The Fate of ^{14}C -Glycerol in the Rice Stem Borer, *Chilo suppressalis* WALKER (Lepidoptera : Pyralidae)

Hisaaki TSUMUKI and Katsuo KANEHISA

*Laboratory of Applied Entomology, Institute for Agricultural and Biological Sciences,
Okayama University, Kurashiki 710, Japan*

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The interconversion between glycogen and glycerol was examined during diapausing and post-diapausing stages by injecting ^{14}C -glycerol.

Radioactive glycerol injected was rapidly incorporated into glycogen in diapausing larvae at 25°C even during increase of glycerol, showing that the interconversion between glycogen and glycerol may easily occur on warmer days in winter. However, this interconversion proceeded in the direction of glycerol synthesis at such low temperature as 4°C.

The isotope injected was incorporated into various tissues to varying degrees, especially it was found predominantly in fat body glycogen.

The degradation rate of ^{14}C -glycerol in diapausing larvae was lower than in post-diapausing larvae. On the other hand, in non-diapausing larvae which were shown to contain no glycerol, ^{14}C -glycerol was rapidly degraded in comparison with hibernating larvae. A cause of no glycerol accumulation in non-diapausing larvae may be attributed to such high activity of glycerol degradation.

INTRODUCTION

In the rice stem borer, changing patterns of glycerol concentrations in haemolymph are closely related to those of glycogen contents in the fat body during hibernation (TSUMUKI and KANEHISA, 1978). The conversion of ^{14}C -glucose into glycerol is higher in diapausing larvae than in post-diapausing types (TSUMUKI and KANEHISA, 1980 a). Consequently, it is conceived that glycogen degraded by increased activity of phosphorylase may be preferentially metabolized by increased activity of α -glycerophosphate dehydrogenase (TSUMUKI and KANEHISA, 1979 a, 1980 b) and then glycerol may accumulate during pre-diapausing to diapausing stages. It is known that glycerol is formed chiefly at the expense of glycogen in many insects (WYATT, 1967). However, it is recently reported that the accumulation of glycerol is not coincident with the disappearance of glycogen in the silkworm eggs (YAGINUMA and YAMASHITA, 1978, 1980). We have not as yet obtained any evidences for the direct interconversions between glycogen and glycerol in rice stem borer larvae.

The present study was undertaken to find the interconversions between glycerol and, glycogen, trehalose and lipid during diapausing and post-diapausing stages using ^{14}C -glycerol as the tracer. Further, ^{14}C -glycerol metabolism in non-diapausing larvae was also investigated.

MATERIALS AND METHODS

Experimental animal. Hibernating larvae and non-diapausing mature larvae were obtained by the method of Tsumuki and Kanehisa (1978). Non-diapausing larvae were reared on rice seedlings at $25\pm 1^\circ\text{C}$ under a long-day photoperiod (16L, 8D). Hibernating larvae were taken from the rice stems in fields as required.

Injection of ^{14}C -glycerol. After anaesthetization with ethyl ether, $1\ \mu\text{l}$ of ^{14}C -glycerol (46 mCi/mM; Radiochemical Centre) was injected and then the larvae were kept at different temperatures. Any $^{14}\text{CO}_2$ gas evolved was trapped by ethanolamine solution (Tsumuki and Kanehisa, 1980 a).

Extraction of carbohydrates, lipid and chitin. Carbohydrates and lipids were extracted by the method of Tsumuki and Kanehisa (1980a). The whole body and tissues were homogenized with 80% ethanol and the sugars and glycogen were extracted with 80% ethanol and 5% trichloroacetic acid (TCA), respectively. The insoluble precipitate after extraction was dried at about 50°C overnight and the lipid was extracted with ethyl ether. The protein remaining in the insoluble residue was digested in 1 M hyamine methanol solution and then the radioactivity in the residue was counted by toluene scintillator (Tsumuki and Kanehisa, 1980a). After the radioactivity was counted, insolubled chitin was separated from the hyamine methanol solution by the filtration and then washed twice with 10 ml portions of toluene and methanol, respectively. The radioactivity incorporated into chitin was again counted by toluene scintillator.

Liquid scintillation techniques. The radioactivity in all fractions was assayed by dioxane scintillator or toluene scintillator with a liquid scintillation counter (Tsumuki and Kanehisa, 1980 a).

RESULTS

Distribution pattern of ^{14}C -glycerol in fractions

To eliminate the effect of anaesthetization by ethyl ether on ^{14}C -glycerol metabolism early after the injection, the treated larvae were kept at 4°C for 8 hr before transference at 25°C . The time needed of the incorporation of ^{14}C -glycerol into CO_2 , trehalose, glycogen, lipid and chitin was examined (Table 1). Radioactive glycerol was rapidly metabolized by diapausing larvae at 25°C but not 4°C . Approximately 40% of injected ^{14}C -glycerol was metabolized for two days after transference at 25°C . Radioactive glycerol was degraded to CO_2 by diapausing larvae at 25°C . It was taken up into various fractions such as carbohydrates, lipid and chitin, and especially into glycogen. The incorporation rate of the isotope into lipid and trehalose was low, but the rate into lipid increased linearly with the elapse of time.

Conversion of ^{14}C -glycerol into $^{14}\text{CO}_2$

The times needed for conversions of ^{14}C -glycerol into CO_2 in diapausing and post-diapausing larvae are shown in Figs. 1, 2. Conversion of the isotope into CO_2 was affected by temperatures. However, radioactivities in CO_2 evolved by diapausing larvae were lower than by post-diapausing larvae at all temperatures tested. That is to say, the metabolic rate of glycerol in diapausing larvae was lower than in post-diapausing larvae.

Table 1. METABOLIC RATE OF ^{14}C -GLYCEROL IN DIAPAUSING LARVAE OF THE RICE STEM BORER

	Percentage of ^{14}C -activity ^a						
	4°C		25°C				
	8 hr	48 hr	1 hr	4 hr	8 hr	24 hr	48 hr
CO_2	0.08	0.57	0.11	0.54	0.84	3.03	5.40
Glycerol	87.22	87.70	87.36	84.70	81.32	72.02	59.53
Trehalose	0.79	0.80	0.73	1.31	1.21	1.24	1.20
Glucose	1.60	1.36	1.68	1.82	1.98	2.22	2.37
Glycogen	3.86	3.16	3.17	4.92	6.55	11.72	18.41
Lipid	0.14	0.15	0.17	0.26	0.32	0.72	1.27
Chitin	0.44	0.40	0.33	0.31	0.33	0.34	0.68
Protein	2.11	1.08	1.17	1.38	1.54	1.79	2.99
Unknown	4.20	4.87	3.90	4.37	5.68	6.54	7.93

^a Percentage to the total radioactivity injected.

Fig. 1. $^{14}\text{CO}_2$ production rate by diapausing larvae injected with ^{14}C -glycerol at various temperatures of the rice stem borer (○), 25°C; (△), 15°C; (×), 4°C.

Incorporation of ^{14}C -glycerol into tissues

To see the mobilization of glycerol among tissues, ^{14}C -glycerol was injected into the haemocoel of diapausing and post-diapausing larvae. The incorporation of the isotope into the haemolymph, alimentary canal, fat body and carcass are shown in Tables 2, 3, in which the radioactivities were analyzed ten days after injection. Different patterns of radioactive incorporation were observed in different tissues. The activity was mainly in the haemolymph and carcass, and low but significant in the fat body and alimentary canal.

Fig. 2. $^{14}\text{CO}_2$ production rate by post-diapausing larvae injected with ^{14}C -glycerol at various temperatures of the rice stem borer (○), 25°C; (△), 15°C; (×), 4°C.

Table 2. DISTRIBUTION PERCENTAGE OF ^{14}C -GLYCEROL AMONG TISSUES OF DIAPAUSING LARVAE OF THE RICE STEM BORER AT VARIOUS TEMPERATURES FOR 10 DAYS

	Percentage of ^{14}C -activity ^a		
	25°C	15°C	4°C
CO ₂	25.7	10.5	3.2
Alimentary canal	2.5	2.3	1.8
Fat body	14.1	6.5	5.5
Haemolymph	28.6	40.2	51.7
Carcass	29.0	40.6	37.8

^a Percentage to the total radioactivity injected.

Table 3. DISTRIBUTION PERCENTAGE OF ^{14}C -GLYCEROL AMONG TISSUES OF POST-DIAPAUSING LARVAE OF THE RICE STEM BORER AT VARIOUS TEMPERATURES FOR 10 DAYS

	Percentage of ^{14}C -activity ^a		
	25°C	15°C	4°C
CO ₂	37.8	27.5	4.3
Alimentary canal	1.8	1.4	1.9
Fat body	13.6	9.8	3.5
Haemolymph	24.7	37.4	59.0
Carcass	22.1	24.3	31.3

^a Percentage to the total radioactivity injected.

Incorporation of ^{14}C -glycerol into tissue constituents was measured in diapausing and post-diapausing larvae (Tables 4, 5). The radioactivities in 80% ethanol fractions were high in all kinds of tissues examined. However, in the fat body the radioactivity in the TCA fraction was high compared with that of the 80% ethanol fraction at 25°C , whereas the activity was low at 4°C . Incorporation of radioactivity into the TCA fraction in diapausing larvae was somewhat lower than in post-diapausing larvae.

The incorporation of radioactivity of main sugar components was measured in haemolymph separated by paper chromatography and the results are shown in Table 6, in which the radioactivity was analyzed ten days after the injection. Radioactive glycerol in haemolymph decreased in both diapausing and post-diapausing larvae with an increase of temperature, while most of the radioactivity in haemolymph was found in glycerol in both types at 4°C . The activity in the haemolymph of diapaus-

Table 4. DISTRIBUTION PERCENTAGE OF ^{14}C -GLYCEROL IN FRACTIONS OF TISSUES OF DIAPAUSING LARVAE OF THE RICE STEM BORER AT VARIOUS TEMPERATURES FOR 10 DAYS

		Percentage of ^{14}C -activity in fraction of ^a			
		80% EtOH	5% TCA	Ethyl ether	Precipitate
25°C	Alimentary canal	1.67	0.68	0.02	0.51
	Fat body	5.84	6.66	0.37	1.06
	Carcass	19.98	6.01	0.51	3.01
15°C	Alimentary canal	2.11	0.21	0.01	0.17
	Fat body	5.47	1.31	0.42	0.42
	Carcass	35.86	2.92	0.55	1.81
4°C	Alimentary canal	1.72	0.05	0.02	0.04
	Fat body	5.26	0.19	0.05	0.11
	Carcass	36.42	0.96	0.11	0.35

^a Percentage to the total radioactivity injected.

Table 5. DISTRIBUTION PERCENTAGE OF ^{14}C -GLYCEROL IN FRACTIONS OF TISSUES OF POST-DIAPAUSING LARVAE OF THE RICE STEM BORER AT VARIOUS TEMPERATURES FOR 10 DAYS

		Percentage of ^{14}C -activity in fraction of ^a			
		80% EtOH	5% TCA	Ethyl ether	Precipitate
25°C	Alimentary canal	0.84	0.40	0.01	0.55
	Fat body	3.57	8.72	0.43	0.86
	Carcass	10.59	5.27	0.85	5.40
15°C	Alimentary canal	1.10	0.13	0.01	0.20
	Fat body	4.28	3.75	0.92	0.82
	Carcass	18.29	1.94	1.24	2.83
4°C	Alimentary canal	1.79	0.05	0.01	0.05
	Fat body	3.11	0.29	0.05	0.06
	Carcass	29.16	0.75	0.30	1.13

^a Percentage to the total radioactivity injected.

Table 6. INCORPORATION PERCENTAGE OF ^{14}C -GLYCEROL INTO TREHALOSE AND GLUCOSE IN HAEMOLYMPH OF DIAPAUSING AND POST-DIAPAUSING LARVAE OF THE RICE STEM BORER AT VARIOUS TEMPERATURES FOR 10 DAYS

		Percentage of ^{14}C -activity		
		25°C	15°C	4°C
Diapause	Trehalose	13.74 ^a (4.07) ^b	3.52(1.41)	1.38(0.71)
	Glucose	2.69 (0.74)	2.20(0.88)	1.85(0.96)
	Glycerol	62.16 (17.81)	84.59(33.99)	90.73(46.94)
Post-diapause	Trehalose	40.21 (9.93)	10.61(3.97)	4.71(2.78)
	Glucose	3.08 (0.76)	4.48(1.68)	2.66(1.57)
	Glycerol	12.86 (3.18)	57.46(21.49)	80.46(47.47)

^a Percentage to total radioactivity spotted on the paper chromatogram.

^b Percentage to the total radioactivity injected.

Table 7. DISTRIBUTION PERCENTAGE OF ^{14}C -GLYCEROL IN FRACTIONS OF NON-DIAPAUSING AND DIAPAUSING LARVAE OF THE RICE STEM BORER AT VARIOUS TEMPERATURES FOR 10 DAYS

	Percentage of ^{14}C -activity ^a			
	Non-diapause			Diapause
	25°C	15°C	4°C	25°C
CO ₂	63.4	36.1	18.9	21.0
Haemolymph	6.1	10.0	30.5	25.7
80% EtOH	18.3	38.9	42.9	26.7
5% TCA	2.8	4.0	5.2	19.8
Ethyl ether	0.9	2.6	0.3	1.6
Chitin	1.9	1.3	0.4	1.3
Protein	6.6	7.0	1.8	4.0

^a Percentage to the total radioactivity injected.

ing larvae was higher than in post-diapausing larvae and ^{14}C -glycerol was converted to trehalose more slowly in diapausing larvae than in post-diapausing types.

^{14}C -glycerol metabolism in non-diapausing larvae

For comparison with diapausing larvae, non-diapausing mature larvae reared at 25°C under a long-day photoperiod were used. The larvae were divided into two parts, haemolymph and the other tissues; the other tissues were further separated into some fractions. The incorporation rates of radioactivities into CO₂, haemolymph and tissue fractions are shown in Table 7. Radioactive glycerol was rapidly converted to CO₂ by non-diapausing larvae. The radioactivity in TCA fractions showed lower levels in non-diapausing larvae than in diapausing ones (Table 4). The incorporation rates of the radioactivity were measured in trehalose and glucose of haemolymph and tissues (Table 8). Radioactive glycerol was very low in non-diapausing larvae. The specific activity of trehalose was more than that of glucose in haemolymph and the tissues of non-diapausing larvae.

Table 8. INCORPORATION PERCENTAGE OF ^{14}C -GLYCEROL INTO TREHALOSE AND GLUCOSE IN HAEMOLYMPH AND TISSUES OF NON-DIAPAUSING AND DIAPAUSING LARVAE OF THE RICE STEM BORER AT VARIOUS TEMPERATURES FOR 10 DAYS

		Percentage of ^{14}C -activity			
		Non-diapause			Diapause
		25°C	15°C	4°C	25°C
Haemolymph	Trehalose	34.57 ^a (2.12) ^b	32.19(3.20)	44.46(13.57)	17.51(4.50)
	Glucose	3.41 (0.21)	3.96(0.39)	2.99(0.91)	3.68(0.95)
	Glycerol	3.70 (0.23)	6.17(0.61)	3.78(1.15)	60.68(15.59)
Tissues	Trehalose	4.34 (0.79)	5.77(2.24)	21.35(9.16)	9.31(2.49)
	Glucose	3.32 (0.61)	2.90(1.13)	4.85(2.08)	3.13(0.84)
	Glycerol	4.27 (0.78)	14.05(5.47)	14.85(6.37)	63.62(16.99)

^a Percentage to total radioactivity spotted on the paper chromatogram.

^b Percentage to the total radioactivity injected.

DISCUSSION

The accumulation and exhaustion of glycerol in the haemolymph have been shown to correlate with the disappearance and reappearance of glycogen in the fat body during hibernation of the rice stem borer (Tsumuki and Kanehisa, 1978). Further, glycerol concentration was affected by environmental temperatures; in diapausing larvae it increased below 20°C and decreased at 25°C (Tsumuki and Kanehisa, 1979 b, 1980 c). In this study, ^{14}C -glycerol was shown to be utilized in glycogen synthesis even in diapausing larvae. These observations show that the interconversion between glycerol and glycogen is readily reversible even when glycerol is increasing. Further, these results suggest that glycerol may be unstable even in diapausing larvae at 25°C and that glycerol formation and degradation may readily occur on warmer days in winter. However, this interconversion may proceed in the direction of glycerol synthesis at temperatures as low as 4°C. Fat body glycogen degraded by phosphorylase activated at low temperature (Ziegler and Wyatt, 1975) may be metabolized by enhanced activity of α -glycerophosphate dehydrogenase during diapausing stages (Tsumuki and Kanehisa, 1980 b) and then glycerol production may be accelerated at a low temperature.

The different pattern of incorporation of ^{14}C -glycerol among the components of tissues implies that the metabolic pathway of ^{14}C -glycerol is different from tissue to tissue. Most ^{14}C -glycerol is converted to glycogen by *Protophormia terranova* at 25°C (Wood and Nordin, 1976). In *Chilo suppressalis*, ^{14}C -glycerol was largely incorporated into the TCA soluble fraction of the fat body at 25°C, showing that glycerol is converted readily to glycogen in the fat body. Then, this observation appears to be able to explain the relationship between haemolymph glycerol and fat body glycogen (Tsumuki and Kanehisa, 1978).

The incorporation of ^{14}C -glycerol into lipid was shown to be low but significant. Since the lipid was extracted after 80% ethanol extraction, the true rate of the incorporation into the lipid fraction may be higher than the results. Radioactive glucose was also incorporated into lipid in the hibernating larvae of the rice stem borer (Tsumuki and Kanehisa, 1980 a). The lipid appeared to be a precursor of glycerol

synthesis as described in silkworm eggs (YAGINUMA and YAMASHITA, 1978, 1980) but the role may be small in comparison with glycogen in the rice stem borer.

The sugars synthesized from ^{14}C -glycerol were mainly trehalose and glucose in haemolymph. The radioactivity of trehalose was higher in post-diapausing larvae than in diapausing ones. Similar phenomena have also been shown in previous experiments in which ^{14}C -glucose was used as a tracer (TSUMUKI and KANEHISA, 1980 a). On the other hand, in non-diapausing larvae the incorporation of ^{14}C -glycerol into trehalose was apparently higher at low temperatures than at high temperature, since trehalose is an energy source (SACKTOR, 1965, 1970; WYATT, 1967) and labelled trehalose may be rapidly consumed as an energy source at high temperatures.

Significant differences in ^{14}C -glycerol degradation between diapausing and post-diapausing larvae were observed. The lower degradation rate of ^{14}C -glycerol in diapausing larvae was coincident with the higher glycerol concentrations during hibernation (TSUMUKI and KANEHISA, 1978).

In non-diapausing larvae which are shown to contain no glycerol, ^{14}C -glycerol was rapidly degraded in comparison with hibernating larvae at all temperatures tested and a trace level of ^{14}C -glycerol injected remained ten days after injection. The failure of glycerol to accumulate in non-diapausing larvae may be attributed to this high activity of glycerol degradation and the low activity of glycerol formation (TSUMUKI and KANEHISA, 1980 a, c).

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