原　著

Absence of Cholesterol Biosynthesis in the Rice Stem Borer, *Chilo suppressalis* Walker. Shoziro Ishii and Chisato Hirano (National Institute of Agricultural Sciences, Nishigahara, Tokyo) Received June 1, 1961. *Botyu-Kagaku*, 26, 71, 1961. (in English)

13. ニカメイガ幼虫におけるコレステロール生合成能の欠除

石井象二郎・平野千里（農林省農業技術研究所） 36. 6. 1 受理

合成飼料によつて飼育する時，ニカメイガ幼虫は生育のために，食物中にコレステロールを要求する．その最適濃度は食物中の 0.1～1.3% である．しかし食物中に過剰のコレステロールが存在しても，幼虫の生育は悪影響を受けない．幼虫は，哺乳動物で認められている各種のステロール前駆物質からコレステロールを生合成し得ない．したがつてニカメイガ幼虫には，コレステロールを生合成する能力が，全く欠除していると考えられる．

Since Hobson's finding[1], it has been established that insects require cholesterol or related sterols in their food materials. The rice stem borer, *Chilo suppressalis* Walker, also requires cholesterol when the larva is reared on a synthetic food medium, under aseptic conditions[2]. From the fact that considerable amount of cholesterol is necessary for normal growth and development of certain insects, it may be assumed that insects lack an ability to synthesize cholesterol from other substances.

Recently, Clark and Bloch[3] reported that the hide beetle, *Dermestes vulpinus*, could not synthesize cholesterol from substances which were known to be cholesterol precursors in mammals. Robbins, Kaplanis, Louloudes and Monroe[4] also recognized that acetate did not act as a precursor of cholesterol in the house fly, when 1-C^{14}-Na acetate was injected into the fly.

On the contrary, Casida, Beck and Cole[5] stated that injection of 1-C^{14}-acetate into the American cockroaches and mice resulted in almost the same percentage recovery of the labeled digitonides.

The present paper deals with the results of feeding tests of the rice stem borer with a synthetic food medium containing cholesterol and/or its precursors, under aseptic conditions.

Experimental

1) Determination of the optimum level of cholesterol in the diet

To determine optimum level of cholesterol in the diet, various amounts of cholesterol were added to the basal diet. Composition of the basal diet used in the present tests is given in Table 1. Preparation of the diet was described in the previous papers.

Table 1. Composition of the basal diet (per flask)

Constituent	Amount
Agar	0.1 g
Cellulose, fibrous	0.3
Casein	1.0
Glucose	0.5
Mineral mixture, Wesson's	0.06
Water	10 ml
B vitamins	
Thiamine hydrochloride	100 μg
Riboflavin	50
Nicotinic acid	100
Pyridoxine hydrochloride	50
Ca-pantothenate	100
Folic acid	10
Choline chloride	2,000
Inositol	1,000
Biotin	10
p-Aminobenzoic acid	100

Cholesterol dissolved in diethylether was added to the basal diet, and the ether was then evaporated on a water bath. The diets put into 100-ml Erlenmeyer flasks were sterilized three times in a Koch's steam sterilizer during successive three days, for thirty, thirty and fifteen minutes, respectively. Procedures of disinfection and inoculation of egg masses were the same as those

described in the previous papers. About 30 eggs were inoculated into a flask. The feeding test was carried out in an incubator at 25°C for thirty days. Number of survived larvae and their mean body weights, at the end of the feeding period, are given in Table 2.

The result of feeding tests with varying concentrations of cholesterol indicated that cholesterol is an indispensable nutrient for the growth of larvae. The larvae grew well by feeding on the diets containing cholesterol at levels of 0.08 to 1.3 per cent of the diet. Minimum optimal concentration of cholesterol seemed to be 0.1 per cent of the diet. It is remarkable that a large amount of cholesterol added in excess to the diet did not significantly affect the larval growth.

After the feeding tests, the residue of the diet containing the highest amount of cholesterol was extracted with 95 per cent ethylalcohol, the extract was condensed, and resulting crystals were obtained by filtration. The crystals showed a positive Liebermann-Burchard's reaction, and produced a digitonide with alcoholic solution of digitonin. These indicate that cholesterol has not been changed during the preparation of the diet and the feeding period.

2) Biosynthesis of cholesterol from sterolprecursors

Several compounds which were known to be precursors of cholesterol in mammals were added to the basal diet (Table 1), with or without cholesterol. Acetic acid, sodium acetate, and glutaric acid dissolved in water, and butyric, isovaleric, and mevalonic (hiochic) acids and squalene dissolved in diethylether were added to the basal diets. Then ether was evaporated on a water bath.

The feeding tests were carried out in the same way as in the previous test, and results are given in Table 3.

The results clearly indicate that the rice stem borer larva was unable to grow on a diet without cholesterol, even if any one of the precursors was added to the diet.

Discussion

The larva of the rice stem borer requires cholesterol in its dietary source, at a level slightly higher than 0.08 per cent of the dry diet. Excess of cholesterol in the diet does not show any adverse effect, and the larvae grow well on the diet in which cholesterol content exceeds 15 per cent.

The minimum optimal level of cholesterol in the diet for the German cockroach, *Blattella germanica*, was considered to be 0.05 per cent[6]. In the larvae of the house fly, optimum level of cholesterol was 50 μg per gram of diet, while the larvae were still able to develop on the diet containing cholesterol at a concentration of 15 per cent of the diet[7]. On the other hand, larvae of *Callosobruchus chinensis* developed on the diet containing cholesterol at levels of 0.01 to 5 per cent, but if content of cholesterol exceeded 6.3 per cent, the larvae could not grow and died[8].

It is of interest that the larvae of the rice stem borer and the house fly grow and develop by

Table 2. The growth response of the rice stem borer larvae on synthetic food media containing various levels of cholesterol.

Cholesterol added to basal diet (mg)	% Cholesterol in diet*	No. of medium	No. of larvae		Mean body weight (mg)
			hatched	survived	
0	—	3	60	21	0.33
0.1	0.005	6	141	79	0.73
0.4	0.020	6	117	103	5.81±0.53
1.6	0.082	6	116	101	44.63±2.63
6.4	0.326	6	136	130	54.22±2.23
25.6	1.289	6	130	127	53.68±2.17
102.4	4.965	6	132	121	39.89±1.67
409.6	17.286	5	124	102	42.92±2.49

*On dry weight basis.

Table 3. Growth response of the rice stem borer larvae to the synthetic diet containing sterol-precursors, with or without cholesterol.

Precursor	With (+) or without (-) cholesterol 10mg	No. of medium	No. of larvae		Mean body weight (mg)
			hatched	survived	
None	−	2	63	6	2.17
	+	2	40	39	46.15
Acetic acid 10 mg	−	2	44	14	0.64
	+	2	49	46	40.46
Naacetate 10 mg	−	2	53	10	0.50
	+	2	40	34	45.91
Butyric acid 10 mg	−	2	67	11	0.45
	+	2	35	30	42.73
Isovaleric acid 10 mg	−	2	33	10	0.50
	+	2	32	28	47.57
Glutaric acid 10 mg	−	2	46	14	0.43
	+	2	54	48	48.15
DL-Mevalonic acid 10 mg	−	3	54	15	0.73
	+	2	46	44	52.18
L-Mevalonic acid (crude)* 10 mg	−	5	128	11	0.37
Squalene 10 mg	−	1	20	9	1.67
	+	1	21	18	15.44
Squalene 20 mg	−	2	55	0	—
	+	2	49	6	3.17

*Isolated from cultured media of *Aspergillus oryzae*, in our laboratory.

feeding on diets which contain a large amount of cholesterol, without remarkable harmful effect on their growth. Fate of the excessive cholesterol consumed by the insects, however, is still unknown.

Generally, most mammals tested have an ability to synthesize cholesterol, and some compounds have been known as the precursors in sterol biosynthesis. Langdon and Bloch[9,10] demonstrated the biosynthesis of squalene from acetate-1-C^{14} in the liver of intact rat, and showed that the resultant squalene-C^{14} acts as an effective precursor of cholesterol. Also butyric and isovaleric acids are utilized for cholesterol synthesis, to an extent equal to or greater than acetate[11,12,13], and such branched chain acids as substituted glutaric acids or β-dimethylacrylic acid may be intermediates in the conversion of acetate to cholesterol[14]. Tavormina, Gibbs and Huff[15] showed that the β-hydroxy-β-methyl-δ-valerolactone(δ-lactone of mevalonic acid) was the major precursor in cholesterol biosynthesis in cell free rat liver homogenates.

In the present experiment, the fact that the larvae failed to grow on a synthetic diet without cholesterol but containing any one of acetic, butyric, isovaleric, glutaric or mevalonic acid, or squalene, indicates that these compounds could not be utilized as precursors for the cholesterol synthesis in the rice stem borer larvae, in vivo. The larva of hide beetle *Dermestes vulpinus* has no ability to synthesize cholesterol from many of the known precursors for mammals, including acetate and mevalonic acid, and can not grow if cholesterol was omitted from the diets [5]. The house fly is also unable to synthesize cholesterol from acetate[6].

The fact that the larva of the rice stem borer can not develop on diets without cholesterol but containing sterol-precursors, and that the larva requires rather high dosages of cholesterol indicate absence of cholesterol synthesis in the larva. It is of interest that the larva failed to grow on the diet containing both squalene and cholesterol. It seems that squalene has some adverse effects on the larva.

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Summary

The rice stem borer larva requires cholesterol as a dietary source for its growth, the optimal level of cholesterol being 0.1 to 1.3 per cent of the diet. Excessive amount of cholesterol, however, has no adverse effect on the larval growth. The sterol-precursors known for mammals were unable to be utilized for synthesis of cholesterol in the larva. These results suggest the absence of cholesterol synthesis in the rice stem borer larva.

Literature

1) Hobson, R. P., *Biochem. J.*, **29**, 2023 (1935)
2) Ishii, S. and Urushibara, H., *Bull. Nat. Inst. Agric. Sci.* (*Tokyo*), **C-4**, 109 (1954)
3) Clark, A. J. and Bloch, K., *J. Biol. Chem.*, **234**, 2578 (1959)
4) Robbins, W. E., Kaplanis, J. N., Louloudes, S. J. and Monroe, R. E., *Ann. Entom. Soc. Amer.*, **53**, 128 (1960)
5) Casida, J. E., Beck, S. D. and Cole, M. J., *J. Biol. Chem.*, **224**, 365 (1957)
6) Noland, J. L., *Arch. Biochem. Biophys.*, **48**, 370 (1954)
7) Levinson, Z. H. and Bergmann, E. D., *Biochem. J.*, **65**, 254 (1957)
8) Ishii, S., *Botyu-Kagaku*, **16**, 83 (1951)
9) Langdon, R. G. and Bloch, K., *J. Biol. Chem.*, **200**, 129 (1953)
10) Langdon, R. G. and Bloch, K., *J. Biol. Chem.*, **200**, 135 (1953)
11) Zabin, I. and Bloch, K., *J. Biol. Chem.*, **185**, 131 (1950)
12) Zabin, I. and Bloch, K., *J. Biol. Chem.*, **192**, 261 (1951)
13) Zabin, I. and Bloch, K., *J. Biol. Chem.*, **192**, 267 (1951)
14) Bloch, K., Clark, L. C. and Harary, I., *J. Biol. Chem.*, **211**, 687 (1954)
15) Tavormina, P. A., Gibbs, M. H. and Huff, J. W., *J. Amer. Chem. Soc.*, **78**, 4498 (1956)

Metabolic Fate of DDT in *Drosophila melanogaster*. III. Comparative Studies.* Masuhisa Tsukamoto (Genetical Laboratory, Faculty of Science, Osaka University, Osaka, Japan). Received July 26, 1961. *Botyu-Kagaku* **26**, 74, 1961 (in English).

14. ショウジョウバエにおける DDT の代謝. III. 誘導体の代謝と他の昆虫での Kelthane の生成. 塚本増久（大阪大学 理学部 生物学教室） 36. 7. 26 受理

DDT から Kelthane への酸化的代謝の機構を知りまた抵抗性と代謝との関係を解明するための1つの手掛りとして，比較的毒性がすくなく，しかも DDT-Kelthane 型の代謝を示すような DDT の誘導体があるかどうかを探索した．その結果，キイロショウジョウバエでは DDT の $-CCl_3$ 部をそれぞれ $-CHCl_2$, $-CH_3$, -H などに置換してもアルコール型の代謝物に酸化されることがわかつたが，フェニール基のパラの -Cl をそれぞれ -H, -OH, $-CH_3$, $-OCH_3$ などで置換した場合にはきわめて速やかに代謝が行われるにも拘らず，アルコール型の代謝物はエーテル可溶性部分中には検出できなかつた．従つてアルキル基の脱塩酸や酸化とは全く異つた別の経路（恐らくフェニール基の）を通つて代謝されるものか，あるいはアルコール型からさらに検出不能な水溶性の形にまで代謝が進むものであろうと推測される．

また DDT-Kelthane 型の代謝はキイロショウジョウバエにのみ見られる特殊な現象ではなくて，ゴキブリやイエバエにおいてもこの酸化型の代謝経路が存在することが明らかとなつたので，むしろ一般に昆虫では DDT の代謝経路としては DDE への脱塩酸と Kelthane への酸化の両方が存在するものであろうと考えられる．

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A list of all the abbreviations used is given in p. 76.