts to the absorption spectrum for the dye in the absence of the substrate.

The familiar starch-iodine color used in the determination of dextrinogenic activity may be viewed as an application of an adsorption indicator. The use of dyestuffs in place of iodine, however, offers an additional approach to the study of the α-amylases.

It is generally accepted that a chain length of about 10 glucose units is required for a red color with iodine, whereas a chain length of 30 units or more yields a blue color. Chains of 4–6 glucose units give no color with iodine (*2*). We have found that a dye such as Congo Red is adsorbed by dextrins that fail to give a color response with iodine. The minimal size of the polysaccharide which will cause a spectral change with Congo Red is about 5 glucose units in length. The size is comparable with the distance between the two amino groups in Congo Red, these groups probably being the foci of attachment to the starch, via hydrogen bonding (*3*). It would appear, then, that in the case of dextrins of low molecular weight, the use of Congo Red would have a distinct advantage over the iodine method.

The adsorption of Congo Red by starch at pH 7 is accompanied by a 20% loss of intensity of the spectral minimum which occurs at a wavelength of 402 mμ. The spectral peak, however, is enhanced about 5%, the peak being shifted slightly toward the red. The adsorption of dye is rapid and reversible. The same may be said for the desorption process, which occurs during the hydrolysis of the starch. During this process the shape of the spectral curve in the vicinity of the minimum is unchanged.

The effect of a sample of filtered saliva on a solution containing 0.05% Lintner's starch and 3×10^{-5} *M* Congo Red was investigated. The saliva had been diluted by factors of 600–9000. The solution also contained 0.01 *M* NaCl and 0.01 μ phosphate buffer at pH 7. The reaction mixture was observed in a Beckman DU quartz spectrophotometer, the temperature of the cuvettes being regulated to ± 0.1° C by means of cooling blocks. Fig. 1 shows that the addition of the starch causes an appreciable decrease in the spectral intensity, but upon the addition of the enzyme to the mixture, the starch gradually loses its combining capacity for the dye until the optical density for dye alone is attained.

A measure of the activity of the amylase is determined by the reciprocal of the time required to effect a given change in optical density. No knowledge of the kinetics of the reaction is necessary. Here one assumes that initially identical starch solutions, which subsequently have the same optical density, contain the substrate in the same state of hydrolysis. This as-

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