general, the average R of neural and liver tissues was somewhat lower than that of fibroblasts.

The mobility of a single surface molecule, the neural cell adhesion molecule (N-CAM), was also measured on chick brain and retina cells (Table 1, experiments 6 and 7). This cell surface molecule has been identified with the use of highly specific antiserums, and its role in cell-cell adhesion and development of neural tissue has been intensively studied in our laboratory (*13*). Despite its role in cell-cell adhesion, the mobility of this specific receptor was similar to that of the more general population of receptors measured using polyspecific anti-brain membrane serum.

Our results indicate that the average D's of a wide variety of surface receptors (but not necessarily all) fall within a narrow range, varying less than twofold under different conditions of cell growth and interaction. This variation is much less than the sixfold decrease in D seen on lectin-induced anchorage modulation (*4*). We conclude that if reversible modulation of receptor mobility is a significant mechanism for signaling cell-cell interactions, it must take place by the specific modulation of a small set of particular individual receptors rather than by general modulation of surface properties.

The differences in the fraction of mobile receptors observed between fibroblasts and the other cells suggest that the distribution of individual receptors in the population between the anchored and free mobility states may be characteristic of differentiation states, cell types, or morphologies. Consistent with this suggestion is the observation that about half of the cells measured in liver tissue showed no apparent recovery. Also, it has been shown that half of human lymphocytes labeled uniformly with a fluorescent monoclonal antibody against HLA antigens show no detectable redistribution of fluorescence after photobleaching, while the other half show redistribution with D of 6.9×10^{-10} cm^2/sec (*14*).

In summary, while Con A binding decreases receptor mobility in a variety of cells, the presence of cells in tissues does not appear to mimic this kind of modulation. However, receptors on about half of the cells in liver tissue labeled with polyspecific antibodies recognizing at least 15 different surface antigens, they were essentially immobile ($D < 5 \times 10^{-12}$ cm^2/sec). In contrast, the same receptors on dissociated liver cells showed values for D and R comparable to other cells. This suggests that naturally occurring modulation may take place by an "all or none" change (or greater than 100-fold decrease) in the mobility of specific receptors, rather than by a sixfold decrease as induced by lectins. Further experiments with monoclonal antibodies or other antibodies of very restricted specificity will be required to test this hypothesis.

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Pesticides: Insecticides and Fungicides Are Chitin Synthesis Inhibitors

Abstract. *Several important groups of fungicides and insecticides are specific inhibitors of chitin synthesis in a* Phycomyces *enzyme system and in insect organ cultures. The recently discovered benzoylphenylurea insecticides, which prevent chitin synthesis in insect tissues, are apparently not direct-acting chitin synthetase inhibitors. These insecticides may prevent insect chitin synthesis by interfering with the proteolytic activation of the chitin synthetase zymogen.*

The biosynthesis of chitin skeletal structures is a promising molecular target for pesticide action, since chitin is restricted in its biological distribution (*1*). The effects of fungicides and insecticides on chitin synthesis have received increasing attention following reports (*2, 3*) that several agricultural chemicals affect insect chitin synthesis [also see review (*4*)].

We have developed biochemical and tissue culture methodologies that allow us to examine the mode of action of compounds that specifically interfere with chitin biosynthesis in insects and fungi. Table 1 lists compounds that are specific inhibitors of a cell-free preparation of chitin synthetase derived from the fungus *Phycomyces*. Specificity is defined as resistance of I_{50} level inhibition to the addition of excess protein (ovalbumin) (I_{50} is the concentration in moles of the compound that produces 50 percent inhibition of control chitin synthetase activity). This test establishes that the specific compounds do not react nonselectively with polypeptide functional groups. Compounds active in the *Phycomyces* system include chlorinated hydrocarbons, triazines, nitrophenols, organophosphates, sulfenimides, and thiolanes. Many of these compounds were not pre-

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Table 1. Compounds that are chitin synthesis inhibitors in the *Phycomyces* and cockroach systems. *Phycomyces* germlings were harvested at mid-log growth phase from a defined minimal medium (*22, 23*) by vacuum filtration and were washed with breakage buffer (0.1*M* tris, *p*H 8.0; 0.02*M* $MgCl_2$; 10 percent glycerol; 0.001*M* EDTA, and 0.001*M* dithiothreitol). The washed germlings were resuspended in breakage buffer and disrupted by grinding with glass beads on a chilled mortar and pestle. The homogenate was centrifuged at 10,000*g* for 10 minutes. The pellet fraction was resuspended in breakage buffer and centrifuged again at 10,000*g* for 10 minutes. The resulting supernatant fraction was discarded, and the washed pellet fraction was resuspended in breakage buffer and used as a cell-free chitin synthetase preparation (*24*). The utilization of the cockroach leg regenerate system for assessing chitin synthesis inhibitors has been described in detail (*4*). The cytotoxicity of compounds was assessed by observing explant cultures in Rose chambers in the presence of an I_{90} to I_{99} concentration of the test material. If no interference with cuticle deposition was observed, the compounds were considered nontoxic.

Compound class	Compounds	I_{50} (mole/liter)	
		Phycomyces	Cockroach
Chloro and fluoro hydrocarbons	Chlordane	3.1×10^{-5}	
	p,p'-DDT	3.1×10^{-5}	7.3×10^{-6}
	Endosulfan	3.0×10^{-5}	
	Dieldrin	1.6×10^{-4}	
	2,2'-Methylenebis[4-chlorophenol]	4.9×10^{-5}	4.0×10^{-6}
Nucleoside and base analogs	Polyoxin D	2.9×10^{-4}	3.4×10^{-9}
	2,4-Dichloro-6-methylpyrimidine	1.6×10^{-4}	
Triazines	Azidotriazine	1.5×10^{-3}	4.0×10^{-9}
Sulfenimides	Captan	3.9×10^{-4}	See (*3*)
Dithiazoliums	Dithiazolium iodide	1.1×10^{-3}	7.7×10^{-8}
Organophosphates	Kitazin-P	4.7×10^{-4}	See (*3*)
Dinitrophenols and benzoylphenylureas	Dinocap	5.0×10^{-5}	
	Dimilin	$> 10^{-2}$	9.4×10^{-11}
	SIR8514	$> 10^{-2}$	2.1×10^{-11}
	SIR6874	$> 10^{-2}$	6.0×10^{-11}
	Penfluron	$> 10^{-2}$	1.6×10^{-11}
Thiolanes	Isoprothiolane	6.6×10^{-5}	1.3×10^{-7}

viously suspected to be chitin synthetase inhibitors. Various chemical structures can block chitin synthetase; most of these compounds are reversible inhibitors and in general do not compete with the substrate for access to the active site of the enzyme. The fungicide polyoxin D is a competitive, substrate-analog type of chitin synthetase inhibitor (*5*). A number of chlorinated hydrocarbons, carbamates, and other compounds demonstrate nonspecific inhibition in the *Phycomyces* system (data not shown).

Benzoylphenylureas (60-40, 60-38, DU119111, SIR8514, SIR6874, and Penfluron), herbicides (2,4-dichlorophenoxyacetic acid and simazine), Dichloran, and 5-fluorouracil have no effect on *Phycomyces* chitin synthetase when tested at their aqueous solubility limits or at a concentration of at least $10^{-2}M$. Chymostatin ($1.1 \times 10^{-5}M$), soybean trypsin-chymotrypsin inhibitor ($1.4 \times 10^{-4}M$), and lima bean trypsin-chymotrypsin inhibitor ($1.2 \times 10^{-4}M$) also have no effect on chitin synthetase activity.

A number of compounds that inhibit chitin synthesis in the *Phycomyces* system also inhibit chitin synthesis in cultured insect tissues (Table 1). The organophosphates kitazin-P and parathion, and the sulfenimide captan are inhibitory in this insect system (*3*). Since none of these compounds produce cytotoxic effects in cockroach organ cultures, they selectively prevent chitin synthesis without affecting the biosynthesis of other cuticular components.

The benzoylphenylureas do not inhibit cell-free preparations of either fungal or insect chitin synthetase (*6, 7*) but are highly active in insect systems. Their potency might therefore be explained by assuming that these compounds do not interact with the large amount of active chitin synthetase present in insect cells but rather affect a cascade event involved in enzyme biosynthesis—namely, the proteolytic activation of the chitin synthetase zymogen. The existence of zymogen forms of chitin synthetase in fungi has been reported (*8*).

Table 2. Inhibition of chymotrypsin activity by 60-40 and 60-38. The activity of chymotrypsin was assayed as described by Leighton *et al.* (*9*). In typical protease assays, 6 to 10 pmole of enzyme in 1 ml of reaction mixture was incubated for 18 hours at 37°C. Experiments (30 to 50 pmole of enzyme in 0.2 ml of 0.05*M* tris, *p*H 8.0, and 0.01*M* $CaCl_2$) included appropriate solvent and enzyme controls. All molar ratio circulations were based on the aqueous solubility limits of the inhibitors.

Molar ratio of inhibitor to enzyme	Time before incubation (hours)	Percent inhibition
	Compound 60-40	
20:1	1	36
20:1	2	57
20:1	4	72
20:1	6	87
10:1	3	43
	Compound 60-38	
60:1	1	3
60:1	2	18
60:1	4	20
60:1	6	32
30:1	3	17

Model experiments show that the benzoylphenylurea 60-40 (Dimilin) and the less effective insecticide 60-38 (*10, 11*) are direct-acting serine protease inhibitors (Table 2). These compounds have a slight preference for chymotrypsin-like proteases (data now shown). Several known chymotrypsin inhibitors (*12–14*) prevent chitin synthesis in the cockroach system (I_{50} values of active inhibitors are chymostatin, $2.3 \times 10^{-7}M$; 2-nitro-4-carboxyphenyl-*N,N*-diphenylcarbamate, $4.0 \times 10^{-6}M$; lima bean trypsin-chymotrypsin inhibitor, $1.2 \times 10^{-6}M$; and soybean trypsin-chymotrypsin inhibitor, $1.9 \times 10^{-6}M$). Leupeptin, antipain, and pepstatin A—inhibitors of trypsin, plasmin, pepsin, renin, and a variety of proteolytic enzymes other than chymotrypsin (*12, 15*)—show no effect in this system at $10^{-5}M$. None of these inhibitors produce cytotoxic effects in cockroach organ cultures.

An unexpected finding is that many currently used insecticides and fungicides are specific chitin synthetase inhibitors (Table 1). Surprisingly, neurotoxins (*16, 17*) and oxidative phosphorylation or respiratory chain inhibitors (*18*) affect the process of chitin biosynthesis. Whether the targets of these inhibitors share common receptor sites, or whether these molecules can disturb membrane structure in a manner that affects the activity of a few cognate polypeptides, is not known. Since many of the inhibitory activities we observe are stereochemically constrained, further quantitative structure-activity studies should aid in the design of more selective and effective chitin synthetase inhibitors. Isoprothiolane is a chitin synthesis inhibitor in both the *Phycomyces* and cockroach systems. It also inhibits carbohydrate uptake in fungal cells (*19*). Its low mammalian toxicity makes it an attractive new compound class for the development of antimycotic and anti-insect agents.

The benzoylphenylureas, which control insect populations at extremely low doses, appear to selectively derange the synthesis of insect chitin-containing

structural elements (*2, 3, 10*). The data presently available indicate that benzoylphenylureas are not direct-acting chitin synthetase inhibitors, but rather that they are direct-acting serine protease inhibitors that block the conversion of chitin synthetase zymogen into active enzyme. Thus a variety of specific chymotrypsin inhibitors, which are not directed against other serine and nonserine active site proteases, are capable of selectively blocking insect chitin synthesis. These data provide evidence for the critical involvement of a chymotrypsin-like protease in insect chitin biosynthesis. Recently, Strauss *et al.* (*20*) implicated a chymotrypsin-like protease in the processing of the pre-segment of human secretory proteins. Furthermore, Green and Ryan (*21*) observed that injury to plant leaf surfaces elicits a hormonally mediated response resulting in the production of large quantities of polypeptide trypsin and chymotrypsin inhibitors. This plant defense system may have the same mode of action as the benzoylphenylureas.

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Disease Resistance: Incorporation into Sexually Incompatible Somatic Hybrids of the Genus *Nicotiana*

Abstract. *Somatic hybrid plants of* Nicotiana nesophila *and* N. stocktonii *with* N. tabacum *(cultivated tobacco) were produced by protoplast fusion. These combinations cannot be achieved with conventional sexual hybridization, yet are important in that the wild* Nicotiana *species are resistant to numerous diseases. Hybridity was verified by chromosome number, isoenzyme analysis, morphological characteristics, and genetic behavior. Local lesion-type resistance to tobacco mosaic virus has been observed in leaves of these somatic hybrid plants.*

Wild species of *Nicotiana* have been used to incorporate disease resistance into cultivated tobacco (*1*). The three *Nicotiana* species of the section *Repandae* (*N. nesophila*, *N. repanda*, and *N. stocktonii*) are among those species resistant to the most diseases of cultivated tobacco. Attempts to crossbreed these wild species with cultivated tobacco by conventional breeding techniques have been unsuccessful (*2, 3*). Two of these species, *N. nesophila* and *N. stocktonii*, have been crossed with *N. tabacum* (*4*) by means of ovule culture in vitro. How-

Table 1. Comparison of morphological characteristics of *N. tabacum* + *N. nesophila* (NN + Su/Su) somatic hybrid plants with the two parental species.

Plant	Flower		Leaf (cm)		Pollen viability (%)
	Color	Length* (cm)	Length of blade*	Maximum width*	
N. tabacum (Su/su)	Dark pink	5.28 ± 0.07	23.00 ± 2.48	7.25 ± 1.23	97.5
NN + Su/Su (somatic hybrids)	Light pink	5.09 ± 0.08	18.25 ± 1.31	8.13 ± 0.32	55.3
N. nesophila (NN)	White	4.95 ± 0.07	13.14 ± 0.46	9.29 ± 0.48	96.5

*Measurement expressed as mean ± standard error with differences significant, with *P* less than .05, for each group of plants.

Table 2. Segregation of the Su locus controlling leaf pigmentation in sexual progeny of *N. tabacum* + *N. nesophila* (NN + Su/Su) somatic hybrid plants.

Sexual cross	Dark green	Light green	Albino
(NN + Su/Su) × *N. tabacum* (su/su)	58	36	0
(NN + Su/Su) × *N. nesophila*	11	14	0
N. tabacum (su/su) × (NN + Su/Su)	3	2	0
(NN + Su/Su) × self	11	35	1

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