acetylcholinesterase in cultivated chick embryonic lung can be increased 2 to 6 times by adding 0.02*M* acetylcholine to the culture medium. Preliminary results from this laboratory indicate that mice treated with anticholinesterase drugs (to increase the level of acetylcholine in tissues) show increased cholinesterase activities in the liver, lung, skeletal muscle, and blood. Indirect evidence that cholinesterase synthesis may be altered in the retina of dark-raised rats is found in reports that ribonucleic acid, required for enzyme synthesis, is decreased in the retinas of animals deprived of light for varying periods (*1, 10, 11*).

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Simazine: Degradation by Corn Seedlings

Abstract. The herbicide 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine) is converted to 2-hydroxy-4,6-bis(ethylamino)-*s*-triazine (hydroxysimazine) in vivo by corn seedlings and in vitro by corn extracts. Hydroxysimazine is considered to be a detoxified form of the herbicide. In vitro conversion was produced by reacting simazine with either a cyclic hydroxamate (2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine) or its glucoside. The latter compounds may mediate at least some of the in vivo conversion in corn.

The herbicide 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine) is used as a selective pre-emergence spray to control annual weeds in corn fields. The tolerance of corn to simazine has been associated with an unidentified nonenzymatic substituent (*1, 2*) which converts simazine to 2-hydroxy-4,6-bis(ethylamino)-*s*-triazine (hydroxysimazine) which is nonphytotoxic. While the formation of hydroxysimazine in vivo has been postulated (*1*), its isolation from treated plants has not been previously reported. The objectives of our investigation were to study the initial steps in the degradation of the simazine molecule both in vivo and in vitro.

Hydroxysimazine is the major degradation product in short-term experiments with intact corn seedlings (*Zea mays* var. Dixie 82). Seedlings were grown in quartz sand (in a greenhouse in 1-quart containers). When they were 6 days old, 400 μg of ring-labeled C^{14}-simazine (specific activity 0.83 mc/mmole) was applied to the surface of the sand. Plants were harvested 10 days after treatment. Degradation products were isolated separately from roots and shoots by solvent fractionation. The distribution of radioactivity in the various fractions is presented in Table 1.

The chloroform-soluble fractions were chromatographed on Whatman 3 MM paper (ascending) in either *i*-amyl alcohol saturated with 3*N* hydrochloric acid or 65-percent 2,6-lutidine; C^{14}-simazine and C^{14}-hydroxysimazine were used as standards. All of the radioactivity in the chloroform-soluble basic fraction chromatographed as hydroxysimazine. The chloroform-soluble nonbasic labeled material was not resolved satisfactorily from the chlorophyll and carotenoid pigments.

The aqueous fractions from the roots and shoots (Table 1) were pooled and passed through an Amberlite IR-120 (H^+) column. The basic components were eluted with 2*N* ammonium hydroxide (72,246 counts per minute). Samples of the eluent were cochromatographed in five solvent systems [65-percent lutidine; *i*-amyl alcohol saturated with 3*N* hydrochloric acid; *n*-butanol, ammonium hydroxide, and water (8:1:1); *n*-butanol, acetic acid, and water (4:1:5); and 30-percent acetic acid on kerosene-soaked paper] with C^{14}-simazine and C^{14}-hydroxysimazine. The major C^{14}-labeled degradation product appeared to be hydroxysimazine.

Roth [as cited by Gysin and Knüsli (*1*)] found a "phenol-like" substance in corn plants which could degrade simazine in vitro. This substance has an R_F of 0.78 in *n*-butanol, acetic acid, and water (4:1:5). A cyclic hydroxamate and its 2-glucoside were recently isolated from corn seedlings.

Table 1. Fractionation of the radioactivity accumulated by corn seedlings treated with C^{14}-simazine. Liquid samples were applied to 1-inch diameter glass sand-blasted planchets for radioactivity determinations. The density of the dried ethanol-insoluble residues was less than 1 mg/cm². Thin layers of the ground residue (30 mg) were counted in 1-inch diameter aluminum planchets, and the counts were corrected for infinite thinness. Results are presented as total counts per minute for ten seedlings. Fresh weight of ten roots was 11.2 g, and fresh weight of ten shoots was 7.6 g.

Fraction	Radioactivity (count/min)	
	Roots	Shoots
Insoluble in 80% ethanol	8,349	2,867
Soluble in 80% ethanol	75,654	52,901
Chloroform-soluble (total)	17,260	12,640
Nonbasic	553	420
Basic	16,707	12,220
Aqueous fraction	46,860	43,120

The hydroxamate's structure has been established as 2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine (*3, 4*). The aglucone has an R_F similar to Roth's simazine-degrading substance (*3, 4*). Both a crystalline sample of this cyclic hydroxamate (*3*) and the 2-glucoside dechlorinate simazine in vitro. The glucoside was isolated from cold acetone-*n*-butanol extracts of 5-day-old corn roots (*Zea mays* var. Dixie 82).

About 2 μmole of the aglucone and 2 μmole of the glucoside were incubated separately with 0.02 μmole of C^{14}-simazine at 37°C in a total volume of 1 ml. After 4 hours the incubation

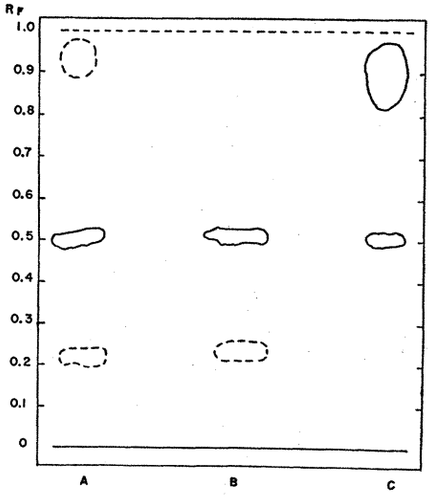

Fig. 1. An autoradiogram made after chromatography of incubation mixtures of (*A*) C^{14}-simazine plus 2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine; (*B*) C^{14}-simazine plus 2-glucosyl-4-hydroxy-3-keto-7-methoxy-1,4-benzoxazine; and (*C*) C^{14}-simazine (upper spot) and C^{14}-hydroxysimazine (lower spot).

mixtures were frozen and lyophyllized. The residues were dissolved in a few drops of ethanol and 0.1*N* hydrochloric acid (1:1) and chromatographed (ascending) on Whatman 3 MM paper with *i*-amyl alcohol saturated with 0.1*N* hydrochloric acid. The autoradiogram (Fig. 1) indicates that most of the C^{14}-simazine is converted to a substance with the R_F of hydroxysimazine by either the aglucone (mixture *A*) or the glucoside (mixture *B*). The results of cochromatography of incubation mixtures in the four other solvent systems mentioned earlier also suggest that the product is hydroxysimazine.

Since hydroxysimazine absorbs maximally at 240 mμ, its formation from simazine by the glucoside and the aglucone was also demonstrated by spectrophotometry. No changes in the ultraviolet spectra attributable to decomposition of the glucoside could be observed when it was incubated with simazine. Hence, a catalytic action for the hydroxamate is suggested.

We have presented evidence of both the in vivo and in vitro conversion of simazine to hydroxysimazine. A cyclic hydroxamate from corn effects this conversion in vitro. This substance may be at least in part responsible for the tolerance of corn to simazine. The reaction can also possibly be mediated by enzymatic or other nonenzymatic systems, or both (*5*, *6*).

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5. An independent observation which relates hydroxamates with the tolerance of certain plants to simazine has been published recently [W. Roth and E. Knüsli, *Experientia* **17**, 312 (1961)].
6. These studies were cooperative investigations of the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture and the North Carolina Agricultural Experiment Station. This report is published with the approval of the North Carolina Experiment Station as paper No. 1325. The sample of C^{14}-simazine was supplied by the Geigy Chemical Corporation, Ardsley, N.Y. We wish to acknowledge the support given these investigations by the National Cotton Council of America and the Geigy Chemical Corporation.

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Identical "Feeding" and "Rewarding" Systems in the Lateral Hypothalamus of Rats

Abstract. Electrodes were implanted in the lateral hypothalamic feeding system; animals were subjected to both feeding and motivational tests. All animals that demonstrated stimulus-bound feeding behavior also showed high self-stimulation rates. As it was impossible to produce the feeding response without simultaneously producing the rewarding effect of hypothalamic stimulation, it was concluded that the feeding system of the lateral hypothalamus is one among a larger group of places where stimulation causes primary rewarding effects. With electrodes in these same areas, food deprivation often caused a major increment in the self-stimulation rate.

The so-called "feeding center" of the lateral hypothalamus and the "satiety center" of the medial hypothalamus are well known (*1*). In neither case are we really dealing with centers, as both feeding and satiety involve systems that traverse the brain (*2*). It is also known that rewarding effects, evidenced by self-stimulation, can be obtained from similar areas of the hypothalamus (*3*). The question remains whether the self-stimulation phenomenon is correlated with the eating or the satiety system. Classical drive-reduction theory in psychology has suggested that reward should be correlated with satiety; thus stimulation at a satiety center might be rewarding (*4*). A less doctrinaire approach might suggest, on the contrary, that feeding activity itself should have a hedonic correlate; thus stimulation at a feeding center might be rewarding.

Approaching the problem more empirically, Morgane has recently found evidence that seems to suggest separation of two different *lateral* hypothalamic areas: one correlated with the *hunger motive* and the other with the *feeding reflex* (*5*). It is not immediately apparent whether stimulation of either of these areas should yield defensive or rewarding reactions.

The present experiment was designed to screen lateral points for eating effects, and then to learn whether points which yield the feeding behavior would also yield positive reinforcement.

Single pairs of electrodes were implanted in the lateral hypothalami of 46 albino rats. The animals were allowed 3 weeks to recover from the operation. In satiated conditions, rats were tested for feeding behavior during bipolar stimulation with an alternating current ranging from 7.5 to 40 μa r.m.s. in steps of 2.5 μa. If a rat began to feed within 2 seconds after the onset of current, continued to feed as long as the current remained on, and ceased feeding as the current was turned off, this was considered to be evidence of stimulus-bound feeding. Each rat was tested for stimulus-bound feeding 10 times a day for 5 days. Animals were considered to be stimulus-bound feeders if they demonstrated the feeding in 40 or more out of the 50 trials. Of the 46 animals prepared, 28 were found to be stimulus-bound feeders.

Each rat was then tested for self-stimulation in a simple Skinner box. Tests were run daily at five different electric current levels ranging from just below to just above threshold for the self-stimulators, but in no case above 40 μa. All rats fell either into a chance category of responding with rates of less than 50 responses in any 8-minute test period, or into the high self-stimulation category with more than 350 responses in the suprathreshold test periods.

Every single animal that demonstrated stimulus-bound feeding behavior also demonstrated self-stimulation. Of the 18 nonfeeding rats, only four stimulated themselves (see Fig. 1).

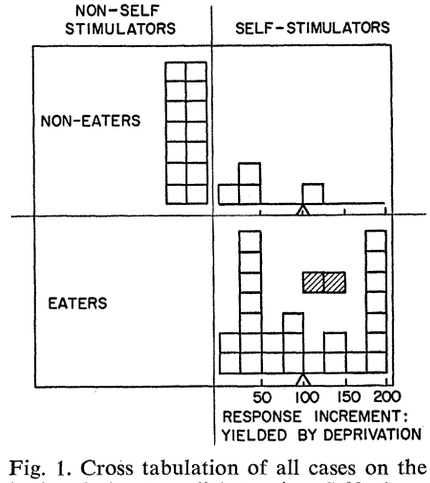

Fig. 1. Cross tabulation of all cases on the basis of the two dichotomies. Self-stimulators are also characterized along an abscissa to indicate the amount of change in self-stimulation output caused by food deprivation. For this latter purpose, animals were run for 8 days; 4 days hungry alternated with 4 satiated. There were five different intervals each day with electric current raised from one to the next. For each animal the mean difference in daily self-stimulation rate was obtained at each current level. For each rat, the highest mean difference was used. Two self-stimulators (see shading) were not tested for drive differences.