

METABOLISM OF ^{14}C -GLUCOSE AND UDP- ^{14}C -G IN HIBERNATING LARVAE OF THE RICE STEM BORER, *CHILO SUPPRESSALIS* WALKER

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INTRODUCTION

Carbohydrates accumulating in diapausing insects have been shown to be trehalose and polyols (Wyatt 1967). Glycogen was abruptly converted to sorbitol and glycerol at the beginning of diapause and these polyols were reconverted to glycogen at the termination of diapause in silkworm eggs (Chino 1958, Yaginuma and Yamashita 1978).

On the other hand, in hibernating larvae of the rice stem borer, the accumulation of glycerol and trehalose in haemolymph was intimately associated with a decline of glycogen content in the fat body during pre-diapausing to diapausing stage. The amounts of these two carbohydrates in haemolymph began to decrease progressively with steadily increasing amounts of glycogen in fat body during post-diapausing stage. However, the change of trehalose concentration was at a low rate in comparison with that of glycerol. Furthermore, changing patterns in the enzyme activities of glycogenetic and glycolytic pathways were coincident with those of carbohydrate contents during hibernation (Tsumuki and Kanehisa 1979, 1980).

However, the details of these interconversions between glycogen and, glycerol and trehalose remain equivocal. Murphy and Wyatt (1965) have shown that trehalose was synthesized from glucose-6-phosphate and uridine-5'-diphospho glucose (UDPG), and glycogen from UDPG in the fat body of the cecropia silkworm.

The present study was carried out to determine the functional relationships between glycogen and, glycerol and trehalose in the tissues of the rice stem borer at various stages of larval life by the use of ^{14}C -glucose and UDP- ^{14}C -G.

MATERIALS AND METHODS

Animal

Hibernating larvae of the rice stem borer, *Chilo suppressalis* Walker, were collected at Okayama Agricultural Experiment Station in Okayama Prefecture.

Chemicals

^{14}C -(U)-glucose (250 mCi/mM) and UDP- ^{14}C -G (273 mCi/mM) were purchased from the Radio Chemical Centre (Amersham, England). All other chemicals were of reagent grade.

Injections of ^{14}C -substances and incubation techniques

After anaesthetization with ethyl ether, 1 μl of ^{14}C -glucose and UDP- ^{14}C -G was individually injected with a micrometer syringe into haemolymph from a leg of the abdomen. After the leg was tied with cotton thread, the larvae were transferred into glass containers as shown in Fig. 1 and the containers were kept at different temperatures

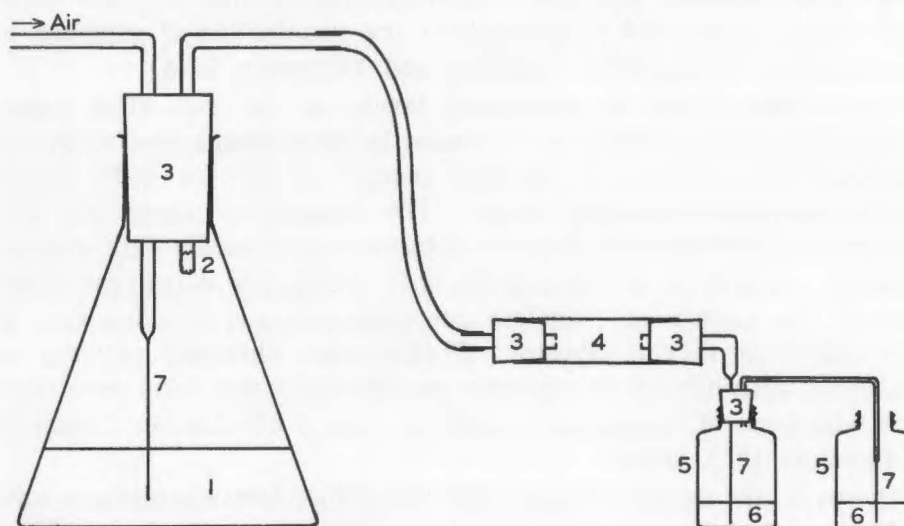


FIG. 1. Instrument of trapping CO_2 gas.

- 1) 10 % NaOH 2) Cotton 3) Rubber stopper 4) Glass container
5) Scintillation vial 6) Ethanolamine solution 7) Glass capillary tube

(Tsumuki and Kanehisa 1975). Any $^{14}\text{CO}_2$ gas evolved was trapped by bubbling air passed over the container through 3 ml of ethanolamine solution (ethanolamine: methyl cellosolve=1:11 v/v) (Crout *et al.* 1966) in scintillation vials (Tsumuki and Kanehisa 1975). The vials were changed at regular intervals.

Extraction of carbohydrates and lipids

The haemolymph, alimentary canal, fat body and carcass were taken out and homogenized in 80 % ethanol. Glycogen was extracted with 5 % trichloroacetic acid (TCA) from the insoluble precipitates in

the ethanol by the method of Tsumuki and Kanehisa (1978). The ethanol and TCA insoluble precipitate was air-dried at about 50°C overnight and then extracted with three 5 ml portions of ethyl ether. The ethyl ether extracts were combined, transferred into a scintillation vial and air-dried at room temperature. After the ethyl ether insoluble residues were broken up, they were digested in 0.5 ml or 1 ml of 1 M hyamine methanol solution at about 50°C .

Liquid scintillation techniques

The radioactivity in all fractions was assayed with an Aloka Liquid Scintillation Counter (Aloka LSC 653). The radioactivity in the 80 % ethanol and 5 % TCA extracts (2 ml) was individually assayed in 10 ml of dioxane scintillator (6 g of PPO, 0.275 g of POPOP and 112 g of naphthalene per 1 l of dioxane) and the radioactivity in CO_2 , lipid and precipitate was assayed in 10 ml of toluene scintillator (4 g of PPO, 0.1 g of POPOP per 1 l of toluene). If two distinct liquid phases were formed between CO_2 absorbent and toluene scintillator, methanol was added to the toluene scintillator.

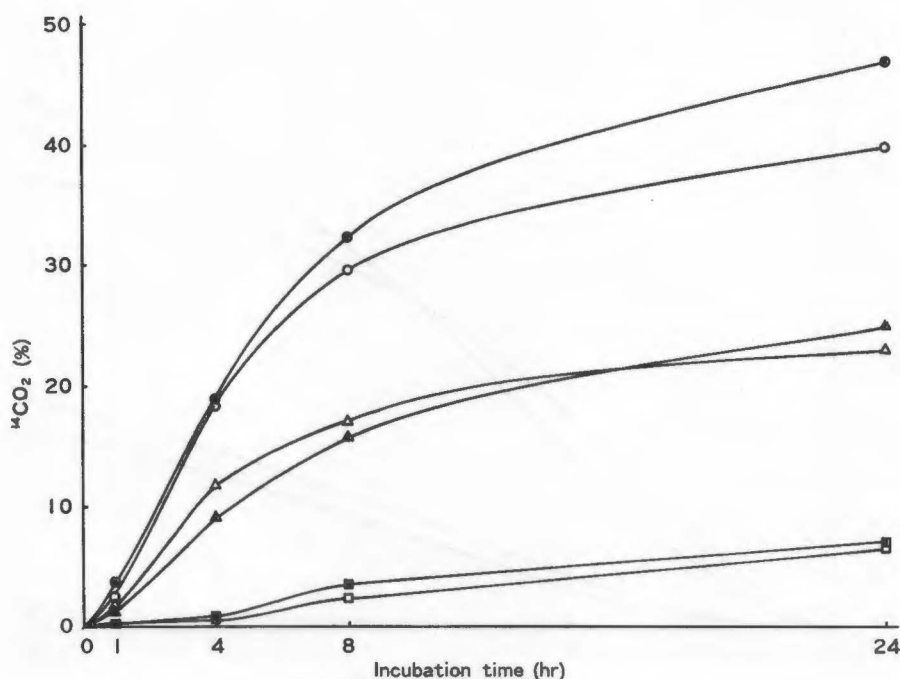


FIG. 2. Production rate of $^{14}\text{CO}_2$ gas by hibernating larvae injected with ^{14}C -glucose at different temperatures.

○: Diapause at 25°C ●: Post-diapause at 25°C △: Diapause at 15°C
 ▲: Post-diapause at 15°C □: Diapause at 4°C ■: Post-diapause at 4°C

Paper chromatography

Ethanol extract was evaporated under reduced pressure and the residue was suspended in a small amount of 80 % ethanol. After centrifugation at 3,000 r. p. m. for 10 minutes, the supernatant was applied on a filter paper. After development, the silver nitrate-positive components were identified (Tsumuki and Kanehisa 1978) and corresponding identical spots on the paper were individually placed in scintillation vials and then 10 ml of toluene scintillator was added to each vial.

RESULTS

CO_2

Substantial quantities of $^{14}CO_2$ were evolved during one day after injection of ^{14}C -glucose and UDP- ^{14}C -G (Figs. 2 and 3). The production rate of $^{14}CO_2$ was affected by environmental temperatures and ^{14}C -substances. No significant differences were shown in $^{14}CO_2$ production between diapausing and post-diapausing larvae, but the production rate appeared to be higher in post-diapausing larvae than in diapausing larvae.

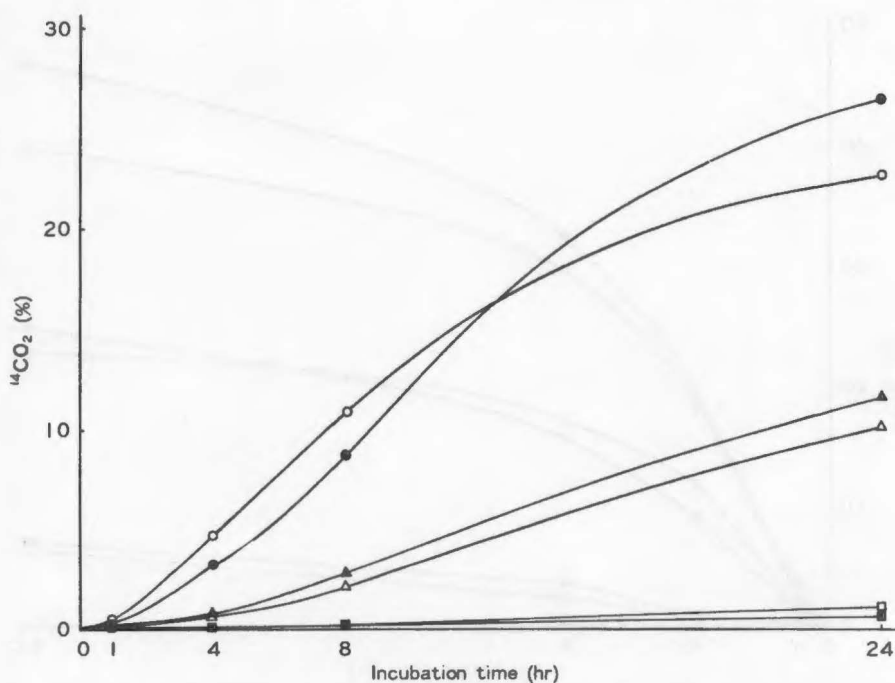


FIG. 3. Production rate of $^{14}CO_2$ gas by hibernating larvae injected with UDP- ^{14}C -G at different temperatures.

○: Diapause at 25°C ●: Post-diapause at 25°C △: Diapause at 15°C
▲: Post-diapause at 15°C □: Diapause at 4°C ■: Post-diapause at 4°C

Incorporation rates of ^{14}C -glucose and UDP- ^{14}C -G into the tissues

Table 1 shows the incorporation of radioactivity into the tissues of diapausing and post-diapausing larvae at different temperatures one day

TABLE 1
The distributions of radioactivities in the tissues of hibernating larvae at different temperatures one day after injection of ^{14}C -glucose and UDP- ^{14}C -G

		Diapause* (%)			Post-diapause** (%)		
		4°C	15°C	25°C	4°C	15°C	25°C
^{14}C -glucose	Haemolymph	30.8	22.6	10.6	24.0	18.1	7.7
	Alimentary canal	2.9	2.7	2.0	2.7	2.9	2.1
	Fat body	12.2	11.8	22.0	9.3	18.8	16.3
	Carcass	47.9	39.7	26.0	57.3	35.2	26.9
UDP- ^{14}C -G	Haemolymph	72.6	50.1	25.6	80.5	52.0	34.5
	Alimentary canal	1.0	2.3	2.1	1.9	1.6	1.6
	Fat body	6.3	10.2	24.5	4.3	11.7	11.6
	Carcass	18.7	27.4	25.4	13.0	22.9	25.8

Percentage of the total radioactivity injected.

* Larvae collected in fields in December.

** Larvae collected in fields from the middle to the end of February.

TABLE 2
The radioactivities recovered from the fractions in the tissues of hibernating larvae at different temperatures one day after injection of ^{14}C -glucose

		Diapause (%)				Post-diapause (%)			
		EtOH	TCA	Ether	Precipitate	EtOH	TCA	Ether	Precipitate
4°C	Alimentary canal	2.0	0.7	0.1	0.2	1.9	0.7	0.1	0.1
	Fat body	8.0	3.2	0.2	0.9	7.5	1.1	0.4	0.3
	Carcass	33.3	12.3	0.4	2.0	38.8	13.4	0.3	4.9
15°C	Alimentary canal	2.0	0.6	0.1	0.1	1.5	1.0	0.1	0.4
	Fat body	5.0	2.8	3.0	1.0	6.4	3.0	7.7	1.6
	Carcass	20.7	16.1	0.9	2.0	20.2	9.4	2.2	3.5
25°C	Alimentary canal	0.9	0.9	0.1	0.2	0.7	1.0	0.1	0.3
	Fat body	4.8	13.9	2.1	1.5	5.7	8.7	3.3	0.3
	Carcass	9.4	13.8	0.8	2.1	9.7	11.8	1.2	4.0

Percentage of the total radioactivity injected.

TABLE 3

The radioactivities recovered from the fractions in the tissues of hibernating larvae at different temperatures one day after injection of UDP- ^{14}C -G

		Diapause (%)				Post-diapause (%)			
		EtOH	TCA	Ether	Precipitate	EtOH	TCA	Ether	Precipitate
4°C	Alimentary canal	0.7	0.3	0.1>	0.1>	1.4	0.4	0.1>	0.1>
	Fat body	4.9	1.2	0.1	0.1	3.6	0.6	0.1	0.1>
	Carcass	14.4	3.7	0.1>	0.5	8.3	4.0	0.1>	0.9
15°C	Alimentary canal	1.3	0.9	0.1>	0.1	1.0	0.4	0.1>	0.2
	Fat body	5.2	4.1	0.7	0.4	5.8	3.4	2.0	0.6
	Carcass	17.4	8.4	0.3	1.3	13.4	7.0	0.6	2.0
25°C	Alimentary canal	0.8	1.1	0.1>	0.2	0.8	0.5	0.1>	0.2
	Fat body	6.1	16.7	0.7	1.1	5.1	5.2	1.0	0.4
	Carcass	12.2	11.1	0.3	1.8	14.9	7.9	0.6	3.1

Percentage of the total radioactivity injected.

after injection of ^{14}C -glucose and UDP- ^{14}C -G into haemolymph. The incorporation rates of the radioactivities in haemolymph into other tissues varied at different temperatures. Furthermore, significant differences in the incorporation rates of the radioactivities in haemolymph into other tissues were shown between ^{14}C -glucose and UDP- ^{14}C -G-injected larvae. Tables 2 and 3 show the results of radioactivity measurements in fractions of the individual tissues. The increasing incorporation rates of the radioactivities into the TCA and ethyl ether fractions, and the precipitates were associated with a decline in the radioactivity of the ethanol fraction. This result indicates that ^{14}C -glucose and UDP- ^{14}C -G were incorporated into glycogen, lipid (Wyatt 1967, Tate and Wimer 1974, Yamashita and Hasegawa 1974) and protein or chitin (Lipke *et al.* 1965, Crompton and Polakis 1969, Tate and Wimer 1974), and that these incorporation rates increased with increase in temperature. Since some kinds of lipids could be extracted with 80 % ethanol, the true radioactivity in the ethyl ether fractions was higher than values obtained in Tables 3 and 4. However, the incorporation rates of radioactivities into lipids may be at low levels.

Incorporation rates of ^{14}C -glucose and UDP- ^{14}C -G into trehalose, glycerol and glucose in the tissues

Ethanol fractions were separated by paper chromatography. The incorporation rates of ^{14}C -glucose and UDP- ^{14}C -G into trehalose, glycerol

and glucose were calculated from the measurements of the relative recovery rate of individually-identified spots based on the recovery of radioactivity applied on the paper chromatogram (Tables 4 and 5). The incorporation rates of ^{14}C -glucose into glycerol exceeded those into trehalose in diapausing larvae. However, these differences were not observed

TABLE 4
Incorporation rate of ^{14}C -glucose into trehalose and glycerol in alimentary canal and fat body of hibernating larvae at different temperatures

		Alimentary canal (%)			Fat body (%)		
		4°C	15°C	25°C	4°C	15°C	25°C
Diapause	Trehalose	11.0	11.3	9.9	7.6	6.3	7.2
	Glucose	5.8	9.8	10.4	5.8	5.5	3.9
	Glycerol	12.4	14.5	13.7	8.0	7.6	7.2
Post-diapause	Trehalose	15.1	9.5	11.0	14.5	13.4	11.0
	Glucose	3.5	2.8	3.9	3.7	6.0	5.6
	Glycerol	10.1	7.1	7.0	7.1	6.0	6.1

Percentage of total radioactivity spotted on the paper chromatogram.

TABLE 5
Incorporation rate of UDP- ^{14}C -G into trehalose and glycerol in alimentary canal and fat body of hibernating larvae at different temperatures

		Alimentary canal (%)			Fat body (%)		
		4°C	15°C	25°C	4°C	15°C	25°C
Diapause	Trehalose	11.3	7.5	11.5	7.4	6.0	4.0
	Glucose	7.9	8.3	6.5	2.6	3.4	1.9
	Glycerol	9.9	11.5	8.6	7.1	4.8	2.8
Post-diapause	Trehalose	23.6	17.6	15.4	34.7	45.7	35.5
	Glucose	12.7	2.9	1.5	6.2	8.5	4.1
	Glycerol	6.7	7.1	5.3	5.3	2.0	4.3

Percentage of total radioactivity spotted on the paper chromatogram.

in larvae injected with UDP- ^{14}C -G. There were significant differences in the levels of radioactivities incorporated into trehalose between diapausing and post-diapausing larvae. Since in such tissues, trehalose, glucose and glycerol formation may be affected by the concentrations of these substances in the haemolymph (Wyatt 1967), the incorporation

rates of radioactivities into trehalose, glucose and glycerol in the haemolymph may be of importance. The incorporation rates of ^{14}C -glucose and UDP- ^{14}C -G into glycerol in the haemolymph of diapausing larvae were higher than those of post-diapausing larvae, and these incorporation rates were accelerated by low temperatures in diapausing larvae in-

TABLE 6
Incorporation rates of ^{14}C -glucose and UDP- ^{14}C -G into trehalose and glycerol in haemolymph of hibernating larvae at different temperatures

		^{14}C -glucose (%)			UDP- ^{14}C -G (%)		
		4°C	15°C	25°C	4°C	15°C	25°C
Diapause	Trehalose	14.3 [*] (4.4 ^{**})	7.3(1.7)	13.8(1.5)	12.3(8.9)	5.9(3.0)	7.8(2.0)
	Glucose	3.4(1.1)	3.3(0.7)	3.3(0.4)	2.5(1.8)	3.3(1.7)	3.0(0.8)
	Glycerol	36.6(11.3)	32.8(7.4)	20.4(2.2)	3.2(2.3)	4.1(2.1)	8.6(2.2)
Post-diapause	Trehalose	10.9(2.6)	20.9(3.8)	30.1(2.3)	6.4(5.2)	18.2(9.5)	21.8(7.5)
	Glucose	29.0(7.0)	3.9(0.7)	4.5(0.3)	1.3(1.0)	6.1(3.2)	4.0(1.4)
	Glycerol	11.1(2.7)	9.6(1.7)	6.6(0.5)	1.3(1.0)	2.9(1.5)	2.1(0.7)

* Percentage of total radioactivity spotted on the paper chromatogram.

** Percentage of total radioactivity injected.

jected with ^{14}C -glucose (Table 6). However, in post-diapausing larvae, no effect of decreased temperature on the incorporation rates of UDP- ^{14}C -G into glycerol was observed. On the contrary, in post-diapausing larvae incorporation of the radioactivities into trehalose increased with increase in temperature.

DISCUSSION

It was suggested that in diapausing and post-diapausing larvae of the rice stem borer, ^{14}C -glucose and UDP- ^{14}C -G were incorporated into tissues and metabolized, but that there were significant differences in the metabolic rates of ^{14}C -glucose and UDP- ^{14}C -G. In comparison with UDP- ^{14}C -G in both diapausing and post-diapausing larvae, ^{14}C -glucose injected into haemolymph was readily metabolized into $^{14}\text{CO}_2$ and incorporated into the other tissues. Most of the radioactivity on the paper chromatogram separated UDP- ^{14}C -G derivatives in the haemolymph was distributed on the starting point and low mobilizing positions except the radioactivity on trehalose, glucose and glycerol spots. Radioactivity was at high levels on the starting point particularly. This fact may indicate that UDP- ^{14}C -G in haemolymph may be rapidly converted to UDP deri-

vatives (Carey and Wyatt 1960), and conjugated substances and glycoside compounds (Tsumuki and Kanehisa 1975) rather than CO_2 and carbohydrates as suggested in this study. However, UDP- ^{14}C -G metabolites in the haemolymph need to be investigated further.

The accumulation of glycerol in the haemolymph during pre-diapausing to diapausing stage was associated with a decline in glycogen content in the fat body of the rice stem borer. The glycerol concentration in the haemolymph began to decrease progressively during post-diapausing stage, while the glycogen content in the fat body steadily increased (Tsumuki and Kanehisa 1978). These phenomena have been supported by research in which the changing patterns of enzyme activities were observed (Tsumuki and Kanehisa 1979, 1980). Furthermore, these observations are also supported by the results obtained in this study. This is to say, the incorporation of ^{14}C -glucose and UDP- ^{14}C -G into glycerol in the haemolymph of diapausing larvae was higher than that of post-diapausing larvae. The changing pattern of radioactivity in glycogen labelled with ^{14}C -glucose was coincident with that in not glycerol but sorbitol in diapausing eggs of the silkworm (Yaginuma and Yamashita 1978). However, in diapausing larvae of the rice stem borer, sorbitol could not be detected (Tsumuki and Kanehisa 1978) and changing pattern of glycogen in the fat body was coincident with that of labelled glycerol in the haemolymph. On the other hand, in non-diapausing larvae, no glycerol formation in the haemolymph was observed and the incorporation of these radioactivities into glycerol was at a trace level (Tsumuki and Kanehisa 1975).

The incorporation rate of ^{14}C -glucose into glycerol exceeded that into trehalose in the haemolymph and that into glycogen in the fat body of diapausing larvae of the rice stem borer at 4°C and 15°C . ^{14}C -glucose was incorporated rather efficiently into trehalose in the presence of UDPG in the locust fat body (Candy and Kilby 1961). In the fat body of the cecropia silkworm, trehalose-6-phosphate synthetase has greater affinity for UDPG in comparison with glycogen synthetase. However, when trehalose accumulates sufficiently to inhibit trehalose-6-phosphate synthetase, the UDPG level rises and allows increased synthesis of glycogen (Murphy and Wyatt 1965). These results are the same as the results obtained in this study. A high trehalose concentration in the haemolymph may inhibit trehalose synthesis and ^{14}C -glucose entering glycolytic and pentose phosphate pathways may accelerate the conversion of glycerol by activating α -glycerophosphate dehydrogenase in the fat body of diapausing larvae (Tsumuki and Kanehisa, in preparation). The radioactivity incorporated into glycogen may be affected by phosphorylase activity (Tsumuki and Kanehisa 1979, 1980). On the other hand,

in post-diapausing larvae, the glycerol concentration decreased with a decrease in α -glycerophosphate dehydrogenase activity (Tsumuki and Kanehisa, in preparation). The trehalose concentration in haemolymph decreased with an increase in trehalase activity in the alimentary canal (Tsumuki and Kanehisa 1979, 1980). Consequently, the incorporation of ^{14}C -glucose and UDP- ^{14}C -G into trehalose exceeded that into glycerol during post-diapausing stage. Yaginuma and Yamashita (1978) have been also shown that in the eggs of the silkworm, the incorporation rate of radioactivity into trehalose increased slightly at the termination of diapause.

There were some significant differences in the incorporation rates of ^{14}C -glucose and UDP- ^{14}C -G into glycogen between diapausing and post-diapausing larvae. These incorporation rates may be affected by glycogen synthetase activity, which is influenced by the stages of larval life in the rice stem borer (Tsumuki and Kanehisa 1979).

SUMMARY

Metabolic relationships between glycogen and, glycerol and trehalose were studied in diapausing and post-diapausing larvae of the rice stem borer, *Chilo suppressalis* Walker, by the use of ^{14}C -glucose and UDP- ^{14}C -G.

^{14}C -glucose and UDP- ^{14}C -G injected into haemolymph were metabolized to form CO_2 , but the metabolism of ^{14}C -glucose greatly exceeded that of UDP- ^{14}C -G. There was no significant difference found in the metabolism to CO_2 between diapausing and post-diapausing larvae.

These radioactivities were distributed in the alimentary canal, fat body and carcass, and were present in glycogen, trehalose, glycerol, lipid, protein and chitin. These incorporation rates were affected by the stage of larval life and environmental temperatures.

The incorporation rate of ^{14}C -glucose into glycerol exceeded that into trehalose in haemolymph and that into glycogen in the fat body of diapausing larvae with high trehalose concentrations in haemolymph at 4°C and 15°C . The incorporation rate into glycerol was accelerated by low temperatures. The incorporation rate into trehalose was higher than that into glycerol in haemolymph of post-diapausing larvae with decreased trehalose.

These observations were coincident with changes of glycerol and trehalose concentrations in haemolymph, and glycolytic and glycogenetic enzyme activities in diapausing and post-diapausing larvae.

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