Complexes of 3-Amino-1,2,4-triazole in Plant Metabolism

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3-Amino-1,2,4-triazole may exert its effects in several ways. Its metal-chelating properties may contribute to reduction in activity of many enzymes. Failure of treated plants to develop certain proteins appears to occur typically. Glycine utilization and histidine metabolism have been shown to be interfered with. A relatively stable glycine-3-amino-1,2,4-triazole derivative has been demonstrated, and the naturally produced derivative aminotriazolylalanine has been identified and characterized. Interference with normal amino acid metabolism can account for the failure of synthesis of plastid proteins that normally occur in green portions of plants. Without the enzymatic and structural protein framework associated with chloroplasts, pigment synthesis cannot occur. Although a glucose-3-amino-1,2,4-triazole adduct has been reported, its importance as a limiting factor in metabolism is questionable. A strong possibility exists that a common precursor of nucleic acid and protein synthesis is interfered with by 3-amino-1,2,4-triazole.

During the mid-1950's, 3-amino-1,2,4-triazole was introduced as a selective herbicide (*1*). One of its earliest uses was as a defoliating and regrowth-inhibiting agent (*12*) in the mechanical harvesting of cotton. The compound increased the effectiveness of many commercial defoliants while adding to the property of regrowth inhibition.

3-Amino-1,2,4-triazole has been used successfully in the control of several woody species. Poison ivy is quite sensitive. Foliage sprays of 3-amino-1,2,4-triazole at the rate of 9 pounds per acre produce an 89% kill of several species of oaks and ashes. Honeysuckle is also easily controlled by applications of 3-amino-1,2,4-triazole. Sassafras and red maple are not very sensitive. Virginia creeper and many ericaceous species are unaffected.

The most characteristic symptom observed following 3-amino-1,2,4-triazole treatments is an almost pure white growth. Neither chlorophyll nor carotenoids are produced in appreciable quantities. Leaf primordia that have already differentiated at the time of treatment may expand almost to full size. In the process of being formed, however, they may be prevented from fully expanding, but will still retain their form. This is in sharp contrast to the drastic formative changes often observed with such herbicides as 2,4-D.

Since field trials demonstrated that 3-amino-1,2,4-triazole is a useful phytocide, basic studies have been conducted to learn more about the entrance, distribution, and transformation of 3-amino-1,2,4-triazole in plants to arrive at some understanding of how the chemical functions as a phytocide. An important recent review of this work is that by Hilton *et al.* (*17*).

Theory and Findings

Tracer studies using 3-amino-1,2,4-triazole and autoradiography indicate that this substance is absorbed through the leaves and roots by members of many plant genera. Translocation both upward and downward may occur quite rapidly. Andersen (*2*) found that radioactivity from 3-amino-1,2,4-triazole-5-C^{14} was distributed throughout nut grass plants $2\frac{1}{2}$ hours after application on a single leaf. The rate of movement of 3-amino-1,2,4-triazole has been said to far exceed that of radioactive 2,4-D. In less than a day after application to leaves, radioactivity is detectable throughout bean, tomato, four o'clock, and maize (*21*). Root growth of *Populus tremula* is noticeably affected within 24 hours after application of 3-amino-1,2,4-triazole to mature leaves (*9*).

Since 1957, it has been known that radioactive 3-amino-1,2,4-triazole is rapidly metabolized (*31*). Racusen (*29*) found in the bean that only 7% of the total radioactivity was in 3-amino-1,2,4-triazole at the end of his sampling period. The only radioactive 3-amino-1,2,4-triazole found was in the treated leaves and in the roots; though large amounts of radioactivity were present in the stem and bud, none of it was in 3-amino-1,2,4-triazole.

The fact that metabolic transformation of 3-amino-1,2,4-triazole is quite rapid and extensive causes considerable difficulty when trying to account for the observed symptoms. The possibility exists that metabolic transformation products play important roles in the phytocidal action of the chemical. But it might equally well be that 3-amino-1,2,4-triazole reacts with certain plant constituents to form toxic compounds that persist and produce the symptoms associated with 3-amino-1,2,4-triazole treatment.

Attempts to elucidate the toxic action of 3-amino-1,2,4-triazole have centered upon respiration studies including glucose adduct formation, metal-binding properties of the compound, porphyrin metabolism, and the metabolism of compounds containing an imidazolyl group.

Respiration Studies. Several investigators have measured respiration of 3-amino-1,2,4-triazole-treated tissues (*12, 20, 25*). 3-Amino-1,2,4-triazole and its salts generally cause an initial stimulation of respiration of *Avena* sections and leaf disks. But, after about 26 hours, the O_2 uptake of treated leaf disks is approximately the same as the controls. Thus far, respiration studies per se have revealed very little that is of positive interpretive value.

Glucose metabolism may, however, be affected. A glucose adduct with aminotriazole has been described by Rogers (*30*) and by Frederick and Gentile (*11*). Infrared absorption spectral analyses indicate that the D-glucose adduct of 3-amino-1,2,4-triazole is an amine glucoside (Figure 1). As such, it could participate in phosphorylating reactions and offer some resistance to phosphorylation by hexokinase. If this were its only

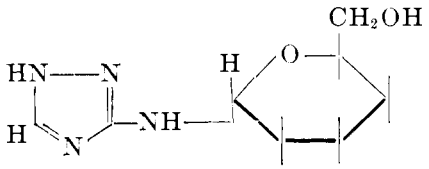

Figure 1. Glucose adduct of 3 amino-1,2,4-triazole as deduced by Frederick and Gentile (10)

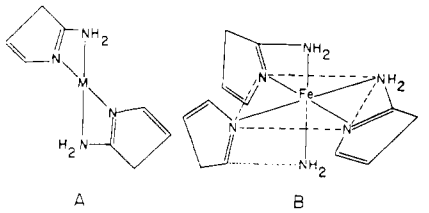

Figure 2. Trivalent (ferric) chelate of amino triazole (10)

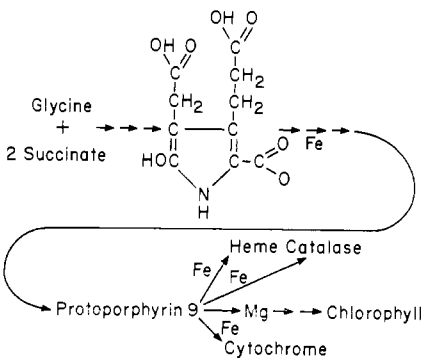

Figure 3. Hypothetical scheme showing the route of synthesis of some of the best known porphyrins

effect on metabolism, however, it would be difficult to account for the typical symptoms.

In test-tube experiments with phosphorylase from *Oscillitoria princeps*, Frederick and Gentile (*10*) have been able to demonstrate that 3-amino-1,2,4-triazole acts as an inhibitor. But very strong 3-amino-1,2,4-triazole solutions are required to block all activity.

Metal Binding. 3-Amino-1,2,4 triazole could exert some of its effects through its ability to form salts upon reaction with many acids and bases. It also reacts with aldehydes and ketones through the amino group and forms stable complexes with several metals such as iron, copper, nickel, and magnesium. On the other hand, 3-amino-1,2,4-triazole treatment is known to result in an accumulation of manganese (*20*).

A ferric chelate can be readily formed, and the author has confirmed Frederick and Gentile's statement that a 3:1 ferric chelate with 3-amino-1,2,4-triazole is produced (Figure 2). Other metallic chelates are also produced (*8*). In none of the author's extensive experiments with local application to leaves, buds, and roots have iron compounds been able to prevent the development of poisoning symptoms following the application of standard amounts of 3-amino-1,2,4-triazole. Some slight relief has been obtained in a few instances. But the 3-amino-1,2,4-triazole-Fe complex itself is effective as a chlorosis-inducing agent. In experiments with corn, McWhorter (*20*) could find no difference in the amount of iron in the leaves of control and 3-amino-1,2,4-triazole-Fe–treated plants. Some of the iron he measured, of course, may have been in complexes.

Porphyrin Metabolism. As pointed out earlier, lack of chlorophyll in new growth follows application of 3-amino-1,2,4-triazole. One could theorize that lack of chlorophyll leads to death. But it must be remembered that it is only the tissue that develops after treatment that shows chlorosis. Is photosynthesis in the old tissue affected? In several experiments with radioactive carbon dioxide, neither the amounts of C^{14} fixed nor the alcohol extractable products receiving label appeared to be altered by the introduction of 3-amino-1,2,4-triazole into the tissue.

Since several steps in the synthesis of chlorophyll are known, it has been relatively easy to learn if 3-amino-1,2,4-triazole blocks any one of them. The scheme shown in Figure 3 has, accordingly, been tested at several points to obtain critical data.

In experiments designed to determine if 3-amino-1,2,4-triazole interferes with the conversion of protochlorophyll to chlorophyll, a technique developed by Smith *et al.* (*32*) was employed. Leaves of dark-grown plants were ground in the dark, and the holochrome was obtained by centrifuging at 105,000 × G. Conversion of protochlorophyll to chlorophyll was observed by means of the Beckman DU spectrophotometer. It occurred equally readily in the presence and absence of 3-amino-1,2,4-triazole.

In a parallel experiment, dark-grown bean plants were subjected to 3-amino-1,2,4-triazole just before bringing them into the light. Treated plants developed some chlorophyll, but soon stopped, whereas the controls produced chlorophyll steadily for 48 hours. These two experiments were interpreted to mean that 3-amino-1,2,4-triazole would not prevent the formation of chlorophyll from preformed protochlorophyll but would stop synthesis of new protochlorophyll. In a further experiment, some light-grown plants were treated with 3-amino-1,2,4-triazole and allowed to remain in the dark until several new leaves had developed. Upon being placed in the light, the leaves turned green. Similar results have been obtained in a repetition of the experiment. Apparently there is an interaction of light and 3-amino-1,2,4-triazole that results in failure to develop new protochlorophyll. Bogorad (*3, 4*) found, additionally, no inhibition of porphyrin synthesis by 3-amino-1,2,4-triazole.

Even though chlorophyll was formed, one might question whether the chloroplast was functional in photosynthesis. An experiment was accordingly designed to give some evidence on this point. At intervals of 0, 1, 4, 8, 15, and 20 hours during the greening process, untreated and 3-amino-1,2,4-triazole–treated etiolated bean plants were supplied with $C^{14}O_2$ for 30 minutes. Paper chromatographic analysis of the $C^{14}O_2$ fixation products showed that photosynthesis began soonest (after 4 hours of illumination) in the untreated leaves, and, for the duration of the experiment, fixed the largest quantity of label into sucrose. An excess of what was tentatively identified as C^{14}-glycolic acid occurred in the 3-amino-1,2,4-triazole–treated plants. Two additional compounds, not found in the controls, became well labeled and are discussed later.

If 3-amino-1,2,4-triazole exerts its effect prior to the production of protoporphyrin 9 (Figure 3), suppression of synthesis of porphyrins other than chlorophyll might occur. Thus heme, catalase, cytochrome, peroxidase, and chlorophyll should all be affected.

Experiments on heme synthesis were carried out with duck blood. Ducks were used because of their availability and the fact that bird erythrocytes retain their nuclei. In these experiments, heme synthesis was actually stimulated by 3-amino-1,2,4-triazole. As yet, these experiments must be regarded as tentative though they have proved to be repeatable. Reports of no similar experiments have come to the author's attention.

Numerous experiments on catalase activity of 3-amino-1,2,4-triazole-treated plants have been carried out. Following application of 3-amino-1,2,4-triazole, catalase activity is sharply reduced in the newly formed leaves. The same is true for cytochrome c. Several reports are now in the literature (*13, 21, 26, 27, 36*) that show inhibition of catalase activity by 3-amino-1,2,4-triazole. Most of these are based on in vitro experiments. Possibly the effect is achieved through chelation of 3-amino-1,2,4-triazole with the iron component of the enzyme. Experiments of Nicholls (*26*), as well as Margoliash and Schejter (*21*), have shown that an irreversible complex is formed. Indeed, this fact has been made use of by Price *et al.* (*27*) in studies on catalase synthesis.

In the production of the pyrrole nucleus, glycine and succinic acid are of key importance. Considerable significance may, therefore, be attached to the discovery that chromatograms detecting free amino acids in 3-amino-1,2,4-triazole–treated plants have much

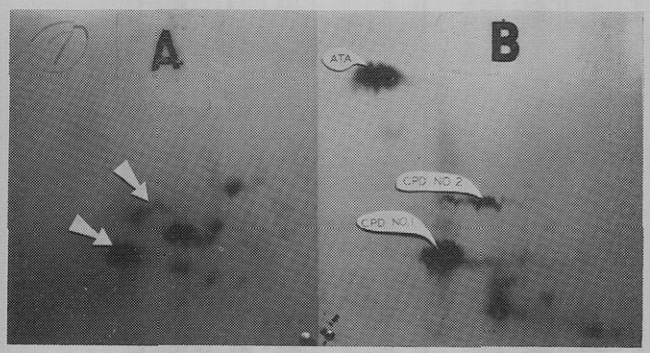

Figure 4. Autoradiograms of chromatographed extracts from plants treated with (*A*) amino triazole and randomly C^{14}-labeled glycine, and (*B*) aminotriazole-5-C^{14} alone

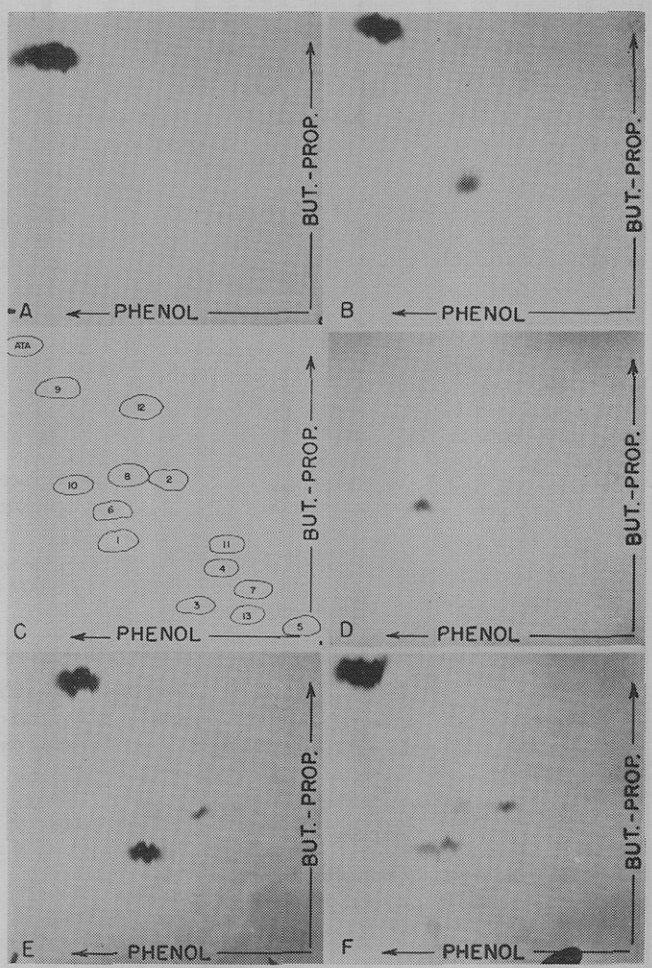

Figure 5. Autoradiograms of two-dimensional chromatograms showing (*A*) radiopurity of stock solution of ATA-5-C^{14}; (*B*) radioactive compounds in extract of bean stem 8 hours after application of ATA-5-C^{14} to one primary leaf; (*C*) chromatographic map with approximate location of various unidentified compounds formed from ATA-5-C^{14} by plants; (*D*) radioactive compounds in extract of stem tips of bean plants 24 hours after application of ATA-5-C^{14} to one primary leaf; (*E*) radioactive compounds in alfalfa stems after exposure to ATA-5-C^{14} for 24 hours in light; (*F*) radioactive compounds in silver maple stems after exposure to ATA-5-C^{14} for 24 hours in light (*5*)

smaller amounts of glycine on them than untreated controls (*5–7*). At the same time glycine disappeared, a new and very distinctive ninhydrin-sensitive spot appeared just below glutamine on chromatograms developed in one direction with phenol-water and the other with butanol–propionic acid–water. The new compound produces a turquoise blue color in the presence of a ninhydrin reagent containing collidine and lutidine and has not been reported to be produced under any other circumstances.

When randomly labeled glycine was supplied to 3-amino-1,2,4-triazole–treated bean tissue, a major portion of the label was in the new compound (Figure 4*A*). Glycine-1-C^{14}, glycine-2-C^{14}, and randomly labeled serine have been used in attempts to find whether the whole glycine molecule or a derivative of it is involved. When 3-amino-1,2,4-triazole and each of the labeled amino acids were added simultaneously to normal bean tips, radioactivity from serine and both carbon atoms of glycine appeared in compounds 1 and 2 (Figure 4*B*). As the 3-amino-1,2,4-triazole concentration increased, the label in both compounds increased while that in malic acid, citric acid, glyceric acid, sucrose, fructose, and alantoin decreased.

Feeding experiments with radioactive 3-amino-1,2,4-triazole have yielded valuable supplementary information. A major portion of the 3-amino-1,2,4-triazole-5-C^{14} label went into compounds 1 and 2. But label from 3-amino-1,2,4 triazole-5-C^{14} went into 12 other compounds as well (Figure 5*C*). Compounds 1, 2, and 8 were the most highly labeled.

Thus, bean plants have the ability to form a complex between 3-amino-1,2,4-triazole and either glycine or serine or a common derivative. Evidently, presence of 3-amino-1,2,4-triazole puts a considerable drain on the glycine and serine pools since nearly half of the total glycine and serine transformed in the presence of those quantities of 0.1*M* 3-amino-1,2,4-triazole taken up by the transpiration stream during the 24 hours following application was in 3-amino-1,2,4-triazole complexes.

Comparisons of the ability of normal and 3-amino-1,2,4-triazole–induced chlorotic leaves to use radioactive glycine, succinate, and glucose were undertaken as a final test of the hypothesis that 3-amino-1,2,4-triazole affects pyrrole synthesis. Some differences were noted, but, on the whole, they were not striking. Only a small amount of label appeared in compound 1. Much of compound 1 was there as was shown by chromatography, but very little label went into it. In the glucose and succinate feedings, the organic acids, particularly malic and citric acids, tended to accumulate label in the chlorotic leaves. Glucose-U-C^{14} supplied some label to both glycine and serine in feeding tests with normal bean tips, but none in the chlorotic leaves. Evidently the compound 1 present was formed before the start of the experiment, and no active synthesis occurred during the time that glycine-U-C^{14} was present in the tissues. Apparently either the tips from the treated plants had lost the ability to make compound 1 or there was little or no 3-amino-1,2,4-triazole present with which to form a complex.

To test the first of the two suggested possibilities, two treated tips were supplied with 3-amino-1,2,4-triazole-5-C^{14} for 4 hours in the light. Tips from treated plants readily produce compound 1 from radioactive 3-amino-1,2,4-triazole. To determine if tips from 3-amino-1,2,4-triazole–treated beans contained unaltered herbicide, several such tips were extracted and chromatographed. No 3-amino-1,2,4-triazole was detected.

Therefore, it may be concluded that treated tips did not transfer label from glycine-U-C^{14} into compound 1 because no 3-amino-1,2,4-triazole was present to form the complex. This conclusion is supported by the results of a translocation study which indicated that unaltered

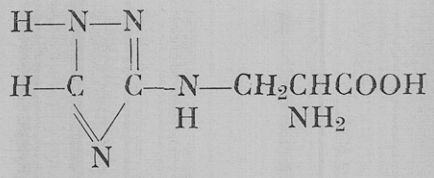

Figure 6. The presumed structure of Massini's (22) compound ATX (3-amino-1,2,4-triazolyl alanine)

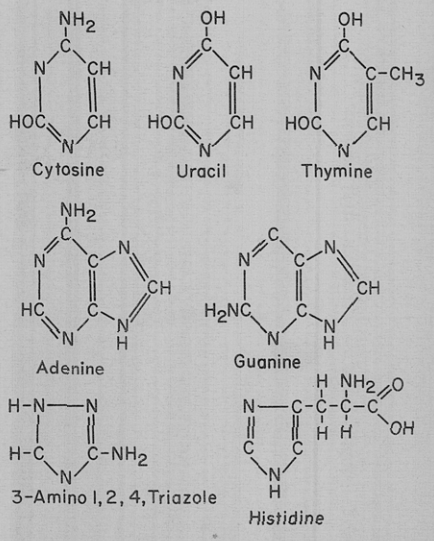

Figure 7. Biologically important compounds containing imidazolyl groups

3-amino-1,2,4-triazole does not reach bean tips following application to a primary leaf.

Since it appeared that chlorotic tips from 3 - amino - 1,2,4 - triazole-treated plants did not contain 3-amino-1,2,4-triazole, tips from 14-day-old, untreated bean plants were supplied with glucose-U-C^{14}, succinate-2,3,-C^{14}, and 0.01*M* 3-amino-1,2,4-triazole simultaneously to see if 3-amino-1,2,4-triazole did affect their use. Succinate utilization was practically the same in the presence of the phytocide as in its absence. But the bean tips receiving 3-amino-1,2,4-triazole and labeled glucose had more activity in phosphorylated sugars and fructose and less in sucrose than did the tips receiving radioactive glucose alone. Perhaps 3-amino-1,2,4-triazole inhibits the incorporation of glucose and fructose into sucrose. No radioactive compound 1 or other 3-amino-1,2,4-triazole complex was detected in these tips, indicating that the metabolic intermediates which complex with 3-amino-1,2,4-triazole are not abundantly labeled from glucose-U-C^{14} and succinate-2,3-C^{14}.

The labeling experiments indicate strongly that 3-amino-1,2,4-triazole does not exist long as an intact molecule in a highly susceptible plant like the bean.

Attempts are being made to characterize the presumed 3-amino-1,2,4-triazole-glycine derivative but much remains to be done. Compound 1 has been shown

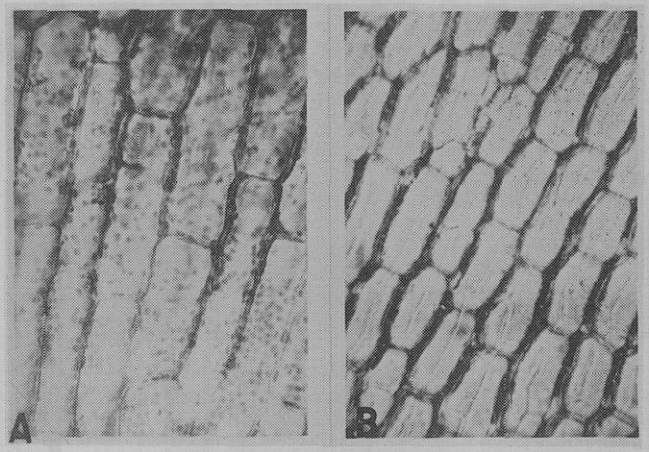

Figure 8. The influence of amino triazole on cell size and plastid formation in *Elodea canadensis* Michx

(A) Untreated control; (B), typical cells from new, colorless leaves produced after transferring the plants to a dilute nutrient solution containing 0.1 mg. of ATA per 400 ml.

Table I. Reaction of Compound 1 and 3-Amino-1,2,4-triazole with Various Indicator Sprays

Color Reagent	Compound 1	3-Amino-1,2,4-triazole
Ninhydrin	Blue green	No visible reaction
Ehrlich's (*p*-dimethyl amino-benzaldehyde)	Yellow	Faint yellow
p-Anisidine	No visible reaction	No visible reaction
Phenol-HCL	Yellow	Yellow
"H-acid" of Rucusen (8-amino-1-naphthol-3,6-disulfonic acid)	Red	Red
Nitroprusside-ferrocyanide	Green	Green

to contain not only the 5-carbon atom of 3-amino-1,2,4-triazole but also carbon atoms from glycine or serine (*7*). Massini (*22*, *23*) analyzed a 3-amino-1,2,4-triazole complex (ATX) and described it as 3-amino-1,2,4-triazolyalanine (Figure 6). There is some doubt about his compound being the same as the author's, however, because the color developed upon spraying with nitroprusside-ferrocyanide is not the same; in addition, the paper electrophoretic behavior pattern is not the same (*22*).

Compound 1 is not volatile. Numerous paper electrophoretograms have been made and run over a pH range from 3.0 to 9.0. Its mobility is greatest in the anionic form. Such data indicate that the derivative is a "zwitterion." On chromatographic columns, it is held by Dowex 50 and can be readily eluted with 1.0*N* NH_4OH. The reaction of compound 1 and 3-amino-1,2,4-triazole with various indicator sprays have been found to be as shown in Table I. Compound 1 is not hydrolyzed by 1.0*N* HCl or 1.0*N* NH_4OH after an hour at 90° C. Even after 24 hours' hydrolysis with 6.0*N* HCl in the autoclave, compound 1 still has up to 76% of its original radioactivity. It is also unaltered after 24 hours in a bean tip. Apparently, it is not readily metabolized (*6*, *14*).

Metabolism of Compounds Containing an Imidazolyl Group. In another approach to the problem of 3-amino-1,3,4-triazole toxicity, the antimetabolite concept was exploited. Complexing could be involved, but the evidence is not as good as the instances cited above.

Examination of the structural formulas in Figure 7 shows that each substance has an imidazolyl group. This fact may be important in explaining 3-amino-1,2,4-triazole toxicity. The biochemical origin of the imidazolyl configuration is still not known though glycine probably contributes to it. There can be no doubt, however, about the importance of the purines, pyrimidines, and histidine in plant metabolism. Accordingly, the effect of 3-amino-1,2,4-triazole on the formation and utilization of each of the compounds deserves systematic investigation.

Possible complexing of precursors in histidine biosynthesis has received attention because of the presence of this amino acid in numerous enzymatic proteins—including catalase (7%). The suggestion has been made (*28*, *33*) that 3-amino-1,2,4-triazole may affect certain enzyme systems involving rupture of N to N and N to C linkages. Furthermore, many proteins do not contain histidide. Thus, it would be possible for a certain amount of growth to take place with only a minimum amount of histidine synthesis. Specific protein deficiencies could show up later.

In experiments with *Euglena*, typical 3-amino-1,2,4-triazole-bleaching symptoms have been retarded with histidine

and one of the precursors of histidine—histidinol. Histidine applications, however, have not, as yet, prevented 3-amino-1,2,4-triazole poisoning in beans. Hilton (*16*) has been able to largely reverse 3-amino-1,2,4-triazole-induced inhibition of growth of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* with histidine. The inhibition could not be reversed to the same extent with D-histidine, L-histidinol, urocanic acid, other L-amino acids, purines, or pyrimidines. Hilton's attempts to demonstrate L-histidine protection against 3-amino-1,2,4-triazole toxicity with seedlings of higher plants yielded negative results.

Sund *et al.* (*35*) found that the effects of 3-amino-1,2,4-triazole on the growth in size of tomato plants can be significantly reduced by the simultaneous application of equimolar amounts of adenine, guanine, hypoxanthine, adenosine, guanosine, or inosine. These compounds had little influence, however, on the inhibition of chlorophyll formation. Weyter and Broquist (*37*) found that inhibition of growth of *Escherichia coli* induced by aminotriazole could be almost completely reversed by adenine. Histidine alone was ineffective, but, in combination with adenine, brought about a reversal that was more complete than with adenine alone. 3-Amino-1,2,4-triazole-inhibited *Torula cremoris* was returned to almost its normal growth rate with the addition of histidine but not by adenine. Wolf (*38*) was able to obtain complete reversal of 3-amino-1,2,4-triazole inhibition of growth of *Chlorella pyrenoidosa* with equivalent weights of adenine, guanine, hypoxanthine, and xanthine.

Measurements are yet to be made on any of these organisms to determine if there is an actual reduction in synthesis of any of their imidazolyl compounds. Preliminary experiments with *Euglena* indicate that synthesis of adenosine monophosphate and cytosine monophosphate are unaffected by quantities of 3-amino-1,2,4-triazole that markedly reduce growth rates. This point is being investigated intensively.

If purine, pyrimidine, and amino acid metabolism are affected by 3-amino-1,2,4-triazole, one can readily account for the failure of new growth of treated plants to become green. Chloroplasts are known to be self-replicating bodies that contain large quantities of protein. Chlorophyll is formed *in situ* and is deposited in monomolecular films on protein layers contained in the plastids. Thus, 3-amino-1,2,4-triazole-treated plants would fail to produce chloroplasts of normal size or perhaps none at all. In experiments carried out in the author's laboratory with Elodea plants transferred to a very weak solution of 3-amino-1,2,4-triazole, the new leaves did indeed lack chloroplasts (Figure 8). *Spirodela* (duckweed) and moss protonemata, however, continued to produce plastids for some time though the plastids were few in number and very reduced in size. Rogers (*31*) and Linser and Kiermayer (*19*) reported similar findings.

One of the few studies of the effects of 3-amino-1,2,4-triazole on cell elongation was done by Jackson (*18*). When 3-amino-1,2,4-triazole and histidine were supplied individually, both 3-amino-1,2,4-triazole and histidine were toxic. But when they were present together they tended to neutralize one another. Jackson's results may be interpreted in at least two ways. Perhaps histidine antagonizes the entrance of 3-amino-1,2,4-triazole into root hairs; then again, 3-amino-1,2,4-triazole may block one or more steps in L-histidine synthesis.

Sund *et al.* (*31*, *35*) and Hilton (*15*) reported that partial relief of 3-amino-1,2,4-triazole-induced chlorosis was obtained in seed plants with riboflavin. Although members of the author's laboratory have tried several times, with a variety of plant materials, they have not been able to duplicate these authors' results. As yet, no one has been able to obtain relief from 3-amino-1,2,4-triazole inhibition in microorganisms with riboflavin additions to the growing medium. In those cases where there has apparently been some reduction in toxicity, some chemical inactivation of 3-amino-1,2,4-triazole or possibly promotion of complex formation may have occurred.

Acknowledgment

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Literature Cited

(1) Alverson, R. A., Ed. "Annotated bibliography of amino triazole," Farm and Home Division, American Cyanamid Co., N. Y., 1957.
(2) Andersen, O., Ph.D. dissertation, University of California, 1957.
(3) Bogorad, L., *J. Biol. Chem.* **233,** 501 (1958).
(4) *Ibid.*, p. 510.
(5) Carter, M. C., Naylor, A. W., *Botan. Gaz.* **122,** 138 (1960).
(6) Carter, M. C., Naylor, A. W., *Physiol. Plantarum* **14,** 20 (1961).
(7) *Ibid.*, p. 62.
(8) Castelfranco, P. A., *Biochim. Biophys. Acta.* **41,** 485 (1960).
(9) Eliasson, L., *Physiol. Plantarum* **15,** 229 (1962).
(10) Frederick, J. F., Gentile, A. C., *Arch. Biochem. Biophys.* **86,** 30 (1960).
(11) *Ibid.*, **92,** 356 (1961).
(12) Hall, W. C., Johnson, S. P., Leinweber, C. L., *Texas Agr. Expt. Sta. Bull.* **789** (1954).
(13) Heim, W. G., Appleman, D., Pyfrom, H. T., *Science* **122,** 693 (1955).
(14) Herrett, R. A., Linck, A. J., *Physiol. Plantarum* **14,** 767 (1961).
(15) Hilton, J. L., *Plant Physiol.* **37,** 238 (1962).
(16) Hilton, J. L., *Weeds* **8,** 392 (1960).
(17) Hilton, J. L., Jansen, L. L., Hull, H. M., *Ann. Rev. Plant Physiol.* **14,** 353 (1963).
(18) Jackson, W. T., *Weeds* **9,** 437 (1961).
(19) Linser, H., Kiermayer, O., *Planta* **49,** 498 (1957).
(20) McWhorter, C. G., *Physiol. Plantarum* **16,** 31 (1963).
(21) Margoliash, E., Schejter, A., *J. Biol. Chem.* **237,** 2359 (1962).
(22) Massini, P., *Acta Botan. Neerl.* **12,** 64 (1963).
(23) Massini, P., *Biochim. Biophys. Acta* **36,** 548 (1959).
(24) Massini, P., "Second U.N. International Conference on the Peaceful Uses of Atomic Energy," Geneva, 1958, Pergamon Press, London, pp. 58–62, 1959.
(25) Miller, C. S., Hall, W. C., *Weeds* **5,** 218 (1956).
(26) Nicholls, P., *Biochem. Biophys. Acta* **59,** 414 (1962).
(27) Price, V. E., Sterling, W. R., Taratola, V. A., Hartley, R. W., Jr., Recheigl, M., Jr., *J. Biol. Chem.* **237,** 3468 (1962).
(28) Rabinowitz, J. C., Pricer, W. W., *Ibid.*, **222,** 537 (1956).
(29) Racusen, D., *Arch. Biochem. Biophys.* **74,** 106 (1958).
(30) Rogers, B. J., "The Hormolog," Amchem Products, Inc., Ambler, Pa., February, 1957.
(31) Rogers, B. J., *Weeds* **5,** 5 (1957).
(32) Smith, J. H. C., Kupke, D. W., Loeffler, J. E., Benitez, A., Ahrne, I., Giese, A. T., in "Research in Photosynthesis," pp. 464–74, Interscience, N. Y., 1957.
(33) Sund, K. A., *Physiol. Plantarum* **14,** 260 (1961).
(34) Sund, K. A., Little, H. N., *Science* **132,** 622 (1960).
(35) Sund, K. A., Paula, E. C., Little, H. N., J. AGR. FOOD CHEM. **8,** 210 (1960).
(36) Tephly, T. R., Mannering, G. J., Parke, R. E., Jr., *J. Pharmacol. Exptl. Therap.* **134,** 77 (1961).
(37) Weyter, F. W., Broquist, H. P., *Biochim. Biophys. Acta* **40,** 567 (1960).
(38) Wolf, F. T., *Nature* **193,** 901 (1962).

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