washed from the cells, its inhibitory effect was reversible. The trypan blue exclusion technique for quantifying the number of viable cells (*13*) showed that viability of controls and lymphocytes incubated for 72 hours in concentrations as high as 10,000 I.U./ml of HCG was identical.

Our studies indicate that HCG is a potent and reversible inhibitor of the response of human lymphocytes to PHA and that the inhibition occurs without cytotoxicity. HCG in concentrations as little as 1 I.U./ml causes some effect, and the effect becomes marked at and above 100 I.U./ml, which is within the range of the concentration of HCG in human serum during the last half of gestation (*14*). More important, however, is the essentially complete inhibition of the lymphocyte activation achieved by 10,000 I.U./ml. The studies of Braunstein *et al.* (*5*) suggest that trophoblasts from the 10-day embryo could produce local concentrations, where the maternal lymphocytes are in actual contact with the trophoblasts, which far exceed 10,000 I.U./ml. These data suggest that maternal lymphocyte immunocompetence during pregnancy may be altered by HCG in a reversible, noncytotoxic manner. Although PHA stimulation of lymphocytes is a standard test in evaluating lymphocyte function, its relation to physiological and pathological stimulation is unclear. It is necessary to further investigate this inhibitory effect of HCG with the use of other systems to evaluate immunocompetence.

EUGENE W. ADCOCK III
FRANÇOIS TEASDALE
CHARLES S. AUGUST, SHEILA COX
GIACOMO MESCHIA
FREDERICK C. BATTAGLIA
MICHAEL A. NAUGHTON
Division of Perinatal Medicine, Departments of Pediatrics, Physiology, and Obstetrics-Gynecology, University of Colorado Medical Center, Denver 80220

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Synergism of Insecticides by Herbicides

Abstract. *The herbicides atrazine, simazine, monuron, and 2,4-D (2,4-dichlorophenoxyacetic acid) enhanced the toxicity of selected insecticides to* Drosophila melanogaster *Meigen,* Musca domestica *L., and larvae of* Aedes aegypti *L. The insecticides—nine organophosphorus compounds, two chlorinated hydrocarbons, and one carbamate—were used at dosages that resulted in low insect mortalities, while the herbicides by themselves were nontoxic. Atrazine was most effective. With increasing amounts of this herbicide and constant amounts of some insecticides, increasing mortalities of fruit flies were observed. Exposure of the insects for 24 hours to carbofuran (0.5 microgram),* p,p'-*DDT [1,1,1-trichloro-2,2-bis*(p-*chlorophenyl)ethane] (4 micrograms), parathion (0.35 microgram), and diazinon (0.2 microgram) alone resulted in mortalities of 7.5, 9.5, 8, and 10.5 percent, respectively. Based on dosage mortality curves obtained with increasing amounts of atrazine, mortalities of 50 percent of the insect populations would have been achieved with 23, 40, 6, and 10 micrograms of atrazine added to the above-mentioned dosages of carbofuran, DDT, parathion, and diazinon, respectively.*

The effects of pesticides or other synthetic chemicals on biological systems have usually been investigated by utilizing one particular test chemical. However, under actual environmental conditions, particularly in agricultural situations, a mixture of synthetic chemicals or their metabolites (or both) is present, and these chemicals may interact in biological systems. For example, detergents increase the persistence and toxicity in soils of the organophosphorus insecticides parathion and diazinon (*1*), reduce the penetration of parathion into pea roots, and inhibit the translocation of lindane into pea greens (*2*). Street (*3*) stressed the ecological significance of pesticide interactions, stating that "DDT and dieldrin are additive at low dosages in inducing testosterone metabolism in pigeon liver." Street *et al.* (*4*) compared the effects of polychlorinated biphenyl compounds (PCB's) and organochlorine pesticides on the induction of hepatic microsomal enzymes. Tsao *et al.* (*5*) reported that Aroclor 5460 greatly increased the residual toxicity

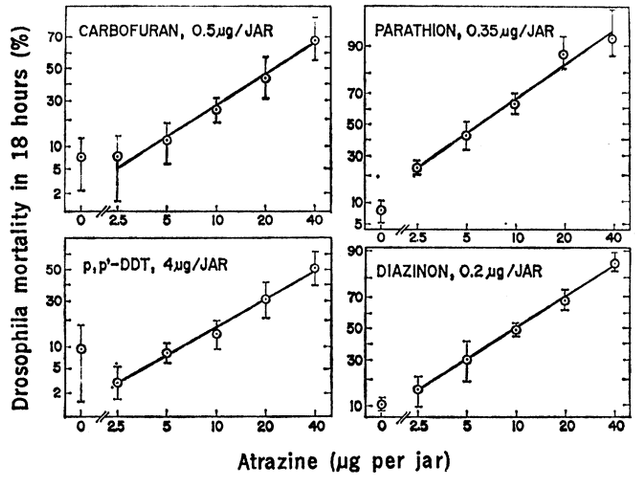

Fig. 1. Effects of increasing dosages of atrazine (0 to 40 µg per jar) in synergizing the toxicities of insecticides applied at constant dosages with the herbicide.

Table 1. Effect of herbicides on the toxicity of parathion and DDT to three insect species. Percent insect mortality is reported for a 24-hour exposure period; values are the means and standard deviations of four or eight replicate tests. In the ratio, $H + I$ is the mortality observed with herbicide plus insecticide, and I is the mortality observed with insecticide alone. For *Drosophila* the herbicide concentration was 40 μg per (4-ounce) bioassay jar; parathion, 0.35 μg per jar; DDT, 2 μg per jar. For *Musca* chemicals were applied topically; the herbicide concentration was 15 μg per fly; parathion, 0.02 μg per fly; DDT, 0.1 μg per fly. For *Aedes* chemicals were applied in water; the herbicide concentration was 10 ppm; parathion, 0.016 ppm; DDT, 0.18 ppm.

Insecticide	No herbicide	Atrazine		Simazine		Monuron		2,4-D	
	Insect mortality (%)	Insect mortality (%)	Ratio $\frac{H+I}{I}$	Insect mortality (%)	Ratio $\frac{H+I}{I}$	Insect mortality (%)	Ratio $\frac{H+I}{I}$	Insect mortality (%)	Ratio $\frac{H+I}{I}$
				Drosophila melanogaster					
None	0	0		0		0		0	
Parathion	9 ± 5.0	48 ± 10*	5.3	23 ± 2†	2.6	24 ± 3.7†	2.7	15 ± 3	1.7
DDT	10 ± 2.5	22 ± 5.4†	2.2	25 ± 13§	2.5	17 ± 7.9	1.7	18 ± 4.5‡	1.8
				Musca domestica					
None	0	0				0		0	
Parathion	11 ± 3.4	45 ± 20*	4.1			40 ± 15*	3.6	15 ± 12	1.4
DDT	8 ± 5.6	25 ± 8.6*	3.1			20 ± 10†	2.5	7 ± 7	0.9
				Aedes aegypti					
None	0	0		0		0		0	
Parathion	15 ± 3.4	80 ± 11*	5.3	67 ± 5.4*	4.5	53 ± 9.4*	3.5	48 ± 8.4*	3.2
DDT	10 ± 8.6	12 ± 11	1.2	10 ± 3.8	1.0	13 ± 12	1.3	17 ± 3.9	1.7

Results significantly different from the control (no herbicide) at the * 0.1 percent level, † 1 percent level, ‡ 5 percent level, § 10 percent level.

of lindane, although it had no toxic effect on *Musca domestica* L. by itself. They also indicated that this polychlorinated polyphenyl compound may have a synergistic effect with lindane. Lichtenstein *et al.* (*6*) demonstrated synergistic effects of PCB's on DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane] and dieldrin in insects, and Fuhremann and Lichtenstein (*7*) showed that PCB's increased in particular the toxicity of the oxygen analogs of organophosphorus insecticides to houseflies. Plapp (*8*) showed that the PCB Aroclor 1254 synergized a carbamate insecticide, carbaryl. Because of these interactions of pesticides with other environmental chemicals in biological systems, we investigated the effects of several herbicides on the toxicity of some commonly used insecticides and on some of their toxic metabolites.

Insects were exposed to herbicides or insecticides, alone or in combinations with each other. The herbicides used were analytical grade atrazine, simazine, monuron, and 2,4-D (2,4-dichlorophenoxyacetic acid). The insecticides and some of their potential metabolites used (see Table 2) were also analytical grade. The three insect species that were exposed to these chemicals were fruit flies (*Drosophila melanogaster* Meigen), a DDT-susceptible strain (CSMA-1948) of houseflies (*Musca domestica* L.), and mosquito larvae (*Aedes aegypti* L.). With *Drosophila*, 3-day-old flies were exposed to dry residues of the chemicals or combinations thereof in 4-ounce (~120-ml) test jars (*9*). Appropriate amounts of atrazine, simazine, or one of the insecticides in chloroform, and of monuron and 2,4-D in acetone were pipetted into the bioassay jars, and the solvents were evaporated. Fifty fruit flies were then placed in each jar containing the dry pesticide residues, and mortality counts were periodically made over a 24-hour exposure period. Each test was replicated four or eight times. With *Musca domestica* the pesticides in 2 μl of solvent were applied topically to the ventral portion of female houseflies. Insecticides or atrazine (or both) were applied in methanol, and monuron and 2,4-D were applied in acetone. Simazine, because of its low solubility in these solvents, was not used with houseflies. Tests were conducted with four or eight replicates, each consisting of 15 houseflies. In experiments with *Aedes aegypti* appropriate amounts of insecticides or herbicides (or both) in chloroform were added to tap water; 2,4-D was added in acetone. The solvents were removed from the water by evaporation under vacuum, the water was adjusted to volume, and 10-ml aliquots were placed in small glass vials. For each test, 15 third-instar mosquito larvae were introduced into the treated water and mortality counts were made over a 24-hour period; tests were replicated four or eight times. The results from all experiments were expressed as the mean and the standard deviation of the percentage mortalities observed after a specific period of exposure to the pesticides. The differences between mortalities due to insecticides and those

Table 2. Increase in toxicity of insecticides to *Drosophila melanogaster* Meigen when atrazine is also present. The results are for 24-hour exposure periods; insect mortality values are means and standard deviations for four or eight replicated tests. The atrazine concentration was 40 μg per (4-ounce) jar. In the ratio, $H + I$ is the observed mortality with herbicide plus insecticide; I is the observed mortality with the insecticide alone.

Insecticide	Insecticide concentration (μg per jar)	Insect mortality (%) with		Ratio $\frac{H+I}{I}$
		No herbicide	Atrazine	
None	0.00	0	0	
Parathion	0.35	9 ± 8.4	77 ± 9.9*	8.6
Paraoxon	0.35	13 ± 2.5	52 ± 2.5*	4.0
Diazinon	0.20	9 ± 3.5	33 ± 11.7‡	3.7
Diazoxon	0.70	14 ± 2.3	82 ± 21†	5.9
Dyfonate	0.35	31 ± 6.8	73 ± 16‡	2.4
Dyfoxon	0.35	12 ± 9.1	33 ± 12§	2.8
Phorate	0.60	22 ± 4.4	52 ± 6.6†	2.4
Phorate sulfoxide	3.5	23 ± 5.3	86 ± 29‡	3.7
Phorate sulfone	2.2	18 ± 3.0	91 ± 14*	5.1
Carbofuran	0.7	43 ± 18	93 ± 4.8‡	2.2
DDT	2.0	17 ± 15	57 ± 12‡	3.4
Dieldrin	0.14	5 ± 4.1	36 ± 4.4*	7.2

Results significantly different from the control (no herbicide) at the * 0.01 percent level, † 0.1 percent level, ‡ 1 percent level, § 5 percent level.

due to insecticides plus herbicides were statistically analyzed by the *t*-test.

In the first experimental series, synergistic effects of herbicides on insecticides were studied by exposing fruit flies, houseflies, or mosquito larvae to parathion, *p,p'*-DDT, one of the four herbicides, or insecticide-herbicide combinations as indicated in Table 1. The insecticide dosages chosen were such that insect mortalities obtained after 24 hours of exposure to insecticides alone were relatively low. The results indicate (Table 1) that in most cases all four herbicides increased the toxicity of the insecticides. This increase was greater with parathion than with DDT. Atrazine was most effective, increasing significantly (at the 0.1 percent level) the toxicity of parathion to fruit flies and mosquito larvae by a factor of 5.3, and to houseflies by a factor of 4.1. Least effective was 2,4-D, which increased insecticide toxicity in only two out of six cases (DDT toxicity with fruit flies and parathion toxicity with mosquito larvae). No synergistic effects on the toxicity of DDT toward mosquito larvae were observed with any of the herbicides. Although enough DDT was added to the water to give a concentration of 0.18 part per million (ppm), this concentration was probably never obtained because of its low water solubility (0.001 ppm). It is, therefore, questionable as to what an extent the larvae had a chance to come into contact with the insecticide.

In the second experimental series, the synergistic effects of atrazine on the toxicity of 12 insecticides were studied with fruit flies. The insecticides included nine organophosphorus compounds, one carbamate, and two chlorinated hydrocarbons (Table 2). Insects were exposed to dry deposits of atrazine, one of the 12 insecticides, or atrazine-insecticide combinations. Atrazine was always applied at 40 μg per bioassay jar and the insecticides in amounts as indicated in Table 2. The toxicity of all the insecticides was significantly increased by atrazine. This increase ranged from a factor of 2.2 to 8.6, depending on the insecticide present. From the data reported above (Tables 1 and 2) it appears that the phenomenon of synergism of insecticides by selected herbicides is rather general, therefore suggesting that the mode of action of the herbicides was not related to the blocking of specific detoxifying enzyme systems within the insect body. Other factors, such as an increase in insecticide penetration through the insect cuticle, could possibly have played a role in these phenomena.

In a third experimental series fruit flies were exposed to fixed amounts of insecticides (carbofuran, 0.5 μg; DDT, 4 μg; parathion, 0.35 μg; or diazinon, 0.2 μg) and increasing amounts of atrazine (from 2.5 to 40 μg per bioassay jar) in order to ascertain a potential dose-response relation of the synergistic effects of the herbicide. Results presented in Fig. 1 indicate that with increasing amounts of atrazine, increasing insect mortalities occurred during the 18-hour exposure period. Exposure of the fruit flies to carbofuran, DDT, parathion, or diazinon alone resulted in mortalities of 7.5, 9.5, 8, and 10.5 percent, respectively. The dosage-mortality curves indicate that 50 percent mortality of the insect populations would have been achieved with the addition of 23, 40, 6, and 10 μg of atrazine, respectively.

Results with fruit flies as shown in Tables 1 and 2 and Fig. 1 were obtained in different tests conducted over a period of several months. Since the susceptibility of the insects to pesticides fluctuates to some extent from week to week, quantitative differences in various data are due to these fluctuations.

This report further illustrates the necessity for continued investigations of the interactions of pesticides with other chemicals in biological systems. Although it is imperative to study the fate and behavior of single environmental chemicals, it is also apparent that these problems must be approached within the concept of a whole system in which chemicals can interact with each other.

E. P. LICHTENSTEIN
T. T. LIANG, B. N. ANDEREGG
Department of Entomology, University of Wisconsin, Madison 53706

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Peroxidase Mediated Antimicrobial Activities of Alveolar Macrophage Granules

Abstract. *The 20,000g pellet obtained by centrifugation of a homogenate of rabbit alveolar macrophages has antibacterial activity in the presence of a hydrogen peroxide–generating system and iodide. Peroxidase activity has been demonstrated in this fraction. Addition of 3-amino-1,2,4-triazole diminished the antibacterial activity of the pellet–hydrogen peroxide–iodide system.*

The precise mechanism (or mechanisms) by which phagocytes inactivate intracellular microorganisms has been a subject of study for almost a century. Renewed interest in this subject is mainly due to the finding of a myeloperoxidase (MPO)–H_2O_2–halide antimicrobial system in the polymorphonuclear neutrophilic leukocytes (PMN). Individually the components of the reaction are devoid of antimicrobial activity at the concentration employed. They appear to function only together (*1*).

To our knowledge, the antimicrobial activities of alveolar macrophages (AM) fractions have not yet been reported. Since these macrophages are generally reported to have either no peroxidase or insignificantly low activity (*2*), possible functioning of a peroxidase–H_2O_2–halide antimicrobial system in these cells has not yet attracted much attention. Species differences in peroxidase activity of mononuclear cells have been demonstrated (*2, 3*). In this communication we demonstrate that the fraction prepared from the homogenate of AM centrifuged at 20,000*g*, which contains most of the peroxidase activity, in the presence of H_2O_2 and I^-, is able to kill *Escherichia coli*. We also show that 3-amino-1,2,4-triazole (AT), an inhibitor of AM peroxidase activity