upon the return of severe wave conditions. Transplants switch to wave-intolerant, soft morphologies in normally sponge-free tide pools that are protected from summer but not winter wave action. Survival is high during the summer (132 of 160 transplants) but low during winter (1 of 56) in these pools. In contrast, overwinter transplant survival is high (26 of 33) in habitats exposed to normal wave action patterns.

Morphological or physiological changes in a wide variety of organisms (such as cold hardening in plants and diapause in insects) can be broadly dichotomized into those providing protection from stressful environments and those appropriate for more benign conditions (*2, 4, 18*). The verbal model proposed above predicts more complex or slower control mechanisms for transitions away from stress tolerance than transitions to a stress-tolerant state in uncertain environments. The experiments reported here indicate that sponge populations quickly adopt a stress-tolerant morphological tactic but delay establishment of an intolerant tactic. This suggests that acclimatory response to environment and the risks inherent in specific acclimatory tactics are closely linked.

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8. Maximum wave forces occurring over a tidal excursion were measured near the mouth of a surge channel where *H. panicea* colonies exhibited high stiffness and strength, and 10 m up the channel where sponge populations were of the weaker type. Measurements over six tides with spherical, spring-loaded wave targets [M. Denny, *Limnol. Oceanogr.* **28**, 1269 (1983)] showed that in the lower part of the channel, wave energies were significantly greater [0.709 $N/cm^2 \pm 0.229$ (standard deviation)] than in the upper section ($0.355\ N/cm^2 \pm 0.248$) (Wilcoxon two-sample test, $P < 0.05$).
9. Sponge clone fragments (10 to 20 cm^3) were moved between environments and allowed to attach to the rock surface by immobilizing them for 4 weeks under plastic mesh bolted to the rock surface. Portions of transplanted clones were left at original sites as controls. In addition, sponges were transplanted within their native sites as further controls. All experiments reported here were conducted concurrently on Tatoosh Island (48°23′N, 124°44′W) from September 1981 through September 1982.
10. Tissue samples were gripped with aluminum spring clamps and pulled (tensometer, courtesy Dr. M. LaBarbera) until failure at a strain rate of $0.025\ sec^{-1}$. Cross-sectional areas and sample lengths were determined by caliper measurements to the nearest 0.1 mm. Stiffness and strength were determined as in (*11*).
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Pargyline Prevents MPTP-Induced Parkinsonism in Primates

Abstract. *1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin which produces permanent parkinsonism in human and nonhuman primates. Treatment of squirrel monkeys with pargyline, a monoamine oxidase (MAO) inhibitor, prevents both clinical and neuropathological evidence of the neurotoxic effects of MPTP. Pargyline also inhibits conversion of MPTP to 1-methyl-4-phenylpyridinium ion (MPP^+), a metabolic step that occurs rapidly after administration of MPTP in animals not treated with pargyline. It is proposed that the conversion of MPTP to MPP^+, possibly involving MAO, may be important for the neurotoxic effects of MPTP to take place, and MPTP itself may not be the neurotoxic agent.*

The neurotoxic effects of MPTP were recognized when a group of drug addicts in northern California injected this substance under the assumption that it was a new "synthetic heroin" (*1*). Within days of using the drug, these patients exhibited virtually all of the motoric features seen in Parkinson's disease, including the classic triad of bradykinesia, rigidity, and tremor. We are now treating seven humans who were exposed to MPTP and have developed moderate to severe, permanent parkinsonism. They have all responded to dopamine agonist and precursor therapy but are now experiencing many of the typical dose-limiting side effects seen with long-term L-dopa therapy in Parkinson's disease (*2*).

Squirrel monkeys given adequate doses of MPTP intraperitoneally also develop a striking parkinsonian syndrome responsive to dopamine precursor and agonist therapy (*3*). Histopathological examination of these animals has revealed selective loss of neurons in the substantia nigra (*3*), the main site of the pathology in Parkinson's disease (*4*). A profound and prolonged decrease in striatal dopamine has been observed in rhesus monkeys given MPTP intravenously (*5*). However, interest in the compound has centered around the fact that MPTP is a true neurotoxin, that is, causing cell death in the substantia nigra, and thereby inducing a permanent clinical syndrome. In this sense, MPTP-induced parkinsonism is much closer to the naturally occurring disease than reversible syndromes induced by pharmacological agents (such as reserpine).

We now present data showing that the MAO inhibitor pargyline, currently prescribed in humans for moderately severe to severe hypertension (Eutonyl, Abbott), blocks the neurotoxic effects of MPTP. Further, we provide evidence that pargyline inhibits conversion of MPTP to MPP^+, a step which we believe is important for this compound to exert its neurotoxicity (*6*). These experiments were carried out after it was noted that pargyline inhibits the conversion of MPTP to MPP^+ in vitro (*7*).

Fourteen male squirrel monkeys (*Saimiri sciureus*), aged 1 to 3 years, were used for these experiments; MPTP was

given intraperitoneally as solutions of the hydrochloride salt. We had already presented evidence that a dosage schedule of 2 mg/kg given at 2-hour intervals four times on a single day consistently produces moderate to severe nerve cell loss in the substantia nigra of these animals (*6*). We have now given eight consecutive animals (monkeys 1 to 8) this "standardized schedule." All monkeys exhibited an acute syndrome ("nodding off," blepharospasm, salivation, increasing bradykinesia, and repeated cycles of abrupt eye opening and generalized tremor). Three animals died in a severely akinetic state within 48 hours of receiving this dosage. The five surviving animals remained severely parkinsonian but responded to both dopamine precursor and agonist therapy. Four were studied neuropathologically between 10 and 44 days after receiving MPTP; all four showed marked nerve cell loss restricted to the substantia nigra. At 6 months, the fifth animal continues to be symptomatic.

The second phase of this experiment consisted of treating animals with pargyline. Animal 9 was first given a single injection of pargyline (50 mg/kg) intraperitoneally, and the standardized schedule of MPTP was then begun one-half hour later. Except for transient "nodding," salivation and tremulousness after each MPTP dose, the animal showed no effects from MPTP (acute or chronic). Animals numbered 10 to 12 were given pargyline orally to see whether this route of administration was effective. After receiving 5 mg/kg daily for 4 days, each was given the standardized dosage of MPTP (5 mg of pargyline was also given orally 1 hour before each of the four doses of MPTP). Prior treatment definitely did not prevent the same acute reactions as those seen in the animals not treated with pargyline. However, all animals receiving the pargyline appeared completely normal within 2 to 3 hours of their last dose of MPTP in marked contrast to animals that were not given pargyline. Animal 10 was killed 4 weeks after receiving pargyline and MPTP, and the brain was subjected to neuropathologic study. In animal 11, the same pargyline-MPTP dosage schedule was repeated 2 weeks after the first dosage was administered, and the animal was killed 1 hour after its last dose of MPTP. The brain was sectioned sagittally, and one-half was submitted for pathology, and the other half was analyzed for MPTP and MPP^+. Animal 12 was killed 1 hour after the last dose of MPTP, and the brain was submitted for analysis. Animals 13 and 14 were given the standardized dosage of MPTP without prior treatment with pargyline and killed 1 hour after the last dose for subsequent studies.

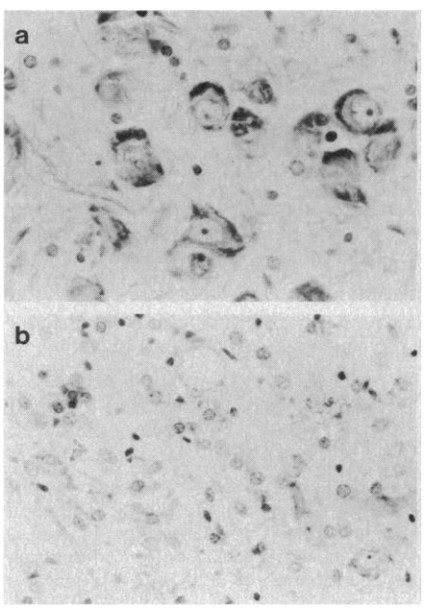

Fig. 1. (a) Substantia nigra, compact zone, from monkey 10, treated with pargyline prior to standardized dosage of MPTP. The nerve cells appear normal and there is no astrocytic and microglial reaction (Luxol fast blue–cresyl violet stain, ×387). (b) Substantia nigra, compact zone, from squirrel monkey 8 with MPTP-induced parkinsonism. The animal was killed on day 44 after the standard MPTP dosage schedule. The substantia nigra shows severe nerve cell loss and marked glial reaction (Luxol fast blue–cresyl violet stain, ×387).

Portions of the brains were analyzed for MPTP and MPP^+. These tissues (including striatum, brainstem, cerebellum, and various areas of cortex) and systemic organs from animals 11 and 12, as well as those from animals 13 and 14, were immediately weighed and homogenized in 10 ml of methanol for each gram of tissue. After addition of deuterated MPTP and MPP^+ as internal standards, homogenates were centrifuged and filtered, and water was added to the methanol extracts. These were assayed for MPTP and MPP^+ by a modification of the stable isotope, combined gas chromatography–mass spectrometry (GC-MS) assay (*6*). MPP^+ and MPTP are easily separated by ether extraction at *p*H 12.0. Under these conditions, MPTP is extracted, while MPP^+, because of its high aqueous solubility, is not. After removal of MPTP, MPP^+ is reduced with sodium borohydride to MPTP, which can be extracted and quantified. The use of deuterated internal standards accounts for extraction and mechanical losses and ensures accurate quantification (coefficient of variation, <10 percent) at low concentrations of MPTP (0.05 μg/g) and MPP^+ (0.10 μg/g).

Histopathological analysis of pargyline-treated animals 10 and 11 showed no evidence of nerve cell damage in the substantia nigra (Fig. 1a). This contrasts dramatically with the striking nigral cell loss in animals treated with MPTP alone (Fig. 1b).

The respective levels of MPTP in animals treated with pargyline (animals 11 and 12) as compared to those not treated (animals 13 and 14) in micrograms per gram of tissue were as follows: 0.96 compared to 0.09 in frontoparietal cortex, 1.2 compared to 0.12 in temporo-occipital cortex, 0.89 compared to 0.09 in cerebellum, and 0.97 compared to 0.14 in basal ganglia. The concentrations of MPP^+ in the tissues were markedly reduced in pargyline-treated animals compared to those not treated (Fig. 2).

Our results thus demonstrate successful blocking of the parkinsonism-inducing effects of MPTP in primates. In our study, pargyline produced a statistically significant protecting effect against a standardized dose of MPTP ($P = 0.006$; the one-sided Fisher's exact probability test) (*8*). Further, in animals examined for pathological effects, there was no evidence of nigral cell loss (Fig. 1). These studies also provide evidence that the acute syndrome of MPTP exposure (which oral pargyline does not block), can be disassociated from its neurotoxic effects on the substantia nigra, suggesting that the acute syndrome may be due (at least in part) to transient alterations in norepinephrine and serotonin (*5*) and perhaps opiate effects as well. Our results also provide evidence that MPTP itself may not be the actual neurotoxic

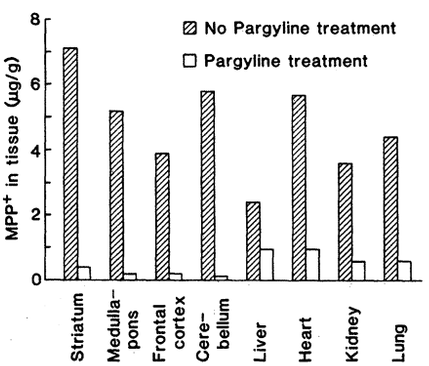

Fig. 2. Concentrations of MPP^+ in brain tissue of animals receiving a standard dose schedule of MPTP with and without pargyline treatment. Animals were killed 1 hour after their last dose of MPTP; values are averages of two animals in each group (monkeys 11 and 12 in treated group, and 13 and 14 in untreated group).

agent, since concentrations of MPTP in the tissue are tenfold higher in pargyline-protected animals than in animals not treated, after each group received toxic amounts of MPTP. Pargyline has also been shown to prevent the dopamine depleting effect of MPTP in mouse striatum (*9*).

The studies reported here also provide evidence for a possible molecular mechanism of action for MPTP. We have shown earlier that removing the double bond at the "4-5" position in MPTP blocks the conversion of MPTP to MPP^+ (*10*) and the toxic effects of the compound. While it is possible that pargyline prevents MPTP neurotoxicity by some means other than preventing the conversion of MPTP to MPP^+ (presumably by blocking MAO activity), we have now demonstrated by two different approaches that blocking this conversion parallels prevention of neurotoxicity. Pargyline actually appears more effective in blocking this conversion in the central nervous system, raising the possibility of a differential effect in the central nervous system as compared to systemic tissues. Our results still do not explain why MPTP is selectively toxic to the substantia nigra, although we suspect that this is in some way related to the high dopamine concentrations in the nigrostriatal system.

Another MAO inhibitor deprenyl (selective for MAO-B) has been used for a number of years as an adjunct in the therapy of Parkinson's disease (*11*). In that deprenyl may be effective in retarding the progress of Parkinson's disease (*12*), and in that MAO inhibitors prevent MPTP-induced parkinsonism, it seems that testing the early use of deprenyl or other MAO inhibitors to alter the course of the disease may be warranted.

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δ-Aminolevulinic Acid–Synthesizing Enzymes Need an RNA Moiety for Activity

Abstract. *When* Chlamydomonas *enzymes that convert glutamate to δ-aminolevulinic acid are separated into three fractions, one of the fractions contains RNA, and the RNA moiety is needed for the enzyme activity.*

δ-Aminolevulinic acid (ALA) is a rate-limiting factor of the chlorophyll biosynthetic pathway. It is synthesized from glutamate in plastids and is the first committed intermediate of the chlorophyll biosynthetic pathway (*1*). The enzymes from barley plastids (*2*) and *Chlamydomonas* cells (*3*) that are responsible for the formation of ALA from glutamate have been isolated and fractionated into three parts by serial affinity chromatography with a Blue Sepharose column and a heme-Sepharose column. The fractions are designated as Blue Sepharose–bound (B), heme-Sepharose–bound (H), and runoff (R). When combined together, the fractions can convert glutamate to ALA, but they are inactive when assayed alone. Cofactors required are adenosine triphosphate (ATP), Mg^{2+}, and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) (*2*, *3*).

The best supported scheme for ALA formation from glutamate is shown in Fig. 1. It had been proposed that glutamate is activated by a kinase using ATP and Mg^{2+} to form glutamate-1-phosphate. Subsequently, a dehydrogenase converts glutamate-1-phosphate to glutamate-1-semialdehyde (GSA). Finally, GSA is converted to ALA (*4*). GSA has been synthesized from *N*-carbobenzoxyglutamatic acid–γ-benzyl ester (*4*), and the structure of its acetal has been verified (*5*). The enzyme GSA aminotransferase (E.C. 5.4.3.8), which converts GSA to ALA, has been purified from barley plastids (*6*). Since none of the other intermediates have been isolated and identified, the exact nature of the proposed reaction sequence remains unclear. However, we have shown that (i) GSA aminotransferase is in the R fraction (*2*, *3*); (ii) the H and B fractions together convert glutamate to a compound that purifies with synthesized GSA on high-performance liquid chromatography (*2*); and (iii) the compound synthesized by the H and B fractions from glutamate, as well as the synthesized GSA, can be converted by the R fraction to ALA (*2*, *3*). This ALA was identified by high-performance liquid chromatography and thin-layer chromatography against authentic samples of ALA.

Under the assumption that H and B

Table 1. Effects of ribonuclease and deoxyribonuclease on the enzyme activity of crude enzyme preparation and H + B fractions. The assay of ALA-synthesizing activity and the method of purifying the product are as described (*2*). The product of the H + B reaction mixture is glutamate-1-semialdehyde. It can be quantitatively recovered by purification procedure used. For more detail, see text. Activity is given as counts per minute per milligram of protein per hour.

Sample	Activity ($cpm \cdot mg^{-1} \cdot hour^{-1}$)
Crude enzyme	100,580
Crude enzyme + ribonuclease*	2,169
Crude enzyme + ribonuclease + ribonucleasin†	93,703
H + B	15,327
H + B + ribonuclease	4,648
H + B + ribonuclease + ribonucleasin	16,076
H + B + deoxyribonuclease‡	13,538
H + B + deoxyribonuclease + ribonucleasin	17,636

*Ribonuclease A (Sigma), 1 μg per milliliter of enzyme assay. †Ribonucleasin (Promega Biotec), 500 units per milliliter of enzyme assay. ‡Deoxyribonuclease I, highest purity (Worthington Diagnostic), 2 μg per milliliter of enzyme assay.