erated areas were, however, more common in tissues from the control mice. (iii) In media containing complete hormone supplement, there was a distinct difference in response between tissues derived from the controls and from the hormone-treated donors. Glands from control animals showed no evidence of growth and resembled tissues cultured in media containing incomplete hormone supplement. In contrast, glands from hormone-treated animals displayed extensive lobuloalveolar development in culture, comparable to that seen in late pregnancy (Fig. 1*D*).

To determine whether mammary glands from control animals could show lobular development after longer periods in vitro, tissues of 3-week-old BALB/c mice were cultured in media containing complete hormone supplement for 12 days. Most of these explants contained degenerated parenchyma. A few, however, showed atypical focal hyperplasias (Fig. 1*B*). The addition of 10 percent blood serum from virgin BALB/c mice to the hormone-supplemented media did not alter these results.

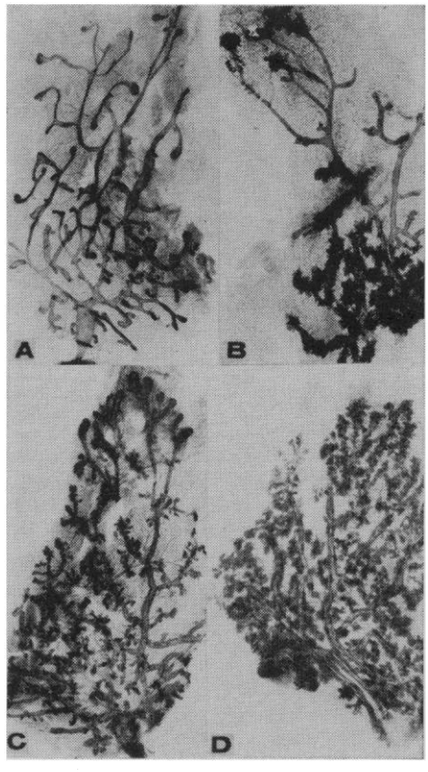

Fig. 1. All photographs show hematoxylin-stained wholemounts at (× 6). *A*, Mammary gland from 4-week-old female mouse. *B*, Mammary gland from 3-week-old control mouse after 12 days of cultivation in insulin, E-17β, progesterone, aldosterone, MH, and STH. The dense areas represent atypical foci of hyperplasia. *C*, Mammary gland from 4-week-old female treated with E-17β, progesterone, MH, and STH for 7 days before the gland was removed. *D*, Mammary gland contralateral to that in *C* after cultivation for 5 days in insulin, E-17β, progesterone, aldosterone, MH, and STH, showing extensive lobuloalveolar development.

Histologic evidence of highly active secretion (distended alveoli, intracellular secretory vacuoles) was seen in tissues from hormone-treated animals cultured for 5 days in complete hormone supplement followed by 5 additional days in secretion-inducing hormones. Tissues from hormone-treated mice cultured for 5 days in complete hormone supplement showed less secretion in the alveoli. This response affords additional evidence that the epithelial structures which grew in mammary cultures from hormone-treated animals behaved like the normal lobules characteristic of pregnancy, inasmuch as non-alveolar parenchyma does not respond to secretion-inducing hormones (*9*).

It appears that the treatment of donor animals with lobuloalveolar mammogens is necessary, under the conditions of culture used in our experiments, for extensive lobuloalveolar differentiation of the mouse mammary gland in defined, hormone-supplemented media. Mammary tissues from control mice did not show similar growth under these conditions. The effect of prior hormone treatment has also been observed in mammary tissues from 4-week-old mice from the strains C3H, C3Hf, and A/Crgl.

Our observations suggest that the hormone treatment in vivo that produced mammary tissues capable of undergoing lobular development in vitro involved the participation of the host in some as yet unknown way, since similar treatment of the glands in vitro was unable to produce tissues capable of growth. Once the tissue changes induced by treatment in vivo are established, however, the conditions for lobular development can be accurately defined.

The conclusion that hormone treatment prior to removal of the glands is necessary for lobular development in vitro is tentative, since appropriate alteration of the culture system might permit such differentiation of untreated tissues entirely in vitro.

It may be possible to use this combined in vivo–in vitro method for the analysis of other similar problems of differentiation. Thus, a lack of understanding of organismal factors initiating morphologic or physiologic changes in tissues need not prevent analyses in vitro of some steps in such transformations. Where the initiation of differentiation can be accomplished in vivo but not in vitro, much might still be learned by "triggering" the mechanism in vivo and studying the subsequent events in vitro.

RUBY R. ICHINOSE
SATYABRATA NANDI
Department of Zoology and
Cancer Research Genetics Laboratory, University of California, Berkeley

References and Notes

1. S. Nandi, *J. Natl. Cancer Inst.* **21**, 1039 (1958).
2. W. R. Lyons, *Proc. Roy. Soc. London Ser. B*, **149**, 303 (1958).
3. J. J. Elias and E. Rivera, *Cancer Res.* **19**, 505 (1959).
4. E. M. Rivera, *J. Endocrinol.*, in press.
5. F. J. A. Prop, *Exptl. Cell Res.* **20**, 256 (1960).
6. E. Y. Lasfargues, *Compt. Rend. Soc. Biol.* **154**, 1720 (1960).
7. J. M. Chen, *Exptl. Cell Res.* **7**, 518 (1954).
8. C. Waymouth, *J. Natl. Cancer Inst.* **22**, 1003 (1959).
9. E. M. Rivera, *Endocrinology* **74**, 853 (1964).
10. Supported by cancer research funds of the University of California and by grants from the National Institutes of Health (CA-05388 and T1 CA-5045). We thank J. J. Elias for his suggestions and C. H. Li for the generous supply of pituitary hormones.

1 May 1964

Amitrole Translocation in Agropyron repens Increased by the Addition of Ammonium Thiocyanate

Abstract. *The mechanism for the enhancement of amitrole activity on quackgrass* (Agropyron repens) *by the addition of ammonium thiocyanate was studied. Absorption and translocation of C^{14}-amitrole after applications to foliage were determined by a direct count of the radioactivity in the wash solution and plant extracts. Regardless of the time of application, the addition of ammonium thiocyanate did not alter the amount of C^{14}-amitrole absorbed. Ammonium thiocyanate (5000 parts per million) greatly increased the amount of carbon-14 translocated. This increase may account for the greater herbicidal effectiveness of the mixture of amitrole and ammonium thiocyanate.*

Amitrole (3-amino-1,2,5-triazole) is an effective herbicide for the control of many species of perennial weeds because it is absorbed by the foliage and translocated throughout the plant (*1*). The herbicidal activity is enhanced by the addition of equimolar amounts of ammonium thiocyanate (NH_4SCN) at a concentration which is nonphytotoxic (*2*). Autoradiographic studies have

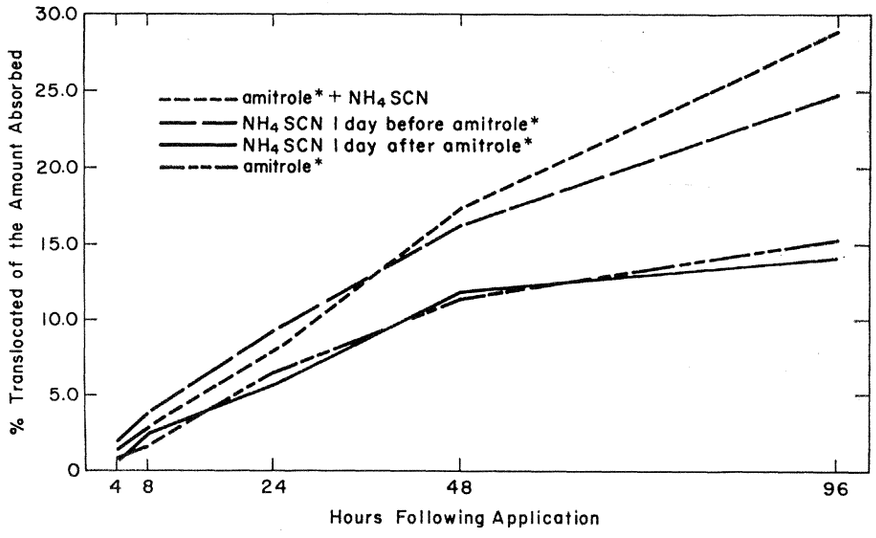

Fig. 1. Translocation of C^{14}-amitrole applied to leaves of quackgrass as influenced by the time of NH_4SCN application. The asterisk indicates the labeled compound. The *F* value for treatments × time is significant at .01 level.

shown that a greater amount of C^{14}-amitrole was distributed throughout the plant when amitrole was combined with NH_4SCN than when amitrole was applied alone (*3*). It was proposed that in the mixture the NH_4SCN lessened the rapid damage to the cells at the absorption site and thereby permitted the foliage to absorb and translocate the amitrole over a longer period of time. Although a greater amount of amitrole was distributed throughout the plant when NH_4SCN was applied concurrently, this increase could not be attributed to either absorption or translocation. Our purpose was to determine whether NH_4SCN was influencing the absorption or translocation of the amitrole in quackgrass.

Actively growing plants were cultured in an aerated full-strength Hoagland's solution. The entire study was conducted at 25°C under a fluorescent light source with an intensity of 10,800 lu/m^2. The plants were grown to the five-leaf stage under a 16-hour light, 8-hour dark cycle. Treatments consisted of seven replications, and trend comparisons were determined by analysis of variance.

A 20-μl droplet of amitrole, labeled with C^{14} in the 5-position of the triazole ring (specific activity, 0.95 mc/mmole) was applied midway between the apex and ligule on the adaxial surface of a recently matured leaf. When labeled amitrole was combined with NH_4SCN, both compounds were applied at a concentration of 5000 parts per million (weight/volume). In the case of split applications, the NH_4SCN was sprayed on the leaf one day prior to, or after, the application of labeled amitrole. Both the labeled amitrole and NH_4SCN solutions were buffered at *p*H 6.2 with a 0.1*M* solution of KH_2PO_4–NaOH.

Amitrole absorption was determined by first washing the treated leaf with 20 ml of 50 percent ethanol and adjusting the volume of the solution recovered to 25 ml. A 1-ml portion of this solution was evaporated on a planchet, and radioassayed with an end-window G-M tube and standard scaler circuit. Preliminary tests showed the recovery of C^{14}-amitrole from the surface of leaves to be greater than 98 percent when the leaves were washed immediately following application.

The amount of C^{14} translocated, based on the total amount of radioactivity in the plant, was determined by first sectioning the plant into three parts: the treated leaf blade, foliage, and roots. These parts were cut in sections (1 cm) and first extracted with 40 ml of hot 70 percent ethanol containing 5 percent formic acid and then with 40 ml of water. The volume of both extracts was adjusted to 50 ml and combined. A 1-ml portion of the combined extracts was evaporated on a planchet and assayed for radioactivity (*4*). With this procedure, 81 to 92 percent of the total radioactivity was recovered.

The NH_4SCN, whether applied before, after, or in combination with C^{14}-amitrole, did not significantly alter the amount of amitrole absorbed. The absorption of amitrole was greatest during the first 4 hours and then decreased between 4 and 96 hours after application. The initial rapid rate of absorption occurred during the time (approximately) required for the treatment droplet to dry. The absorption 96 hours after application (percentage of the amount applied) was as follows: amitrole alone, 19.1; amitrole plus NH_4SCN, 20.6; NH_4SCN application 1 day before amitrole, 22.9; and NH_4SCN 1 day after amitrole, 20.1.

Translocation studies indicated that NH_4SCN, depending on the time of application, altered the amount of C^{14} translocated out of the treated leaf (Fig. 1). When NH_4SCN was applied in combination with labeled amitrole or 1 day before amitrole application, the amount of C^{14} translocated to both the foliage and roots was greater than when labeled amitrole was applied alone or 1 day before the application of NH_4SCN. These differences in the amount of C^{14} translocated became progressively greater with time and were particularly pronounced between 48 and 96 hours after application. The NH_4SCN applied in combination with labeled amitrole or 1 day before approximately doubled the amount of C^{14} translocated 96 hours after application.

These data indicate that NH_4SCN did not alter the amitrole absorption process but markedly increased the translocation of amitrole. Since this response was only produced when NH_4SCN was applied in combination with amitrole or 1 day prior to amitrole application, it appears possible that NH_4SCN is predisposing the amitrole translocation process. Numerous translocation studies (*5*) on amitrole alone have indicated that the bulk of the absorbed amitrole was bound at the application site, and unavailable for transport. Thus, NH_4SCN might lessen this binding phenomena and consequently permit more amitrole to be translocated.

W. F. Donnalley
S. K. Ries
Department of Horticulture, Michigan State University, East Lansing

References and Notes

1. B. J. Rogers, *Weeds* **5**, 5 (1957).
2. Amchem Products, Inc., Technical Data Sheet H-78, April 1959.
3. A. S. Crafts and W. W. Robbins, *Weed Control* (McGraw-Hill, New York, ed. 3, 1962), p. 314.
4. P. Massini, *Acta Botan, Neerl.* **10**, 99 (1961).
5. O. Anderson, *Weeds* **6**, 370 (1958).
6. A. S. Crafts and S. Yamaguchi, *Hilgardia* **27**, 421 (1958).
7. L. L. Jansen, *Plant Physiol.*, in press.
8. Supported by a grant from Amchem Products, Inc., Ambler, Pa. Journal Article No. 3346 from the Michigan Agricultural Experiment Station.

5 June 1964