

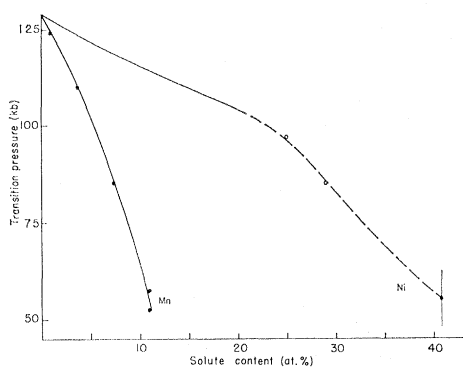


Fig. 1. Transition pressures for the Fe-Mn and Fe-Ni alloy systems. The dashed line indicates the range of the new Fe-Ni data; vertical bar shows an estimated error for the lowest Fe-Ni pressure.

Transition pressures for the two alloy systems appear in Fig. 1; the data for the solid portion of the Fe-Ni curve have been reported (8), and the dashed portion is a hand-fit to the new experimental points shown. The behavior of the Fe-Mn system is more dramatic, as the manganese solute is less dilatory than the nickel in lowering the transition pressure. Confidence in this result has been reinforced by the results of an associated study of the Fe-Mn system by the interaction-zone technique. This technique (8) is less precise than pin measurements, but, with constant initial pressure, the interaction-zone thickness (and thus the transition pressure) decreases with roughly the same dependence on solute content as Fig. 1 shows.

The solute concentrations were limited to the indicated ranges by the need for keeping the alloys entirely

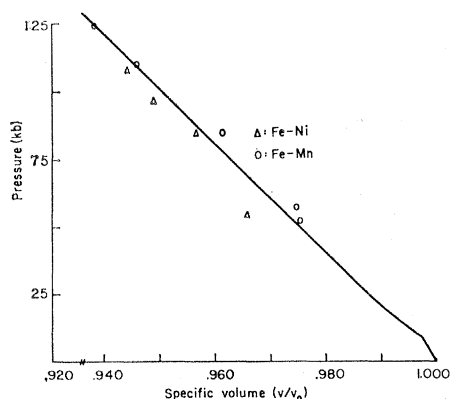


Fig. 2. Pressure-volume states for the onset of the polymorphic transition for the Fe-Mn system, and for Fe-Ni alloys containing more than 20 percent Ni by weight. The Hugoniot of pure iron (solid line) is included for comparison.

in the *bcc* phase. It is interesting that the transition pressures at the limiting compositions for both these systems are about 55 kb.

With the normal explosive lens systems available, it is difficult to obtain shock waves with acceptable planarity at pressures less than about 150 kb (in iron, or in materials with shock impedances close to that of iron); thus the initial pressure in all these experiments was some 150 kb. With such a relatively high input pressure, the effects of the plastic-I wave on the free surface can be seen only briefly before the arrival of the plastic-II wave at the surface. Few pins are contacted in that short time, and the relevant velocity is poorly determined. An estimate of the transition-pressure error caused by the uncertainty in free-surface velocity is shown in Fig. 1 for the lowest Fe-Ni transition pressure; data above 100 kb are attended by more-normal errors of 2 to 3 percent.

A pressure-volume plot of the transition points appears in Fig. 2. The solid line is the Hugoniot (the locus of dynamic *P-V* states) for pure iron up to the transition. All the Fe-Mn data are shown, but for clarity only data for Fe-Ni alloys containing more than 20 percent Ni by weight are included in the plot. The data suggest that, as the amount of solute is increased, the Fe-Ni alloys become more compressible and the Fe-Mn alloys become slightly less compressible than iron. For both alloy systems these trends must reverse at some point because the Hugoniot of nickel is known to lie somewhat above the iron curve (nickel is less compressible) (9), and static-pressure results indicate that manganese is considerably more compressible than iron (10). These facts are not surprising, as neither element has a *bcc* structure.

This report is the last on our pin-technique investigations of polymorphism in binary iron alloys. When previous results (3) are combined with these data, it is clear that by selecting the proper binary iron alloy one may obtain transition pressures in a continuous range from 55 to about 600 kb.

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References and Notes

1. D. Bancroft, E. L. Peterson, F. S. Minshall, *J. Appl. Phys.* **27**, 291 (1956).
2. R. L. Clendenen and H. G. Drickamer, *J. Phys. Chem. Solids* **25**, 865 (1964); T. Takahashi and W. A. Bassett, *Science* **145**, 483 (1964).
3. T. R. Loree, C. M. Fowler, E. G. Zukas, F. S. Minshall, *J. Appl. Phys.* **37**, 1918 (1966).
4. F. S. Minshall, *ibid.* **26**, 463 (1955).
5. This is point 2 in (3, fig. 1).
6. M. H. Rice, J. M. Walsh, R. G. McQueen, in *Solid State Physics*, F. Seitz and D. Turnbull, Eds. (Academic Press, New York, 1958), vol. 6, p. 1.
7. This is point 3 in (3, fig. 1).
8. C. M. Fowler, F. S. Minshall, E. G. Zukas, in *Response of Metals to High Velocity Deformation* (Proc. A.I.M.E. conf. 9, 275). (Interscience, New York, 1960).
9. J. M. Walsh, M. H. Rice, R. G. McQueen, F. L. Yarger, *Phys. Rev.* **108**, 196 (1957).
10. P. W. Bridgman, *Proc. Amer. Acad. Arts Sci.* **76**, 55 (1948).
11. Work performed under AEC auspices.

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Malathion Degradation by *Trichoderma viride* and a *Pseudomonas* Species

Abstract. *Malathion* was found to be metabolized quickly by a soil fungus, *Trichoderma viride*, and a bacterium, *Pseudomonas* sp., which were originally found in soils from northern Ohio that had been sprayed heavily with insecticides. Results of a survey of the breakdown capabilities of 16 variants of *T. viride* revealed that certain colonies from this species had a very marked ability to breakdown malathion through the action of a carboxylesterase(s). The enzymes can be made soluble by preparing the acetone powder suspension.

Malathion is an important selective insecticide in the control of various pest insects. The pattern of its degradation has been extensively studied in insects and mammals (1). In brief, mammals are more resistant to this insecticide than most insects are because they have a superior ability to break the malathion molecule at the carboxylester sites. This point became even more evident when insects highly resistant to malathion were found to possess additional enzymes that can hydrolyze the carboxylester bonds of malathion (2). Studies of microbial degradation of organophosphorus insecticides by Ahmed and Casida (3) revealed that phorate and other dialkyl phenylphosphates and phosphorothioates could be degraded through the action of both *Pseudomonas fluorescens* and *Thiobacillus thiooxidans*. From a comparison of autoclaved and nonauto-

clayed wet and dry soils Lichtenstein and Schulz (4) concluded that the breakdown of parathion was brought about by the comparative numbers and metabolic activities of soil microorganisms. Malathion is known to be an insecticide of relatively short half-life (5), although the exact reasons for it have not been fully investigated.

Soil samples were obtained from six locations in each of two apple orchards in northeastern Ohio (Tope's orchard, Fredericksburg and Snyder's orchard, Wooster) and from the factory yards of Shell Chemical Corporation near Denver, Colorado. To study the total ability of these soil samples to break down malathion, a 10-g portion of each soil sample was first incubated for 24 hours at 30°C with 20 mg of C¹⁴-malathion (labeled at 1,2-succinyl carbon moiety) that had previously been applied to the inner surface of the sample flasks as thin films with the aid of acetone as a carrier. The samples were shaken vigorously to ensure good spreading of malathion in the soil.

Microorganisms were isolated from soil samples by adding 1 g of soil to 99 ml of sterile water and shaking the mixture vigorously for 3 minutes. From this stock, serial dilutions of 10⁻² and 10⁻³ were made in sterile petri dishes and mixed with soil-extract agar of the type described by Allen (6). Plates were incubated at 30°C for 4 days, then examined, and individual colonies were selected and streaked singly on soil-extract agar. Following incubation an additional and similar transfer was made to insure culture purity. For malathion incorporation, each isolate was inoculated into 10 ml of a solution of yeast extract and mannitol as described by Fred and Waksman (7), maintained at 30°C for 57 hours, and then incubated with 2 µg of C¹⁴-malathion for 24 hours at 30°C to assess its degradation capability.

Each soil sample that had previously been treated with C¹⁴-malathion was extracted twice with 5 ml of chloroform. The chloroform phase was washed first with 10 ml of 0.2 percent trichloroacetic acid (final pH 2) and then with 10 ml of sodium phosphate buffer (0.67M), pH 7.

Culture media (7) was similarly treated with 0.1 ml of trichloroacetic acid and immediately extracted twice with 5 ml of chloroform. The solvent phase was washed with 10 ml of buffer

Table 1. Degradation of C¹⁴-malathion by various soil samples. Data are expressed in percentage of the total malathion originally added.

Treatment	Malathion remaining (%)	Diethyl malate (%)	Carboxyl-esterase products (%)	Desmethyl malathion (%)	Other hydrolysis products (%)	Unidentified metabolites (%)
<i>Soil sample, Tope</i>						
None	13.8	0.4	50.7	19.3	15.8	0
Streptomycin	9.9	2.7	60.9	5.6	20.8	0.1
Propylene oxide	26.4	24.9	25.9	3.2	18.9	0
Autoclaved	99.6	0	0.3	0.1	0	0
<i>Soil sample, Snyder</i>						
None	27.0	0.3	41.2	8.9	22.9	0
Streptomycin	21.9	.3	41.6	18.8	15.9	1.2
Propylene oxide	61.8	5.7	26.9	2.0	3.6	0
Autoclaved	99.6	0	0.2	0.1	0	0
<i>Soil sample, Shell</i>						
None	71.9	0	25.2	0.8	1.9	0
Streptomycin	80.5	0	17.0	.1	2.0	0.4
Propylene oxide	99.8	0	0.5	.9	0	0
Autoclaved	99.6	0	.4	0	0	0

Table 2. Degradation of C¹⁴-malathion by various microorganisms isolated from Ohio soil samples. Data are expressed in percentage of the total malathion originally added.

Source	Malathion remaining (%)	Diethyl malate (%)	Carboxyl-esterase products (%)	Desmethyl malathion (%)	Other hydrolysis products (%)	Unidentified metabolites (%)
<i>Pseudomonas</i> sp.						
Tope soil	2.0	3.1	80.6	11.3	3.2	0
<i>Trichoderma viride</i>						
Snyder soil (B)*	25.5	19.0	37.3	17.3	1.2	0
Snyder soil (C)	12.9	11.5	41.9	31.5	1.7	0.5
Snyder soil (E)	11.1	3.7	50.6	34.0	0.6	0
Pure culture†	18.6 ± 11.9	3.0 ± 2.0	27.3 ± 11.9	37.9 ± 8.5	11.2 ± 5.1	1.8 ± 1.6

* The letters B, C, and E indicate different cultural colonies. † Variants kindly supplied by Dr. M. P. Backus. Results from the 16 different colonies of *T. viride* are expressed in average ± standard deviation.

as above. All fractions (chloroform, water pH 7, and water pH 2) were radiometrically assayed to find the extent of malathion breakdown. The chloroform phase was further analyzed by means of thin-layer chromatographic technique to separate various metabolites (2).

Results of soil experiments (Table 1) indicated that both Tope and Snyder samples had a high capability for degrading malathion while the Shell sample showed relatively low activity. That malathion degradation was caused by microorganisms is apparent from the low capability of autoclaved soil samples to break down this insecticide. Colonies of microorganisms, 10 from Tope samples and 13 from Snyder, were isolated as previously described.

The test results indicated that one isolate from the Tope sample and three from the Snyder sample had exceptionally high capabilities for degradation of malathion. The three promising colonies of microorganisms from the Snyder sample were later identified

as *Trichoderma viride*, a fungus species. The microorganism isolated from the Tope sample was found to belong to the bacterial genus *Pseudomonas*. It is a short, polar-flagellated, Gram-negative rod that did not produce pigment. Final identity of the species has not been achieved. Table 2 shows the degradation activities of these four isolates of microorganisms. It was also possible to isolate the soluble esterases from these microorganisms by first treating the washed cells with acetone at approximately -50°C, making dry acetone powder, and then extracting the enzymes with 1.2 percent NaCl solution that was buffered at pH 7 with sodium phosphates. All esterase actions could be blocked with 10⁻⁶M diisopropyl fluorophosphate. To ascertain whether *T. viride* is generally an active microorganism in degrading malathion and to determine whether closely related organisms are even more active, 16 variants of this species were obtained from the collection of Dr. M. P. Backus, University of Wisconsin.

The result (Table 2) indicates that the majority of varieties are indeed very active in degrading malathion through carboxylesteratic hydrolysis as well as by desmethylation processes.

The precise mechanisms of the degradation of malathion by these microorganisms are uncertain at this time. The fact, however, that the carboxylic acid derivatives of malathion constitute the major portion of malathion metabolites strongly suggests the presence of powerful carboxylesterases in these microorganisms. Some of the variants of *T. viride* showed high desmethylation activities, which suggests another degradation pathway in these organisms. The result of the metabolite analysis indicated that the conversion of malathion to the more toxic analog, malaoxon, did not take place in these microbial preparations; this suggests that these microorganisms lack proper oxidative systems.

Trichoderma viride is a very common species of fungus in the soil, as indeed are many species of the genus *Pseudomonas*, and their presence should be of great interest from the viewpoint of eliminating some insecticides. It is

equally conceivable that the toxic properties of a compound might be extended by altering the populations of these microorganisms in the soil.

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References and Notes

1. H. R. Krueger and R. D. O'Brien, *J. Econ. Entomol.* **52**, 1063 (1959); F. W. Seume and R. D. O'Brien, *Toxicol. Appl. Pharmacol.* **2**, 495 (1960).
2. F. Matsumura and A. W. A. Brown, *J. Econ. Entomol.* **54**, 1176 (1961); F. Matsumura and C. J. Hogendijk, *Entomol. Exp. Appl.* **7**, 179 (1964).
3. M. K. Ahmed and J. E. Casida, *J. Econ. Entomol.* **51**, 59 (1958).
4. E. P. Lichtenstein and K. R. Schulz, *ibid.* **57**, 618 (1964).
5. F. A. Gunther and R. C. Blinn, *Ann. Rev. Entomol.* **1**, 167 (1956).
6. O. N. Allen, *Experiments in Soil Bacteriology* (Burgess, Minneapolis, rev. ed., 1951).
7. E. B. Fred and S. A. Waksman, *Laboratory Manual of General Microbiology* (McGraw-Hill, New York, 1928).
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Dominant Hemispherectomy: Preliminary Report on Neuropsychological Sequelae

Abstract. *The first reported case of continuing survival 6 months after left hemispherectomy for glioma in a right-handed adult shows diminishing psychological impairment. Different degrees of initial impairment and in subsequent recovery of propositional speech, verbal comprehension, reading, writing, and other functions indicate quantitative rather than qualitative differences in adult left- and right-hemispheric functions.*

We describe here the first reported case of an adult (E.C., a 47-year-old male who was right-handed and right-eyed) who is continuing to improve in many functions more than 7 months after removal of the entire left cerebral hemisphere because of glioma. The initial symptoms of tumor and the effects of hemispherectomy confirmed that the left hemisphere was "dominant" for language. In this preliminary report we summarize salient changes during the first 7 months after hemispherectomy.

Beginning in November 1964, E.C. experienced increasingly frequent attacks of speechlessness and seizures in the right arm and right face. Neuropsychologic studies showed right-sided manual and ocular dominance, consistent with a family history of right-

handedness (both parents, two siblings, and his two children). A tumor removed from the left sensory-motor area on 31 March 1965 was identified as a glioblastoma multiforme. Subsequent progressive right-sided weakness indicated recurrence of the tumor and a left hemispherectomy was performed by one of us (C.W.B.) on 7 December 1965. (In summary, the surgical report stated that the corpus callosum was split lateral to the anterior cerebral artery, all branches of the artery being coagulated and divided. After cutting through the thalamus posteriorly and the basal ganglia anteriorly, the entire hemisphere was removed in one piece.)

Immediately following hemispherectomy, the patient had a right hemiplegia, right hemianopsia, and severe

aphasia. Ability to follow simple verbal commands indicated normal hearing and some comprehension of speech. Tests on 31 May 1966 showed normal hearing at 250 to 2000 cy/sec in each ear and a moderate bilateral loss at 4000 cy/sec in responses to pure-tone stimuli delivered through a hand-held receiver.

The patient spontaneously articulated words and short phrases fairly well immediately after the operation. However, he could not repeat single words on command or communicate in "propositional" speech until February 1966. Although he is still unable to speak voluntarily most of the time, occasional propositional speech continues to increase, along with ability to repeat successfully longer sentences on command in fewer trials. In May 1966 the patient was asked questions to test his comprehension. His replies to questions about the weather and an appointment showed that he understood what was asked, and he gave the correct number of years when asked how long he had lived in his house. In the fifth postoperative month, E.C. showed sudden recall of whole familiar songs, and he now sings with little hesitation and with few errors in articulation.

Learning to print single words with his left hand required several practice sessions in occupational therapy, but, in May 1966, the patient was able to say and print correctly the word "cow" when he was shown a picture and asked to write the name of the object. Writing, however, has shown little subsequent improvement. Improving comprehension of spoken words was reflected in E.C.'s increasing scores in the Peabody picture vocabulary test (PPVT). On 25 January 1966, he slowly but correctly selected four of the first six items from six pages, each with four pictures to choose from, before indicating he was tired; on 3 June he correctly selected 85 of 112 items. The marked improvement in PPVT scores shows an increasing attention span and capacity for prolonged testing, as well as increasing verbal comprehension which was evident in conversation and in performances on other psychological tests.

On 23 May 1966 five colored pens (red, blue, green, yellow, and black) were placed before E.C. and he was asked to pick up the yellow pen. At first he selected the red one at his extreme left, but when it was carefully explained that this was a test to see if