Department of Zoology, The George S. Wise Center for Life Sciences, Tel Aviv University, Tel Aviv, Israel

The effect of fatty acids and their alpha-fluoro analogs on the feeding response and development of the hidebeetle *Dermestes maculatus* Deg.

By E. COHEN and H. Z. LEVINSON[1]

Abstract

Larvae and adults of the species *Dermestes maculatus* were attracted by palmitic and stearic acids and repelled by a lower homolog, caprylic acid. Lauric acid, which strongly deterred larvae, was phagostimulatory to adults. Palmitic and stearic acids enhanced larval growth and metamorphosis, whereas caprylic and lauric acids were inhibitory. The above effects parallel the feeding response.

Larvae differed from adults in their response to alpha fluoro fatty acids. The former were invariably repelled by the fluoro analogs, whereas the latter were stimulated to feed on a diet containing alpha fluoro laurate and alpha fluoro stearate. It appears that mature stages were incapable of distinguishing between fatty acids and their respective fluoro derivatives. Sodium fluoro acetate was highly toxic to hidebeetle larvae, while alpha fluoro fatty acids appeared relatively nontoxic, inhibiting growth at a dietary level of 1.0 % only. This moderate effect was ascribed to inhibition of beta oxidation, whereby the lethal fluoro acetate was not released.

Introduction

The hide beetle, *Dermestes maculatus*, is adapted to develop on food highly rich in proteins and lipids, such as hides and skins (HINTON 1945). The beetles display a remarkable feature, to tolerate and consume excessive dietary fat which other insects could not survive. Although it might be expected that the animals are dependent on a lipid source for growth and metamorphosis, it was shown that they can be readily raised on a fat-free semi-synthetic diet (E. COHEN, in preparation). Moreover, the above diet was supplemented with antibiotics to eliminate any microbial source of lipids.

An improved growth of several insects by dietary fatty acids has been reported. Palmitic and oleic acids were found to be phagostimulatory to the beetles *Tribolium confusum* and *Ctenicera aeripennis destructor* respectively (LOSCHIAVO 1965; DAVIS 1968). The polyenes linoleic and linolenic acids appear to be gustatory attractants of the ant *Solenopsis saevissima* (VINSON et al. 1967). The attractance and repellence properties of various fatty acids were reviewed by DETHIER (1947). The present paper deals with growth and

[1] Present address: Max-Planck-Institut für Verhaltensphysiologie, 8131 Seewiesen, BRD.

Z. ang. Ent. 76 (1974), 98–105

ISSN 0044-2240 / ASTM-Coden: ZANEAE

feeding response of *D. maculatus* larvae and adults towards several fatty acids. Antimetabolites to fatty acids were prepared by substitution of fluorine for hydrogen at the alpha position to the carboxyl. The fluoro compounds appeared to be relatively nontoxic to fungi (GERSHON and PARMEGIANI 1967) and were tested in comparison to their respective fatty acids.

Materials and methods

Insect material

Stock cultures of *Dermestes maculatus* were maintained on a diet comprised of *sieved fish meal* (20 mesh) and yeast extract powder at a ratio of 18:1, 10 % casein (NBCo) and bacon. Drinking water was introduced to adults in order to promote oviposition, particularly when considerable quantities of newly laid eggs were required. The eggs were collected and cleaned, and newly hatched larvae were removed for experimental purposes. Insects in stock as well as in experiments were kept at 28 ± 0.5° and 60 % RH.

Semi synthetic diets

In all the experiments, larvae and adults fed a synthetic diet prepared according to LEVINSON et al. (1967). Casein Hammersten was used when rate of development was assayed, while with the food preference tests, the above was replaced by casein (NBCo) which yielded a coherent dietary texture.

Development experiments

Newly hatched larvae were separately raised on a synthetic diet mixed with various concentrations of either fatty acids or their alpha-fluoro derivatives. A development index was calculated using the following three parameters: 1. Weight of larvae after 20 days of growth, 2. Number of days to reach 50 % pupation, 3. Percentage of adult emergence.

Food preference tests

Small glass tubes (4 mm diameter, 20 mm length), were filled with the tested diets and weighed. One side was sealed by parafilm paper, and the tubes were attached to the bottom of a petri dish (9 cm diameter) by a piece of parafilm. The tubes were arranged radially at equal distances, with the open side towards the center of the dish. Either 3 medium size larvae (14–18 mg), or 5–6 day-old females, were introduced and after a period of 3–4 days for larvae and 6–8 days for adults, the tubes were reweighed and rate of food ingestion was determined. In this type of experiment, females were used since preliminary observation indicated that males consume smaller quantities of food than females. This is most likely due to the nutritional requirements of females for egg maturation.

Fluorine determination

Larvae were maintained either on a diet containing 0.1 % alpha-fluoro stearic acid or a control diet. Total fluorine was measured according to SINGER and ARMSTRONG (1959) after treating dried insect material with sodium hydroxide.

Preparation of sodium fluoro acetate

One gr of sodium hydroxide was dissolved in 5.0 ml water, followed by the addition of 20.0 ml methanol. Subsequently, 2.0 ml of ethyl-fluoroacetate were carefully added, keeping the temperature below 35°. Thirty min. later, the methanol and water were evaporated and the residue of sodium fluoro acetate ($CH_2FCOONa$) was washed in hexane and dried in a desiccator.

Results

The data recorded in Table 1 reveal that both mature and immature stages of *D. maculatus* preferred to consume a diet containing the highest concentration of bacon used (25.0 %). Larvae ingested 111.2 mg and adults 58.0 mg, about 9- and 5-fold of the controls comparatively. This uncommon feeding response might be attributed to the hidebeetle adaptation to its fat-rich habitat. The above results prompted the assay of several fatty acids and it became evident from Table 2 that high dietary levels of lauric (C 12), palmitic (C 16) and stearic (C 18) acids encouraged food intake by adults while a lower homolog, caprylic acid (C 8) acted as a phagorepellent at 5.0 % in the food. On the other hand, the larvae were deterred by low dietary levels of caprylic and lauric acids, whereas the long chain fatty acids, palmitic and stearic acids, appeared to be phagostimulatory. The above-mentioned discrepancy was even more pronounced where alphafluoro derivates of fatty acids were assayed. As in the case of n-fatty acids, the adults were stimulated to consume dietary F-lauric and F-stearic acids, but were repelled, to a large extent, by caprylic acid and drastically by sodium fluoro acetate. All the fluoro compounds were invariably phagorepellents to larvae. The difference in response between *D. maculatus* larvae and adults towards fatty acids and their fluoro analogs, especially the stimulatory action of F-lauric and F-stearic acid to adults, is quite surprising. However, it became evident that the fluoro compounds did not alter the gustatory response of adults, whilst larvae could discriminate between them and their corresponding acids.

Table 1

Gustatory response of *Dermestes maculatus* larvae and adults to bacon fat

Bacon Concentration (%)[1]	Average food consumption (mg) Larvae	adults
0.0	13.4	12.0
1.0	15.3	14.0
25.0	111.2	58.0

[1] Melted fraction of bacon at 80°. 20 repetitions at each concentration.

The data recorded in Table 3 evaluates the nutritional effects of fatty acids and their fluoro analogs on growth and metamorphosis of the hidebeetle. Sodium fluoro acetate is a well known toxicant to insects as well as to other organisms, acting on the Krebs cycle. It was toxic to *D. maculatus* larvae at a dietary level of 0,1 %, and lethal at higher concentrations. The respective sodium acetate improved development at all levels tested. Caprylic acid reduced development at 0.1 % concentration in the diet, while lauric acid was less detrimental at the above level, suppressing growth at 5.0 % only. The long chain compounds, palmitic and stearic acids, enhanced development and metamorphosis at 0.1 % in the diet. This is in good agree-

Table 2

Gustatory response of *Dermestes maculatus* to various free fatty acids and their alpha fluoro analogs

Concentration %	A. Fatty acid: Caprylic acid L	Caprylic acid A	lauric acid L	lauric acid A	palmitic acid L	palmitic acid A	stearic acid L	stearic acid A
Control	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.5	0.41	0.95	0.46	—	1.88	3.28	0.97	2.31
1.0	0.23	0.95	0.32	2.45	1.28	3.29	1.00	1.88
5.0	0.19	0.47	0.38	1.18	6.34	9.60	3.05	5.82

Concentration %	B. alpha fluoro analogs of fatty acids: Sodium-F-acetate L	Sodium-F-acetate A	F-caprylic acid L	F-caprylic acid A	F-lauric caid L	F-lauric caid A	F-palmitic acid L	F-palmitic acid A	F-stearic acid L	F-stearic acid A
Control	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—	1.00	1.00
0.5	0.34	0.58	0.63	0.52	0.01	2.54	0.49	—	0.95	2.25
1.0	0.10	0.42	0.40	0.53	0.00	—	0.34	—	0.59	—
5.0	0.13	0.21	0.39	0.59	0.00	4.31	0.10	—	0.37	1.77

Compounds were mixed in the semi-synthetic diet and the quantity of food consumption served to evaluate the degree of attraction or repellence. For more details see Materials and methods. Numbers are based on a multiple of control values.
L = larvae, A = adults. 20 repetitions for each concentration.

Table 3

Effects of fatty acids and their alpha-fluoro derivatives on the development of *D. maculatus*

Compound	Dietary concentration – % 0.0	0.01	0.1	1.0	5.0
Sodium fluoro acetate	1.00	0.82	0.00	lethal	lethal
Sodium acetate	1.00	1.13	1.29	—	1.39
Caprylic acid	1.00	0.98	0.51	—	0.00
F-caprylic acid	1.00	0.76	0.67	0.00	0.00
Lauric acid	1.00	0.90	0.72	0.68	0.00
F-Lauric acid	1.00	0.90	0.79	0.00	0.00
Palmitic acid	1.00	1.18	1.24	1.09	—
F-Palmitic acid	1.00	1.06	0.43	0.00	—
Stearic acid	1.00	1.12	1.23	0.93	—
F-stearic acid[1]	1.00	0.77	0.58	0.00	—

Numbers are multiples of the control index of development. Index of development = $\frac{\text{Average larval weight (20 days)}}{\text{Number of days to 50 \% pupation}} \times$ % of adult emergence. 0.00 – Growth was drastically retarded and larvae did not reach pupation within 60 days. – 20 larvae were used for each concentration.

[1] Larvae were raised on a diet containing 0.1 % alpha fluoro stearic acid, pupae were removed and the fluorine content was measured. Treated animals contained 0.5 µg fluorine per pupae while the value for the control was 0.02 µg fluorine per animal. Most of the fluorine was organic since by omission of sodium hydroxide (see Materials and methods), no fluorine could be detected.

ment with the food preference results summarized in Table 2. The improved development is probably associated with phagostimulatory action of several fatty acids. The alpha fluoro derivatives appeared to inhibit growth at a dietary concentration of 0.1 % but apparently are relatively nontoxic. Although larval development was drastically suppressed at a dietary level of 1.0 %, no mortality was recorded (index value = 0.00). Since fluoro acetate was lethal to *D. maculatus*, it is suggested that this compound was not liberated from alpha-fluoro fatty acids. F-palmitic and F-stearic acids were slightly more inhibitory to larval growth than the lower homologs, F-caprylic and F-lauric acid, at a 0.1 % concentration. The above might be related to better absorption and incorporation of long chain compounds into larval tissues.

Fluorine content of pupae obtained from larvae which had been fed a 0.1 % concentration of fluoro stearic acid was 0.5 μg per pupa, while the control value was 0.02 μg per animal. Since inorganic fluorine could not be detected, it is believed that organic fluoro compounds, probably the original fluoro stearate, were incorporated into the insect tissues.

Discussion

An established method in raising stock colonies of *Dermestes maculatus* is the addition of bacon to the fish meal and yeast extract powder mixture (Levinson et al. 1967). Preference tests using elevated concentrations of melted bacon demonstrated that mature as well as immature stages preferred to consume from the highest level (25.0 %). This unique response might be ascribed to the insect adaptation to develop on fat rich food (Hinton 1945). When, in the above tests, bacon was replaced by free fatty acids, it became evident that *D. maculatus* was attracted to a large extent by palmitic and stearic acids and preferred to ingest from the tube containing the highest level (5.0 %). The phagostimulatory effects of the above acids were reflected by an increased ovipostion (Cohen and Levinson 1972), probably due to increased food reserves accumulated during the preoviposition period. It was also observed by the above authors that several dietary fatty acids were incorporated into triglycerides of eggs and insect tissues. It has been previously reported that wheat germ oil and soybean oil encouraged feeding of locusts and silkworms respectively (Dadd 1960; Ito 1961). Triglycerides present in high concentration in the mycelium of *Nigrospora sphaerica* attracted *Tribolium confusum* (Starratt and Loschiavo 1972). The latter resembles the positive response of *D. maculatus* to bacon, which is largely composed of triglycerides (Hilditch 1956). Fatty acids were reported to stimulate feeding of several insects. Palmitic and oleic acids were phagostimulatory to the flour beetle *T. confusum* (Loschiavo 1965) and to *Ctenicera aeripennis destructor* (Davis 1968), respectively. Polyunsaturated acids were implicated in attracting the ant *Solenopsis saevissima* (Vinson et al. 1967).

It should be noted that larvae and adults differed in their response towards short chain fatty acids. Adults were attracted by lauric acid whereas larvae were strongly repelled. Adults were deterred by 5.0 % dietary caprylic acid and larvae were discouraged even by a level of 0.5 %. Short chain

fatty acids (C 6–C 12) are detrimental to insects as well as to microorganisms. LEVINSON and ASCHER (1954) revealed that caproic (C 6), caprylic (C 8) and capric (C 10) acids were toxic to the housefly *Musca vicina*, and this was confirmed by QURAISHI and THORSTEINSON (1965b) for *Musca domestica*. WYSS et al. (1945) observed the increasing fungistatic as well as fungicidic action of fatty acids up to C 11 and suggested that the solubility extent was the limiting factor. Similar information regarding the fungistatic activity was obtained by LEVINSON and ASCHER (1954). HOUSE and GRAHAM (1967) demonstrated the toxicity of capric acid to *T. confusum*, suggesting its use to control the pest in infested wheat meal. The above acid was detrimental to mosquito larvae also (QURAISHI and THORSTEINSON 1965a).

Alpha fluoro fatty acids were invariably phagorepellents towards immature stages of *D. maculatus*. It was surprising to notice a difference in the adult response. F-lauric and F-stearic acids appeared to be phagostimulatory, similar to the unsubstituted fatty acids, despite a comparatively less positive response. It is evident that the adult gustatory receptors did not discriminate between the fatty acid and the fluoro substituted compound.

Sodium fluoro acetate, which is a well-known toxic agent, displayed a pronounced phagodeterrent property towards both mature and immature stages.

When the nutritional effects of fatty acids and their respective analogs were examined, it became clear that the fluoro compounds were relatively nontoxic, though they severely suppressed larval growth and inhibited pupation at a dietary level of 1.0 %. In this context, it is worthy to note that alpha fluoro palmitic and alpha fluoro stearic acids reduced development even at 0.01 % and 0.1 %, respectively. This may be linked to better absorption and solubility of the corresponding long chain molecules in fats. The above interpretation is supported by PATTISON (1959), who pointed to the increased toxicity of long chain homologs of omega fluoro fatty acids compared to the short acids. Growth suppression or promotion by various fatty acids are in good agreement with their phagostimulatory or phagorepellent effects. Thus palmitic and stearic acids, being consumed to a greater extent, enhanced growth and reduced time required for pupation.

Dietary fluoro acetate was highly toxic at a concentration of 0.1 %, inducing mortality at 1.0 %. It has been previously reported that cholesteryl fluoroacetate was toxic to *Musca vicina* at a level of 0.2 % in the diet, probably since ester hydrolysis resulting in the formation of the lethal fluoro acetate occurred (BERGMANN and LEVINSON 1966). Fluoro acetate interferes with the energy producing processes, via incorporation into fluoro citric acid, yielding a strong inhibitor of aconitase in the Krebs cycle (e. g. GOLDMAN 1969). PATTISON (1959) surveyed the action of aliphatic fluoro compounds and arrived at the conclusion that even-numbered fatty acids, in which fluorine was substituted for hydrogen at the omega position, were highly toxic compared to the odd-numbered homologs. The former were able to yield fluoro acetate via beta oxidation. In further investigations, PATTISON et al. (1965) observed that 6-fluoro hexanoic acid was toxic to mice whereas 2,6-difluoro hexanoic acid was nontoxic. They inferred that beta oxidation was prevented by fluorine at the alpha position. GERSHON et al. (1967) prepared alpha fluoro fatty acids which were nontoxic compared to fluoro acetate. When these compounds and n-fatty acids were tested as to their

antifungal action, GERSHON and PARMEGIANI (1967) observed the maximal activity of C 4–C 10 and C 8–C 14 for fatty acids and fluoro derivatives, respectively. Overall, both types of molecules were inefficient fungicides. The above mentioned information, however, favours the supposition that alpha fluoro fatty acids did not result in the toxic fluoro acetate because beta oxidation was blocked. The same is supported by the chemosterilizing effect of fluoro stearic acid on *D. maculatus* (E. COHEN, unpublished information). Females, raised on a diet containing 1.0 % of alpha F-stearate, laid non-viable eggs. Since fatty acids were found to be incorporated into oocytes, and short chain fatty acids acted as sterilizing agents (COHEN and LEVINSON 1972), it is assumed that F-stearic acid entered the egg lipids. Moreover, the above molecule was most likely incorporated into body tissues since only organic fluorine was detected in the insect body when larvae were maintained on dietary fluoro stearate (table 3). It is postulated that embryos were unable to utilize the lipid reserves since beta oxidation was inhibited. Keeping in mind that alpha fluoro analogs are relatively nontoxic, they might be of ecological significance in controlling insects.

Acknowledgements

The authors wish to thank Dr. I. SHAHAK, Department of Organic Chemistry, the Hebrew University of Jerusalem, for providing the alpha fluoro fatty acids. We are also greatly indebted to Dr. I. GEDALIA, Department of Preventive Dentistry-chemistry, "Hadassa" Medical School, Jerusalem, for measuring the fluorine content of insect tissues. This work was supported by Grant No. FG-Is-213 U.S. Department of Agriculture.

Zusammenfassung

Die Wirkung von Fettsäuren und ihrer Alpha-Fluoro-Analogen auf die Nahrungsaufnahme und Entwicklung des Speckkäfers Dermestes maculatus Deg.

Larven und Imagines von *Dermestes maculatus* Deg. wurden von Palmitic- und Stearic-Säure angezogen und von der kürzeren Homologen, Caprylic-Säure, abgestoßen. Lauric-Säure wirkte stark abstoßend auf die Larven und phagostimulatorisch auf die Imagines. Palmitic- und Stearic-Säure beschleunigten den Wuchs der Larven und die Metamorphose während Caprylic- und Lauric-Säure verzögernd wirkten.

Die Larven reagierten anders als die Imagines auf Alpha-Fluoro-Fettsäuren. Die Larven wurden von Fluoro Analogen immer abgestoßen, während die Imagines von Alpha-Fluoro-Laurat und Alpha-Fluoro-Stearat enthaltender Nahrung zur Nahrungsaufnahme stimuliert wurden.

Anscheinend sind die Imagines nicht fähig zwischen Fettsäuren und ihren respectiven Fluoro-Derivaten zu unterscheiden. Sodium-Fluoro-Acetat wirkte sehr giftig auf die Larven. Alpha-Fluoro-Fettsäure wirkte auf die Larven verhältnismäßig wenig giftig. Nahrung mit einem Zusatz von 1,0 % dieser Säure verzögerte nur das Wachstum. Diese schwache Wirkung ist der Hemmung der Beta-Oxidation zuzuschreiben, die das Freisetzen des tödlichen Fluoro-Acetats verhindert.

References

BERGMANN, E. D.; LEVINSON, Z. H., 1966: Utilization of steroid derivatives by larvae of *Musca vicina* (Macq.) and *Dermestes maculatus* Deg. J. Insect physiol. 12, 77–81.

COHEN, E.; LEVINSON, Z. H., 1972: The effects of fatty acids on reproduction of the hidebeetle *Dermestes maculatus* (Dermestidae: Coleoptera). Life Sci. 11, 293–299.

DADD, R. H., 1960: The nutritional requirements of locusts. I. Development of synthetic diets and lipid requirements. J. Insect physiol. 4, 319–347.

DAVIS, G. R. F., 1968: Phagostimulation and consideration of its role in artificial diets. Bull. Ent. Soc. Am. 14, 27–30.

DETHIER, V. G., 1947: Chemical Insect Attractants and Repellents. New York: The Blakiston Co.

GERSHON, H.; PARMEGIANI, R., 1967: Organic fluorine compounds. II. Synthesis and antifungal properties of 2-fluoro fatty acids. J. Med. chem. 10, 186–188.

— SCHULMAN, S. G.; SPEVAK, A. D., 1967: Organic fluorine compounds. III. Action of perchloryl fluoride on substituted ethyl cyanocetates and animal toxicities of the fluorinated products. J. Med. chem. 10, 536–541.

GOLDMAN, P., 1969: The carbon-fluorine bond in compounds of biological interest. Science 164, 1123–1130.

HILDITCH, T. P., 1956: The Chemical Constitution of Natural Fats. New York: John Wiley and Sons. Inc.

HOUSE, H. L.; GRAHAM, A. R., 1967: Capric acid blended into foodstuff for control of an insect pest, *Tribolium confusum* (Coleoptera: Tenebrionidae). Can. Ent. 99, 994–999.

ITO, T., 1961: Nutrition of the silkworm *Bombyx mori*. IV. Effects of ascorbic acid. Bull. Seric. Expt. Sta. 19, 345–373.

LEVINSON, Z. H.; ASCHER, K. R. S., 1954: Chemicals affecting the preimaginal stages of the housefly. IV. The fatty acid period. Riv. Parassitol. 15, 111–119.

— BARELKOVSKY, J.; BAR ILAN, A. R., 1967: Nutritional effects of vitamin omission and antivitamin administration on development and longevity of the hidebeetle *Dermestes maculatus* Deg. (Coleoptera, Dermestidae). J. Stored Prod. Res. 3, 345–352.

LOSCHIAVO, S. R., 1965: The chemosensory influence of some extracts of brewer's yeast and cereal products on the feeding behaviour of the confused flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae). Ann. Ent. Soc. Am. 58, 576–588.

PATTISON, F. L. M., 1959: Toxic Aliphatic Fluorine Compounds. Amsterdam: Elsevier Publ. Co.

— BUCHANAN, R. L.; DEAN, F. H., 1965: The synthesis of -monofluoroalkanoic acids. Canad. J. Chem. 43, 1700–1713.

QURAISHI, M. S.; THORSTEINSON, A. J., 1965a: Effect of synthetic "queen substance" and some related chemicals on immature stages of *Aedes aegypti*. J. Econ. Entomol. 58, 185–187.

— — 1965b: Toxicity of some straight chain saturated fatty acids to housefly larvae. J. Econ. Entomol. 58, 400–402.

SINGER, L.; ARMSTRONG, W. D., 1959: Determination of fluoride in blood serum. Analyt. chem. 31, 105–109.

STARRATT, A. N.; LOSCHIAVO, S. R., 1972: Aggregation of the confused flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae), elicited by fungal triglycerides. Can. Ent. 104, 757–759.

VINSON, S B.; THOMPSON, J. L.; GREEN, H. B., 1967: Phagostimulants for the imported fire ant *Solenopsis saevissima* Var. Richteri. J. Insect Physiol. 13, 1729–1736.

WYSS, O.; LUDWIG, B. Y.; JOINER, R. R., 1945: The fungistatic and fungicidal action of fatty acids and related compounds. Arch. Biochem. 7, 415–425.