INFORMATION TO USERS

While the most advanced technology has been used to photograph and reproduce this manuscript, the quality of the reproduction is heavily dependent upon the quality of the material submitted. For example:

- Manuscript pages may have indistinct print. In such cases, the best available copy has been filmed.
- Manuscripts may not always be complete. In such cases, a note will indicate that it is not possible to obtain missing pages.
- Copyrighted material may have been removed from the manuscript. In such cases, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, and charts) are photographed by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each oversize page is also filmed as one exposure and is available, for an additional charge, as a standard 35mm slide or as a 17"x 23" black and white photographic print.

Most photographs reproduce acceptably on positive microfilm or microfiche but lack the clarity on xerographic copies made from the microfilm. For an additional charge, 35mm slides of 6"x 9" black and white photographic prints are available for any photographs or illustrations that cannot be reproduced satisfactorily by xerography.

Order Number 8721870

The relationships between temperature acclimation, neural lipid saturation, and the toxicity of allethrin to the American cockroach, *Periplaneta americana*

Baldus, Tom James, Ph.D.

Iowa State University, 1987

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark __✓__.

1. Glossy photographs or pages _____
2. Colored illustrations, paper or print _____
3. Photographs with dark background _____
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages ✓
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Dissertation contains pages with print at a slant, filmed as received _____
16. Other _____

University
Microfilms
International

The relationships between temperature acclimation, neural lipid saturation, and the toxicity of allethrin to the American cockroach, *Periplaneta americana*

by

Tom James Baldus

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Zoology
Major: Zoology (Physiology)

Approved:

Signature was redacted for privacy.
In Charge of Major Work

Signature was redacted for privacy.
For the Major Department

Signature was redacted for privacy.
For the Graduate College

Iowa State University
Ames, Iowa

1987

TABLE OF CONTENTS

GENERAL INTRODUCTION

Acclimation has been defined as the laboratory-induced adaptive responses that occur during the lifetime of an organism (Hochachka and Somero, 1984). Only the genetic information present at the beginning of the organism's life can be utilized to develop biochemical adaptations according to this definition; therefore, only phenotypic changes occur in acclimation. The adaptive response may involve changes in amounts or types of macromolecules, adjustments in medium surrounding the macromolecules, or adjustments in rates of enzyme function. These acclimative changes are important for preservation of structural integrity of macromolecules, and for maintenance of adequate supplies of ATP, metabolic intermediates, and mechanisms for regulation of metabolic rate. If an animal can successfully acclimate, it can restore its system to a steady-state function (compensatory adaptation); it could also gain a new ability to benefit from its environment (exploitative adaptation) (Hochachka and Somero, 1984).

The physiological and biochemical changes involved in temperature acclimation have been studied in many groups of animals, especially fish (Das and Prosser, 1967; Hochachka and Somero, 1968, 1984), and insects (Anderson and Mutchmor, 1968). In goldfish, warm acclimation reduces the rate of protein synthesis in gill and liver (Das and Prosser, 1967);

it also results in more highly saturated lipids in the brain (Roots and Johnston, 1968; Cossins et al., 1977). In Tribolium confusum, the confused flour beetle, and Musca domestica, the housefly, temperature acclimation can affect locomotion, oxygen consumption, and ATPase activity (Anderson and Mutchmor, 1971). In the American cockroach, Periplaneta americana, many physiological parameters are affected by temperature acclimation, including enzyme activity (Hoffmann, 1985), action potential extinction temperature, chill-coma temperature (Staszak and Mutchmor, 1973), conduction velocity and firing rate of specific motor neurons (Bradfisch et al., 1982), and saturation level of lipids (Munson, 1953).

Other parameters such as age, sex, species, and stage of development of the insect have long been known to affect the toxicity of an applied insecticide (Hadaway and Barlow, 1957; Sun, 1960). Test conditions such as application method, type of solvent, and post-treatment temperature can also affect toxicity. According to DeVries and Georghiou (1979), the temperature effect may depend on the toxin's mode of action. Allethrin, a synthetic pyrethroid that alters the permeability of voltage-gated sodium channels in the axonal membrane of squid and crayfish giant axons (Narahasi, 1985), is more toxic to American and German cockroaches at lower post-treatment temperatures, whereas

other pyrethroids (cypermethrin and deltamethrin) are more toxic at higher post-treatment temperatures (Gammon et al., 1981; Scott and Matsumura, 1983). Clearly, many factors should be considered when selecting dose levels for field application or when attempting to compare results of laboratory tests.

Since temperature acclimation definitely affects the physiology of insects, and the physiology of the insect influences its susceptibility to an applied insecticide, it seems reasonable to expect that temperature acclimation as well as post-treatment temperature could affect toxicity. However, compared to studies of post-treatment temperature effects, very few studies have examined the relationships between acclimation temperature and toxicity. Of these, several indicate that acclimation temperature has no effect on toxicity. Potter and Gillham (1946) applied DDT and pyrethroids to flour beetles, Hadaway and Barlow (1957) applied DDT and dieldrin to mosquitoes and houseflies, and Rai (1967) applied several organophosphates, chlorinated hydrocarbons, carbamates, and pyrethrins to locusts; no effect of acclimation temperature on toxicity was found in any of these studies. However, others have found that temperature acclimation can affect the toxicity of DDT (Munson, 1953), allethrin and malathion (Baldus, 1984), and endrin, toxaphene, and heptachlor (Jamil, 1984) applied to

the American cockroach.

Although the mechanisms by which pre- or post-treatment temperatures affect toxicity are not clearly understood, several workers have speculated that lipid changes may be important. Osborne and Smallcombe (1983) claim that changes in lipids of the nerve cell membrane (over generations) affected the binding of lipophilic toxins in the housefly, leading to resistance (decreased toxicity). Munson (1953) and Baldus (1984) speculated that changes in saturation levels of P. americana lipids were in part responsible for differences in toxicity. In insects, fatty acid composition can change in response to diet (Nation and Bowers, 1982; Cookman et al., 1984), starvation (Stanley-Samuelson and Dadd, 1983), and temperature acclimation (Danks and Tribe, 1979; Downer and Kallapur, 1981). Lipid changes, in turn, may affect membrane permeability (Hazel, 1973), receptor affinity (Kilian et al., 1980), and (to some extent) enzyme activity (Brattsten et al., 1986).

Clearly, lipid changes affect the physiology of animals. It is not surprising, then, that many workers have attempted to correlate such changes, including changes in fatty acid composition, with the effects of other variables on animal function. The fatty acid composition of lipids has been determined in intact animals (Nakasone and Ito, 1967; Stanley-Samuelson and Dadd, 1983), individual tissues

and organs (Nelson et al., 1967; Stanley-Samuelson and Pipa, 1984), and even in cell lines of insects (Stanley-Samuelson et al., 1986).

To test the idea that temperature acclimation, changes in lipid composition, and allethrin toxicity are interrelated, experiments were designed to determine:

1. if acclimation and post-treatment temperatures affect the toxicity of allethrin applied to adult P. americana;
2. if temperature acclimation affects the saturation level and fatty acid composition of neural and non-neural lipids of adult P. americana;
3. if effects of temperature treatments on toxicity are correlated with effects on fatty acid composition.

Explanation of Dissertation Format

This dissertation is composed in the alternate format. The sections (Parts I-II) of this dissertation are complete manuscripts modified to conform to the specifications of the Iowa State University Thesis Office. Each part consists of an introduction, materials and methods, results, discussion, and references. A general introduction precedes Part I and a general summary follows Part II.

SECTION I. THE EFFECTS OF ACCLIMATION AND POST-TREATMENT TEMPERATURE ON THE TOXICITY OF ALLETHRIN TO THE AMERICAN COCKROACH, *PERIPLANETA AMERICANA*

INTRODUCTION

Many factors affect the toxicity of an applied insecticide. The influence of age, sex, and species has been reported by Sun (1960) and Hadaway and Barlow (1957). An insect's activity, weight, respiratory and metabolic rates, as well as the solubility, penetration rate, and detoxification pathways of the toxin can all affect the insecticide's LC_{50} (concentration of the toxin that kills 50% of the test insects).

The effect of post-treatment temperature on toxicity has been extensively investigated; according to DeVries and Georghiou (1979), the effect depends on the pesticide's mode of action. Pyrethroids are normally more toxic at lower post-treatment temperatures (Adams and Miller, 1980; Sparks et al., 1982), exhibiting what is commonly called a negative temperature coefficient. However, recent studies by Gammon et al., (1981) and Scott and Matsumura (1983) indicate that allethrin and structurally related pyrethroids exhibit a negative temperature coefficient while others, such as cypermethrin and deltamethrin, exhibit a positive temperature coefficient for mortality. The underlying mechanism and structural components responsible for the post-treatment temperature's effect on toxicity are poorly understood. According to Gammon (1978), post-treatment temperature may play a role in determining the mode of

action of allethrin. Temperatures above 26°C enhance allethrin-induced repetitive firing while temperatures below 26°C enhance blockage of impulse conduction in the isolated cockroach nerve cord (Narahashi, 1971; Gammon, 1979).

The effects of pre-treatment temperature on toxicity are less well-established. Of the few studies with insects, most indicate that acclimation temperature has no effect on toxicity (Potter and Gillham, 1946; Hadaway and Barlow, 1957; Rai, 1967). However, Munson (1953) found that DDT was more toxic to Periplaneta americana nymphs acclimated for at least 2 weeks to 34°C than to those acclimated to 23°C. In contrast, allethrin was less toxic to adult American cockroaches acclimated for 7-10 days at 30°C than to those acclimated at 14 or 22°C (Baldus, 1984). Jamil (1984) found that the toxicity of chlorinated hydrocarbons to Periplaneta americana was influenced by an acclimation period of 40 days; high or low temperature acclimation reduced mortality when the insects were exposed to the insecticide at their acclimation temperature. According to Thomas and Rice (1986), 6 days of temperature acclimation affected the metabolism and tissue retention of toluene in the Dolly Varden char Salvelinus malma, resulting in increased toxicity at 4° compared to 12°C. How temperature acclimation renders insects and other animals more or less susceptible to a toxin is not clearly understood. In fish,

acclimative effects on metabolism of the toxin may be important (Thomas and Rice, 1986). In Periplaneta americana, temperature acclimation can affect many factors including rate of oxygen consumption (Das and Singh, 1974), fat body protein content (Singh and Das, 1980), and enzyme activity (Hoffmann, 1985). The nervous system also exhibits acclimative effects (Anderson and Mutchmor, 1968); if the active site of an insecticide is affected by temperature acclimation, it seems reasonable that the toxicity of the compound would also be affected.

The active site of allethrin, the insecticide used in this research, is currently being debated. In giant axons of the American cockroach, concentrations of allethrin high enough to block impulse conduction (10^{-6} g/ml) inhibit sodium-activation and potassium-activation mechanisms (Narahashi, 1965). In more recent work with American cockroach giant axons, Laufer et al. (1984) found that 8 pyrethroids (including s-bioallethrin, the most potent isomer of allethrin) induce transient modifications of sodium channels that are in the active state. In a recent analysis of the debate, Narahashi (1985) stated that sodium channels are the major target site of pyrethroids in squid and crayfish giant axons. Allethrin acts by slowing the sodium channel inactivation mechanism, and, like other pyrethroids, prolongs the inward sodium current, thereby

increasing the depolarizing after-potential. This effect is directly responsible for the repetitive neural activity in frog myelinated nerve fibers induced by these insecticides (van den Bercken and Vijverberg, 1983). Allethrin may also affect transmitter release by inhibiting an ATP-ase (Clark, 1981). The post-synaptic effects (interactions with acetylcholine receptors) of allethrin at motor endplates have been investigated in the electric ray, Torpedo ocellata by Abbassy et al. (1982).

In this paper, the toxicity of allethrin to adult male P. americana subjected to various pre- and post-treatment temperature conditions is investigated. Although these experiments do not provide direct information about allethrin's active site, toxicity changes are indicative of other physiological and biochemical changes, and they may suggest additional experiments that will help elucidate the nature of toxicity-related acclimative changes.

MATERIALS AND METHODS

The immersion method, in which insects are 'dunked' in the test solution, was used because it allows rapid and uniform application of insecticide to large numbers of insects (Craufurd-Benson, 1938), and because it generally gives reproducible results, standard deviations similar to those for the more time-consuming topical application methods, steeper dose-response curves, and narrower confidence limits for LC_{50}s (Cornwell, 1976). Technical grade allethrin (purity = 90.75%) was generously donated by McLaughlin Gormley King Company, Minneapolis, Minnesota. Periplaneta americana adult males were removed from a colony that had been established 25 years earlier and which had never been exposed to pesticides. The cockroaches were acclimated for 7-8 days at either 14, 22, or 30 ± 1°C and 55-65% relative humidity in Freas Model 805 incubators. Each replicate of 10 insects was kept in a 1.9 liter mason jar and provided with regular Purina® dog chow and water ad libitum.

Test insects were anesthetized by exposure to CO_2 for 10 seconds, immersed in the insecticide solution for 30 seconds, poured out onto paper towels to drain, and after one minute returned to 1.9 liter mason jars containing food and water. Three replicates were used per test, each consisting of 10 males ranging in weight from .71 to .93 g

(average = .85 g). On each day that tests were conducted, a control replicate was treated with solvent solution minus insecticide. Mortality counts were made 24 hours after treatment as suggested by Sawicki and Farnham (1964) and Flynn and Schoof (1970). In previous work with allethrin and malathion (Baldus, 1984), a replicate consisted of 10 males and 10 females. Insecticide test concentrations were chosen to give a wide range of partial mortality values for the combined-sex replicates. Because male and female mortalities differed markedly, the data from the earlier study were deemed unsuitable for use in the present research, where only males are used. In addition to limiting the study to males, experiments described here used a slightly modified testing procedure and apparatus in order to increase the precision of the measurements.

In all experiments, a 7-8 day pre-treatment acclimation at 14, 22, or 30°C was followed by treatment at room temperature (22°C). In one series of experiments, all replicates were held for 24 hours at 22°C before mortality counts were made. In a second series of experiments, the acclimation temperature and the post-treatment temperatures were the same.

Five insecticide concentrations causing between 10 and 90% mortality were used in generating each dose-response curve. A computer program based on the statistical

procedures of Litchfield and Wilcoxon (1949) was used to fit curves by least-squares regression, to calculate slopes, to determine LC_{50}s and their confidence intervals, and to test for homogeneity by the chi-squares method. The probits used to convert the dose-response relationships to linear form are probability units, defined by Finney (1971) as standard deviation units plus 5 (i.e., standard deviation units −2, −1, 0, +1, and +2 correspond to probits 3, 4, 5, 6, and 7).

RESULTS

Chi-square analysis (Table 1) indicates that each allethrin dose-response curve is within the statistical limits for homogeneous response ($P \leq 0.05$). According to the statistical program used (Litchfield and Wilcoxon, 1949), 3 degrees of freedom are possible when 5 concentrations are used to generate a dose-response curve; a total chi-square of less than 7.81 indicates homogeneous response (indicating that the line representing the response fits the data well). Table 1 shows the temperatures, concentrations of allethrin, and pooled mortalities from 3 replications per test used to calculate the 6 LC_{50}s in Table 2; the same data were used to generate the dose-response curves in Figs. 1 and 2. Data from individual, unpooled replicates are recorded in Appendix A (Tables A.1 and A.2).

The results in Table 2 demonstrate that temperature acclimation had a significant effect on the toxicity of allethrin. LC_{50}s are all significantly different; allethrin was three times as toxic to cockroaches acclimated to 14° as to those acclimated to 30°C (confirming the negative temperature coefficient). The LC_{50} for cockroaches acclimated at 22°C was greater than that for 14°-acclimated cockroaches but less than the LC_{50} for cockroaches acclimated at 30°C. The slope of the dose-response curve increased significantly with acclimation temperature; the

slope for 30°-acclimated cockroaches was approximately 6 times as high as that for 14°C-acclimated cockroaches. Fig. 1 shows that by plotting dose-response data on log-probit axes, the normal sigmoidal response is transformed into a straight line. The close agreement between observed (data points) and expected mortalities (solid lines) can easily be seen. The significant increase in slope with increased acclimation temperature is also apparent in Fig. 1.

Fig. 2 illustrates that there is also close agreement between observed and expected mortality when pre- and post-treatment temperatures are the same. However, the dose-response curves are parallel. Although post-treatment temperature did not affect the slope of the dose-response curve, it did have a significant effect on allethrin LC_{50}s (Table 2). Lower post-treatment temperature combined with lower acclimation temperature resulted in increased toxicity, while higher acclimation combined with higher post-treatment temperature decreased allethrin's toxicity. The net result of combined pre- and post-treatment temperature effects was a 24-fold increase in toxicity at lower temperatures. The LC_{50} for cockroaches acclimated and post-treated at 22°C was again intermediate compared to the LC_{50}s from the 2 other temperatures. Control mortality averaged only 3.6% (Table A. 3), so adjustments in dose-response data were not necessary.

Table 1. Statistical analysis of tests of allethrin's toxicity to *Periplaneta americana* adult males

Accl temp (°C)	Post-tr temp (°C)	Conc. (ppm)	Mortality Totals 3 reps (n=30)	Mortality Totals expected (n=30)	Chi-squares
14	22	.50	5	4.892	.003
	22	1.00	10	9.376	.060
	22	2.00	15	15.053	.000
	22	3.00	16	18.457	.850
	22	5.00	24	22.326	.490
					1.403
22	22	2.00	3	3.637	.127
	22	3.00	10	9.156	.112
	22	4.00	16	14.503	.299
	22	5.00	17	18.781	.452
	22	8.00	26	25.837	.007
					.997
30	22	5.00	6	7.12	.231
	22	6.00	16	15.576	.024
	22	6.50	20	19.476	.040
	22	7.00	25	22.679	.974
	22	8.00	25	26.843	1.202
					2.471
14	14	.50	8	7.501	.044
	14	.75	14	14.429	.025
	14	1.00	19	19.628	.058
	14	1.25	23	23.126	.003
	14	1.50	26	25.409	.090
					.220
30	30	10	3	6.525	2.434
	30	12	12	8.761	1.691
	30	14	13	10.892	.641
	30	22	16	17.703	.400
	30	24	19	18.982	.000
					5.166

Table 2. Influence of acclimation and post-treatment temperature on allethrin's toxicity to Periplaneta americana adult males*

Acclimation temp (°C)	Post-treatment temp (°C)	LC_{50} (ppm)	95% Confidence limits	Slope
14	22	1.988 A	1.458-2.780	1.638 A
22	22	4.104 B	3.568-4.746	3.745 B
30	22	5.931 C	5.538-6.257	9.637 C
14	14	.774 A	.643- .892	3.558 A
22	22	4.104 B	3.568- 4.746	3.745 A
30	30	18.411 C	15.574-23.437	2.945 A

* LC_{50}s or slopes followed by the same letter are NOT significantly different.

Figure 1. Allethrin dose-response curves for Periplaneta americana adult males acclimated at 14°, 22°, and 30°C and post-treated at 22°C. Each point (observed value) is the pooled value for 3 replicates of 10 insects each. Lines represent expected values and were drawn by the method of least-squares. Mortality counts were made 24 hours after treatment with allethrin

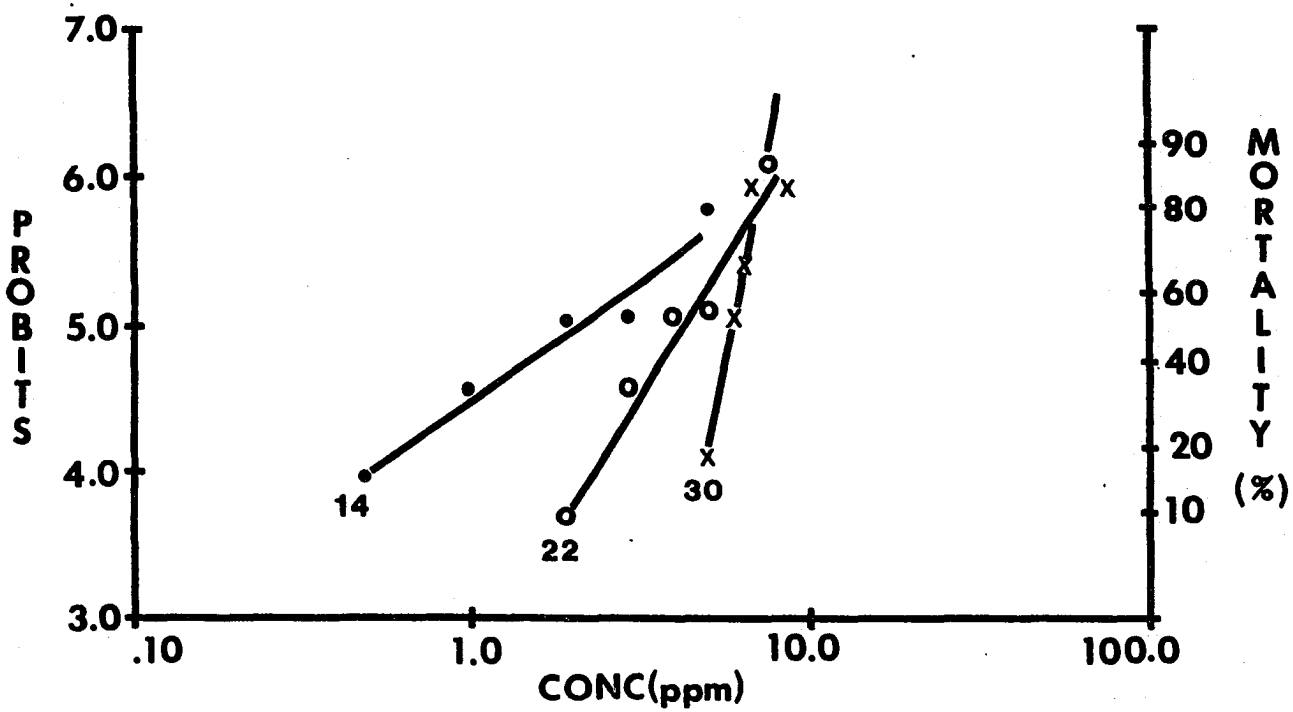
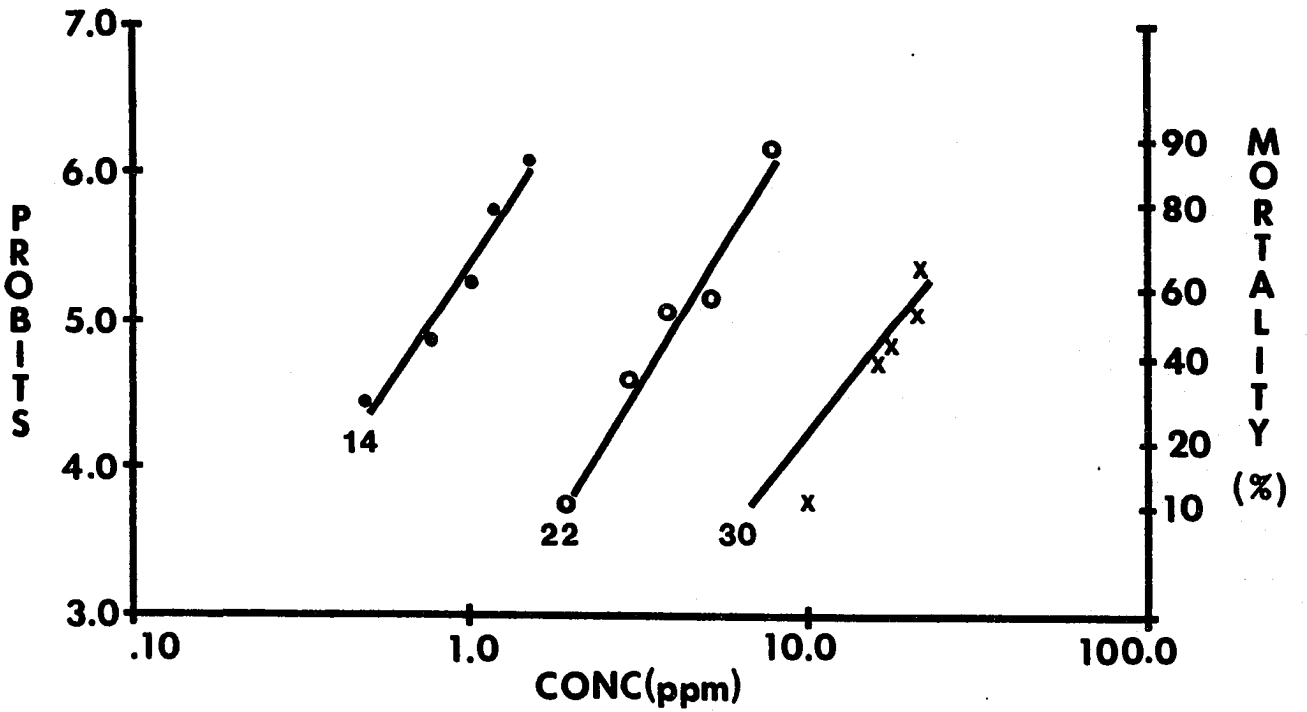

7.0
6.0
5.0
4.0
3.0
PROBITS
90
80
60
40
20
10
MORTALITY
(%)
.10
1.0
10.0
100.0
CONC(ppm)
14
22
30

Fig. 2. Allethrin dose-response curves for Periplaneta americana adult males acclimated at 14°, 22°, and 30°C (post-treatment = acclimation temperature). Each point (observed value) is the pooled value for 3 replicates of 10 insects each. Lines represent expected values and were drawn by the method of least-squares. Mortality counts were made 24 hours after treatment with allethrin


7.0
6.0
5.0
4.0
3.0
PROBITS
90
80
60
40
20
10
MORTALITY
(%)
14
22
30
.10
1.0
10.0
100.0
CONC(ppm)

DISCUSSION

Temperature acclimation (pre-treatment temperature) and post-treatment temperature influence the toxicity of allethrin to the American cockroach. Toxicity is negatively correlated with both, with acclimation temperature alone causing up to a 3-fold increase in toxicity. When acclimation and post-treatment temperature are combined, a 24-fold increase in toxicity occurs over the range 30° to 14°C.

Several workers have studied the effects of post-treatment temperature alone on toxicity. Using the housefly, Musca domestica, and several pyrethroids, Adams and Miller (1980) observed the negative temperature effect. DeVries and Georghiou (1979) found that lower post-treatment temperature doubled bioresmethrin's toxicity to the house fly. Sparks et al. (1982) also observed increased toxicity at lower post-treatment temperature when the cabbage looper, Trichoplusi ni, was topically treated with the pyrethroids permethrin (7.5-fold), deltamethrin (3.2-fold), and fenvalerate (2.0-fold). Since either one of the temperature treatments, (pre- or post-) results in a 2, 3 (our results), or 7.5-fold change in pyrethroid toxicity, the 24-fold change that we observed when combining low pre- and post-treatment temperatures seems to be of reasonable magnitude.

Because of effects on toxicity, an insect's temperature regime before exposure to a toxicant must be controlled just as carefully as the post-treatment temperature if results from different laboratories are to be comparable. Information on temperature-toxicity relationships could also aid in proper dose selection in the field. Proper dose selection is an increasingly important factor in pest treatment, since insects treated but not killed can facilitate development of resistance in a population.

The underlying mechanism of temperature's effect on toxicity is not clearly understood. Lower post-treatment temperature enhances blockage of impulse conduction by allethrin and increases hyperexcitation of the cockroach's peripheral nervous system, probable factors in allethrin's greater toxicity at low post-treatment temperatures (Gammon, 1978, 1979; Narahashi, 1979). Pre-treatment (acclimation) temperature may act through the same mechanism to a lesser or greater degree resulting in toxicity changes of a different magnitude.

The toxicity trend obtained in this study together with relevant information in the literature suggest the possibility that changes in an insect's lipids may be important to toxicity. In this study, toxicity increased in an orderly manner (as acclimation temperature decreased). In many animals, lipids also change in an orderly manner;

low acclimation temperature usually results in more unsaturated lipids. Munson (1953) found this to be true for whole-body lipids of the American cockroach. It has also been demonstrated that lipophilic toxins are more soluble in unsaturated than in saturated lipids (Munson, 1953). Pradhan et al. (1952), found that DDT was more soluble in lipids with lower melting points (presumably less-saturated).

Since it has been shown that radiolabeled pyrethroids (Soderlund, 1979) and pyrethrins (Zeid et al., 1983) reach the nervous system of American cockroaches, it seems reasonable to suggest that any change in the cockroach's nervous system that affects the solubility and binding of the toxin could affect its toxicity. Osborne and Smallcombe (1983) claim that changes in lipids of the nerve cell membrane (over generations) affected the binding of lipophilic toxins in the housefly, eventually leading to resistance. Richards (1943) claims that the lipids of nerve sheaths are important factors in the penetration of neurotoxins in insects.

It seems possible that acclimative changes in the saturation level of lipids near allethrin's active site could affect toxicity. Lipids in the axonal membrane and neural fat body sheath of the cockroach (which, according to Lane (1974) is formed by patches of cells surrounding the

nervous system) seem to be likely candidates. These speculations led to another series of experiments in which gas-liquid chromatography was used to determine the fatty acid composition and saturation level of neural as compared to non-neural lipids as a function of thermal acclimation (Baldus and Mutchmor, 1987). Future investigations may reveal the nature and precise location of the toxicity-related acclimative changes found in these experiments.

REFERENCES

Abbassy, M. A., Eldefrawi, M. E., and Eldefrawi, A. T. 1982. Allethrin interactions with the nicotinic acetylcholine receptor channel. Life Sci. 31, 1547-1552.

Adams, M. E. and Miller, T. A. 1980. Neural and behavioral correlates of pyrethroids and DDT-like poisoning in the housefly, Musca domestica L. Pest. Biochem. Physiol. 13, 137-147.

Anderson, R. L. and Mutchmor, J. A. 1968. Temperature acclimation and its influence on the electrical activity of the nervous system in three species of cockroaches. J. Insect. Physiol. 14, 243-251.

Baldus, T. J. 1984. The effects of temperature acclimation on the toxicity of allethrin and malathion to the American cockroach, Periplaneta americana. M. S. thesis. Iowa State University, Ames, Iowa.

Baldus, T. J. and Mutchmor, J. A. 1987. The effects of temperature acclimation on the fatty acid composition of the nerve cord and fat body of the American cockroach, Periplaneta americana. Comp. Biochem. Physiol. A. (In press).

Clark, J. M. 1981. Pyrethroid inhibition of neural ATPases. Ph.D. dissertation. Michigan State University, East Lansing, Michigan.

Cornwell, P. B. 1976. The Cockroach. Vol. 2. Associated Business Programmes, Ltd., London.

Craufurd-Benson, H. J. 1938. An improved method for testing liquid contact insecticides in the laboratory. Bull. Ent. Res. 29, 41-56.

Das, A. B., and Singh, S. P. 1974. Thermal acclimation in the rate of endogenous oxygen consumption of the fat body and coxal muscle of Periplaneta americana (Linn). Environ. Physiol. Biochem. 4, 139-146.

DeVries, D. H., and Georghiou, G. P. 1979. Influence of temperature on the toxicity of insecticides to susceptible and resistant house flies. J. Econ. Entomol. 72, 48-50.

Finney, D. J. 1971. Probit analysis. 3rd ed. Cambridge University Press, London. 333 pp.

Flynn, A. D., and Schoof, H. F. 1970. Immersion technique for measuring susceptibility of house flies and cockroaches to insecticides. J. Econ. Entomol. 63, 883-886.

Gammon, D. W. 1978. Neural effects of allethrin on the free walking cockroach, Periplaneta americana: an investigation using defined doses at 15° and 32°C. Pestic. Sci. 9, 79-91.

Gammon, D. W. 1979. An analysis of the temperature-dependence of the toxicity of allethrin to the cockroach. In Neurotoxicology of Insecticides and Pheromones. (pp. 97-117) (Edited by T. Narahashi). Plenum Press, New York.

Gammon, D. W., Brown, M. A., and Casida, J. E. 1981. Two classes of pyrethroid action in the cockroach. Pestic. Biochem. Physiol. 15, 181-191.

Hadaway, A. B., and Barlow, F. 1957. The influence of temperature and humidity upon the action of insecticides: II. Temperature during the pre-treatment period. Ann. Trop. Med. Parasitol. 51, 194-200.

Hazel, J. R. 1973. The regulation of cellular function by temperature-induced alterations in membrane composition. In Effects of Temperature on Ectothermic Organisms. (pp. 56-67) (Edited by W. Wieser). Springer-Verlag, New York.

Hoffmann, K. H. 1985. Metabolic and enzyme adaptation to temperature. In Environmental Physiology and Biochemistry of Insects. (pp. 1-32) (Edited by K. H. Hoffmann). Springer-Verlag, Berlin.

Jamil, K. 1984. Differential response of American cockroach Periplaneta americana L. to chlorinated hydrocarbon insecticides when acclimatized to different temperatures. Entomology. 9, 201-207.

Lane, N. J. 1974. The organization of insect nervous systems. In Insect Neurobiology. (pp. 1-71) (Edited by J. E. Treherne). American Elsevier Publishing Co., New York.

Laufer, J., Roche, M., Pelhate, M., Elliot, M., Janes, N. F., and Sattelle, D. B. 1984. Pyrethroid insecticides: actions of deltamethrin and related compounds on insect axonal sodium channels. J. Insect Physiol. 30, 341-349.

Litchfield, J. T., Jr. and Wilcoxon, F. 1949. A simplified method of evaluating dose-effect experiments. J. Pharma. Exp. Ther. 96, 99-113.

Munson, S. C. 1953. Some effects of storage at different temperatures on the resistance of the American roach to DDT. J. Econ. Entomol. 46, 756-760.

Narahashi, T. 1965. The physiology of insect axons. In Physiology of the Insect Central Nervous System. (pp. 1-20) (Edited by J. E. Treherne and J. W. Beament). Academic Press, New York.

Narahashi, T. 1971. Effects of insecticides on excitable tissues. Adv. Insect Physiol. 8, 1-93.

Narahashi, T. 1979. Nervous system as a target site of pheromones and insecticides. In Neurotoxicology of Insecticides and Pheromones. (pp. 1-4) (Edited by T. Narahashi). Plenum Press, New York.

Narahashi, T. 1985. Nerve membrane ionic channels as the primary target of pyrethroids. Neurotoxicology 6, 3-22.

Osborne, M. P., and Smallcombe, A. 1983. Site of action of pyrethroid insecticides as revealed by the kdr resistance factor. In Pesticide Chemistry: Human Welfare and the Environment. (pp. 103-107) (Edited by J. Miyamoto). Pergamon Press, Oxford.

Potter, C., and Gillham, E. M. 1946. Effects of atmospheric environment before and after treatment on the toxicity of contact poisons to insects. Ann. Appl. Biol. 33, 142-159.

Pradhan, S., Nair, M. R., and Krishnaswami, S. 1952. Lipid solubility as a factor in the toxicity of contact insecticides. Nature 170, 619-620.

Rai, B. K. 1967. Temperature coefficient of insect susceptibility to insecticides. Indian J. Exp. Biol. 5, 151-155.

Richards, A. G. 1943. Lipid nerve sheaths in insects and their probable relation to insecticide action. J. New York Entomol. Soc. 50, 55-65.

Sawicki, R. M. and Farnham, A. W. 1964. A dipping technique for selecting house-flies, Musca domestica L., for resistance to insecticides. Bull. Entomol. Res. 55, 541-546.

Scott, J. G., and Matsumura, F. 1983. Evidence for two types of toxic actions of pyrethroids on susceptible and DDT-resistant German cockroaches. Pestic. Biochem. Physiol. 19, 141-150.

Singh, S. P., and Das, A. B. 1980. Biochemical changes in tissue composition of Periplaneta americana (Linn.) acclimated to high and low temperatures. J. Therm. Biol. 5, 211-218.

Soderlund, D. M. 1979. Pharmacokinetic behavior of enantiomeric pyrethroid esters in the cockroach, Periplaneta americana L. Pestic. Biochem. Physiol. 12, 38-48.

Sparks, T. C., Shour, M. H. and Wellemeyer, E. G. 1982. Temperature-toxicity relationships of pyrethroids on three lepidopterans. J. Econ. Entomol. 75, 643-646.

Sun, Y. P. 1960. Pre-test conditions which affect insect reaction to insecticides. In Methods of Testing Chemicals in Insects. (pp. 1-9) (Edited by H. H. Shepard). Burgess Publishing Co., Minneapolis, Minnesota.

Thomas, R. E., and Rice, S. D. 1986. Effect of temperature on uptake and metabolism of toluene and naphthalene by Dolly Varden char, Salvelinus malma. Comp. Biochem. Physiol. 84C. 83-86.

Van den Bercken, J., and Vijverberg, H. P. 1983. Interaction of pyrethroids and DDT-like compounds with the sodium channels in the nerve membrane. In Pesticide Chemistry: Human and the Environment. (pp. 115-121) (Edited by J. Miyamoto). Pergamon Press, Oxford.

Zeid, M. M., Dahm, P. A., Hein, R. E., and McFarland, R. H. 1953. Tissue distribution, excretion, of $C^{14}O_2$ and degradation of radioactive pyrethrins administered to the American cockroach. J. Econ. Entomol. 46, 324-336.

SECTION II. THE EFFECTS OF TEMPERATURE ACCLIMATION ON THE FATTY ACID COMPOSITION OF THE NERVE CORD AND FAT BODY OF THE AMERICAN COCKROACH, *PERIPLANETA AMERICANA*

INTRODUCTION

Lipids play a variety of important biological roles in insects and other animals, but the fatty acid composition of only a few insects has been determined. These include the African migratory locust, Locusta migratoria (Fawzi et al., 1961), the silkworm moth, Bombyx mori (Nakasone and Ito, 1967), the American cockroach, Periplaneta americana, and the horse-lubber grasshopper, Taeniopoda equis (Stanley-Samuelson and Dadd, 1983). Whole-body lipid extracts contain mostly even-numbered saturated and monounsaturated fatty acids from 14 to 18 carbons in length; a small proportion of polyunsaturates from 18 to 22 carbons in length also occurs. Palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acids comprise about 98% of the total fatty acids in whole-body lipid extracts.

The fatty acids in individual organs and tissues have also been determined, including the nerve cord, brain (Stanley-Samuelson and Dadd, 1983), fat body (Nelson et al., 1967), reproductive tissue, and salivary glands of P. americana (Stanley-Samuelson and Pipa, 1984). In fish, the fatty acid compositions of whole brains (Roots and Johnston, 1968), brain synaptosomes (Cossins et al., 1977), olfactory axons, and trigeminal axons (Friedman et al., 1986), have been determined. Lee and Gonsoulin (1979) have described the fatty acid content of nerve cords, ganglia, and brains

of the horseshoe crab, Limulus polyphemus.

The fatty acid composition of animals is influenced by many factors, including temperature acclimation. The fatty acids of frogs (Baranska and Wlodawer, 1969), and fish (Roots and Johntson, 1968; Friedman et al., 1986) are affected by temperature acclimation, as is the fatty acid composition of the fruit fly Drosophila melanogaster (Keith, 1966) and the blowflies Calliphora erythrocephala and Sarcophaga bullata (Danks and Tribe, 1979). In all these studies, low temperature acclimation resulted in a greater degree of unsaturation of lipids.

Temperature acclimation may also affect the toxicity of insecticides. Munson (1953) found that DDT was more toxic to warm- than to cold-acclimated P. americana nymphs. Baldus (1984) and Baldus and Mutchmor (1987) found that a 7-8 day period of temperature acclimation affected the toxicity of allethrin to P. americana adult males. Several researchers have suggested that the effects of acclimation on toxicity may be due to acclimative changes in the insect's lipids. Munson (1953) postulated that increased levels of unsaturated lipids in the bodies of cold-acclimated cockroaches protected the insect by binding DDT at non-target site locations. Baldus and Mutchmor (1987) speculated that acclimative changes in the lipids of the neural fat body sheath (Lane, 1974) and/or the axonal

membrane might influence the toxicity of allethrin. Because lipid changes near allethrin's active site would probably have a different effect on toxicity than changes at non-target sites, both non-neural and neural organs were used for comparative purposes. In the present study, evidence is presented which shows that the fatty acid composition of fat bodies and nerve cords (including the neural fat body sheath) of P. americana adult males is affected differently by acclimation at 14, 22, or 30°C, and that the changes are correlated with acclimative changes in the toxicity of allethrin.

MATERIALS AND METHODS

Adult male P. americana (average weight=0.85 g) were acclimated for one or three weeks at either 14, 22, or 30 ± 1°C and 55-65% relative humidity in Freas Model 805 incubators. Nerve cords and fat bodies were dissected in ice-cold saline (Stanley-Samuelson and Dadd, 1983), blotted lightly on filter paper, and weighed. Initially the saturation level of extracted lipids was measured by iodine numbers obtained by titration. However, it was soon apparent that greater precision was necessary; therfore, gas-liquid chromatographic techniques were adopted. For the chromatographic analysis, portions of the 10 nerve cords or 4 fat bodies comprising one sample were removed to ensure a final mass of 45 to 50 mg (3 samples were analyzed at each temperature). The sample was ground in a glass homogenizer containing 2 ml of chloroform/methanol (2:1) and filtered before removal of non-lipid contaminants by a modified Folch wash (Folch et al., 1957).

Methyl esters of the fatty acids were prepared by adding 3 ml of absolute methanol and 10 drops (approximately 0.3 ml) of concentrated sulfuric acid to approximately 4 ml of sample. After 2 hours at 60°C, the reaction was stopped by addition of 3 ml distilled water. Methyl esters were removed with 3 pentane washes (Stanley-Samuelson and Dadd, 1981). The methyl ester sample was dried completely under

nitrogen and taken up in 1.0 ml pentane. A four microliter aliquot of the concentrated sample was injected into a Hewlett Packard 5700 Series gas chromatograph equipped with a 3.1m X 2mm inside diameter glass column packed with 10% Silar^{R} 10C on 100/120 mesh Gas Chrom^{R} QII (Alltech Associates, Inc.). A 2m column was used in preliminary runs, but a 3.1m column afforded much better separation of fatty acids, and was used to obtain all of the chromatographic data reported herein. Measured flow rates were: nitrogen 15, hydrogen 29, and air 200 ml/minute. Injector and detector were at 250°C, and samples were run isothermally at 165°C for 50 minutes.

Peaks were identified by comparison of the retention times with those of authentic standards interspersed between test runs. Identities were confirmed by spiking samples with individual standards and then noting the effect on the heights of specific peaks. Areas under peaks were determined by a model 3380 electronic integrator connected to the chromatograph. Fluctuation in detector response was checked by daily injection of a mixture of 10 standard fatty acids (10 ppm each); maximal day-to-day variation in weight-% was 2%. Examples of typical chromatograms obtained from lipid extracts are shown in Appendix B (Figs. B.1 and B.2).

Statistical analysis included ANOVA to determine variability, F tests to identify treatment effects, and least significant difference and Student's t tests to identify significant differences between groups.

RESULTS

The acclimative changes in lipid composition appeared to be complete after a 1-week period; a one-week acclimation period had the same effect on the relative amounts of saturated and unsaturated fatty acids as a three-week period, with respect to both direction and magnitude of change (Tables 1 and 2). For any particular fatty acid, temperature acclimation influenced its weight-% within one week; an additional 2 weeks of acclimation did not increase or decrease the difference in weight-% found at the 3 temperatures. The additional 2 weeks of temperature acclimation did cause a general change in the weight-% of some fatty acids without affecting the relative differences produced by the three acclimation temperatures; palmitic acid decreased while linoleic acid increased at all temperatures in both organs. Saturated and unsaturated fatty acid totals for 3-week compared to 1-week acclimated cockroaches were not significantly different at any temperature for either organ. Because acclimative changes in lipid composition were essentially complete after one week, the following account is based on the one-week tests.

A total of fifteen fatty acids were detected in nerve cord extracts, and 11 were identified (Fig. 1 and Table 1). Total weight-% for unknowns was less than 2% at any acclimation temperature (Table 1). The predominant fatty

acids included oleic (40%), palmitic (22%), linoleic (17%), stearic (10%), arachidonic (3%), and palmitoleic (2%); these 6 comprised 94% of the weight-% of total detectable fatty acids. Ten of the 15 were significantly ($P \leq 0.05$) affected by acclimation temperature (Table 1). Low temperature acclimation caused a systematic decrease in the amounts of 3 saturated fatty acids (myristic, pentadecanoic, and palmitic), resulting in the following rank order for weight-%: 14<22<30°C (see Fig. 2). The weight-% of 3 unsaturated fatty acids (linoleic, eicosadienoic, and arachidonic) increased in response to low temperature acclimation, resulting in the opposite rank order (14>22>30°C). By examining the changing column heights in Fig. 2, one can more easily visualize the decrease in saturated and the increase in unsaturated fatty acids at lower acclimation temperatures. The 5 remaining identified fatty acids (palmitoleic, margaric, stearic, oleic, and linolenic) followed no consistent pattern with regard to weight-% and acclimation temperature. The effects of cold-acclimation are readily seen when data are normalized so that weight-% at 30°C equals 1.0. Figures 3, 4, and 5 show that certain saturated fatty acids decrease (Fig. 3), certain unsaturated fatty acids increase (Fig. 5), and other fatty acids follow no consistent pattern (Fig. 4). The net effect of a decrease in weight-% of the saturated fatty

acids myristic, pentadecanoic, palmitic, and margaric (Fig. 3) and an increase in the unsaturated fatty acids linoleic, eicosadienoic, and arachidonic (Fig. 5) is increased unsaturation of neural lipids at lower acclimation temperatures. The total weight-% of unsaturated fatty acids reflects this net effect; the total was 68% at 14° and 64% at 30°C.

A total of seventeen fatty acids were detected in fat body extracts, and 11 were identified (Fig. 6 and Table 2). Total weight-% for unknowns was less than 1.5% at any acclimation temperature (Table 2). The predominant fatty acids included oleic (40%), palmitic (25%), linoleic (18%), stearic (10%), palmitoleic (3%), and myristic (1%); these 6 comprised 97% of the weight-% of total detectable fatty acids. Compared to nerve cords, fat bodies had higher weight-percentages of short-chain saturated fatty acids (myristic and palmitic) and significantly less (lower weight-percentages) long-chain polyunsaturated fatty acids (eicosadienoic and arachidonic). Six of the 17 detectable fatty acids were significantly affected by acclimation temperature (Table 2); these included myristic, palmitic, margaric, stearic, oleic, and linoleic. Myristic was the only identified fatty acid from fat body extracts whose weight-% followed a consistent pattern (14<22<30°C). No clear pattern of weight-% in the other fatty acids could be

detected (Table 2). For example, pentadecanoic, palmitic, stearic, and arachidonic weight-percentages were lowest at 22°C while palmitoleic, oleic, linoleic, and eicosadienoic weight-percentages were highest at 22°C. Normalized data for fat body fatty acids also show that most saturated (Fig. 7) and unsaturated (Fig. 8) fatty acids follow no consistent pattern with temperature acclimation. The random changes in fat body fatty acids with temperature acclimation displayed in Figs. 7 and 8 are in marked contrast to the consistent (i. e., directional) changes in nerve cord fatty acids shown in Figs. 3 and 5.

In addition to differences in individual fatty acids and different responses to temperature acclimation, total weight-% of unsaturated fatty acids was also different in the 2 organs. Nerve cord unsaturates averaged 67% for the 3 acclimation temperatures, while the fat body average was 63%.

Table 1. Weight-% of individual fatty acids from nerve cord extracts of *Periplaneta americana* adult males*

Code	Acid	Accl temp (1 wk) 14°C (n=3)	22°C (n=3)	30°C (n=3)	Accl temp (3 wk) 14°C (n=2)	22°C (n=2)	30°C (n=2)
14:0	myristic	.51C	.71B	.94A	.37A	.66A	.70A
15:0	pentadecanoic	.05A	.10A	.17A	.00A	.00A	.08A
16:0	palmitic	18.22B	19.47B	22.44A	17.08A	18.51A	20.28A
16:1	palmitoleic	1.63B	2.23A	2.09AB	1.32A	2.00A	1.66A
17:0	margaric	.49AB	.43B	.62A	.39B	.47AB	.56A
	unknown	.38A	.50A	.43A	.28B	.45A	.34AB
18:0	stearic	11.02A	8.63B	10.34A	10.36AB	9.25B	10.68A
18:1	oleic	40.20	43.15A	40.23B	41.34A	42.43A	38.42B
18:2	linoleic	18.95A	18.18AB	17.21B	19.64A	19.33A	20.24A
	unknown	.27A	.00A	.00A	.00A	.04A	.00A
18:3	linolenic	.66A	.44A	.68A	.00A	.28A	.18A
	unknown	.00B	.21A	.18A	.00A	.12A	.12A
20:2	eicosadienoic	.90A	.75A	.56B	.93A	.67B	.68B
20:4	arachidonic	5.66A	3.98B	3.27B	6.64A	4.70A	4.93A
	unknown	.94A	1.10A	.88A	1.54A	1.08A	1.15A

*Within the same acclimation period, means followed by the same letter are not significantly different.

Table 2. Weight-% of individual fatty acids from fat body extracts of Periplaneta americana adult males*

Code	Acid	Accl temp (1 wk) 14°C (n=3)	22°C (n=3)	30°C (n=3)	Accl temp (3 wk) 14°C (n=2)	22°C (n=2)	30°C (n=2)
14:0	myristic	.86B	.90B	1.35A	.62A	1.20A	1.16A
	unknown	.20A	.15A	.10A	.18A	.03B	.00B
15:0	pentadecanoic	.15A	.10A	.19A	.12A	.16A	.10A
16:0	palmitic	23.00B	22.31B	25.41A	21.59A	21.92A	24.86A
16:1	palmitoleic	2.38A	2.82A	2.61A	1.79B	3.40A	2.02B
17:0	margaric	.69A	.47B	.65A	.56A	.36A	.48A
	unknown	.35A	.46A	.41A	.26B	.48A	.24B
18:0	stearic	12.23A	7.37B	9.77AB	11.50A	7.18B	10.81A
18:1	oleic	43.59AB	45.36A	40.48B	45.94A	44.16A	40.62B
18:2	linoleic	14.80B	18.72A	17.65AB	16.39A	19.86A	18.76A
	unknown	.02A	.02A	.00A	.00A	.00A	.00A
18:3	linolenic	.37A	.37A	.42A	.19C	.37A	.30B
	unknown	.14A	.19A	.18A	.08A	.14A	.06A
20:2	eicosadienoic	.07A	.13A	.09A	.07A	.14A	.08A
20:4	arachidonic	.34A	.12A	.16A	.20A	.12AB	.03B
	unknown	.05A	.00A	.00A	.00A	.00A	.00A
	unknown	.63A	.41A	.39A	.51A	.38A	.37A

*Within the same acclimation period, means followed by the same letter are not significantly different.

Figure 1. Fatty acid profile from nerve cord extracts of Periplaneta americana adult males after a 7-8 day period of temperature acclimation at 30°C. Number below each vertical bar is code for chain length: number of double bonds. Number above each bar is average weight-% from 3 samples

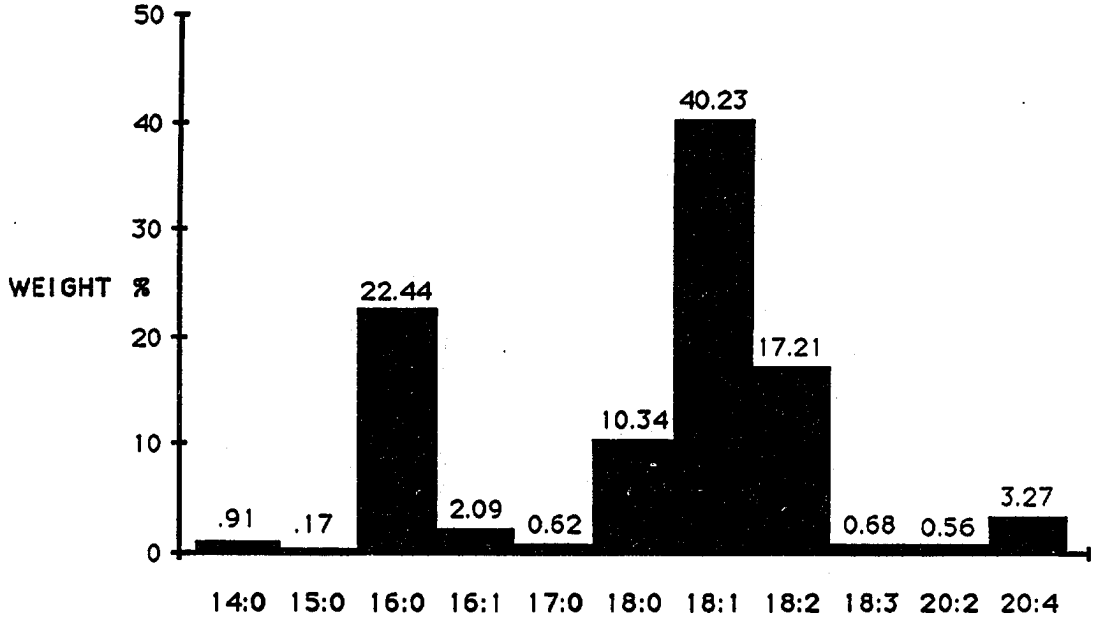

WEIGHT %
50
40
30
20
10
0
.91
.17
22.44
2.09
0.62
10.34
40.23
17.21
0.68
0.56
3.27
14:0
15:0
16:0
16:1
17:0
18:0
18:1
18:2
18:3
20:2
20:4

Figure 2. Weight-% of 3 saturated and 3 unsaturated fatty acids in nerve cord extracts of Periplaneta americana adult males after a 7-8 day period of temperature acclimation at 14, 22, or 30°C. All values are means from 3 samples. The range of S. E. M. values was .040 to .564 weight-%

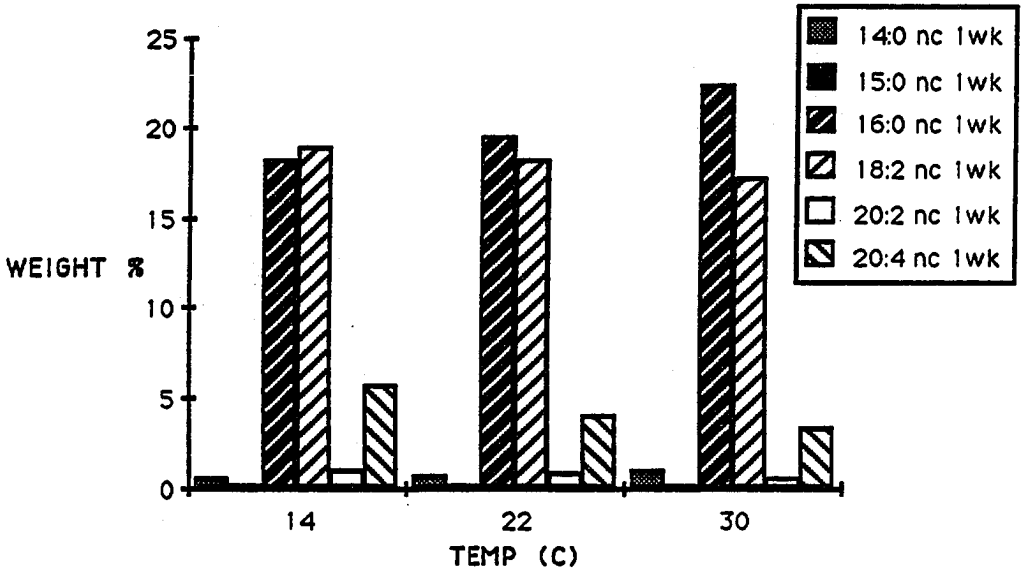

14:0 nc 1wk
15:0 nc 1wk
16:0 nc 1wk
18:2 nc 1wk
20:2 nc 1wk
20:4 nc 1wk
WEIGHT %
25
20
15
10
5
0
14
22
30
TEMP (C)

Figure 3. Normalized data (weight-% divided by weight-% at 30°C) for 4 saturated fatty acids from nerve cord extracts of Periplaneta americana adult males after a 7-8 day period of temperature acclimation at 14, 22, or 30°C. (means from 3 samples)

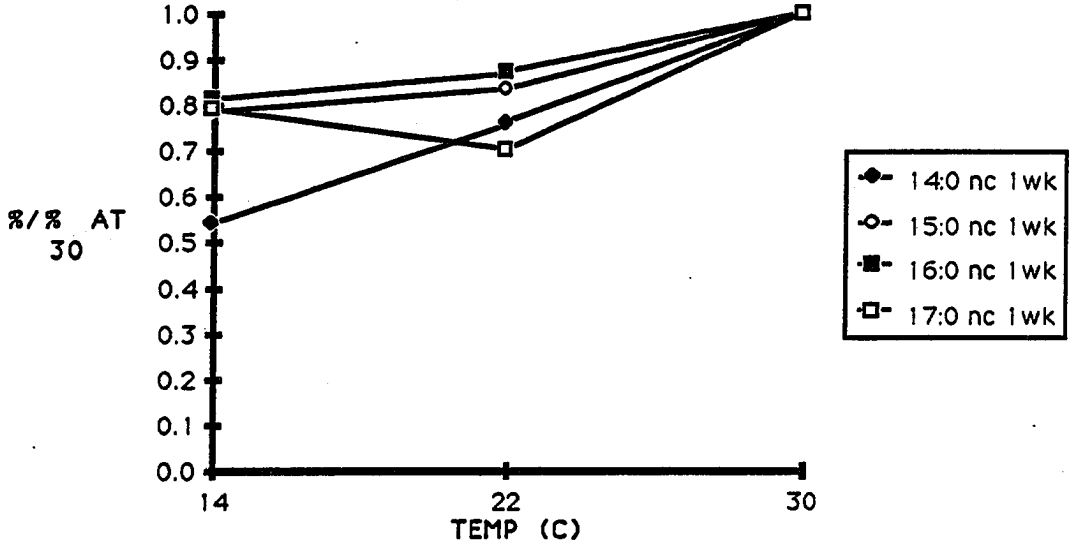

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
%/% AT 30
14
22
30
TEMP (C)
14:0 nc 1wk
15:0 nc 1wk
16:0 nc 1wk
17:0 nc 1wk

Figure 4. Normalized data (weight-% divided by weight-% at 30°C) for fatty acids from nerve cord extracts of Periplaneta americana adult males after a 7-8 day period of temperature acclimation at 14, 22, or 30°C. (means from 3 samples)

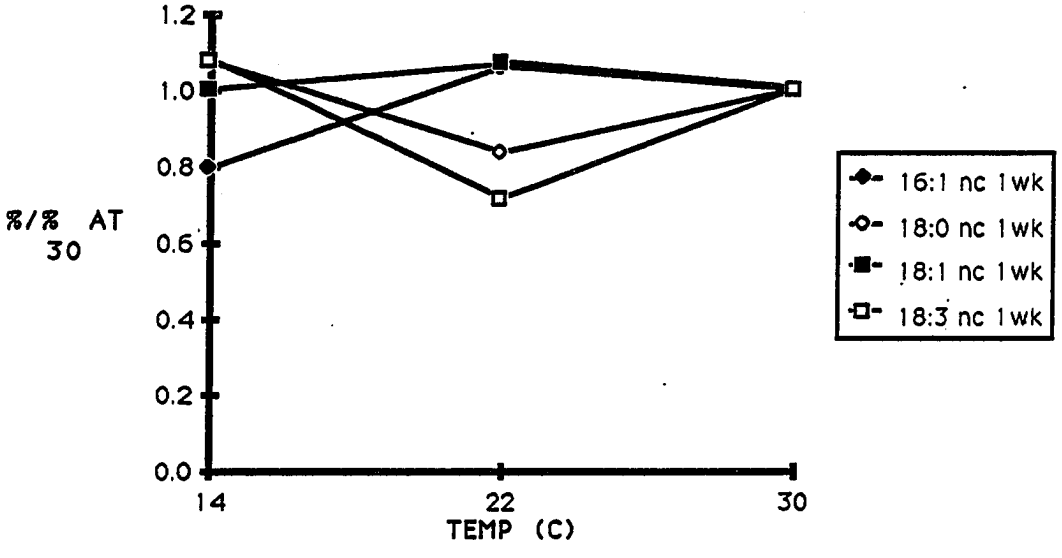

1.2
1.0
0.8
0.6
0.4
0.2
0.0
%/% AT 30
14
22
30
TEMP (C)
16:1 nc 1wk
18:0 nc 1wk
18:1 nc 1wk
18:3 nc 1wk

Figure 5. Normalized data (weight-% divided by weight-% at 30°C) for 3 polyunsaturated fatty acids from nerve cord extracts of Periplaneta americana adult males after a 7-8 day period of temperature acclimation of 14, 22, or 30°C. (means from 3 samples)

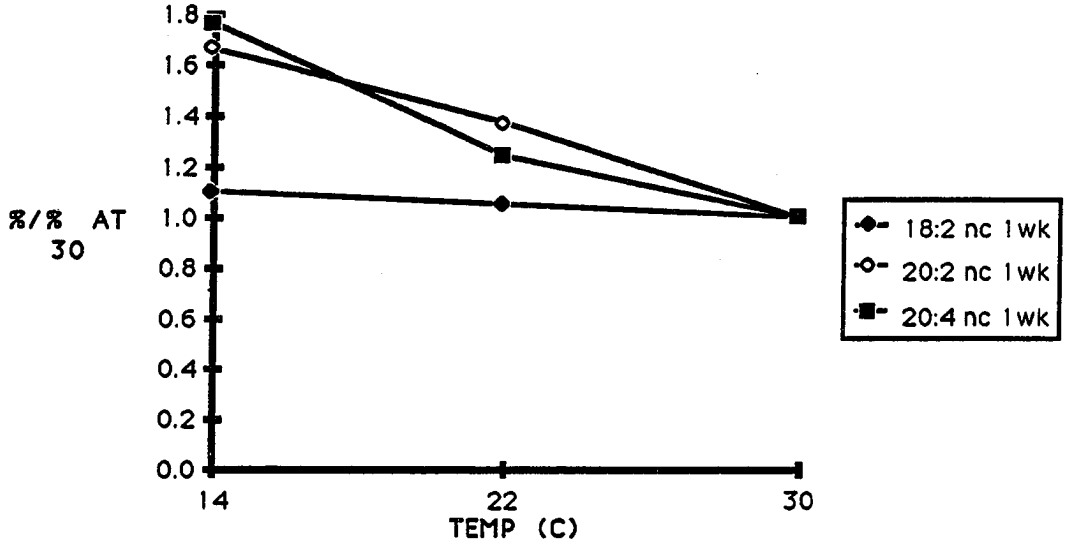

%/% AT 30
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
14
22
30
TEMP (C)
18:2 nc 1wk
20:2 nc 1wk
20:4 nc 1wk

Figure 6. Fatty acid profile from fat body extracts of Periplaneta americana adult males after a 7-8 day period of temperature acclimation at 30°C. Number below each vertical bar is code for chain length: number of double bonds. Number above each bar is average weight-% from 3 samples

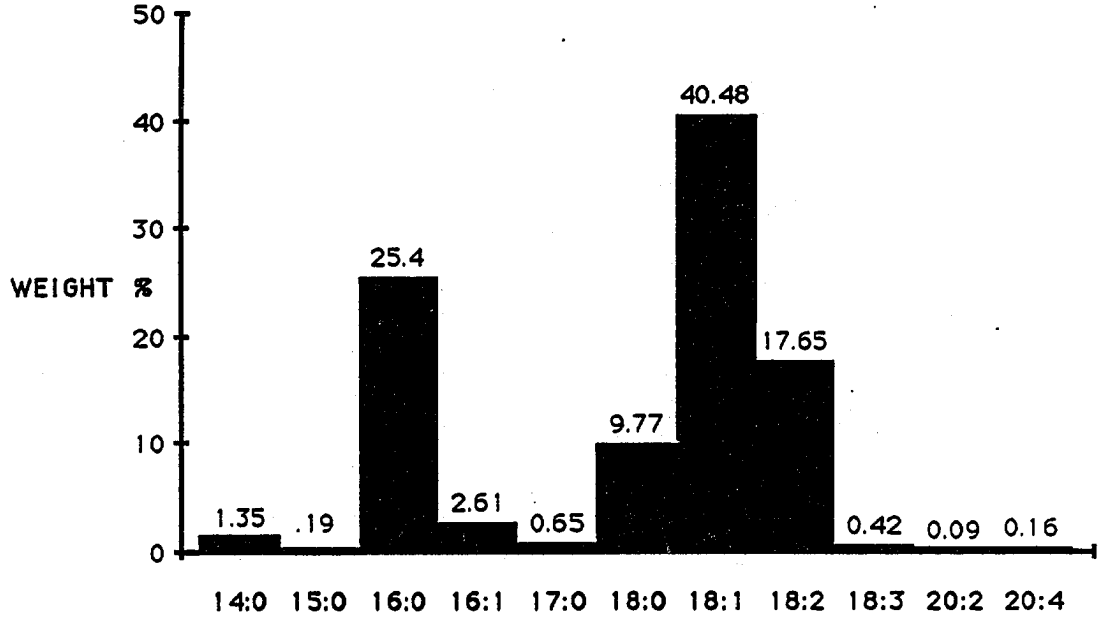

WEIGHT %
50
40
30
20
10
0
1.35
.19
25.4
2.61
0.65
9.77
40.48
17.65
0.42
0.09
0.16
14:0
15:0
16:0
16:1
17:0
18:0
18:1
18:2
18:3
20:2
20:4

Figure 7. Normalized data (weight-% divided by weight-% at 30°C) for 5 saturated fatty acids from fat body extracts of Periplaneta americana adult males after a 7-8 day period of temperature acclimation at 14, 22, or 30°C. (means from 3 samples)

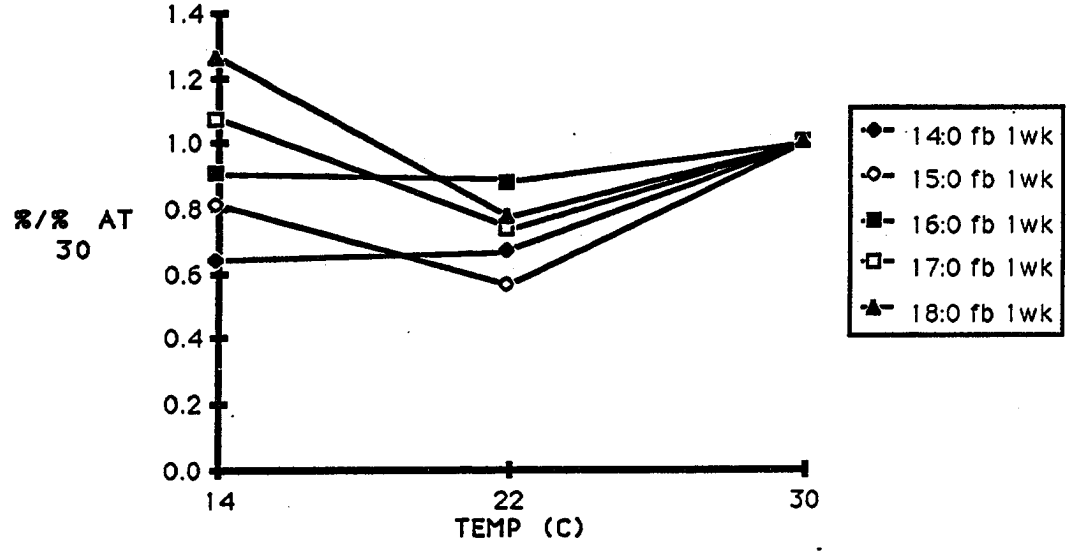
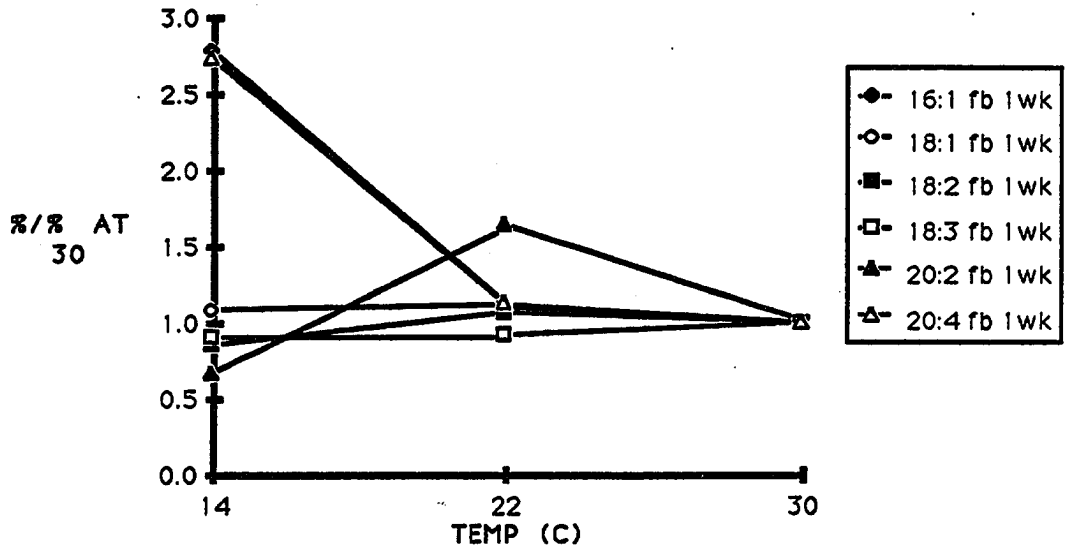

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
%/% AT 30
14
22
30
TEMP (C)
14:0 fb 1wk
15:0 fb 1wk
16:0 fb 1wk
17:0 fb 1wk
18:0 fb 1wk

Figure 8. Normalized data (weight-% divided by weight-% at 30°C) for 6 unsaturated fatty acids from fat body extracts of *Periplaneta americana* adult males after a 7-8 day period of temperature acclimation at 14, 22, or 30°C. (means from 3 samples)


3.0
2.5
2.0
1.5
1.0
0.5
0.0
%/% AT 30
14
22
30
TEMP (C)
16:1 fb 1wk
18:1 fb 1wk
18:2 fb 1wk
18:3 fb 1wk
20:2 fb 1wk
20:4 fb 1wk

DISCUSSION

Relatively few researchers have investigated the fatty acid composition of individual tissues and organs of insects. Of the studies done with P. americana, Nelson et al. (1967) determined the fatty acid composition of the mono-, di-, and triglycerides of the fat bodies from adult males. Our results (also from adult males) from unfractionated lipid extracts of fat bodies include the same 3 lipid classes. The close agreement between our results and those of Nelson et al. (1967) can be seen by listing their results followed by ours (in parentheses) for several fatty acids: 14:0=1% (.90-1.4), 16:0=25% (22-25), 16:1=2% (2.4-2.8), 18:0=8% (7.4-12.1), 18:1=44% (41-45), 18:2=16% (15-19).

The fatty acid profile of nerve cords obtained in this study can also be compared to unfractionated extracts of nerve cords plus brains from unsexed adult P. americana (Stanley-Samuelson and Dadd, 1983). Close agreement can again be seen for several fatty acids: saturated plus monoenes=72% (72-77), 18:2=17% (17-19), 20:2=0.2% (0.5-0.9),20:4=2% (3.2-5.6). Our values for saturated fatty acids plus monoenes are also comparable to those found for goldfish brain and horseshoe crab nerve cords: Cossins et al. (1977) found that the major phospholipids of synaptosomal membranes isolated from goldfish brain contain

42-79% saturated + monoenic fatty acids, depending on acclimation temperature and class of phospholipid. The saturated plus monoenes in nerve cord phospholipids of the horseshoe crab comprised 67% of the total fatty acids (Lee and Gonsoulin, 1979).

There are few studies on the effects of temperature acclimation on the fatty acid composition of individual tissues and organs of insects. A 4-day period of low temperature acclimation resulted in an increase in long chain unsaturated fatty acids in phospholipids of flight muscle mitochondria in blowflies (Danks and Tribe, 1979). The effects of temperature acclimation on fatty acid composition of insect nervous systems has apparently never been investigated.

Acclimative studies have been done for several species of fish. Warm-acclimation resulted in increased levels of saturated fatty acids in phospholipids of garfish axons (Friedman et al., 1986) and goldfish brain synaptosomes (Cossins et al., 1977). The garfish were acclimated for 4-8 weeks and the goldfish for 21 days. In our studies, a relatively short (7-8 day) period of temperature acclimation affected the fatty acids of nerve cords from the American cockroach (a significant result in light of the above studies).

In speculating about the mechanism for the negative

temperature coefficient that exists between acclimation temperature and allethrin toxicity to P. americana adult males (Baldus and Mutchmor, 1987), we suggested that the neural lipids of cold-acclimated cockroaches might be more unsaturated than the lipids of warm-acclimated cockroaches. The results of this study lend support to that idea since, in nerve cord extracts, low temperature acclimation resulted in a decrease in 3 saturated fatty acids and an increase in 3 unsaturated fatty acids (see Fig. 2). When the increased toxicity of allethrin at low acclimation temperatures (Baldus and Mutchmor, 1987) is examined in light of the present research, a positive correlation between toxicity and degree of unsaturation of neural lipids is apparent. Although we present no direct evidence for a causal relationship between the two, it seems possible that increased amounts of unsaturated neural lipids would bind more allethrin, since lipophilic toxins are more soluble in unsaturated than in saturated lipids (Munson, 1953). If increased binding in the nervous system did, indeed, occur, then increased toxicity should follow.

In conclusion, a relatively short (7-8 day) period of temperature acclimation affects the fatty acid composition of nerve cord extracts in P. americana adult males in an orderly fashion, resulting in increased unsaturation at low temperatures. The same is not true of fat body lipids.

This increased unsaturation of neural lipids is positively correlated with allethrin's increased toxicity. These experiments are some of the first to investigate the effects of temperature acclimation on insect neural lipids; they are also among the very few to report that the nerve cord and fat body respond quite differently to temperature acclimation. In light of the above discussion, these findings seem quite significant.

REFERENCES

Baldus, T. J. 1984. The effects of temperature acclimation on the toxicity of allethrin and malathion to the American cockroach, Periplaneta americana. M. S. thesis. Iowa State University, Ames, Iowa.

Baldus, T. J., and Mutchmor, J. A. 1987. The effects of acclimation and post-treatment temperature on the toxicity of allethrin to the American cockroach, Periplaneta americana. Comp. Biochem. Physiol. C. (In press).

Baranska, J., and Wlodawer, P. 1969. Influence of temperature on the composition of fatty acids and on lipogenesis in frog tissues. Comp. Biochem. Physiol. 28, 553-570.

Cossins, A. R., Friedlander, M. J., and Prosser, C. L. 1977. Correlations between behavioral temperature adaptations of goldfish and the viscosity and fatty acid composition of their synaptic membranes. J. Comp. Physiol. 120, 109-121.

Danks, S. M., and Tribe, M. A. 1979. Biochemical changes in blowfly flight muscle mitochondria following temperature acclimation. J. Therm. Biol. 4, 183-195.

Fawzi, M., Osman, H., and Schmidt, G. H. 1961. Analyze der Korperfette von Imaginalen Wanderheuschrecken der Art Locusta migratoria migratoriodes L. (Orth.). Biochem. Z. 334, 441-450.

Folch, J., Lees, M., and Sloane-Stanley, G. H. 1957. A simple method for the isolation and purification of total lipides from animal tissue. J. Biol. Chem. 226, 497-509.

Friedman, K. J., Easton, D. M., and Nash, M. 1986. Temperature-induced changes in fatty acid composition of myelinated and non-myelinated axon phospholipids. Comp. Biochem. Physiol. 83B, 313-319.

Keith, A. D. 1966. Analysis of lipids in Drosophila melanogaster. Comp. Biochem. Physiol. 17, 1127-1136.

Lane, N. J. 1974. The organization of insect nervous systems. In Insect Neurobiology. (pp. 1-71) (Edited by J. E. Treherne). American Elsevier Publishing Co., New York.

Lee, R. F., and Gonsoulin, F. 1979. Lipids from nerve tissues of the horseshoe crab, Limulus polyphemus. Comp. Biochem. Physiol. 64B, 375-379.

Munson, S. C. 1953. Some effects of storage at different temperatures on the resistance of the American roach to DDT. J. Econ. Entomol. 46, 756-760.

Nakasone, S., and Ito, T. 1967. Fatty acid composition of the silkworm, Bombyx mori L. J. Insect Physiol. 13, 1237-1246.

Nelson, D. R., Terranova, A. C., and Sukkestad, D. R. 1967. Fatty acid composition of the glyceride and free fatty acid fractions of the fat body and hemolymph of the cockroach, Periplaneta americana (L.). Comp. Biochem. Physiol. 20, 907-917.

Roots, B. I., and Johnston, P. V. 1968. Plasmalogens of the nervous system and environmental temperature. Comp. Biochem. Physiol. 26, 553-560.

Stanley-Samuelson, D. W., and Dadd, R. H. 1981. Arachidonic and other tissue fatty acids of Culex pipiens reared with various concentrations of dietary arachidonic acid. J. Insect. Physiol. 27, 571-578.

Stanley-Samuelson, D. W., and Dadd, R. H. 1983. Long-chain polyunsaturated fatty acids: patterns of occurrence in insects. Insect Biochem. 13, 549-558.

Stanley-Samuelson, D. W., and Pipa, R. L. 1984. Phospholipid fatty acids from exocrine and reproductive tissues of male American cockroaches, Periplaneta americana (L.). Arch. Insect. Biochem. Physiol. 1, 161-166.

GENERAL DISCUSSION

Results of the present research indicate that (compared to warm-acclimation) a 7-8 day period of low temperature acclimation can cause up to a 3-fold increase in the toxicity of allethrin applied to adult male P. americana. When low acclimation temperature is followed by low post-treatment temperature, a 24-fold increase in toxicity occurs. The importance of controlling post-treatment temperature in toxicity studies has been firmly established by others, but pre-treatment (acclimation) temperature has been largely ignored. According to this study and others, however, (Baldus, 1984; Jamil, 1984; Thomas and Rice, 1986) acclimation temperature should also be controlled if toxicity results from different laboratories are to be accurately compared. Environmental temperature should also be considered when selecting dose levels for field or greenhouse application of insecticides.

Because most insecticides are non-selective, and insect species acclimate differently, temperature acclimation could be used strategically in the greenhouse for more effective pest control. If a given acclimation temperature rendered a pest insect more susceptible to a toxin without affecting beneficial insects (and other predators), an advantage could clearly be gained. Manipulation of post-treatment temperature for the same advantage has been suggested by

others (Everson and Tonks, 1981).

The underlying mechanisms for the effects of temperature on insecticide toxicity are not clearly understood. According to Gammon (1978, 1979), lower post-treatment temperature enhances blockage of impulse conduction by allethrin in nerve cord giant axons and also enhances hyperexcitation of the peripheral nervous system of the cockroach; he postulates that the peripheral effects are the probable reason for allethrin's greater toxicity at lower post-treatment temperatures.

Very few studies have investigated the mechanisms involved in temperature acclimation's effects on toxicity; a complete explanation is, therefore, not available. Possible explanations exist that can not be excluded on the basis of these studies. An increased detoxification rate as a result of higher acclimation temperature may be possible, but the significance of altered detoxification rates as a mechanism of resistance (changes in toxicity) has not been established (Soderlund et al., 1983). Since the rearing room temperature was normally about 30°C, and 30°C-acclimated cockroaches were the least susceptible to allethrin, it is possible that increased toxicity was due to a temperature stress. Two lines of evidence, however, seem to invalidate this possibility. First, controls in this study displayed low mortality rates (3.6%) with no differences between

acclimation temperatures. In addition, a previous study showed that no significant differences in toxicity occurred when malathion was applied to adult male cockroaches acclimated at 3 different temperatures (Baldus, 1984).

The hypothesis that changes in lipids of the neural fat body sheath and/or the axonal membrane are partly responsible for the effects of acclimation temperature on the toxicity of allethrin to adult male P. americana was previously suggested (Baldus, 1984; Baldus and Mutchmor, 1987). The results of the present research are consistent with that hypothesis. Temperature acclimation resulted in systematic changes in the proportions of 6 different fatty acids in nerve cord extracts, resulting in increased unsaturation at lower acclimation temperature. Since lipophilic toxins are more soluble in unsaturated lipids (Munson, 1953), more allethrin could bind to the neural lipids of cold-acclimated cockroaches, resulting in greater toxicity.

The same fatty acid changes that may contribute to increased susceptibility to allethrin, may otherwise have served an adaptive function for the insect. The degree of unsaturation of fatty acids is important in membrane fluidity and in providing the proper environment for correct membrane function (Bell et al., 1986). The fatty acid changes, therefore, may be a form of compensatory

adaptation, according to the definition of Hochachka and Somero (1984), that help restore membrane fluidity to a steady-state of function. Maintenance of the steady-state of an insect's nervous system is certainly very important for survival, while fat body function seems less critical. It seems logical, then, that the fatty acid composition of the nerve cord may change in a compensatory manner whereas fat body lipids might remain unchanged. Possible methods by which these fatty acid changes could be achieved in the insect include de novo synthesis, preferential uptake from hemolymph, and modification (shortening or elongation) of existing fatty acids. Certain highly unsaturated fatty acids may even have specific functions in membranes of neural tissues (Dratz and Deese, 1986); the evidence, however, is only circumstantial. Recently, Takenaka et al. (1987), found definite evidence that fatty acids can suppress the sodium current in internally perfused squid giant axons. The effect was shown to depend on chain length and chemical structure of the fatty acid.

The present research demonstrates that a relatively short (7-8 day) period of temperature acclimation affects the neural lipids and the susceptibility of the American cockroach to allethrin. Because increased unsaturation of lipids from nerve cords is positively correlated with increased toxicity of allethrin at low acclimation

temperatures, and because very few studies of this type have been done, the results seem significant from both physiological and toxicological points of view.

ADDITIONAL REFERENCES

Anderson, R. L., and Mutchmor, J. A. 1968. Temperature acclimation and its influence on the electrical activity of the nervous system in three species of cockroaches. J. Insect. Physiol. 14, 243-251.

Anderson, R. L., and Mutchmor, J. A. 1971. Temperature acclimation in Tribolium and Musca at locomotory, metabolic, and enzyme levels. Insect Physiol. 17, 2205-2219.

Baldus, T. J. 1984. The effects of temperature acclimation on the toxicity of allethrin and malathion to the American cockroach, Periplaneta americana. M. S. thesis. Iowa State University, Ames, Iowa.

Baldus, T. J., and Mutchmor, J. A. 1987. The effects of acclimation and post-treatment temperature on the toxicity of allethrin to the American cockroach, Periplaneta americana. Comp. Biochem. Physiol. C. (In press).

Bell, M. V., Henderson, R. J., and Sargent, J. R. 1986. The role of polyunsaturated fatty acids in fish. Comp. Biochem. Physiol. 83B, 711-719.

Bradfisch, G. A., Drewes, C. D., and Mutchmor, J. A. 1982. The effects of cooling on an identified reflex pathway in the cockroach (Periplaneta americana), in relation to chill-coma. J. Exp. Biol. 96, 131-141.

Brattsten, L. B., Gunderson, C. A., Fleming, J. T., and Nikbaht, K. N. 1986. Temperature and diet modulate cytochrome P-450 activities in southern armyworm, Spodoptera eridania (Cramer), caterpillars. Pestic. Biochem. Physiol. 25, 346-357.

Cookman, J. E., Angelo, M. J., Slansky, F., and Nation, J. L. 1984. Lipid content and fatty acid composition of larvae and adults of the velvetbean caterpillar, Anticarsia gemmatalis, as affected by larval diet. J. Insect Physiol. 30, 523-527.

Cossins, A. R., Friedlander, M. J., and Prosser, C. L. 1977. Correlations between behavioral temperature adaptations of goldfish and the viscosity and fatty acid composition of their synaptic membranes. J. Comp. Physiol. 120, 109-121.

Danks, S. M., and Tribs, M. A. 1979. Biochemical changes in blowfly flight muscle mitochondria following temperature acclimation. J. Therm. Biol. 4, 183-195.

Das, A. B., and Prosser, C. L. 1967. Biochemical changes in tissues of goldfish acclimated to high and low temperatures-I. Protein synthesis. Comp. Biochem. Physiol. 21, 449-467.

DeVries, D. H., and Georghiou, G. P. 1979. Influence of temperature on the toxicity of insecticides to susceptible and resistant house flies. J. Econ. Entomol. 72, 48-50.

Downer, R. G., and Kallapur, V. L. 1981. Temperature-induced changes in lipid composition and transition temperature of flight muscle mitochondria of Schistocerca gregaria. J. Therm. Biol. 6, 189-194.

Dratz, E. A., and Deese, A. J. 1986. The role of docosahexaenoic acid (22.6w3) in biological membranes: examples from photoreceptors and model membrane bilayers. In Health Effects of Polyunsaturated Fatty Acids in Seafoods. (pp. 319-351) (Edited by A. P. Simopoules; R. R. Kifer; and R. E. Martin). Academic Press, Inc., Orlando, Florida.

Everson, P. R., and Tonks, N. V. 1981. The effect of temperature on the toxicity of several pesticides to Phytoseiulus persimilis (Acarina:Phytoseiidae) and Tetranychus urticae (Acarina:Tetranychidae). Can. Entomol. 113, 333-336.

Gammon, D. W. 1978. Neural effects of allethrin on the free walking cockroach, Periplaneta americana: an investigation using defined doses at 15° and 32°C. Pestic. Sci. 9, 79-91.

Gammon, D. W. 1979. An analysis of the temperature-dependence of the toxicity of allethrin to the cockroach. In Neurotoxicology of Insecticides and Pheromones. (pp. 97-117) (Edited by T. Narahashi). Plenum Press, New York.

Gammon, D. W., Brown, M. A., and Casida, J. E. 1981. Two classes of pyrethroid action in the cockraoch. Pestic. Biochem. Physiol. 15, 181-191.

Hadaway, A. B., and Barlow, F. 1957. The influence of temperature and humidity upon the action of insecticides: II. Temperature during the pre-treatment period. Ann. Trop. Med. Parasitol. 51, 194-200.

Hazel, J. R. 1973. The regulation of cellular function by temperature-induced alterations in membrane composition. In Effects of Temperature on Ectothermic Organisms. (pp. 56-67) (Edited by W. Wieser). Springer-Verlag, New York.

Hochachka, P. W., and Somero, G. N. 1968. The adaptation of enzymes to temperature. Comp. Biochem. Physiol. 27, 659-668.

Hochachka, P. W., and Somero, G. N. 1984. Biochemical Adaptation. Princeton University Press, Princeton, New Jersey.

Hoffman, K. H. 1985. Metabolic and enzyme adaptation to temperature. In Environmental Physiology and Biochemistry of Insects. (pp. 1-32) (Edited by K. H. Hoffman). Springer-Verlag, Berlin.

Jamil, K. 1984. Differential response of American cockroach Periplaneta americana L. to chlorinated hydrocarbon insecticides when acclimatized to different temperatures. Entomology. 9, 201-207.

Kilian, P. L., Dunlap, C. R., and Mueller, P. 1980. Reconstitution of acetylcholine receptor from Torpedo californica with highly purified phospholipids: effect of -tocopherol, phylloquinone, and other terpenoid quinones. Biochem. Biophys. Res. Commun. 93, 409-414.

Munson, S. C. 1953. Some effects of storage at different temperatures on the resistance of the American roach to DDT. J. Econ. Entomol. 46, 756-760.

Nakasone, S., and Ito, T. 1967. Fatty acid composition of the silkworm, Bombyx mori L. J. Insect Physiol. 13, 1237-1246.

Narahasi, T. 1985. Nerve membrane ionic channels as the primary target of pyrethroids. Neurotoxicology 6, 3-22.

Nation, J. L., and Bowers, W. S. 1982. Fatty acid composition of milkweed bugs Oncopeltus fasciatus and the influence of diet. Insect Biochem. 12, 455-459.

Nelson, D. R., Terranova, A. C., and Sukkestad, D. R. 1967. Fatty acid composition of the glyceride and free fatty acid fractions of the fat body and hemolymph of the cockroach, Periplanta americana (L.). Comp. Biochem. Physiol. 20, 907-917.

Osborne, M. P., and Smallcombe, A. 1983. Site of action of pyrethroid insecticides as revealed by the kdr resistance factor. In Pesticide Chemistry: Human Welfare and the Environment. (pp. 103-107) (Edited by J. Miyamoto). Pergamon Press, Oxford.

Potter, C., and Gillham, E. M. 1946. Effects of atmospheric environment before and after treatment on the toxicity of contact poisons to insects. Ann. Appl. Biol. 33, 142-159.

Rai, B. K. 1967. Temperature coefficient of insect susceptibility to insecticides. Indian J. Exp. Biol. 5, 151-155.

Roots, B. I., and Johnston, P. V. 1968. Plasmalogens of the nervous system and environmental temperature. Comp. Biochem. Physiol. 26, 553-560.

Scott, J. G., and Matsumura, F. 1983. Evidence for two types of toxic actions of pyrethroids on susceptible and DDT-resistant German cockroaches. Pestic. Biochem. Physiol. 19, 141-150.

Soderlund, D. M., Sanborn, J. R., and Lee, P. W. 1983. Metabolism of pyrethrins and pyrethroids in insects. In Progress in Pesticide Biochemistry and Toxicology Vol. 3. (pp. 401-435) (Edited by D. H. Hutson; and T. R. Roberts). John Wiley and Sons, Ltd., New York.

Stanley-Samuelson, D. W., and Dadd, R. H. 1983. Long-chain polyunsaturated fatty acids: patterns of occurence in insects. Insect Biochem. 13, 549-558.

Stanley-Samuelson, D. W., and Pipa, R. L. 1984. Phospholipid fatty acids from exocrine and reproductive tissues of male American cockroaches, Periplaneta americana (L.). Arch. Insect. Biochem. Physiol. 1, 161-166.

Stanley-Samuelson, D. W., Keddie, B. A., and Volkman, L. E. 1986. Fatty acid composition of whole bodies, specific tissues and cell lines of two lepidopteran insects. Comp. Biochem. Physiol. 85B, 369-373.

Staszak, D. J., and Mutchmor, J. A. 1973. Influence of temperature on chill-coma and the electrical activity of the central and peripheral nervous systems of the American cockroach, Periplaneta americana. Comp. Biochem. Physiol. 45A, 909-923.

Sun, Y. P. 1960. Pre-test conditions which affect insect reaction to insecticides. In Methods of Testing Chemicals in Insects. (pp. 1-9) (Edited by H. H. Shepard). Burgess Publishing Co., Minneapolis, Minnesota.

Takenaka, T., Horie, H., and Hori, H. 1987. Effects of fatty acids on membrane currents in the squid giant axon. J. Membrane Biol. 95, 113-120.

Thomas, R. E., and Rice, S. D. 1986. Effect of temperature on uptake and metabolism of toluene and naphthalene by Dolly Varden char, Salvelinus malma. Comp. Biochem. Physiol. 84C, 83-86.

ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. John A. Mutchmor, and my committee members Drs. Donald C. Beitz, Joel R. Coats, James R. Redmond, and Kenneth C. Shaw for their guidance throughout this study. I also thank Dr. Philip G. Haydon for assistance with computer-generated figures, Dr. Donald C. Beitz for advice on lipid chromatography, Dr. David F. Cox for statistical analysis, and Mr. Daniel M. Symonik for technical assistance with gas-liquid chromatography.

Special thanks go to my wife, Debra Kay, for her everlasting patience, constant encouragement, and continual emotional and financial support. I also thank my son, Derek Thomas, for providing me with many delightful hours of much-needed diversion.

APPENDIX A: DATA WITH CHI-SQUARE TEST OF HOMOGENEITY

These tables contain the dose-response records of the individual replications that were used to construct the regression lines for allethrin. A Commodore PET 2001 computer calculated the expected values by the method of least squares and the chi-square value by

$$\frac{\text{observed \% - expected \%}}{\text{expected \% (100-expected \%)}} \times \text{number of insects in the test.}$$

Table A.1. Statistical analysis of allethrin tests on *Periplaneta americana* adult males (post-treatment temp = 22C)

Accl temp (°C)	Test#	Conc (ppm)	Mortality (n=10)	Totals 3 reps (n=30)	Totals expected (n=30)	Chi-square
14	1	.50	2	5	4.892	.003
	2		1			
	3		2			
	4	1.00	4	10	9.376	.060
	5		3			
	6		3			
	7	2.00	7	15	15.053	.000
	8		5			
	9		3			
	10	3.00	5	16	18.457	.850
	11		5			
	12		6			
	13	5.00	8	24	22.326	.490
	14		8			
	15		8			1.403
22	16	2.00	1	3	3.637	.127
	17		1			
	18		1			
	19	3.00	4	10	9.156	.112
	20		4			
	21		2			
	22	4.00	6	16	14.503	.299
	23		6			
	24		4			
	25	5.00	6	17	18.781	.452
	26		6			
	27		5			
	28	8.00	9	26	25.837	.007
	29		9			
	30		8			.997
30	31	5.00	3	6	7.120	.231
	32		1			
	33		2			
	34	6.00	7	16	15.576	.024
	35		4			
	36		5			
	37	6.50	6	20	19.476	.040
	38		7			
	39		7			

Table A. 1. (Continued)

Accl temp (°C)	Test#	Conc (ppm)	Mortality (n=10)	Totals 3 reps (n=30)	Totals expected (n=30)	Chi-square
	40	7.00	8	25	22.679	.974
	41		9			
	42		8			
	43	8.00	8	25	26.843	1.202
	44		8			
	45		9			2.471

Table A.2. Statistical analysis of allethrin tests on Periplaneta americana adult males (acclimation= post-treatment temp)

Accl temp (°C)	Test#	Conc (ppm)	Mortality (n=10)	Totals 3 reps (n=30)	Totals expected (n=30)	Chi-square
14	1	.50	4	8	7.501	.044
	2		2			
	3		2			
	4	.75	6	14	14.429	.025
	5		4			
	6		4			
	7	1.00	6	19	19.628	.058
	8		7			
	9		6			
	10	1.25	9	23	23.126	.003
	11		7			
	12		7			
	13	1.50	9	26	25.409	.090
	14		8			
	15		9			.220
22	16	2.00	1	3	3.637	.127
	17		1			
	18		1			
	19	3.00	4	10	9.156	.112
	20		4			
	21		2			
	22	4.00	6	16	14.503	.299
	23		6			
	24		4			
	25	5.00	6	17	18.781	.452
	26		6			
	27		5			
	28	8.00	9	26	25.837	.007
	29		9			
	30		8			.997
30	31	10.00	1	3	6.525	2.434
	32		1			
	33		1			
	34	12.00	6	12	8.761	1.691
	35		3			
	36		3			
	37	14.00	6	13	10.892	.641
	38		4			
	39		3			

Table A. 2. (Continued)

Accl temp (°C)	Test#	Conc (ppm)	Mortality (n=10)	Totals 3 reps (n=30)	Totals expected (n=30)	Chi-square
	40	22.00	5	16	17.703	.400
	41		6			
	42		5			
	43	24.00	6	19	18.982	.000
	44		7			
	45		6			5.166

Table A.3. Control mortalities from allethrin test on *Periplaneta americana* adult males

Accl temp	Post-treatment temp	Test#	Mortality (n=10)	Mortality (%)
14	14	1	0	10
	14	2	0	0
	14	3	1	10
	14	4	0	0
	14	5	1	10
	22	6	0	0
	22	7	0	0
	22	8	1	10
	22	9	0	0
	22	10	0	0
22	22	11	1	10
	22	12	0	0
	22	13	0	0
	22	14	0	0
	22	15	1	10
30	22	16	0	0
	22	17	0	0
	22	18	0	0
	22	19	0	0
	22	20	2	20
	30	21	0	0
	30	22	0	0
	30	23	0	0
	30	24	1	10
	30	25	0	0
				$\overline{\text{Mean}=3.0}$

APPENDIX B: TYPICAL GAS-LIQUID CHROMATOGRAMS

These 2 figures are photo-copies of actual recordings of fatty acid methyl esters from lipid extracts of the American cockroach. To obtain each recording, a four microliter aliquot of the concentrated sample was injected into a Hewlett Packard 5700 Series gas chromatograph.

Fig. B.1. Typical gas-liquid chromatogram of fatty acid methyl esters from lipid extracts of nerve cords from adult male P. americana. The number associated with each peak is retention time in minutes

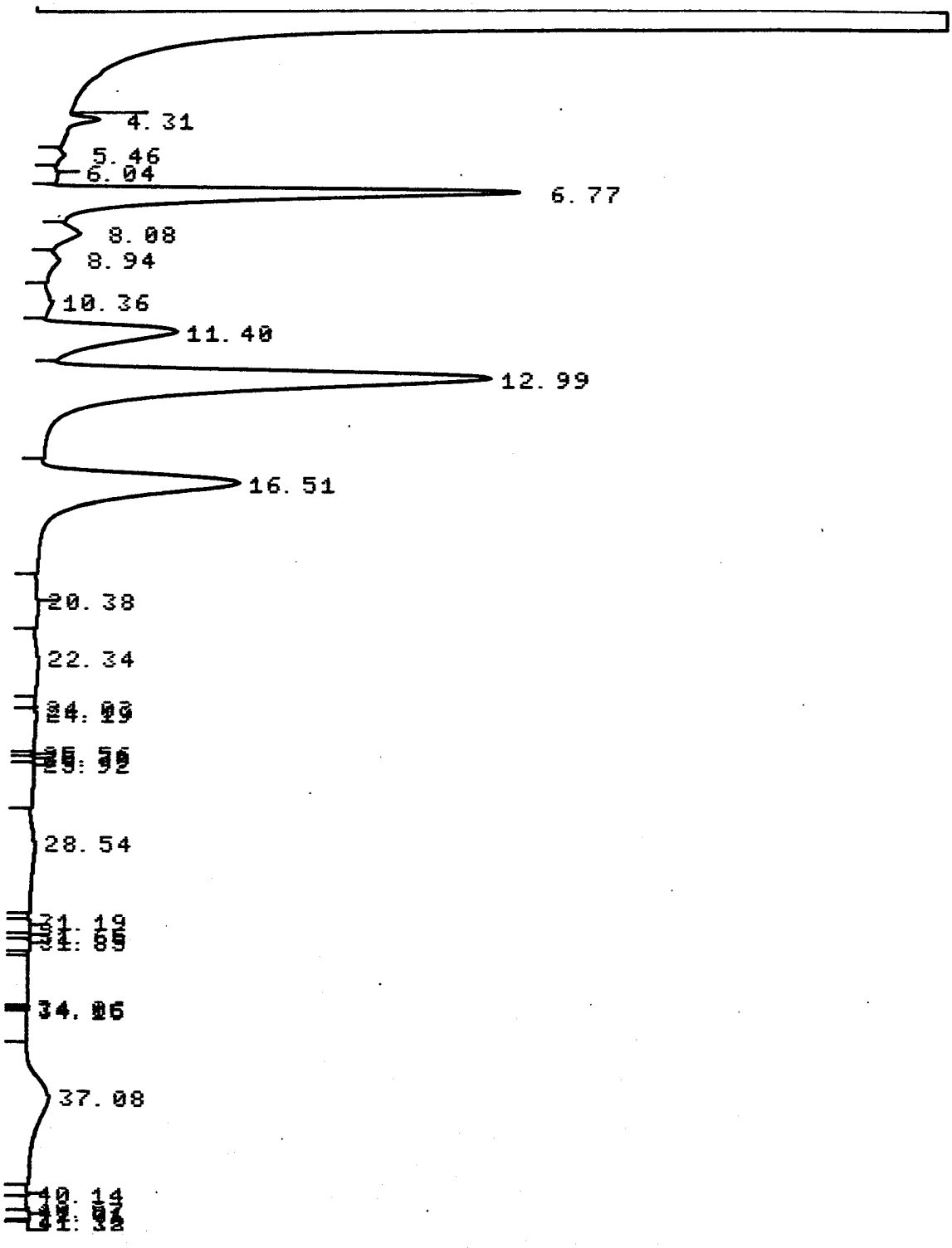
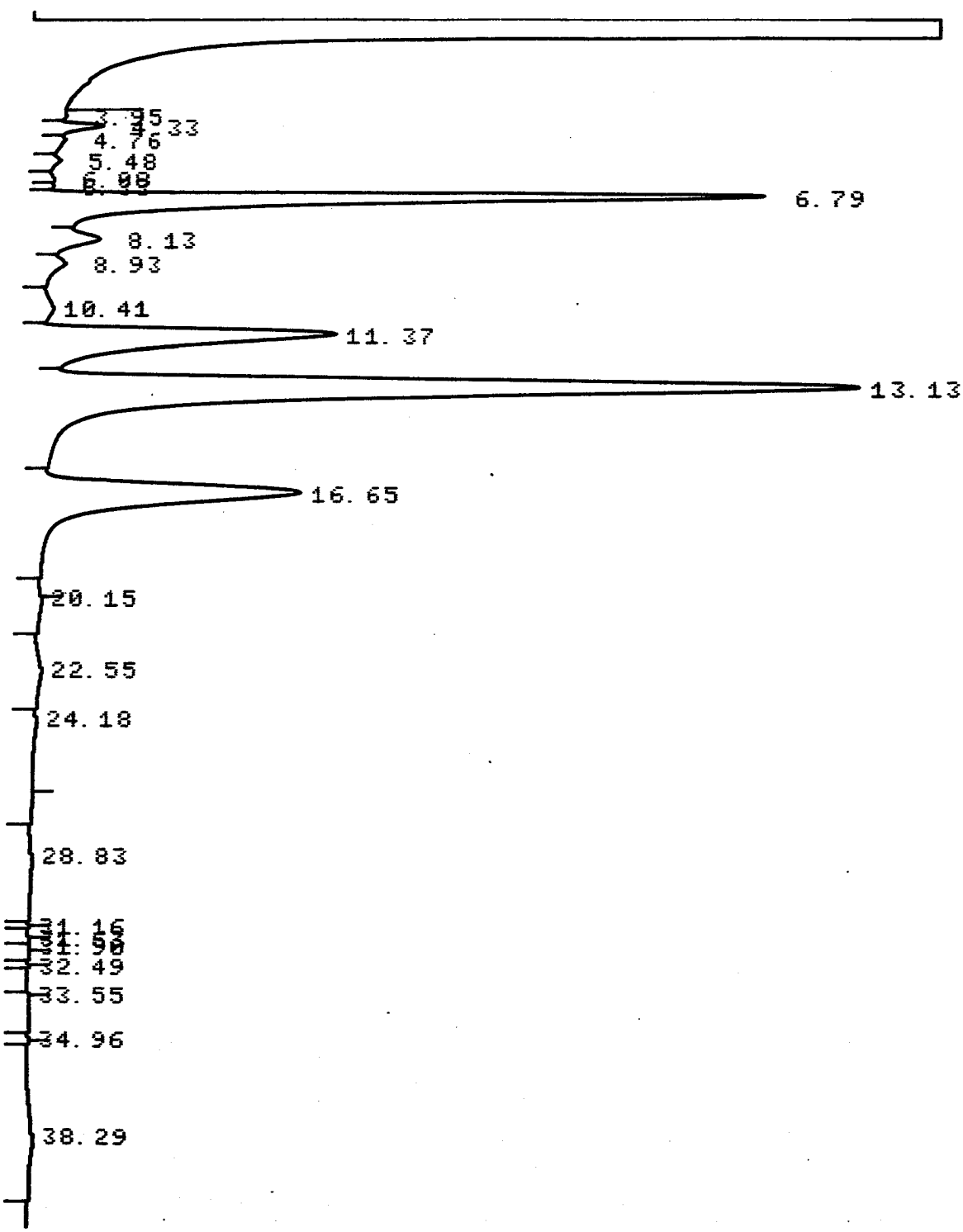

4.31
5.46
6.04
6.77
8.08
8.94
10.36
11.40
12.99
16.51
20.38
22.34
28.54
37.08

Fig. B.2. Typical gas-liquid chromatogram of fatty acid methyl esters from lipid extracts of fat bodies from adult male P. americana. The number associated with each peak is retention time in minutes


4.76
5.48
6.79
8.13
8.93
10.41
11.37
13.13
16.65
20.15
22.55
24.18
28.83
32.49
33.55
34.96
38.29