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Utilization of Sterols in Clothes Moths, *Tinea pellionella* and *Tineola bisselliella*[1] Shoziro Ishii and Sachio Kawahara* (Pesticide Research Institute, College of Agriculture, Kyoto University, Kyoto) Received October 1, 1966. *Botyu-Kagaku*, 31, 153. 1966.

22. イガおよびコイガのステロール要求

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イガ *Tinea pellionella* とコイガ *Tineola bisselliella* はいずれも羊毛害虫として知られている．イガは羊毛を含む動物質食物しか寄主とし得ないが，コイガは動物質，植物質両方を寄主とすることができる．各種の飼育試験の結果，イガとコイガとではステロールの要求に相違があることがわかった．すなわちイガは食物中のステロールがコレステロールでないと成育しないが，コイガはコレステロールの他に植物ステロールである β-シトステロール，スティグマステロールをも利用する．このステロール要求の差が寄主の範囲を規定している．イガ類のステロール要求を利用して，コレステロールを含まぬ米ぬかで飼育したコナマダラメイガ *Ephestia cautella* 幼虫のステロールを，ステロール源とした飼料でイガを飼育すると成育することから，コナマダラメイガ幼虫は植物ステロールからコレステロールへ変えることを証明した．一方ガスクロマトグラフにより米ぬかステロールと，コナマダラメイガステロールを定量し，化学的にもこの変換を裏付けた．

The case-bearing clothes moth, *Tinea pellionella* and the weebbing clothes moth, *Tineola bisselliella* are known to be serious pest insects of woolen products. In experimental conditions, the webbing clothes moth is able to rear by feeding plant marerials such as rice bran, while case-bearing clothes moth is not by feeding them.

It is of interest to clarify why the webbing clothes moth can develop by feeding either plant or animal origin product, and the case-bearing clothes moth can not develop by feeding plant products.

a) Feeding tests on rice bran and fish meal.

Rice bran and fish meal were used for food of both clothes moths. These two food materials were mixed at various proportions. Five eggs collected from the rearing of both moths were transferred in small vials (16 mm × 60 mm) containing 1 g of rice bran and/or fish meal. Each experiment was replicated two or three times. The feeding experiments were carried out at 25°C. Number of adults emerged and the developmental period from egg to adult were recorded. The results are given in Table 1.

The results clearly indicated that the webbing clothes moths can develop by feeding either fish meal or rice bran even though the latter food was not so suitable, while the case-bearing clothes moth can develop by feeding only the fish meal. If rice bran was mixed with fish meal at a ratio of 1:1, larvae of the case-bearing clothes moth could not develop and died.

b) Improvement of amino acid composition in rice bran.

In order to improve amino acid composition of

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Table 1. Results of feeding tests of clothes moths on mixture of fish meal and rice bran

Mixture of fish meal : rice bran	*Tinea pellionella*		*Tineola bisselliella*	
	Period (day)	Percentage adult emergence	Period (day)	Percentage adult emergence
10 : 0	59	100	39.5	100
9 : 1	62.5	79.9	36.2	50.0
7.5 : 2.5	57.7	73.3	35.5	60.0
5.0 : 5.0	—	0	36.2	73.3
2.5 : 7.5	—	0	44.7	46.6
1.0 : 9.0	—	0	39.5	59.9
0 : 10	—	0	68.0	13.3

Table 2. Feeding tests of clothes moths on rice bran mixed with milk casein

Mixture of rice bran : milk casein	*Tinea pellionella*		*Tineola bisselliella*	
	Period	Percentage adult emergence	Period (day)	Percentage adult emergence
10.0 : 0	—	0	68.0	13.3
9.0 : 1.0	—	0	60.5	59.9
7.5 : 2.5	—	0	52.5	19.9
5.0 : 5.0	—	0	51.5	79.9
2.5 : 7.5	—	0	43.5	19.9
1.0 : 9.0	—	0	41.7	100.0
0 : 10.0	—	0	—	0

rice bran, milk casein was added at various proportions to rice bran. However, the improved diets were still unsuitable for the development of the case-bearing clothes moth as shown in Table 2.

c) Sterol requirement.

It was assumed that utilization of sterols may be different between two species of clothes moths. Feeding tests were carried out using synthetic food media in order to prove this assumption. The composition of basal diet modified from the diet for *Tineola bisselliella* (Fraenkel and Blewett 1946)[2], is shown in Table 3.

Table 3. Composition of basal diet

Casein (N. B. C., vitamin free)	50 parts
Glucose	50
Minerals, Wesson's	2
Water	10
Vitamins	
Thiamine HCl	25.0 μg/g
Riboflavin	12.5
Nicotinic acid	25.0
Pyridoxine HCl	12.5
Ca-pantothenate	25.0
Folic acid	2.5
Choline chloride	500.0
Inositol	250.0
Biotin	2.5
p-Aminobenzoic acid	25.0

Sterols to be tested were dissolved in ether and then added to the basal diet at a concentration of 0.1% in dry weight basis.

Kind of sterols tested and results of feeding tests are shown in Table 4.

Rice bran sterol and *Ephestia* sterol were isolated from rice bran and larvae of the almond moth *Ephestia cautella*, respectively.

From the feeding tests, it is evident that the webbing clothes moth can utilize both cholesterol and phytosterol, while the case-bearing clothes moth can not utilize phytosterol but utilize only cholesterol.

The fact that the both clothes moths can develop by feeding on the synthetic medium added *Ephestia* sterol, suggests that cholesterol would be contained in *Ephestia* sterol,

Table 4. Results of feeding tests of clothes moths on synthetic food media containing different kind of sterols

Sterols	*Tinea pellionella*		*Tineola bisselliella*	
	Number of adult emerged*	Period (day)	Number of adult emerged	Period (day)
Cholesterol	5	82.3	7	53.8
β-sitosterol	0	—	3	44.3
Stigmasterol	0	—	4	54.5
Lanosterol	0	—	0	—
Fish meal sterol	2	86.0	7	53.5
Rice bran sterol	0	—	3	62.5
Ephestia sterol	6	82.0	10	44.4

* Ten eggs for each experiment

Gas liquid chromatographic analysis of rice bran sterol and *Ephestia* sterol using two column systems, SE-30 and QF-1, indicated that *Ephestia* sterol contained a large amount of cholesterol which would be converted from phytosterol in *Ephestia* larvae as shown in Fig. 1 and Table 5.

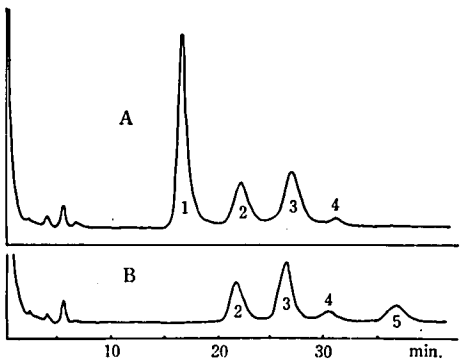

Fig. 1 Gas chromatograms of *Ephestia* and rice bran sterols using QF-1 column.
A : *Ephestia* Sterols, B : Rice bran sterols.
1 : cholesterol, 2 : campesterol,
3 : β-sitosterol, 4 : unknown 1,
5 : unknown 2

Table 5. Gas liquid chromatographic analysis of rice bran sterol and *Ephestia* sterol*

Steroles	Rice bran		*Ephestia*	
	QF-1	SE-30	QF-1	SE-30
Cholesterol	0.19%	0.47%	57.84%	60.39%
β-Sitosterol	55.12	59.61	28.24	26.93
Campesterol	19.17	10.78	12.33	8.94
Unknown 1	8.17	5.73	1.59	1.83
Unknown 2	17.35	19.16		
Unknown 3		4.24		1.90

* Carried out by courtesy of Dr. J. N. Kaplanis, U. S. D. A., Beltsville, Md.

d) Level of cholesterol requirement.

As cholesterol was considered to be suitable for the develoment of the both clothes moths, minimum concentration of cholesterol in the diet was determined. Basal diet used for feeding tests was as same as the previous one, and known amounts of cholesterol dissolved in ether were added to the basal diet. Concentration of cholesterol added and results of feeding tests are given in Table 6.

The both clothes moths developed by feeding

Table 6. Requirement of cholesterol level in synthetic food media for the development of clothes moths

Concentration of cholesterol	*Tinea pellionella*		*Tineola bisselliella*	
	No. of adult emerged*	Period (day)	No. of adult emerged	Period (day)
0.1 %	5	82.0	7	53.8
0.04	2	85.0	8	51.0
0.02	3	88.6	4	57.5
0.01	4	85.0	1	54.0
0.005	0	—	0	—

* Ten eggs for each experiment,

diets contained more than 0.01% of cholesterol in the basal diet.

Discussion

Two kinds of clothes moths, the case-bearing clothes moth, *Tinea pellionella*, and the webbing clothes moth, *Tineola bisselliella*, are different in their food habit even though both species are known to be insect pests of woolen products. The case-bearing clothes moth larva develop by feeding only animal products, while the webbing clothes moth larva develops by feeding either animal or plant products.

From the results of feeding of the both clothes moths on the mixture of rice bran and fish meal, *Tinea* larvae could not develop when rice bran was added more than 50 per cent, while *Tineola* larvae developed by feeding the mixture of various proportions. It shows that rice bran is either insufficient a certain nutrient for the growth, or inadequete in the proportion of nutritional requirements of *Tinea* larva.

In order to improve amino acid composition of rice bran, milk casein was added to rice bran in various proportions, but none of *Tinea* larvae developed. These experimental evidences sus pected that sterol requirement may be different between both clothes moths. Feeding tests on synthetic food media containing different kind of sterol were carried out. The results clearly indicated that *Tinea* larvae developed by feeding diets containing cholesterol and *Ephestia* sterol, and could not develop on diets containing β-sitosterol and rice bran sterol. On the other hand, *Tineola* larvae developed on plant and animal sterols.

The fact that sterols isolated from *Ephestia* larvae fed on rice bran is suitable for the development of *Tinea* larvae suspected that *Ephestia* sterols contained cholesterol, and that *Ephestia* larvae converted phytosterol to cholesterol.

Results of gas liquid chromatography indicated that rice bran sterols composed of β-sitosterol, campesterol, and some unknown sterols which could not be identified, wheares *Ephestia* sterols contained a large amount of cholesterol in addition to β-sitosterol, campesterol and unknown sterols. It is clear the larva of *Ephestia cautella* has an ability to convert phytosterol to cholesterol.

Simultaneously Mitsui (1965)[4] studied sterol requirement of several stored product insects including clothes moths, and he found that *Tineola* larva can utilize cholesterol, β-sitosterol and ergosterol, while *Tinea* larva can utilize only cholesterol.

The conversion of phytosterol to cholesterol in phytophagous insects has been suggested in the silkworm, *Bombyx mori by* Bergmann (1934)[1], and recently it was confirmed by Ikekawa *et al* (1966)[3] in the Silkworm and in the virginia pine sawfly *Neodiprion pratti* by Schaefer et al (1965)[5]. Dealkylation from phytosterol to cholesterol would be taken place in *Ephestia* larva.

Cholesterol requirement for the clothes moths was considered to be more than 0.01% in the diet.

Summary

Larvae of two related clothes moths, the case-bearing clothes moth, *Tinea pellionella*, and the webbing clothes moth, *Tineola bisselliella* are different in sterol requirement. The webbing cothes moth utilizes both cholesterol and phytosterols such as β-sitosterol and stigmasterol, while the case-bearing clothes moth utilizes only cholesterol.

This is the principal reason, that the webbing clothes moth can develop by feeding either plant or animal products.

Sterols isolated from larvae of the almond moth, *Ephestia cautella*, which had been fed on rice bran were used as sterol source for the two species of clothes moths. The *Ephestia* sterol was utilized by larvae of both species of insects. These results expected that the *Ephestia* sterol contains cholesterol. Results of gas liquid chromatographic analyses clearly showed that the *Ephestia* sterol contained a large amount of cholesterol. These experimental evidences indicated that *Ephestia* larva has an ability to convert phytosterol to cholesterol.

Minimum concentration of cholesterol for the growth of the both species was considered to be 0.01%.

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Enzymatic Hydrolysis of Monofluoroacetanilides in Insects. Kazuhiko Ando and Toshiie Nakamura (Agricultural Chemicals Research Laboratories, Sankyo Co. Ltd., Yasu-Cho, Shiga-Ken) Received October 22, 1966. *Botyu-Kagaku*, 31, 157. 1966. (with English Summary, 161).

23. Monofluoroacetanilides の昆虫体における酵素的加水分解*

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Monofluoroacetanilides の殺虫作用機構について検討した結果，昆虫に対しても温血動物に対すると同様に作用することがわかった．すなわちまず酵素的加水分解によってモノフルオロ酢酸と相当するアニリン類を生じ，生じたモノフルオロ酢酸が中毒の原因となると考えられる．さらにこの加水分解酵素は昆虫と温血動物とではかなりその性質を異にし，殊に昆虫のそれは温血動物のそれよりも Dipterex, triphenylphosphate などの阻害剤の影響を受けにくいということがわかった．

1. 緒　　言

Monofluoroacetanilides は強い殺虫作用を持つフッ素化合物であり[2]，特に吸汁口を持つ昆虫およびハダニ類に対して優れた効力を有することが知られている[7]．これら化合物の温血動物体における毒性発現機構については，最近中村らにより一連の研究が行なわれた．すなわち monofluoroacetanilides は温血動物体内ではまず酵素的加水分解を受けてモノフルオロ酢酸と相当するアニリン類を生じ，これが毒性発現の第一段階となると考えられる[9,10]．この加水分解酵素は Fluoroacetanilides amidohydrolase（以下 Fluoroacetanilidase と略称する）と仮称され，マウスおよびニワトリ肝臓の酵素についてその諸性質が明らかにされている[11,12]．さらに fluoroacetanilidase 阻害剤[13]をもちいての毒性軽減およびその場合の殺虫効力への影響について興味ある事実が見出された[14]．温血動物体内ではかくして生じたモノフルオロ酢酸は，既に Peters らによって明らかにされているように fluorocitrate に生合成され，これが TCA-cycle 中の aconitase を阻害することにより中毒作用を起こすものであり，その結果中毒した動物体内には多量のクエン酸の蓄積がみられるということが知られている[6,15,16,17]．

モノフルオロ酢酸誘導体の昆虫に対する殺虫作用機構についてはこれまでにあまり検討が行なわれていないが，Matsumura and O'Brien[5] は monofluoroacetamide および Na-fluoroacetate で中毒したイエバエ，ワモンゴキブリの体内にはやはり多量のクエン酸の蓄積がみられることを明らかにしており，モノフルオロ酢酸誘導体の殺虫作用は基本的には温血動物に対する作用と変わらないものであることを示唆している．また同時にワモンゴキブリおよびマウスホモジネートの monofluoroacetamide の加水分解活性を比較測定して，選択毒性の観点からの考察も試みている．

著者らは monofluoroacetanilides の殺虫作用機構を究明する目的でまづ昆虫体における fluoroacetanilidase の存在および中毒後のクエン酸蓄積の有無を確認し，monofluoroacetanilides は昆虫体においても酵素的加水分解を受け，温血動物におけると同様に毒作用を発現すると考えられることを明らかにした．さらに特に選択毒性の観点から昆虫の fluoroacetanilidase と温血動物のそれについて若干の比較検討を試み興味ある結果を得たのでここに報告する．

2. 実 験 材 料

(1) 供試化合物

monofluoroacetanilide (FAn) : m. p. 74.0～74.5 °C, 純度 F⁻値換算99.0%, および monofluoroaceto-*p*-bromoanilide (FBA) : m. p. 136.5～137.0°C, 純度 F⁻値換算99.2%, の2種類を使用した．いずれもダイキン工業（株）より提供を受けた．酵素阻害剤としてもちいた dimethyl (2, 2, 2-trichloro-1-hydro-

* 本研究の内容は1966年3月31日，昭和41年度日本応用動物昆虫学会大会（京都）において発表された．