blocking effect directly on the muscle. This effect is significantly inferior to the inhibitory effect observed when the muscle is stimulated via the nerve. These observations suggest that the target for these compounds is to be found in the nerve muscle junction.

The effect of the analogues **III** and **IV** on the twitch tensions is of the same order of magnitude as demonstrated for **I** and **II**. It is therefore suggested that the mode of action of **III** might correspond to that of **I** and **II**. The mode of action of **IV** seems to be more complex since this compound is the only one tested that shows an inhibitory effect on the action potential in the frog nerves.

The effects of the open-chain analogues on the in vitro preparations lend no support to the hypothesis that the dithiolane moiety is the active principle and that active analogues are metabolized into dithiolanes[15].

The effect level for charatoxin compares well to that of the structurally related nereistoxin on the sartorius nerve muscle preparation as it appears from figure 2. Since both compounds show insecticidal potency with similar symptoms of intoxication[6] it is indicated that further studies on the insecticidal properties of charatoxin analogues may prove fruitful.

Many questions remain to be answered regarding the precise site of action of charatoxin and its analogues. The results presented here clearly point at the cholinergic transmission as the region of action. But nothing is known about the specificity of the observed inhibition. Further studies on e.g. binding affinity to acetylcholine receptors, and a more detailed electrophysiological investigation of the effect on the membrane potentials should therefore be carried out.

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Influence of posttreatment temperature on the toxicity of pyrethroid insecticides to susceptible and resistant larvae of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.)

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Summary. The organophosphorus insecticides chlorpyrifos, leptophos, and phosfolan and the carbamate methomyl was found to be more toxic to larvae of a susceptible strain of *Spodoptera littoralis* (Boisd.) when the posttreatment temperature was increased from 20 to 35 °C. In contrast, the pyrethroids permethrin, fenvalerate, deltamethrin, cypermethrin, and flucythrinate were more toxic at 20 °C than at 35 °C. This effect was more pronounced in the pyrethroid-resistant strains. Evidently, resistance levels were reduced at low temperature. However, the application of piperonyl butoxide or DEF in combinations with the tested pyrethroids on R-strains resulted in reducing the effect of temperature.

Several factors influence insecticide toxicity, one being temperature. It has been shown that the toxicity of natural pyrethrins and DDT correlated negatively with increasing posttreatment temperature[1-3]. On the other hand organophosphates have shown a positive temperature coefficient[2,4,5]. Carbamates were reported to have a slightly negative temperature coefficient[6,7], although methomyl manifested greater toxicity at higher temperatures in some insect species[4,8,9].

Recent developments in pyrethroid chemistry have resulted in synthesis of relatively stable compounds with high toxicity to insects. Some evidence indicates that synthetic pyrethroids have a negative temperature coefficient similar to the natural pyrethrins. This has been reported for the effect of pyrethroids on houseflies[6,10], the cabbage looper[8], crickets[11], and tobacco cutworms[9].

In Egypt, several synthetic pyrethroids have been introduced for the chemical control of cotton pests. The present work describes the effect of posttreatment temperature on the toxicity of some pyrethroids, in comparison with other insecticides, to larvae of a susceptible and 3 pyrethroid-resistant strains of the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.).

Materials and methods. 4 different strains of *S. littoralis* were used; a laboratory susceptible strain (S-FM), a flucythrinate resistant-strain (R-CB), a deltamethrin-resistant strain (R-DM), and a fenvalerate-resistant strain (R-FN). The S-FM strain was originally obtained from Faiyum Province in 1966 and was reared in the laboratory without any exposure to insecticides. The resistant R-CB, R-DM, and R-FN strains were originally collected from Shrquiya Governorate in 1980. The R-CB strain was subjected to continuous selection for 15 generations, while R-DM and R-FN strains were selected for 22 successive generations. Selection procedure and the history of these strains have already been described[12].

Technical grade samples of different insecticides were used as supplied by the manufacturers. Flucythrinate [(RS)-α-cyano-3-phenoxybenzyl(S)-2-(4-difluoremthoxyphenyl)-3-methylbutyrate] was 89.6% pure. The other tested insecticides were all > 95% purity. The LD_{50} value for each insecticide was estimated by topical application using an Arnold hand microapplicator (Burkard). 4th instar larvae (30–35 mg/larvae) were treated with 0.5 µl of the insecticide solution in acetone containing the required dose (µg), on the dorsal side of the thorax. 5 replicates of ten larvae were made for each insecticide dose. 6 doses causing mortalities of circa 10–90% were tested for each insecticide. Treated larvae were transferred to Petri dishes, supplied with fresh castor bean leaves, and kept in an incubator at 20 and 35 °C. Mortality counts were recorded after 24 h and corrected according to Abbott[13] using controls treated with acetone only (mortalities were not more than 5%). The data were subjected to probit analysis by the method of Finney[14] to estimate the LD_{50} values (µg/g body weight).

Results and discussion. As shown in table 1, the toxicity of the pyrethroid insecticides to 4th instar larvae of S-FM strain was higher than that of the other carbamate and organophosphorus compounds. Regarding the effect of posttreatment temperature, table 1 indicates that the 3 organophosphorus compounds and the carbamate methomyl were more toxic at 35 °C than at 20 °C. This agrees with other reports of a positive temperature correlation for these compounds[2,4,5,8,9]. With respect to the pyrethroid insecticides, all became increasingly toxic with decreasing temperature. The effect was more obvious with permethrin and fenvalerate, for which the toxicity increased by 7.01 and 6.47 times, respectively, at 20 °C compared with 35 °C. Flucythrinate showed relatively little increase of activity with decreasing temperature, as its relative toxicity at 20 °C was 2.02. With cypermethrin and deltamethrin a moderate increase in toxicity at 20 °C (4.4–4.83 times) were recorded. The present data agree with those obtained on houseflies[6,10] the cabbage looper[8], crickets[11], and tobacco cutworms[9].

With respect to the R-strains, table 2 indicate that the effect of temperature on the toxicity of pyrethroid was more pronounced than in S-FM strain (table 1). The increase of deltamethrin toxicity at 20 °C was 8.23 times compared with only 4.4 times in case of S-FM strain. The respective values for fenvalerate were 10.12 and 6.47, and for flucythrinate were 2.86 and 2.02. Similar data were obtained on houseflies with CP47412 [1,1-di(p-chlorophenyl)-2,2-dichlorocyclopropane], lindane, and bioresmethrin[6]. Investigations in our laboratory indicated that both oxidative and hydrolytic mechanisms contribute, at least in part, to the resistance of *S. littoralis* larvae against deltamethrin, fenvalerate, and flucythrinate[12,15]. When these compounds were combined with piperonyl butoxide (oxidase inhibitor) or DEF (esterase inhibitor) in the ratio of 1:5 (insecticide:synergist), the effect of temperature on their toxicities was obviously reduced. For example, the relative toxicity value for deltamethrin at 20 °C was 8.23. The same values for the compound in combination with piperonyl butoxide or DEF were only 4.31 and 2.8, respectively.

The present data could be explained, at least in part, on the basis of decreased activity of detoxification enzymes at low temperature[5,6,15]. The effect of temperature was more pronounced in R-strains because detoxification enzymes are supposed to be abundant in these strains. Consequently, the effect of temperature was reduced by the application of piperonyl butoxide or DEF because detoxification enzymes were partially inhibited by the synergists. Temperture might also affect the absorption of insecticides[5], activity of insecticides at their site of action[6,17], nerve-blocking action[16,18], and sensitivity of sense organs[19]. The overall effect of temperature is the outcome of these numerous temperature-dependent interactions.

Table 2 also shows that the level of resistance is influenced by the temperature at which the insects are tested. The resistance levels of R-DM, R-FN, and R-CB strains to deltamethrin, fenvalerate, and flucythrinate at 35 °C were 21.21, 14.9, and 26.68-fold, respectively. At 20 °C, the respective values were only 11.3, 9.52, and 18.85-fold. Similarly, a field strain of the same insect 4-fold resistant to permethrin at 30 °C was only 1.4 fold resistant at 15 °C[6]. Thus, more realistic levels of resistance can be arrived at if the experiments are performed at a temperature similar to that of the normal field environment.

The practical application of the present data indicates that most synthetic pyrethroids should not be applied during hot weather or when a severe prolonged increase in temperature is expected. This is especially true for fenvalerate, permethrin, deltamethrin, cypermethrin, and probably for flucythrinate.

Table 1. The influence of posttreatment temperature on the toxicity of various insecticides to 4th instar larvae of S-FM strain of *S. littoralis*

Compound	LD_{50} (µg/g b.wt) 35 °C	20 °C	Relative toxicity[a]
Permethrin	0.61	0.087	7.01
Fenvalerate	1.1	0.17	6.47
Deltamethrin	0.066	0.015	4.4
Cypermethrin	0.29	0.06	4.83
Flucythrinate	0.85	0.42	2.02
Methomyl	2.10	2.48	0.85
Chlorpyrifos	1.81	3.52	0.51
Leptophos	1.78	4.10	0.43
Phosfolan	3.64	5.10	0.71

[a] Relative toxicity = $\frac{LD_{50} \text{ at } 35\,^{\circ}C}{LD_{50} \text{ at } 20\,^{\circ}C}$

Table 2. The effect of posttreatment temperatures on the toxicity of 3 pyrethroids to 4th instar larvae of different R-strains of *S. littoralis* when applied alone or in combination with piperonyl butoxide of DEF

Compound	Strain	Temperature	Compound alone LD_{50}[a]	RT[b]	RL[c]	Compound + piperonyl butoxide LD_{50}	RT	Compound + DEF LD_{50}	RT
Deltamethrin	R-DM strain	35 °C	1.4		21.21	0.41		0.29	
		20 °C	0.17	8.23	11.33	0.095	4.31	0.103	2.8
Fenvalerate	R-FN strain	35 °C	16.4		14.9	5.70		5.92	
		20 °C	1.62	10.12	9.52	1.21	4.71	1.41	4.19
Flucythrinate	R-CB strain	35 °C	22.68		26.68	3.59		3.91	
		20 °C	7.92	2.86	18.85	2.31	1.55	2.05	1.9

[a] µg/gm b.wt.

[b] Relative toxicity = $\frac{LD_{50} \text{ at } 35\,^{\circ}C}{LD_{50} \text{ at } 20\,^{\circ}C}$ [c] Resistance level = $\frac{LD_{50} \text{ of R-strain}}{LD_{50} \text{ of S-strain}}$

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Calcium dobesilate (Doxium) as a prostaglandin synthetase inhibitor in pregnant human myometrium in vitro

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Summary. This comparative study on the effect of calcium dobesilate and indomethacin on prostaglandin biosynthesis was performed on microsomal fractions of pregnant human myometrium. Both drugs inhibited prostaglandin synthesis, indomethacin being more potent. Calcium dobesilate inhibited, in a dose-dependent manner, the synthesis of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and TXB_2. Its inhibitory action is comparable to that of etamsylate.

There is a surprisingly large number of compounds known to inhibit the enzymes of the arachidonate metabolic pathway. It was demonstrated in our previous study that etamsylate (diethylammonium-1,4-dihydroxy-3-benzenesulfonate) inhibits in vitro the synthesis of prostaglandins (PGs) in the microsomal fraction of pregnant human myometrium[1].

Calcium dobesilate (calcium 2,5-dihydroxybenzenesulfonate) is also a benzenesulfonate derivative that has proved to be effective in the treatment of chronic venous insufficiency[2–4] and diabetic retinopathy[5–7]. The chemical resemblance of calcium dobesilate to etamsylate has prompted us to undertake a similar study on PG synthesis, making a comparison with indomethacin, which is a commonly used PG synthetase inhibitor.

Materials and methods. Myometrial strips from pregnant women were obtained by excision from the edge of the surgical incision in lower uterine segment caesarian sections. The procedure and the assay of PG-synthetase activity were the same as described in our etamsylate study[1].

The developing-solvent system used in thin layer chromatography for the isolation of PG metabolites after incubation of (1–^{14}C) arachidonic acid with microsomes of pregnant human myometrium allowed the separation of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and TXB_2. The incubation media also contained different concentrations of calcium dobesilate (0.01, 0.1, 1.0, 5.0 and 10.0 mM) obtained from OM Laboratories, Geneva, Switzerland. The activity of PG synthetase was calculated as pmol of PG formed per 30 min per mg of protein.

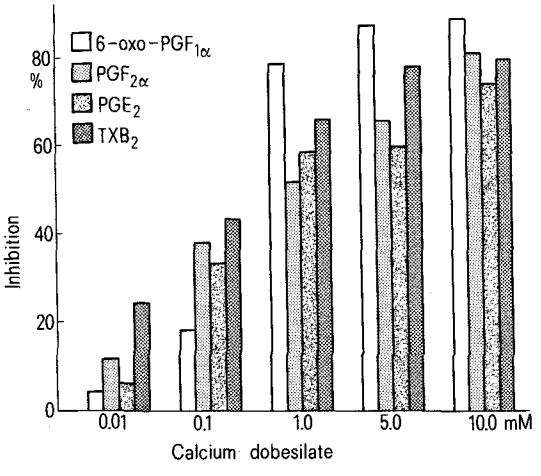

Figure 1. Inhibition of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and TXB_2 synthesis during incubation with Ca dobesilate. Each value represents the average of duplicate experiments (variation 4–6%).

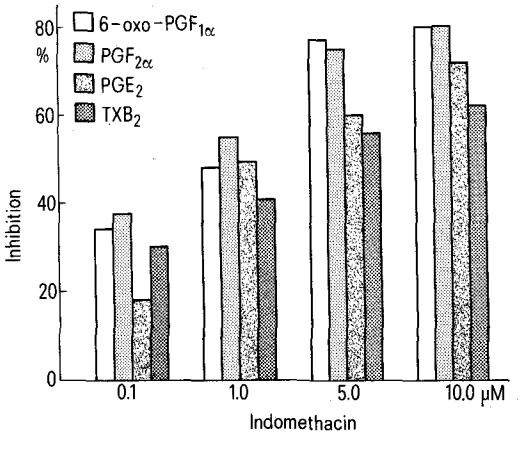

Figure 2. Inhibition of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and TXB_2 synthesis during incubation with indomethacin. Each value represents the average of duplicate experiments (variation 4–6%).