

cells of algae and amebae (23)? In the model system of Nirenberg and his associates, single strandedness appears to be an essential feature for messenger activity; that is, polyU (polyuridylic acid) is active, but when polyA (polyadenylic acid) is added along with it, the polyU is ineffective (24). From the fact that the base ratio of messenger RNA is supposed to be similar to that of DNA, it would appear that both complementary strands of the DNA are copied and are present. If so, how then does the accepted scheme solve the problem of removing the unwanted strand?

An alternative possibility would be that each single strand of the DNA had a base pair relation of adenine = thymine and guanine = cytosine. In the absence of any reason for such a postulation (such as base-pairing in the case of double strands) this possibility is not attractive at the present time. It should be noted that the situation does not appear to apply for the DNA which is supposed to serve as a template for ribosomal RNA (25). Another discussion of the limitations of the current theory in terms of experimental data derived with HeLa cells has recently appeared (26).

In conclusion, certain points should be clearly stated. This paper is not an attempt to disprove the validity of the messenger concept. The messenger concept is a very powerful tool with which to view new data and plan further experimental approaches. The messenger concept is, at the same time, only a working hypothesis and one which no doubt will have to be altered as more information is acquired. A potentially undesirable situation is developing, where the distinction between working hypothesis and fact is becoming diffuse. The process of protein synthesis with respect to its direction, control, utilization of energy, and integration with the rest of the cell's activities is not likely to be completely understood until the various implicated reactions are viewed in their relation to cellular organization. The problem should be approached in many different ways. Simple schemes representing our current thinking should not be treated as established patterns.

RICHARD W. HENDLER
Laboratory of Biochemistry,
National Heart Institute,
Bethesda 14, Maryland

References

1. P. C. Zamecnik, *Biochem. J.* **85**, 257 (1962); M. Calvin, *Am. Inst. Biol. Sci. Bull.* **13**, 29 (1962); J. D. Watson, *Science* **140**, 17 (1963).
2. F. H. C. Crick, in *Progress in Nucleic Acid Research* (Academic Press, New York, 1963).
3. R. B. Roberts, *Proc. Natl. Acad. Sci. U.S.* **48**, 1245 (1962); R. W. Eck, *Science* **140**, 477 (1963).
4. N. W. Pirie, *Nature* **197**, 568 (1963); J. R. S. Fincham, *Ann. Rev. Biochem.* **38**, 343 (1959).
5. F. H. C. Crick, L. Barnett, S. Brenner, R. J. Watts-Tobin, *Nature* **192**, 1227 (1962).
6. R. W. Hendler, *ibid.* **193**, 821 (1962).
7. S. Brenner, F. Jacob, M. Meselson, *ibid.* **190**, 576 (1961).
8. F. Jacob and J. Monod, *J. Mol. Biol.* **3**, 318 (1961).
9. E. Volkin and L. Astrachan, in *The Chemical Basis of Heredity* (Johns Hopkins Press, Baltimore, 1957), p. 686.
10. L. Astrachan and E. Volkin, *Biochim. Biophys. Acta* **29**, 536 (1958).
11. J. McCarthy, R. J. Britten, R. B. Roberts, *Biophys. J.* **2**, 57 (1962).
12. Y. Kitazume, M. Ycas, W. S. Vincent, *Proc. Natl. Acad. Sci. U.S.* **48**, 265 (1962).
13. R. W. Hendler and J. Tani, in preparation.
14. F. Gros, H. Hiatt, W. Gilbert, C. G. Kurland, R. W. Risebrough, J. D. Watson, *Nature* **190**, 581 (1962).
15. A. Tissieres and J. D. Watson, *Proc. Natl. Acad. Sci. U.S.* **48**, 1061 (1962).
16. S. H. Barondes and M. W. Nirenberg, *Science* **138**, 810 (1962).
17. J. Hurwitz, J. J. Furth, M. Malamy, M. Alexander, *Proc. Natl. Acad. Sci. U.S.* **48**, 1222 (1962).
18. C. Levinthal, A. Keynanaand, A. Higa, *ibid.*, p. 1631.
19. A. Tsugita, H. Fraenkel-Conrat, M. W. Nirenberg, J. H. Matthaei, *ibid.*, p. 816.
20. M. W. Nirenberg, J. H. Matthaei, O. W. Jones, *ibid.*, p. 104.
21. D. Nathans, G. Notani, J. H. Schwartz, N. D. Zinder, *ibid.*, p. 1424.
22. B. R. Chatterjee and R. P. Williams, *Biochem. Biophys. Res. Commun.* **9**, 72 (1962).
23. H. Chantrenne, in *The Biosynthesis of Proteins* (Pergamon, New York, 1962).
24. M. W. Nirenberg and J. H. Matthaei, *Proc. Natl. Acad. Sci. U.S.* **47**, 1588 (1962); M. F. Singer, O. W. Jones, M. W. Nirenberg, *ibid.* **49**, 392 (1963).
25. S. A. Yankofsky and S. Spiegelman, *ibid.* **48**, 1466 (1962).
26. H. Harris, H. W. Fisher, A. Rodgers, T. Spencer, J. W. Watts, *Proc. Roy. Soc. London, Ser. B* **154**, 177 (1963).

6 August 1963

Simazine: Degradation by Soil Microorganisms

Abstract: *A soil fungus, Aspergillus fumigatus Fres., is effective in the degradation of the herbicide 2-chloro-4,6-bis(ethylamino)-s-triazine (simazine). The degradation of both ring- and chain-labeled (C^{14}) simazine was observed in an unamended and an amended (sucrose) basal medium. A loss of C^{14} occurred in all culture solutions containing either ring- or chain-labeled simazine, but the decrease in activity observed was greater with chain-labeled than with ring-labeled simazine. Chromatographic evidence indicates that A. fumigatus may possess a degradation mechanism unlike that which occurs in corn plants.*

The herbicide 2-chloro-4,6-bis(ethylamino)-s-triazine (simazine) is used for selective weed control in crops. Although several investigators (1) have suggested that soil microorganisms are at least partly responsible for the degradation of simazine in soil, it has not been clearly demonstrated that soil microorganisms can utilize simazine as a source of carbon or nitrogen. The nature of the metabolic products from the degradation of simazine by soil microorganisms is unknown, but in some higher plants 2-hydroxy-4,6-bis(ethylamino)-s-triazine (hydroxysimazine) has been detected as a major metabolic product (2, 3).

In the present investigation soil microorganisms that could degrade simazine were isolated by enrichment of a soil solution. Organisms utilizing simazine as a sole or supplemental source of carbon were *Aspergillus flavipes* (Bainier and A. Sartory) Thom and Church, *A. fumigatus* Fres., *A. ustus* (Bainier) Thom and Church, *Fusarium moniliforme* Sheldon, *F. oxysporum*

Schlect., *Penicillium purpurogenum* Stoll, *P. sp.*, *Rhizopus stolonifer* (Ehr. ex Fr.) Vuill, *Stachybotrys sp.*, *Trichoderma viride* (Pers. ex Fr.), three species of *Streptomyces*, and four bacterial isolates believed to belong in the genus *Arthrobacter*. In comparative studies *Aspergillus fumigatus*, an organism not previously reported by other workers, was most effective in the degradation of simazine. The objective of this investigation was to observe, in an amended and an unamended basal medium with pure cultures of *A. fumigatus*, the degradation of both ring- and chain-labeled (C^{14}) simazine.

The unamended basal medium contained 0.2 g of K_2HPO_4 , 0.3 g of NH_4NO_3 , 0.2 g of $CaSO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, and 1 mg of $FeSO_4 \cdot 7H_2O$ in 1000 ml of distilled H_2O . The amended basal medium contained 0.1 g of sucrose as a supplemental carbon source. Sufficient amounts of simazine (4) labeled with C^{14} in the chain or ring (specific activity 4.97 and 5.06 $\mu c/mg$, respectively) were added to these media so that the

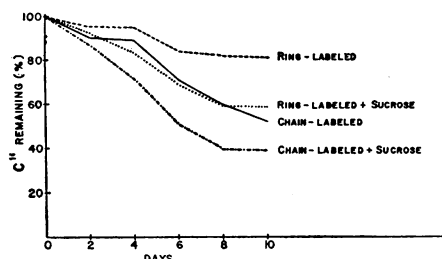


Fig. 1. Degradation of ring- and chain-labeled simazine in liquid cultures of *A. fumigatus*.

final concentration of simazine was 5 parts per million. After sterilization of the media by filtration, 100 ml portions were placed in sterile flasks and inoculated with 1 ml of a spore suspension. Duplicate flasks without the spore suspension served as controls. The cultures were incubated at 25°C on a reciprocating shaker. At 24-hour intervals a 5-ml sample of the culture solution was collected. Three 1-ml portions of each sample were transferred to stainless steel planchets and the residual radioactivity was determined. The radioactivity (counts per minute) of culture solutions obtained in this manner was corrected for background and concentration changes caused by evaporation and expressed as percentage of the value for sterile controls. The remaining 2-ml of the sample was concentrated and chromatographed descendingly on Whatman No. 1 filter paper with butanol, acetic acid, and water (4:1:5). Chromatograms prepared in

this manner were first scanned with a 4-pi strip scanner and then exposed to "no-screen" x-ray film for 2 weeks.

The data revealed significant decreases in the radioactive carbon remaining in culture solutions containing labeled simazine (Fig. 1). The loss of activity in culture solutions containing chain-labeled simazine was greater than that observed in solutions containing ring-labeled simazine. Losses of radioactivity were even greater from culture solutions containing sucrose as a supplemental carbon source. Radioactive chromatograms from both ring- and chain-labeled simazine culture solutions revealed a major shift in peaks of radioactivity, with the occasional formation of a double peak. Simazine (R_F 0.94) was transformed into a compound (R_F 0.87) not previously reported, which was distinguishable from hydroxysimazine (R_F 0.78) (5). A small amount of substance having the same R_F value as hydroxysimazine was also present in both ring- and chain-labeled solutions, and its concentration increased somewhat throughout the incubation period.

However, a smaller amount of this same substance was also present in the sterile control solutions as a chemical impurity. A more complete separation of the new compound (R_F 0.87) from simazine was obtained when the radioactive spots from the original chromatograms were eluted with the mixture of butanol, acetic acid, and water and rechromatographed descendingly on Whatman No. 1 filter paper with isoamyl alcohol saturated with 0.1N HCl. A portion of the eluate was cochromatographed with authentic hydroxysimazine to determine whether the new compound was distinguishable from hydroxysimazine. When these chromatographs were scanned, simazine and two additional compounds having R_F values of 0.81 and 0.22, respectively, were detected (Fig. 2). Known solutions of simazine and hydroxysimazine were also chromatographed in this system. The R_F value of simazine was 0.91 in this solvent system, whereas hydroxysimazine had an R_F value of 0.62.

A degradation scheme was proposed by Gysin and Knüsli (6) who suggested that the transformation of 2-chloro-4,6-bis(ethylamino)-s-triazine to 2-hydroxy-4,6-bis(ethylamino)-s-triazine preceded cleavage of the triazine ring and the subsequent evolution of CO_2 . Recent investigations (3) with C^{14} ring-labeled

simazine clearly demonstrated that hydroxysimazine is an early major degradation product in higher plants and that the conversion of simazine to hydroxysimazine is a nonenzymatic reaction. Corn and certain other plants contain a benzoxazinone derivative which catalyzes the conversion of simazine to hydroxysimazine. Presumably, in corn plants, the hydroxysimazine is further degraded when the carbon in the 2-position of the ring is removed in the form of CO_2 . The results of this experiment indicate that simazine is degraded by *Aspergillus fumigatus*, and that the decrease in activity with chain-labeled simazine is approximately twice that occurring with ring-labeled simazine.

It is not known how much of the decrease in activity can be attributed to physical adsorption of the triazine on the organism, but subsequent studies indicate that metabolism is the dominant factor in this decline, since no visible growth occurs in the basal medium in the absence of a carbon source. Although it is not known how *A. fumigatus* degrades simazine, conceivably the organism could carry out a dechlorination reaction similar to that observed in higher plants. From the results obtained in this experiment, however, it seems probable that this organism possesses a different mechanism for the degradation of simazine. Whether ring cleavage occurs and, if so, whether it precedes dealkylation or deamination of the side chains by this organism is not known at the present time.

DONALD D. KAUFMAN

PHILIP C. KEARNEY

THOMAS J. SHEETS

Crops Research Division,
U.S. Agricultural Research Service,
Beltsville, Maryland

References and Notes

1. O. C. Burnside, E. L. Schmidt, R. Behrens, *Weeds* 9, 477 (1961); J. Guillemat, M. Charpentier, P. Tardieux, J. Pochon, *Ann. Epiphyties* 11, 261 (1960); R. Waefler, *Soil Microflora and Herbicides*, (Geigy, Basel, Switzerland, 1961); J. J. Reid, *Proc. Northeast Weed Control Conf.* 14, 19 (1960); M. T. H. Ragab and J. P. McCollum, *Weeds* 9, 72 (1961).
2. M. Montgomery and V. H. Freed, *Weeds* 9, 231 (1961); H. H. Funderburk, Jr., and D. E. Davis, *ibid.* 11, 101 (1963).
3. R. H. Hamilton and D. E. Moreland, *Science* 135, 373 (1962); W. Roth and E. Knüsli, *Experientia* 17, 312 (1961); P. Castelfranco, C. L. Foy, D. B. Deutsch, *Weeds* 9, 580 (1961).
4. Samples of C^{14} -simazine were supplied by the Geigy Chemical Corporation, Ardsley, N.Y.
5. The values obtained for simazine and hydroxysimazine closely agree with those obtained by other workers.
6. H. Gysin and E. Knüsli, *Advan. Pest Control Res.* 3, 289 (1960).

15 August 1963

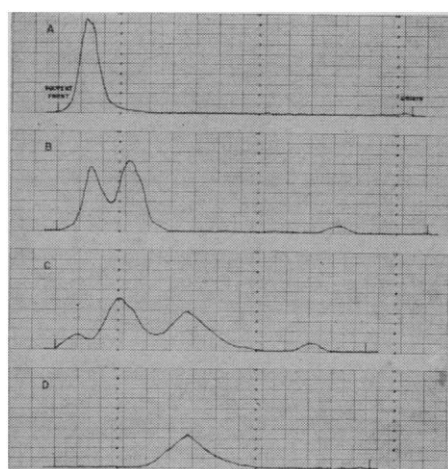


Fig. 2. Chromatographic scans of (A) C^{14} -labeled simazine, (B) C^{14} -labeled simazine and new compounds, (C) C^{14} -labeled simazine and new compounds cochromatographed with C^{14} -labeled hydroxysimazine, and (D) C^{14} -labeled hydroxysimazine, in a mixture of isoamyl alcohol and 0.1N HCl.