are sprayed and the complex combinations of agents which may be involved in any one episode. Thus to the ecological havoc wrought by the defoliation campaign (*1*, *2*) and the potential teratogenic (*2*, *3*) and mutagenic (*3*) effects must be added direct effects on the exposed population, including prolonged asthenia and skin disorders.

The possible potentiating effects of eye irritation in an environment where trachoma is endemic must also not be overlooked. Nor to our knowledge has there yet been any substantial study of the potential carcinogenic effects of the chemical agents. It is difficult to obtain an estimate of the potential population thus at risk following the chemical war. Figures provided by the Democratic Republic of Vietnam and the Peoples Republic of China speak of more than 1.5×10^6 people having been poisoned (*11*), and the scale on which the chemicals have been applied (*2*) would certainly make this figure a feasible one.

Although the defoliation campaign has been largely phased out in Vietnam, we cannot be sanguine that we are merely cataloging past damage that is not being repeated elsewhere. The use of chemical defoliants by the Portuguese against guerrillas in Angola is authenticated (*12*), and there have been repeated reports that the chemicals are still in use on a large scale elsewhere in Indochina, notably Laos (*13*), despite the reductions in their use in Vietnam itself. It is urgent that clear information on the effects of chemical sprays on humans and lower animals be obtained, in view of the continued use of these agents in Indochina and elsewhere.

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References and Notes

1. G. H. Orians and E. W. Pfeiffer, *Science* **168**, 544 (1970); A. H. Westing, *BioScience* **21**, 893 (1971); *ibid.*, p. 1225; Committee on Commerce, *Effects of 2,4,5-T on Man and the Environment* (Serial 91-60, Government Printing Office, Washington, D.C., 1970).
2. Herbicide Assessment Commission of the AAAS, preliminary report 1970, in *War Related Civilian Problems in Indochina*, part 1, Vietnam (Senate Judiciary Committee, Washington, D.C., 1971), pp. 113–131; R. T. Cutting, T. H. Phuc, J. H. Ballo, M. W. Benenson, C. H. Evans, *Congenital Malformations in Hydatiform Moles and Stillbirths in the Republic of Vietnam 1960–1969* (Government Printing Office, Washington, D.C., 1970).
3. Ton That Tung, Trinh Kim Anh, Bach Qúoc Tuyên, Dao Xuân Tra, Nguyên Xuân Huyên, *Vietnam. Stud. (Hanoi)* **19**, 53 (1971).
4. M. F. Kahn, in *CBW: Chemical and Biological Warfare*, S. Rose, Ed. (Beacon, Boston, Mass., 1969), p. 87; A. Lacombe, J. P. Farriaux, G. Fontaine, P. M. Muller, *Rev. Pediat.* **3**, 207 (1967).
5. Our visit was at the invitation of the Committee for the Dissemination of Science and Technology of the Democratic Republic of Vietnam, and under the auspices of the World Federation of Scientific Workers. Its details have been reported elsewhere [S. Rose and H. Rose, *New Sci.* **49** (No. 735), 134 (1971).
6. A. W. Galston, in *CBW: Chemical and Biological Warfare*, S. Rose, Ed. (Beacon, Boston, Mass., 1969), p. 62.
7. Herbicide abbreviations are as follows: 2,4-D, 2,4-dichlorophenoxyacetic acid; Agent Orange, a mixture of 2,4-D and 2,4,5-trichlorophenoxyacetic acid; Agent White; a mixture of 2,4-D and picloram; Agent Blue, dimethyl arsenic acid; and CS, *o*-chlorobenzylmalononitrile.
8. H. Bauer, K. H. Schulz, U. Spiegelberg, *Arch. Gewerbepath. Gewerbehyg.* **18**, 538 (1961).
9. R. J. Lifton, *Death in Life: Survivors of Hiroshima* (Random House, New York, 1967); L. Eitinger, *Concentration Camp Survivors in Norway and Israel* (Allen & Unwin, London, 1964).
10. N. P. Goldstein, P. H. Jones, J. R. Brown, *J. Amer. Med. Ass.* **171**, 1306 (1959).
11. Stockholm International Peace Research Institute, *The Problem of Chemical and Biological Warfare* (Humanities, New York, 1971), vol. 1, pp. 176–177.
12. The relevant press reports on this are compiled in A. Humbaraci, *Threats to the Ecology of Southern Africa* (Zambian Information Office, Rome, 1971).
13. K. Boupha, *Report to the International Conference on Medicine and the Indochina War, Paris (1971)*.
14. We thank Ton That Tung, Nguyên Xuân Huyên, and the patients of Hospital A for their cooperation in making this survey; and A. H. Westing for helpful discussions.

19 January 1972; revised 16 June 1972 ■

Development of a Receptor on a Foreign Nerve Fiber in a Pacinian Corpuscle

Abstract. *When the sensory fiber of a Pacinian corpuscle (in cat mesentery) is transected (at the inferior mesenteric nerve) transduction fails within 30 hours: the nerve ending produces no generator potentials in response to mechanical stimulation. Electrically elicited nerve impulse conduction continues for at least another 18 hours. A transducer mechanism develops on a regenerating nerve fiber when this fiber enters the denervated corpuscle. Such transducer development takes place on myelinated fibers from the inferior mesenteric nerve, which normally supplies corpuscles, as well as on myelinated hypogastric nerve fibers, which normally do not go to corpuscles, including fibers larger than the original corpuscle afferents.*

The mechanoreceptor Pacinian corpuscle develops by an interaction between a sensory nerve cell and an epithelial or fibroblastic (lamella) cell: as the nerve cell's axon (myelinated) grows into the embryonic target tissue, lamella cells there begin to proliferate and eventually to envelop the axon terminal forming a laminated capsule around it (*1*) (Fig. 1, I). In the course of this development, a highly sensitive receptor mechanism differentiates on the axon terminal, capable of transducing mechanical into electrical energy (*2*). This transducer mechanism is distinct from the mechanism of nerve impulse conduction: it produces a local, graded electrical potential (generator potential) in response to mechanical stimulation (strain) (*3*), is insensitive to tetrodotoxin (*4*), and has a high temperature coefficient (*5*). The transducer differentiation appears to be restricted to the nonmyelinated terminal portion, where the axon is in intimate contact with the lamella cells. The remainder of the axon inside and outside the capsule is insensitive to mechanical stimuli of the order of magnitude to which the terminal responds (*6*), and the lamella cells do not partake in the transduction (*7*).

Is the differentiation of the transducer mechanism a specific property of the Pacinian corpuscle axon, or are other axon types capable of such differentiation if they interact with the lamella cells?

This question and related ones are asked here for the Pacinian corpuscle of the cat's mesentery. The approach is to cut the nerve supply of the corpuscle in the adult animal and to reinnervate it with a foreign nerve fiber after the original one has degenerated.

The Pacinian corpuscle of the cat's mesentery (mesocolon) is well suited for a study of this kind. These sense organs, about 1 mm long and 0.5 mm thick, are visible to the naked eye; their afferent axons, which often run separate from each other over several centimeters, are easily seen in the translucent mesocolon and dissected with low-power optics (Fig. 2). The capsule survives after many months of denervation if its blood supply is intact.

To denervate the corpuscle, the in-

ferior mesenteric (IM) nerves were cut at their exits from the IM ganglia (on the average 3 cm away from the corpuscles). The IM artery was left intact. It was thus easy to denervate all corpuscles in the mesocolon at one time without disrupting their blood supply. For reinnervation, the severed nerve ends were joined as shown in Fig. 1, IIa and III. For foreign innervation of the corpuscles, the hypogastric nerve was severed and the central stump was grafted to the distal end of the IM nerve (Fig. 1, IIb). The hypogastric normally innervates the bladder (*8*); the bladders contained no Pacinian corpuscles (*9*). The central stump of the IM nerve was tied off to minimize stray innervation. Tests of mechanical and electrical responsiveness of the corpuscles and their axons were made in vitro (26° to 27°C): the mesocolon was excised and mounted in a bath of Krebs solution and the axons were dissected free for electrical recording and stimulation (Fig. 1, IV). For mechanical stimulation, an electrically driven piezoelectric crystal provided displacement pulses of peak amplitudes continuously variable between 0 and 100 μm (*10*).

Transection of the IM nerve led to degeneration of all corpuscle axons: 72 hours after the transection the axons no longer responded to electrical stimulation anywhere along their course between the IM ganglion and the corpuscle, and the axons no longer stained with methylene blue inside the corpuscle, but instead took the Marchi-Stewart (*11*) stain typical of degenerating nerves. The transducer mechanism was the first to fail. Within 30 hours of nerve transection, all corpuscles failed to produce generator potentials in response to mechanical stimulation. Action potentials continued to be produced by electrical stimulation of the extracorpuscular axon for at least 48 hours in all cases.

Out of 11 IM nerve reunions, 6 resulted in reinnervation: axons were found conducting electrically elicited action potentials to corpuscles 30 to 40 days after nerve union. In these cases of successful nerve regrowth, 8 out of 56 tested corpuscles generated potentials in response to mechanical stimulation (Table 1). The conduction velocities in the reinnervating fibers ranged from 3.2 to 8.8 m/sec as against 20 to 33 m/sec in the original axons. The velocities and the spike durations in the reinnervating fibers correspond to myelinated fibers of about 1 to 2.5 μm in diameter as against 6 to 10 μm (*12*). This is what one would expect if the corpuscles were reinnervated by the same class of IM axons as the original ones; regenerating axons are smaller than original ones (*13*).

The hypogastric nerve grafts were successful in all of eight operations; axons running to corpuscles were found

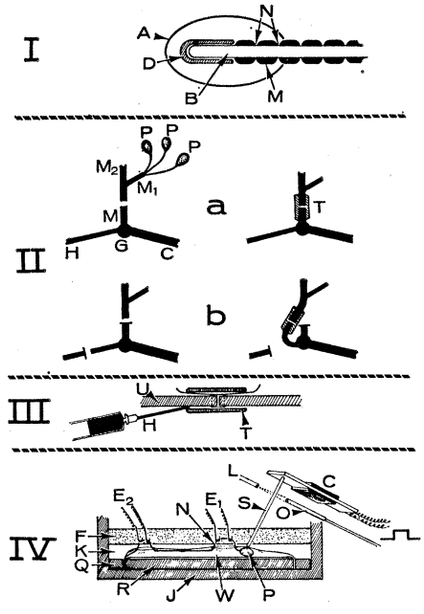

Fig. 1. (I) Diagram of an adult Pacinian corpuscle. (*B*) Nonmyelinated axon terminal, the site of transduction; (*N*) first and second intracorpuscular Ranvier nodes, sites of nerve impulse conduction; (*M*) myelinated axon; (*D*) a lamella cell. The capsule (*A*) consists of many concentric lamella cells. (II) Diagram of nerve transections (left) and unions (right). (*C*) Celiac root of inferior mesenteric (IM) ganglion (*G*); (*M*) IM nerve. This nerve collects all corpuscle (*P*) axons in the mesocolon via branch M_1 (branch M_2 innervates the intestine); (*H*) hypogastric nerve to bladder. (a) Inferior mesenteric nerve reunion. (b) Hypogastric-IM nerve union. The transverse bars represent sites of nerve ligation. (III) Diagram of nerve union procedure. Silk threads are passed through the nerve stumps (*U*) and the stumps are drawn together into a polycarbonate (Nucleopore) membrane tube (*T*). Blood is then injected (*H*) into the tube; the clot fibrin serves to guide the regenerating axons. (IV) Set-up for mechanical and electrical stimulation. (*J*) Lucite box with Krebs (*K*) and silicone (*F*) fluids; (*W*) mesocolon; (*N*) axon of a corpuscle (*P*); (E_1) and (E_2) recording and stimulating electrodes of reversible functions; (*C*) piezoelectric crystal for mechanical stimulation; (*S*) glass stylus transmitting crystal's deflection to corpuscle; (*L*) miniature light; and (*O*) photodiode for photoelectric monitoring of deflections (*10*). Drawings not to scale.

to respond to electrical stimulation 45 to 47 days after the hypogastric-IM nerve union. Out of 64 corpuscles with such foreign innervation, 23 generated potentials in response to mechanical stimulation (*14*). The sensitivity was of the same order as that of normally innervated corpuscles and of corpuscles reinnervated by IM nerve. The conduction velocities in the corpuscle fibers ranged from 1.6 to 34 m/sec, corresponding to myelinated fibers of 0.5 to 10 μm in diameter (*12*) (Table 1).

The possibility has to be considered that some of the corpuscles might have been reinnervated by stray fibers from the IM nerve in the hypogastric graft experiments. Such innervation must have been rare indeed. In three control animals, with a total of 34 mesocolonic corpuscles, in which the IM nerve was handled as in the experiments and the cutting and grafting of the hypogastric nerve was omitted, not a single corpuscle was reinnervated after 34 to 48 days. That corpuscles were in fact innervated by foreign fibers is particularly clear in three cases [Nos. 112(b) and 143, (a) and (b), in Table 1] in which the conduction velocities were 33 to 34 m/sec, corresponding to fiber diameters of about 10 μm. These fibers are much larger than those in IM reinnervation (Table 1), and, since regenerating fibers in general are considerably smaller than their predecessors (*13*), the hypogastric fibers from which these new corpuscle fibers grew out must have been larger than the original corpuscle afferents (*15*).

A series of experiments were performed to find out whether the regenerating axon, particularly its tip, is mechanosensitive before the axon enters the capsule. We could not visualize the outgrowing axon in vivo; the axon was obscured by the old nerve sheath in which it grew. It was clear, however, from histological preparations that the fibers reached the corpuscle via the sheath 20 to 35 days after the nerve reunions. We therefore applied mechanical stimuli on the sheath 1 mm away from the corpuscle during this time span. Occasionally action potentials were thus elicitable, but this required stimuli (displacements) of the order of 10^1 μm in contrast with the 10^{-2} μm that sufficed to excite the axon terminal inside the capsule. This enormous difference in sensitivity is not simply due to mechanical differences between the sheath and capsule: the responsiveness of the axon terminal immediately after

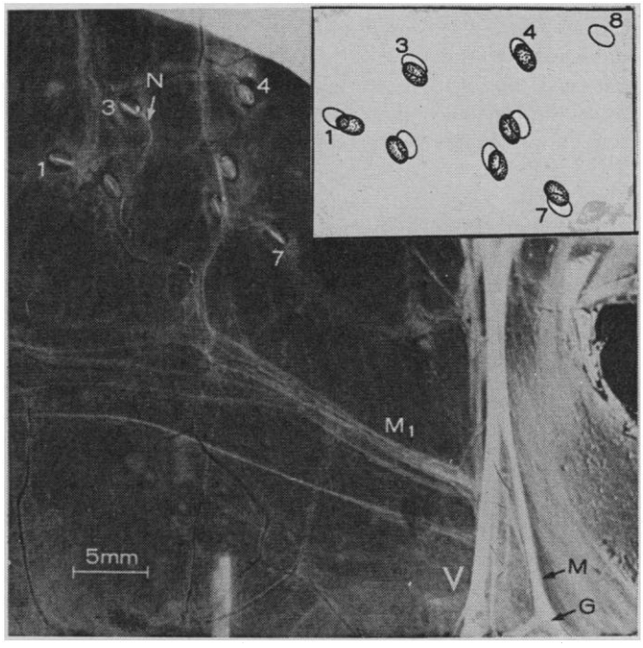

Fig. 2. Photograph in vivo of the entire working area showing the mesocolon with seven corpuscles (1 to 7), the IM ganglion (*G*), the IM nerve (*M*), and the artery (*V*). Some of the corpuscle axons (*N*) are seen through the mesocolon running separately over a length. All sensory axons go to the IM ganglia (*G*) via branch M_1 of the IM nerve. The inset gives the corpuscle locations before denervation and after reinnervation. The corpuscles are traced (gray) from the left photograph just before IM nerve transection and from a photograph 33 days thereafter (outlines), at the time receptor reinnervation was tested. The relative positions of corpuscles 1 to 7 are sensibly the same. See text concerning corpuscle 8. The slight changes in position are attributable to differences in stretch of the mesentery. Corpuscles 1, 3, 4, 7, and 8 were functional after reinnervation.

its capsule is cut away, or after the terminal is subsequently (artificially) wrapped in mesenteric tissue, is of the same high order as that of the terminal inside the capsule (*7, 10*). The response pattern was also quite different: a single step of displacement produced trains of action potentials rather than one single action potential, the typical response to stimulation of the intracorpuscular axon terminal (*16*). Moreover, the responsiveness of the extracorpuscular axons was irreversibly lost after a few stimuli, often after just one. For all these reasons, the responses elicited along the (extracorpuscular) length of a regenerating axon appear to have been due to injury depolarization, rather than to a specialized transducer action.

Table 1. New corpuscle innervation.

Animal	Time after nerve union (days)	Number of corpuscles tested	Number of corpuscles mechano-responsive	Axon conduction velocity (m/sec)*
		Reinnervation by inferior mesenteric nerve reunion†		
71	30	7	1	‡
74	30	14	0	2.9
76	32	8	1	3.3
79	40	8	2	8.8
81	40	9	0	0.8
140	33	8	4	(a) 2.8
				(b) 3.2
				(c) 5.2
		Innervation by hypogastric nerve graft		
112	47	8	2	(a) 23.8
				(b) 32.9
113	47	6	0	2.0
115	44	15	6	1.6
117	44	6	2	(a) 2.4
				(b) 12.7
118	47	4	0	33.3
120	48	10	5	(a) 8.4
				(b) 17.0
123	45	9	5	(a) 14.4
				(b) 15.4
				(c) 24.5
143	35	8	3	(a) 34.0
				(b) 33.6
				(c) 16.8

* Conduction velocities were not determined in some responsive corpuscles. The mean conduction velocity of the original corpuscle axons was 28.5 m/sec ± 4.4 standard deviation. † Not listed are the data of five animals in which there was no evidence of nerve regrowth. ‡ Action potentials were conducted by this axon, but the axon stretch available was too short for accurate velocity measurement.

The results thus reveal that a fully functioning transducer process can develop on the ending of a regenerating myelinated fiber, even a foreign one, when it enters the capsule. Such a transducer development may possibly involve the formation of new molecules or molecular arrangements, or the unlocking or activation of molecular structures preexisting all along the axon. A good possibility is that the transducer differentiation on the nerve ending is the result of an interaction between the nerve cell and the lamella cells of the capsule, that is, an exchange of molecular information at close range between these cells. The lamella cells make junctions with the nerve fiber (*17*), but there is as yet no evidence of whether the junctions are of a kind that allows passage of molecules between the cells (*18*).

It is not clear whether the lamella cells were from the old capsule or from a newly formed one around the regenerating nerve. The latter seems unlikely in most cases at least: in one animal, the number and relative positions of four excitable corpuscles, checked 10, 19, 27, and 33 days after nerve transection, remained unchanged after reinnervation by inferior mesenteric fibers. [The number and positions of three other capsules not mechanoresponsive also were constant (Fig. 2).] Only one excitable corpuscle was found

after reinnervation which had not been seen before. It is not certain, however, whether this corpuscle had arisen anew; the corpuscle lay in fat and may have escaped our notice earlier.

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References and Notes

1. N. Cauna and G. Mannan, *J. Anat.* **93**, 271 (1959); V. Bozilov, *Folia Morphol. Warsaw* **24**, 251 (1965).
2. W. R. Loewenstein, in *Handbook of Sensory Physiology*, vol. 1, *Principles of Receptor Physiology*, W. R. Loewenstein, Ed. (Springer-Verlag, New York, 1971), p. 269.
3. J. A. B. Gray and M. Sato, *J. Physiol. London* **122**, 610 (1953); W. R. Loewenstein, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 29 (1965).
4. W. R. Loewenstein, C. A. Terzuolo, I. Washizu, *Science* **142**, 1180 (1963).
5. N. Ishiko and W. R. Loewenstein, *J. Gen. Physiol.* **45**, 105 (1961); D. Inman and P. Peruzzi, *J. Physiol. London* **155**, 280 (1961).
6. W. R. Loewenstein, *J. Neurophysiol.* **24**, 150 (1961).
7. Destruction of the lamella cells in the adult corpuscle does not impair transduction [W. R. Loewenstein and R. Rathkamp, *J. Gen. Physiol.* **41**, 1245 (1958)].
8. A. J. Harris, *J. Comp. Neurol.* **79**, 1 (1943).
9. This was assured by careful scrutiny of the bladders of the animals used for the hypogastric graft experiments. The bladders were first examined in vivo under the dissecting microscope before the nerve operations. The bladder is translucent; had any Pacinian corpuscles been there, they would have been spotted even more easily than in the mesentery. After the experiments the bladders were stained with methylene blue and sliced up for further systematic microscopic scrutiny.
10. W. R. Loewenstein and M. Mendelson, *J. Physiol. London* **177**, 377 (1965).
11. R. J. C. Stewart, *J. Pathol. Bacteriol.* **43**, 339 (1936).
12. H. Grundfest, *Annu. Rev. Physiol.* **2**, 213 (1940). The spike durations were < 1.2 msec in all cases. Fiber diameters were calculated assuming

$$\frac{d\text{Velocity}}{d\text{Diameter}} = 6 \times 10^6 \text{ sec}^{-1} \text{ (37°C)}$$

[J. B. Hursch, *Amer. J. Physiol.* **127**, 131 (1939)]. Velocity data were multiplied by 1.8 to correct to 37°C [J. Tasaki and M. Fujita, *J. Neurophysiol.* **11**, 311 (1948)].
13. L. Guth, *Physiol. Rev.* **36**, 441 (1956).
14. The experiments with hypogastric fibers were done after those with IM fibers, when we had become better at joining severed nerves. This probably accounts for the higher incidence of hypogastric corpuscle innervation.
15. C. Dijkstra's [*Z. Mikrosk. Anat. Forsch.* **34**, 75 (1933)] elegant histological studies on transplanted Grandry corpuscles of the duck skin suggest that these receptors may receive foreign axons too. However, these studies, purely histological, do not show whether the reinnervated receptors were functioning [see also M. Jacobson, *Developmental Neurobiology* (Holt, Rinehart & Winston, New York, 1971), p. 288].
16. The response to a sustained step of compression of the axon terminal is a single action potential even after removal of the capsule, the mechanical high-pass filter of this sense organ; see (*10*).
17. B. L. Munger, in *Handbook of Sensory Physiology*, vol. 1, *Principles of Receptor Physiology*, W. R. Loewenstein, Ed. (Springer-Verlag, New York, 1971), p. 523.
→ W. R. Loewenstein, *Ann. N.Y. Acad. Sci.* **137**, 441 (1966); *Develop. Biol.* **19** (Suppl. 2), 151 (1968); E. Furshpan and D. D. Potter, *Curr. Top. Develop. Biol.* **3**, 95 (1968).
19. We thank Drs. M. Jacobson and S. J. Socolar for valuable discussion. Supported by research grants from the National Institute of Neurological Diseases and Stroke and from the National Science Foundation. Part of this work was done at the Cell Physics Laboratory, Physiology Department, Columbia University, College of Physicians and Surgeons.

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Field and Vegetable Crop Mutants with Increased Resistance to Herbicides

Abstract. *Wheat mutants with increased seedling resistance to terbutryn (2-tert-butylamino-4-ethylamino-6-methylthio-s-triazine) and tomato mutants with increased resistance to diphenamid (N,N-dimethyl-2,2-diphenylacetamide) were selected by the experimenters out of populations grown from seeds treated with ethyl methanesulfonate. Induced mutations may thus provide a tool for breeding crop cultivars with increased resistance to certain herbicides.*

There is extensive use of selective herbicides, that is, weed killers which control the weed population within a certain agricultural crop without causing economic injury to the crop. Their selectivity may be due to differences between the crop and the weeds in one or more of the following processes: uptake, translocation, inactivation, or breakdown of the herbicide. However, these differences often are small and dependent on climatic and soil factors. Consequently, slight changes in dosages, as well as effects of herbicide-environment interaction may result in damage to the crop or insufficient weed control. Moreover, no appropriate herbicides have yet been found for the control of the important weed species of some crops.

Intercultivar differences in resistance to herbicides have been reported (*1*). We investigated the possibilities of breeding crop cultivars with improved resistance to herbicides by means of induced mutations.

Mutations were induced in the spring wheat cultivar 'Alpha' and in the tomato cultivar 'VF 145-B 7879' by soaking the seeds at room temperature in 8 m*M* ethyl methanesulfonate (EMS) for 15 hours, and in 64 m*M* EMS for 24 hours, respectively. The M_2 populations contained plants with variegated leaves or other morphologic abnormalities. Seedling resistance of wheat to terbutryn (2-*tert*-butylamino-4-ethylamino-6-methylthio-*s*-triazine) and of tomato to diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide) was tested in the M_3 through M_5 and M_2 through M_4 generations, respectively (*2*). Seedlings were grown in loamy sand soil in undrained square plastic pots (17 by 17 by 7 cm). Terbutryn [1 part per million (ppm), by weight], or diphenamid (40 ppm) were applied by mixing them thoroughly with the soil prior to potting, on the day before sowing the seeds. In the replicated trials, six to ten seedlings of each mutant line, and an equal number of seedlings of the original cultivar, were grown in two parallel rows in each pot.

Screening of 50,000 M_3 plants, descending from 2,800 M_2 families, yielded 588 relatively resistant wheat seedlings. No seedlings resistant to the herbicide were found in a parallel screening of 10,000 plants of the original cultivar. Seven lines from two families were selected in M_4 by the experimenters (Fig. 1) and were then tested in a trial with ten replications in the M_5 generation. Every day, throughout the period of increasing seedling mortality, the percentage of surviving seedlings was significantly higher for

Table 1. Effect of diphenamid, added to soil prior to seed sowing, on the fresh weight of 25-day-old tomato seedlings.

Plant	Weight per seedling (mg)	
	Untreated	Treated
Original cultivar	728	433
M_4 lines	674	503
Standard error	26.7	26.7

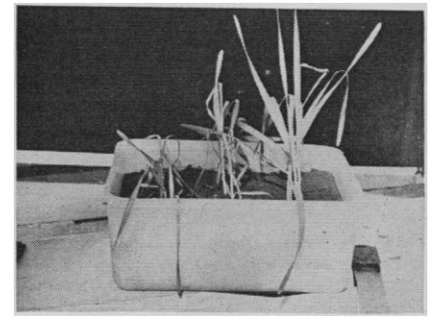

Fig. 1. Wheat seedlings grown in soil treated with terbutryn. Right, resistant M_4 line; center, original cultivar; left, susceptible M_4 line.