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Comparative Effects of Piperonyl Butoxide and *N*-(4-Pentynyl)phthalimide on Mammalian Microsomal Enzyme Functions[1]

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Methylenedioxyphenyl (MDO) derivatives, such as piperonyl butoxide (PB), markedly enhance the insecticidal effects of pyrethrins, but are less effective with synthetic pyrethrin analogs such as allethrin; *N*-(4-pentynyl)-pthalimide (NPP) synergizes with allethrin strongly and with pyrethrins weakly. In the present studies, both PB and NPP synergists produced marked and similar inhibition of hepatic microsomal enzyme function in mice, as measured indirectly by prolongation of hexobarbital narcosis and zoxazolamine paralysis, and as measured directly by inhibition of *ortho* hydroxylation of biphenyl, hydroxylation of hexobarbital, demethylation of aminopyrine, and by stimulation of *para* hydroxylation of biphenyl.

Although natural pyrethrins (Figure 1) are efficient insecticides and of low toxicity to mammals, their use has been limited by high costs and restricted availability.[3] This limitation has been partly offset by use of methylenedioxyphenyl (MDO) derivatives, such as piperonyl butoxide (PB),[4] which markedly enhance activity of pyrethrins. While allethrin, a synthetic pyrethrin analog,[5] is equally insecticidal as nonsynergized natural pyrethrins, it is, however, less susceptible to MDO synergism.[6] Recently, synthetic alkynylphthalimides, particularly *N*-(4-pentynyl)phthalimide (NPP), have been shown to synergize with allethrin strongly and with natural pyrethrins weakly (Figure 1).[7] Other synthetic acetylene derivatives, such as propynyl aryl ethers, also synergize with carbamate insecticides; the mode of action of alkynyl compounds has been attributed to a specific interaction of the acetylenic moiety with SH groups of active enzymes or the essential metal cofactors of the mixed function oxidases.[8]

Putatively, synergists, such as PB, act by blocking detoxifying microsomal enzymes in insects.[3] PB also inhibits drug-hydroxylating systems in mammalian liver,[9] accounting presumably for the synergistic acute toxicity induced in mice by combined administration of PB with various drugs and environmental pollutants.[10] The apparent selective synergism of natural and synthetic pyrethrins by PB and NPP, respectively, could reflect different effects of these agents on the microsomal detoxification of the pyrethroids in insects. Accordingly, we have attempted to determine whether these two classes of synergists act differently on hepatic microsomal enzyme functions in mammals.

Method.—PB and NPP were tested on microsomal enzyme functions in mice with three substrates: aminopyrine, hexobarbital, and biphenyl; biphenyl provides a differential measure with respect to alteration of the specific hydroxylations at *ortho* and *para* positions.[11] Groups of 3 adult (30 g) male Swiss mice (ICR/Ha), maintained on Purina chow and water *ad libitum*, were injected ip with 0.1-ml suspensions of PB or NPP in 1% Tween-20 (v/v) at 10, 40, 160, and 640 mg/kg. Test and control mice were sacrificed 1 hr later by cervical dislocation; crude microsomal fractions (10,000*g*/15 min) from liver homogenates in 0.25 *M* sucrose–phosphate buffer at pH 7.4 were prepared and stored 24–48 hr at −30° prior to test. The 4 enzyme activities were assayed concurrently and also on occasion independently at different times after storage on each individual fraction from control and treated groups. Experiments were replicated between 3 and 8 times. In the case of the *ortho* hydroxylation of biphenyl with NPP, 5 pooled samples of livers were each prepared and assayed in duplicate. Enzyme activities referred to the amount of substrates, expressed as OD or as calculated weight, hydroxylated per hr per 250-mg aliquot of liver tissue. Log dose–response regressions were calculated on the basis of the differences between mean activities of test and control groups; the dose-dependent effects of PB and NPP were expressed in terms of slope values (Figure 2 and Table I).

Additionally, the effects of each synergist on the duration of hexobarbital narcosis and zoxazolamine paralysis in groups of 8 mice were tested at fourfold increments, ranging from 2.5 to 640 mg/kg, by previously described techniques.[12]

Results and Discussion

Both synergists produced similar patterns of inhibition of *para* hydroxylation of biphenyl, of hydroxylation of hexobarbital, and of dealkylation of aminopyrine (Figure 2 and Table I). Comparable weak stimulation

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(3) P. S. Hewlett, *Chem. Ind.*, **22**, 701 (1968).

(4) H. Wachs, *Science*, **105**, 1530 (1947).

(5) M. Schechter, N. Green, and F. LaForge, *J. Amer. Chem. Soc.*, **71**, 3165 (1949).

(6) J. E. Moore, *Advan. Chem. Ser.*, **53** (1966).

(7) J. L. Neumeyer and H. H. Incho, German Patent 1,217,693; *Chem Abstr.*, **65**, 8830*g* (1966).

(8) R. M. Sacher, R. L. Metcalf, and T. R. Fukuto, *J. Agr. Food Chem.*, **16**, 779 (1968).

(9) H. Jaffe, K. Fujii, M. Sengupta, H. Guerin, and S. S. Epstein, *Life Sci.*, **7**, 1051 (1968).

(10) S. S. Epstein, J. Andrea, P. Clapp, and D. Mackintosh, *Toxicol. Appl. Pharmacol.*, **11**, 442 (1967).

(11) P. J. Creaven and D. V. Parke, *Biochem. Pharmacol.*, **15**, 7 (1966).

(12) K. Fujii, H. Jaffe, and S. S. Epstein, *Toxicol. Appl. Pharmacol.*, **13**, 431 (1968).

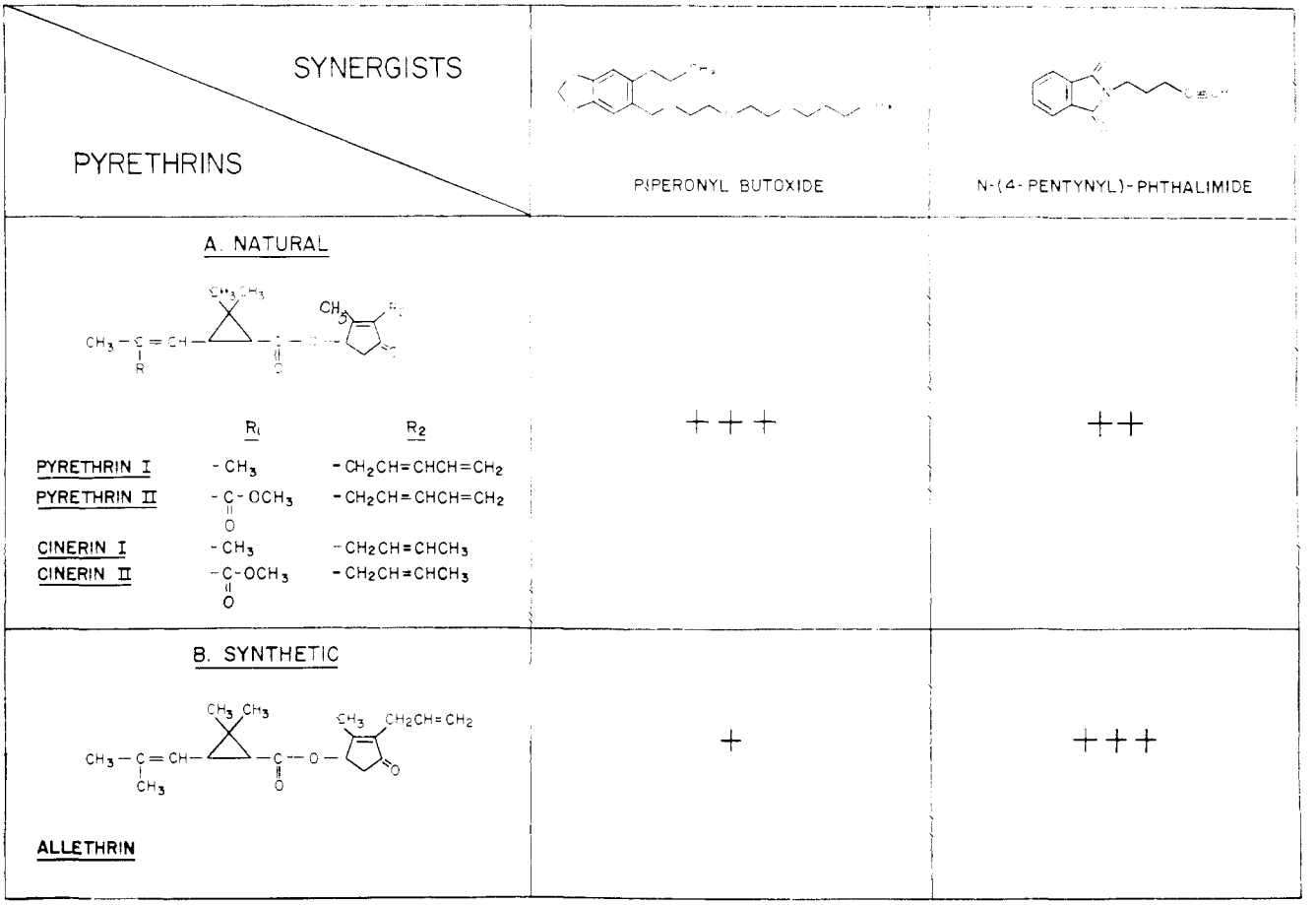

Figure 1.—Selective synergism of piperonyl butoxide and *N*-(4-pentynyl)phthalimide on natural and synthetic pyrethrins: strong (+++); moderate (++); weak (+).

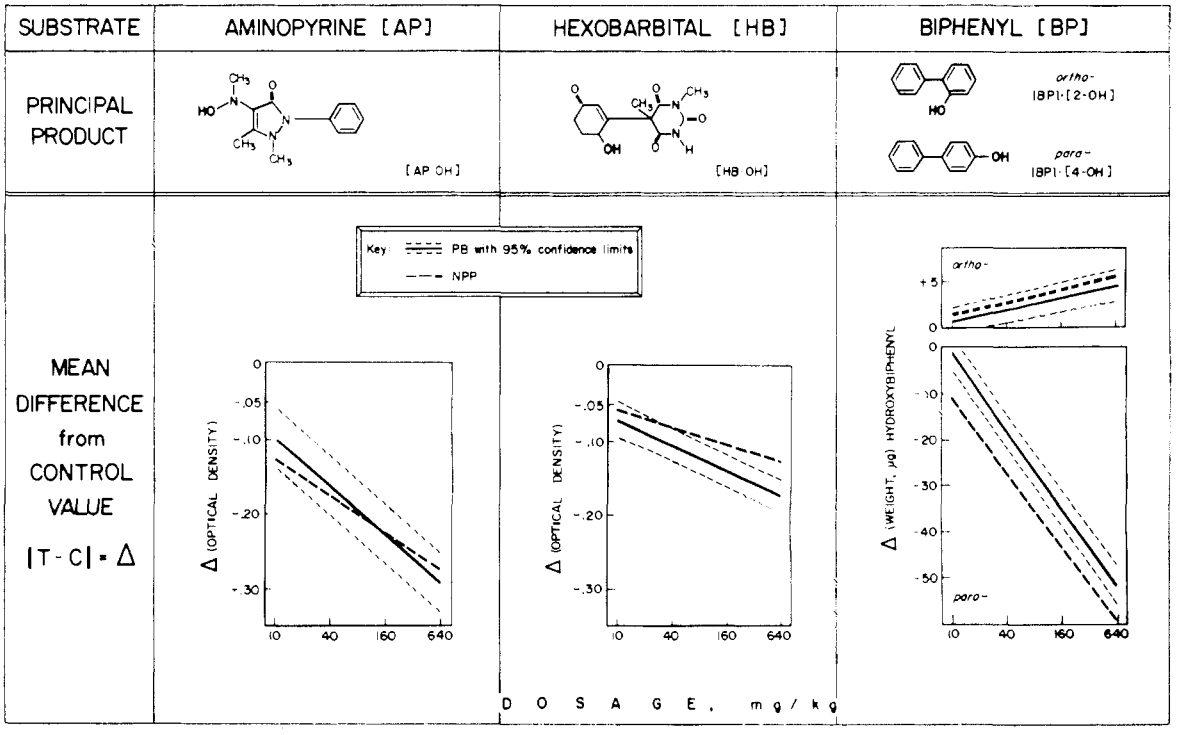

Figure 2.—Dose-response characteristics of piperonyl butoxide and *N*-(4-pentynyl)phthalimide on liver microsome hydroxylations of aminopyrine, hexobarbital, and biphenyl. All ordinates refer to amount of substrate hydroxylated per hr per 250-mg aliquot of liver tissue. Calculated regression lines, slope values, and number of replicates are presented in Table I.

Table I

Effect of Pyrethrin Synergists on Microsomal Enzyme Functions

Substrate	Microsomal enzyme function	Parameter measured	Slope values[a] ± S.D. (No. of observations) N-(4-pentynyl)-phthalimide	Piperonyl butoxide	Effect of enzyme function
Biphenyl	[2-OH]	$\Delta(W)_{ug}$	1.45 ± 0.41[b]	+1.3 ± 0.36 (5)	Stimulation
	[4-OH]		−16.2 ± 1.41 (7)	−16.9 ± 1.82 (5)	
Aminopyrine	[AP-OH]	$\Delta(OD) \times 10^2$	−5.0 ± 0.95 (7)	−6.5 ± 0.90 (5)	Inhibition
Hexobarbital	[HB-OH]		−2.4 ± 0.75 (8)	−3.4 ± 0.56 (3)	

[a] Slope values calculated on the basis of the differences between the means of test and control groups of 3 animals each at 4 dosages [10, 40, 160, and 640 mg/kg]. [b] Based on duplicate assays on 5 pooled samples from 2 animals each.

of *ortho* hydroxylation of biphenyl was induced by both PB and NPP (Table I and Figure 2).

Marked and similar prolongation of the duration of hexobarbital narcosis and of zoxazolamine paralysis in mice was induced by both synergists. Slope values of the calculated dose–response functions in the hexobarbital assay were 0.58 ± 0.04 and 0.52 ± 0.06 for NPP and PB, respectively; for the zoxazolamine assay, corresponding values were 0.38 ± 0.14 and 0.21 ± 0.04 for NPP and PB, respectively.

While the inhibition of mammalian microsomal enzyme functions by MDO synergists, as determined biochemically and by pharmacological assay, generally conforms with structure–activity correlations that have been reported for these pyrethrum synergists,[9] manifestly the inhibition affected to a comparable extent by PB (one of the most potent MDO synergists) and the nonmethylenedioxyphenyl synergist NPP indicates that intrinsically the microsomal systems are susceptible perhaps to more than one mode of blockade. The specific mode of action of synergists thus may not necessarily reflect competitive inhibition as has been suggested for blockade of the drug-detoxification process in insect and mammalian systems.[13] Concurrent suppression of the *para* hydroxylation of biphenyl and concomitant stimulation of *ortho* hydroxylation has been previously reported and postulated to be due to a ligand-induced allosteric modification of the enzyme moiety.[14] As has been demonstrated here, both PB and NPP are equally effective inhibitors of the *para* hydroxylation and have similar stimulatory effects on the *ortho* hydroxylation of biphenyl; and the hypothesis that synergists may produce an allosteric effect on the microsomal components would allow for the apparent lack of structural specificity of these agents such as the two classes represented by PB and NPP. However, these results do not appear to be in line with the selective synergism of these two compounds on natural and synthetic pyrethrins in insects (Table I). This comparative study should be extended directly to assay of comparable microsomal fractions derived from various insects, for, patently, the metabolism of pyrethroids must differ markedly in mammals wherein they are essentially inocuous agents, presumably through rapid detoxification, and in insects wherein they manifest a high degree of toxicity.

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(13) J. E. Casida, J. L. Engle, E. G. Essac, F. X. Kamienski and S. Kuwatsuka, *Science*, **153**, 1130 (1966).

(14) J. Jaffe, K. Fujii, H. Guerin, M. Sengupta, and S. S. Epstein, *Biochem. Pharmacol.*, **18**, 1045 (1969).