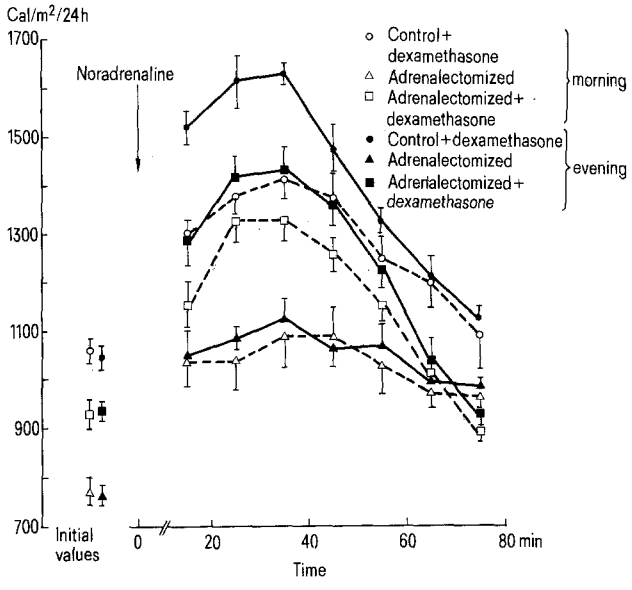

Fig. 3. The effect of dexamethasone on the heat production under the influence of noradrenaline in adrenalectomized and the control animals. Morning experiment: △—△, adrenalectomized; □—□ adrenalectomized + dexamethasone; ○—○, control + dexamethasone. Evening experiment: ▲—▲, adrenalectomized; ■—■ adrenalectomized + dexamethasone; ●—● control + dexamethasone. Mean ± SEM of 12 animals.

was registered in the control animals treated with noradrenaline. In these animals the diurnal fluctuations of the sensitivity to this hormone were markedly expressed, the differences between morning and evening experiments being significant ($p < 0.01$).

The evidence that in adrenalectomized animals the diurnal fluctuation of the sensitivity to injected noradrenaline was not restored after the treatment with dexamethasone suggests that this phenomenon is not linked to the presence of glycocorticoids only, but probably to the circadian fluctuation of the adrenocortical activity.

In conclusion, from the results reported here, taken together with our previous findings[1], it may be assumed that the diurnal fluctuation of the sensitivity to injected noradrenaline is the consequence of thyroid-adrenal interaction. The phenomenon is probably linked to the circadian fluctuation of adrenocortical activity, but as shown, the thyroid hormones are markedly involved in its expression.

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A wide spectrum of resistance to pyrethroid insecticides in *Musca domestica*

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Summary. Selection of a strain of house flies with the pyrethroid bioresmethrin has resulted in a wide range of cross resistance to 15 other pyrethroid insecticides.

In the past 30 years, over 364 species of arthropods have developed resistance to 1 or more insecticides[1]. One of the principal approaches for countering resistance involves the synthesis of new insecticides that are effective on these resistant strains. At the same time, society requires that new insecticides be safe to mammals and that they not persist in the environment. The natural pyrethrins, extracts from the flower of *Chrysanthemum cinerariaefolium,* are safe to mammals, but they are too unstable for field use. Elliott has succeeded in synthesizing many analogs of the compounds, which are referred to as synthetic pyrethroids[2-5]. The mammalian/insect selectivity ratio for pyrethroids was reported to be 4500 as compared to 16, 33, and 91 for carbamates, organophosphates, and organochlorines, respectively[6], and as a class, these compounds are relatively unstable and do not accumulate in the environment[6]. The requirements that they should not be affected by existing resistance and that they should not be prone to induce resistance to themselves are somewhat more difficult to satisfy, but encouraging results were given by Keiding when he reported that a mixture of natural pyrethrins plus piperonyl butoxide (p.b.) had been used for 20 years to control house flies on Danish farms without any resistance developing[7]. In the last 3 years, however, a few cases of resistance to both natural pyrethrins and synthetic pyrethroids have been reported with the following levels of resistance or cross resistance in field-selected populations: *Musca domestica,* 105× toward bioresmethrin[7]; *Aedes aegypti,* 30× toward permethrin[8]; *Boophilus microplus,* 10× toward cypermethrin[9]; *Spodoptera littoralis,* 4× toward permethrin; and *S. exigua,* 2000× toward permethrin (J.S. Holden, personal communication). In addition, laboratory selections have resulted in 75× resistance to natural pyrethrins plus p.b. in *Blattella germanica*[10], >4000× resistance to trans-permethrin in *Culex pipiens quinquefasciatus*[11], and 250× resistance to natural pyrethrins and 100× resistance to resmethrin in *Musca domestica*[12].

Resistance to pyrethroids could be due to detoxification of individual insecticides at sites susceptible to enzymatic attack. Such is often the case with carbamates and organophosphates. Another possibility is that resistance to all members of the pyrethroid group could be developed by the reduction of sensitivity at the active site, as is presumed to be the case with resistance to cyclodienes[13]. The type of resistance that can be induced by a particular pyrethroid is thus a most important question: if it is of the metabolic type, it could be circumvented by appropriate alteration of molecular structure, but if it is of the insensitivity type, solutions become untenable since resistance may extend to all other pyrethroids. Here we are reporting that, in our pyrethroid-resistant strain of *Musca domestica* (strain SBC), resistance appears to be of the latter type. This suggestion is

based on the fact that selection of the strain with bioresmethrin has resulted in resistance to all of the 16 pyrethroids that we have tested.

Strain SBC was derived from a fenthion-selected colony of *M. domestica* possessing 600× resistance to fenthion and high levels of cross resistance to several other organophosphates. The strain was selected with bioresmethrin until the resistance ratio (RR) was circa 80×, at which time it was tested with a wide variety of pyrethroids by means of topical applications of 1 µl volumes of an acetone solution of the chemicals to 3-day-old female flies. Parallel tests were performed on the susceptible (NAIDM) strain. Treated flies were held at 15 °C and mortality was recorded after 24 h. The insecticides tested varied from allethrin, one of the earliest pyrethroids, to some of the most recent ones, such as cypermethrin, decamethrin, and fenvalerate. Information on cross resistance to these newer pyrethroids is important since some of these compounds are being developed for commercial use. Various structural and isomeric analogs were also included, thus allowing for the possibility of different rates of metabolic detoxification.

The table lists the LD_{50} values for the susceptible (S) and resistant (R) strains as well as the computed RR's. Allethrin contains many molecular sites which are susceptible to metabolic detoxification, hence its rather high S-LD_{50} of 0.18–0.22 µg/female. Many of the newer pyrethroids have fewer susceptible sites: the S-LD_{50}'s of cis-permethrin and cis-decamethrin are extremely low (0.00048 and 0.0006, respectively), suggesting a high degree of recalcitrance to detoxification. The overall biodegradation rate is greatest for primary alcohol esters of trans-substituted acids since they undergo rapid hydrolytic and oxidative attack[14].

If resistance is due to increased metabolism of the insecticide, then compounds that can be most easily metabolized might be expected to display higher resistance than is shown by the more stable compounds. However, this is not the case: the strain was as resistant to the metabolically stable pyrethroids as it was to those which could be easily metabolized. For example, there was little difference in RR between pairs of cis and trans isomers. In addition, the strain was only 14× resistant to allethrin and cismethrin, while it was 48, 35 and 63× resistant to the metabolically stable cis-cypermethrin, cis-decamethrin, and fenvalerate, respectively.

Cross resistance of the bioresmethrin-selected (SBC) strain of *Musca domestica* to synthetic pyrethroids

	LD_{50} (µg/♀)		
	Susceptible strain (NAIDM)	Resistant strain (SBC)	Resistance ratios (RR)
Bioresmethrin	0.0060	0.51	86
R-Allethrin	0.22	4.2	19
S-Allethrin	0.18	2.5	14
Cismethrin	0.031	0.42	14
cis-Ethano resmethrin	0.0084	0.28	33
trans-Ethano resmethrin	0.0023	0.073	32
cis-Permethrin	0.0057	0.13	23
trans-Permethrin	0.011	0.39	35
cis-Cypermethrin	0.00048	0.023	48
trans-Cypermethrin	0.0013	0.057	44
cis-Decamethrin	0.0006	0.021	35
trans-Decamethrin	0.0014	0.058	41
Fenvalerate	0.006	0.40	63
S3206*	0.0091	0.33	36
trans-Ethanocyphenothrin	0.0016	0.0335	21
cis-Ethanocyphenothrin	0.0180	> 0.1	> 6

* (R,S)-α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate.

Some preliminary investigations with insecticide synergists have shown that metabolism plays only a limited role in the strain's resistance. The esterase inhibitor DEF® failed to synergize bioresmethrin, while p.b., an MFO inhibitor, produced only 7.8× synergism, leaving a 14× level of unsuppressed resistance in the strain.

Early studies on DDT-resistant house flies revealed that, in certain strains, at least part of the DDT resistance was due to a nonmetabolic mechanism conferring delayed knockdown[15]. These strains were also cross-resistant to natural pyrethrins. Later studies on the genetics of this mechanism led to the mapping of gene *kdr*[16,17], and it was suggested that this gene acts by way of decreased nerve sensitivity since it confers broad cross resistance to both pyrethroids and DDT. A similar finding was reported for a DDT-resistant strain of *Boophilus microplus,* which was found to be cross-resistant to 7 pyrethroids[9]. The existence of the *kdr* gene in the SBC strain remains to be investigated.

Thus, selection with bioresmethrin has resulted in resistance to a wide range of pyrethroids regardless of their metabolic stability, and this resistance cannot be eliminated by the use of metabolic inhibitors. It is clear that this type of resistance could have serious consequences in the field: use of a single pyrethroid could result in the development of resistance to the entire class.

A similar type of resistance also exists in the cyclodiene group, where selection with 1 cyclodiene is known to result in resistance to all other members of the group. There are several important differences between cyclodiene and pyrethroid resistance, however. Cyclodiene resistance is characterized by being monofactorial, nonsynergizable, and of a high phenotypic expression (100–1000× RR's), and therefore, 100% kill is not obtainable even with very high dosages[13,18,19]. In contrast, pyrethroid resistance appears to be polyfactorial, somewhat suppressible by synergists, and of a lower phenotypic expression, and the resistant insects can still be killed, at least in the laboratory, by increasing the dosage of the insecticide.

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