familial xenografts were exchanged (*5*).

Graft rejection may be mediated by coelomocytes which seem to congregate around and in xenografts reaching maximum numbers approximately 5 days after grafting (*6*). Moreover, that some of these cells were observed during healing suggests that cell involvement in initial general nonspecific reactions (for example, healing of wounds) was associated with all transplants. Thus, with xenograft rejection and autograft acceptance, one may infer specificity in coelomocyte recognition of the difference between "self versus not self" antigens. To test the capacity of worms to distinguish between these antigens, an autograft and xenograft were transplanted simultaneously to the same graft bed of a *Lumbricus* host. Cells involved in nonspecific reactions during the early phases of healing were thus confronted simultaneously with auto- and xenogeneic antigens. Both grafts healed together; the xenograft was destroyed later, but the autograft remained permanently viable.

Table 3. Statistical analyses of graft survival times (data from Tables 1 and 2). The .05 significance level was accepted as indicating significant population difference. The (W) indicates use of Wilcoxon matched pairs signed rank test, all others analyzed with the Mann-Whitney *U* test (two-tailed) (*8*); +5 indicates an interval of 5 days between the first- and second-set grafting.

Direction of grafting compared	*P*
**Lumbricus* → *Eisenia* **Lumbricus* → *Eisenia* xenograft ↑ ↑ autograft	.32
Allolobophora → *Lumbricus* *Eisenia* → *Lumbricus*	<.00003
Eudrilus → *Lumbricus* *Eisenia* → *Lumbricus*	<.00003
Allolobophora → *Lumbricus* *Eisenia* → *Lumbricus*	.0001
Eudrilus → *Eisenia* *Lumbricus* → *Eisenia*	.002
Eudrilus → *Eisenia* *Allolobophora* → *Eisenia*	.00014
Allolobophora → *Eisenia* *Lumbricus* → *Eisenia*	.40
Eisenia* → *Lumbricus* **Eisenia* → *Lumbricus* ← *Eisenia	.38
Allolobophora* → *Lumbricus* +5 *Eisenia* → *Lumbricus* ← *Eisenia* ↑ +5 *Allolophora	.19
+5 **Eisenia* → *Lumbricus* ← *Eisenia*	.96
+5 **Eisenia* → *Lumbricus* ← *Eisenia* ↑ +5 *Allolobophora*	
+5 *Eisenia* → *Lumbricus* ← *Eisenia**	.96
+5 *Eisenia* → *Lumbricus* ← *Eisenia** ↑ +5 *Allolobophora*	
**Eisenia* → *Lumbricus* +5 **Eisenia* → *Lumbricus* ← *Eisenia* ↑ +5 *Allolobophora*	.00014
Eisenia* → *Lumbricus* +5 *Eisenia* → *Lumbricus* ← *Eisenia ↑ +5 *Allolobophora*	<.00003
Eisenia* → *Lumbricus* ← *Eisenia* ↑ +5 *Allolobophora +5	<.00003 (W)
Eisenia* → *Lumbricus* ← *Eisenia* *Eisenia* → *Lumbricus* ← *Eisenia +5	<.00003
**Eisenia* → *Lumbricus* ← *Eisenia* ↑ +5 *Allolobophora* +5	.0013 (W)
Eisenia → *Lumbricus* ← *Eisenia** ↑ +5 *Allolobophora*	

* Indicates which samples were compared.

The experiment which indicated the high degree of specificity of earthworm immune competence involved transplants from *Eisenia* and *Allolobophora* to *Lumbricus* hosts. Two *Eisenia* transplants showed a shortened rejection time, while a single *Allolobophora* transplant was destroyed independently; a control *Allolobophora* graft on a *Lumbricus* host was rejected at the same time. A host *Lumbricus* with two xenotransplants grafted simultaneously but in different locations showed a survival time equivalent to a single *Eisenia* graft to *Lumbricus*.

The earthworm's ability to reject transplants, in itself, had no apparent survival value in evolution. Graft destruction has only provided a convenient model for demonstrating biological specificity. Yet, in the context of the phylogeny of immunity we have revealed, for the first time, a primitive immune system in an invertebrate that may be cell-mediated and prototypic. The apparent inability of earthworms to synthesize substances in response to various bacterial antigens suggests that humoral immunity evolved in other invertebrate phyla (*7*) and primitive vertebrates (*4*). Further clarification of anamnestic responses to tissue transplants would confirm our views that at least two of the parameters of adaptive immunity, namely specificity and memory, did not evolve exclusively with the lower vertebrates.

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Pathotoxin-Induced Disease Resistance in Plants

Abstract. *Primary leaves of bean plants treated with nonphytotoxic concentrations of the pathotoxin victorin were rendered highly resistant to two plant viruses. Leaves treated with higher concentrations of victorin became necrotic. These effects on plants that are resistant to victorin and to the fungus that produces it lend support to the hypothesis that activation of a defensive self-repair mechanism may account for resistance to this highly selective pathotoxin.*

Pathotoxins are substances of biological origin which play causal roles in plant diseases (*1*). A well-known example is victorin, a pathotoxic product of *Helminthosporium victoriae* Meehan and Murphy, the fungus which causes Victoria blight of oats (*2*). Very small quantities of victorin applied to susceptible oats cause pathological changes in permeability, transpiration, and respiration which are followed by visible disease symptoms and death of the plants (*2*, *3*). Much larger quantities cause similar pathological changes in the physiology of resistant oat tissues, but, in these, lethal effects do not follow (*4*). This ability of resistant tissues to respond and recover led to the hypothesis that activation of a defensive self-repair mechanism may account for resistance to victorin (*5*). Once activated, such a defense mechanism might render plants resistant to other pathogenic agents. To test this possibility we studied the effect of victorin on the reaction of the bean *Phaseolus vulgaris* L. cv Pinto to two viruses which induce necrotic lesions on primary leaves.

The source of victorin was a single lot of culture filtrate which, undiluted, contained 10,000 unit/ml in the standard root-growth test (*6*). When used in the crude form, the filtrate was diluted

100-, 1,000-, or 10,000-fold with distilled water to yield test solutions which contained, respectively, 100, 10, or 1 unit/ml. A portion of the crude filtrate was desalted and deproteinized by the method of Luke and Wheeler (*7*). This partly refined material was diluted and tested at the same concentrations as the crude filtrate. Portions of crude and refined victorin, detoxified as described (*3*) and diluted to the same extent as the active solutions, served as controls.

Bean plants were grown in Vermiculite irrigated with half-strength Hoagland's solution. When the primary leaves were partially expanded, but before the first trifoliate leaf had begun to unfurl, the plants were cut off at the base of the stem and placed in the test solutions. The plants were allowed to take up the test solutions for 36 hours (12 hours of light at 8800 lu/m^2, 12 hours dark, 12 hours light), at 23°C, in a controlled environment chamber. The primary leaves were then dusted with carborundum and inoculated with suspensions of purified alfalfa mosaic virus (AMV) or tobacco mosaic virus (TMV), at concentrations which would produce about 50 to 150 lesions per half-leaf on the controls.

Plants treated with 10 units of victorin per milliliter were rendered highly resistant to both AMV and TMV. In most tests, leaves from plants treated with victorin were virtually free of lesions (Fig. 1A), whereas more than 100 lesions per half-leaf were produced on controls (Fig. 1B). In every test, treatment with victorin resulted in a reduction in numbers of lesions of more than 90 percent (Table 1).

Table 1. Effect of prior treatment with victorin (10 unit/ml for 36 hours) on the response of Pinto bean primary leaves to alfalfa mosaic virus and tobacco mosaic virus.

Experiment	Replications (No.)	Victorin	Lesions per half-leaf	
			Victorin (av. No.)	Control (av. No.)
		Alfalfa mosaic		
1	3	Crude	5	69
2	2	Crude	6	83
3	4	Crude	3	58
4	4	Refined	1	42
5	3	Refined	<1	53
6	3	Refined	<1	116
		Tobacco mosaic		
1	3	Crude	0	73
2	3	Crude	4	93
3	3	Refined	0	133
4	3	Refined	0	63

Extensive necrosis developed on leaves of plants treated with 100 units of victorin per milliliter whether or not the leaves were subsequently inoculated with virus (Fig. 1C). This made accurate counts of virus-induced lesions impossible, but protection appeared to be complete because virus-inoculated leaves showed no symptoms not found on controls. No significant differences in numbers of lesions were found among plants treated with 1 unit/ml, those treated with detoxified victorin, and those merely held in water.

The sensitivity of bean leaves to victorin varied with their stage of development. Primary leaves 2 to 3 days younger than those shown in Fig. 1 were usually not injured when treated with 100 units of victorin per milliliter, and this concentration was also required to induce significant resistance to the two viruses. Leaves 2 to 3 days older than those in Fig. 1 were much more sensitive and were often injured when treated with 10 units of victorin per milliliter, the lowest concentration which markedly reduced virus symptoms. A similar increase in sensitivity with age has been observed in victorin-treated first leaves of oats (*5*).

Victorin is capable of activating a defense mechanism in beans which is highly effective against two viruses. This and the increased sensitivity of older leaves, which are presumably less capable of self-repair, support the hypothesis that resistance to victorin depends on the ability of the plant to repair the initial damage caused by this pathotoxin.

Systemic resistance to plant viruses, similar to but less complete than that caused by victorin, has been induced by infection with other viruses (*8, 9*) or with fungal pathogens (*10*). As victorin itself is pathogenic (*1*), our results might be considered another example of resistance to one pathogen induced by prior exposure to another pathogen. Necrosis caused by the initial infection, possibly a diffusible product of the necrotic process, has been associated with resistance induced by prior infection with viruses and fungi (*9, 10*). Victorin,

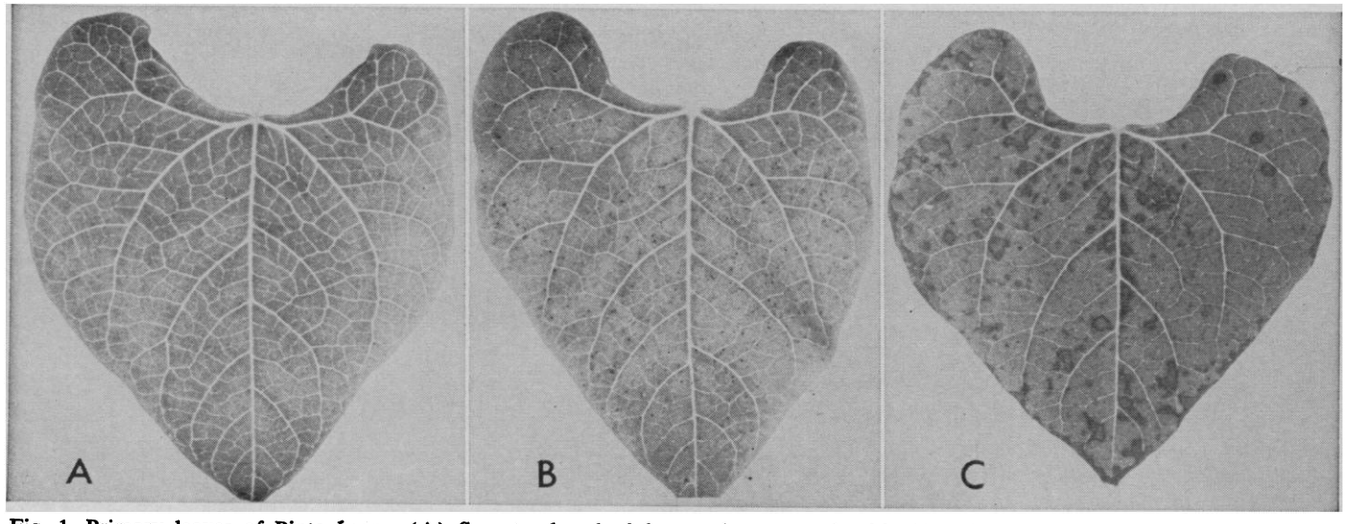

Fig. 1. Primary leaves of Pinto beans. (A) Symptomless leaf fro m plant treated with 10 units of victorin per milliliter for 36 hours and then inoculated with tobacco mosaic virus (TMV). (B) Control leaf treated with detoxified victorin and inoculated with TMV. Minute dark spots are virus lesions. (C) Necrosis caused by treatment with 100 units of victorin per milliliter in a leaf not inoculated with virus.

however, induced virtually complete resistance at concentrations that did not cause necrosis. This may mean that different mechanisms of resistance are involved or, because victorin at higher concentrations causes necrosis, it may be that actual necrosis is not required but only the initiation of a process which leads to it.

Although victorin and Victoria blight have served as a model system for extensive investigations of pathogenesis and the nature of disease resistance in oats (*1*, *11*), this is the first report of an effect of this pathotoxin on a plant which is not a member of the grass family. Failure to detect such effects in earlier investigations can be attributed in part to the highly selective nature of victorin; the 100 unit/ml required to injure bean leaves is 100,000 times higher than the 0.001 unit/ml which causes 50 percent inhibition of root growth in susceptible oats (*6*). Low sensitivity of the very young seedlings used in previous attempts to demonstrate effects of victorin on plants other than susceptible oats (*6*) may also have been a factor.

The extreme instability of purified preparations of victorin makes an accurate estimate of its potency impossible. However, the refined preparation used in this study contained, on a dry weight basis, 1 mg of solids per milliliter. Disease resistance was induced by a thousand-fold dilution of this preparation or at a concentration of solids of 1 μg/ml. Because our refined preparation was not pure, the actual potency must be much higher. This evidence of high potency, even to resistant tissues, and the fact that victorin strongly inhibits auxin-induced cell elongation (*12*) suggest that this pathotoxin has great potential for investigation of physiological processes in plants. In particular, victorin should provide a useful model system to study nonspecific defense mechanisms in higher plants.

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m-Hydroxyphenylacetic Acid Formation from L-Dopa in Man: Suppression by Neomycin

Abstract. *The increased excretion of* m-*hydroxyphenylacetic acid in the urine of patients with parkinsonism being treated with* L-*dopa was reduced by gut sterilization with neomycin. The* p-*dehydroxylation step is thus brought about solely by the action of gut flora; the pathway is unlikely to be involved in the events within the brain leading to the therapeutic benefit effected by* L-*dopa.*

A substantial proportion of patients with parkinsonism obtain more therapeutic benefit from L-dopa (dihydroxyphenylalanine) than from any drug previously available (*1*). While it has been assumed that clinical improvement stems from dopamine generation within the central nervous system, Calne *et al.* (*2*) have pointed out that such a chemical transformation is likely to be rapid (*3*) whereas the time course of the therapeutic response to the drug is slow (*1*). The possibility cannot therefore be ruled out that the clinical effect derives not from dopamine replacement but from the buildup of some minor metabolite unconnected with the main route of dopa degradation. Therefore we charted minor pathways of dopa metabolism revealed by the large doses of drug employed (up to 8 g/day).

The existence of one such pathway, terminating in an increased urinary output of *m*-hydroxyphenylacetic acid (*m*-HPAA), was noted during a trial of dopa in patients with parkinsonism (*2*). DeEds *et al.* (*4*), who made a similar observation after feeding DL-dopa to rabbits, considered that *m*-HPAA might derive from the *p*-dehydroxylation of an intermediate in the reaction sequence, 3,4-dihydroxyphenylacetic acid. However, the possibility that the transformation occurs at some other stage, perhaps by *p*-dehydroxylation of dopa itself, of dopamine, or even of dihydroxyphenylpyruvic acid, with the remaining metabolic steps taking place after absorption of the dehydroxylated product, cannot be ruled out. A human stool suspension can bring about *p*-dehydroxylation in vitro of a variety of phenolic acids (*5*). If *p*-dehydroxylation of the catechol moiety by gut flora (*6*) were an essential step in the production in vivo of *m*-HPAA from L-dopa in man, gut sterilization with neomycin might decrease the urinary output of *m*-HPAA.

Six patients with idiopathic parkinsonism, receiving their maximum tolerated oral dosage of L-dopa (Fig. 1), were given oral doses of neomycin (1 g) daily. L-Dopa metabolism is unlikely to differ in healthy subjects and subjects with parkinsonism (*2*). Urine samples were collected before and during day 3 of neomycin treatment. The *m*-HPAA was isolated from urine saturated with salt (3 ml diluted to 10 ml with 0.01*N* HCl) at *p*H 2.0 by extracting twice (25 ml) with ethyl acetate. Portions (20 and 25 ml, respectively, pooled) of the extracts were evaporated to dryness under vacuum at 40° to 50°C, and the methyl ester–trimethylsilyl ether derivative was prepared. This was separated from other phenolic acid derivatives by isothermal (190°C) gas chromatography (*7*) on a Pye Panchromatograph with a 210-cm 10 per-

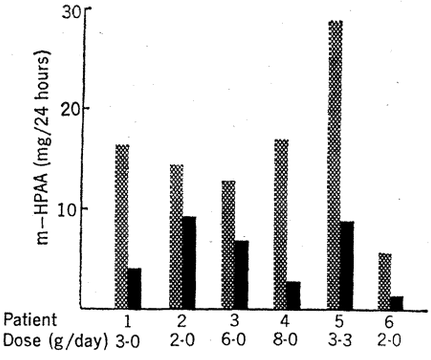

Fig. 1. Excretion of *m*-hydroxyphenylacetic acid (*m*-HPAA) before (hatched columns) and during (solid columns) day 3 of oral administration of neomycin (1 g/day). The subjects were six patients with parkinsonism being treated orally with L-dopa at the dosage shown.