Change in Major Carbohydrate Contents in Diapausing and Nondiapausing Pupae of the Fall Webworm, *Hyphantria cunea*[1]

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최만연 · 부경생 : 흰불나방(*Hyphantria cunea* D.)의 휴면용과 비휴면용에서 탄수화물 함량의 변화

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ABSTRACT Carborhydrate contents were compared between diapausing and non-diapausing pupae of the fall webworm, *Hyphantria cunea* DRURY. Glycogen content in the whole body of diapausing pupae kept at −5±1°C was less than that of those left at room temperature, while the amounts of trehalose and sorbitol showed the reverse trend. The osmotic pressure of haemolymph was higher in diapausing pupae than that in non-diapausing pupae. The mean oxygen consumption rate of diapausing pupae was 4~6.7 times less than that of non-diapausing, normal pupae.

INTRODUCTION

For successful survival against adverse environmental conditions, especially during the overwintering period, body glycogen is converted into blood sugars or sugar alcohols such as trehalose, glycerol, etc., in insects(3, 4, 16). These mono-and disaccharides and polyols are accumulated in overwintering insects and act as an antifreeze by increasing blood osmolarity. But the mechanism of their cold tolerance is not fully understood yet(8, 18). Their interconversion are temperature-dependent (7, 9) and composition and quantities of blood sugars and alcohols differ among diapausing insects (8, 12).

Several diapausing pupae were found to accumulate large amounts of glycerol (16, 17). In diapausing embryos of *Bombyx morri*, glycerol and sorbitol appeared at the expense of glycogen (4). The conversion of glycogen to polyols was found to be accomplished anaerobically, although reconversion at the end of diapause required oxygen (8).

In overwintering prepupae of the polar sawfly (*Trichiocampus populi*), on the other hand, trehalose was accumulated (3). A temperature-dependent interconversion between fat body glycogen and haemolmyph trehalose was demonstrated in diapausing pupae of the silkworm (*Philosamia cynthia pryeri*) and *Leguminivora glycinivorella* (9, 14). During diapause of these insects, almost all fat body glycogen was converted into haemolymph trehalose. From these and other data, Hayakawa and Chino (1982) classified diapausing insects into two types, sugar alcohol or trehalose-accumulating types.

This study was carried out to comare carbohydrate contents between non and diapausing pupae of the fall webworm, *Hyphantria cunea* Drury.

MATERIALS AND METHOD

Insects

To produce the non-diapausing pupae, *Hyphantria cunea* larvae were reared on *Morus alba* leaves at 25°C under LD(light:dark) 16 : 8. The diapausing pupae were obtained from larvae reared at 25°C under less than LD 12 : 12 and also collected from field late in September.

Extraction of carbohydrate in pupae

Ten pupae were homogenzed with 3ml of

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50% (v/v) ethanol in glass homogenizer, and it was later washed twice with 1ml ethanol. The washings were added to the original homogenate. After centrifugation at 3000g for 15min, the supernatant was separated from the sediment. The sediment was mixed with 3ml of 50% ethanol again. After the second centrifugation at the same condition, the sediment was mixed with 2ml of 50% ethnol, and third centrifugation was performed at the same condition. Then, the supernatants were added to the original one.

Determination of sugar and sugar alcohol

The supernatant was concentrated undernitrogen gas to make a final volume of about 1~2ml. The concentrated supernatant was used for sugar and sugar-alcohol determination with a HPLC (column:Lichrosorb NH2(10μm), Refractive index detector, PU 4010 pump, PYEUNICAM).

Acetonitrile-water mixture(80 : 20) was used as the elution solvent. The sugar and sugar-alcohol were determined by standard peak measurement.

Determination of glycogen

The separated sediment was mixed with 10ml of 10%(W/V) trichloroacetic acid(TCA). The mixture was boiled at 100°C for 15min and then centrifuged at 3000g for 15min. The supernatant was separated from the the sediment. The sediment was mixed with 5ml of 10% TCA, and the second supernatant was obtained in the same way as the first. The supernatant was added to the original one. The glycogen content in aliquot of the supernatant was determined by the anthrone/sulphuric acid method(13, 15), using glucose as a standard. The green color was read on the spectrophotometer (PU 8600 Series UV/VS, Single beam spectrophotometer, Philips) at 620nm.

Determination of haemolymph osmotic pressure

Haemolymph osmotic pressure was determined with an osmometer (Model: Advanced wide range Osmometer 3WII). Fifty microliters of haemolymph collected from 6th instar larvae and pupae were dissolved in 1ml of 0.1M phosphate buffer (pH 7.0). The osmolarity of this solution and of the buffer solution was determined on the osmometer, and osmolarity of the blood was calculated from their difference and the dilution factor.

Determination of the oxygen consumption

Oxygen consumption was measured with a Warburg manometer (13 position, circular model) at 25°C (5).

RESULTS

The quantities of major carbohydrates (glycogen, trehalose and sorbitol (+glucose)) found in diapausing pupae were higher than those of non-diapausing pupae (Table 1). In diapausing pupae, about 40~50% of total carbohydrates were in smaller molecules, such as trehalose, sorbitol, etc. This proportion increased when the pupae were kept at lower temperature (Table 1, Fig. 1). But other mono- and disaccharides (xylose, fructose, sucrose, inositol, etc.) were not significantly different between diapausing pupae kept for 2 months at two different temperatures(Fig. 1). Also glycerol was not a major component either (Fig. 1). Contents of glycogen and total carbohydrates in diapausing pupae kept for 5 months at -5 ± 1°C decreased gradually, while those of trehalose and sorbitol (+glucose) did not change (Table 2). This means that the decrease was due to glycogen and other monosaccharides. The glycogen contents of diapausing pupae kept in field rapidly decreased during January (Fig. 2), and remained at about the same level up to March. Thereafter its level started to increase, apparently at the expense of other mono- and disaccharides.

Table 1. Contents of major carbohydrate contents in naturally induced diapausing pupae of *Hyphatria cunea* (mg/10 individuals)

Pupae[a]	Conditions	Glycogen	Trehalose	Sorbitol (+glucose)	Others[b]
NP	1~2 days after pupation	14.0	0.4	2.5	2.0
	5 days after pupation	9.7	0.7	0.8	1.4
	11 days after pupation	8.5	2.0	0.4	1.0
DP	left at room temp.(non-heated)for about 1 month	19.8	4.5	4.7	4.1
	kept at −5±1°C for about 1 month	15.8	5.7	6.8	4.9

[a] NP: non-diapausing pupae (initial weight; 117±5.7mg/pupae),
DP: diapausing pupae (initial weight; 175±15.3mg/pupae)
[b] others=fructose+inositol+glycerol+xylose+unknown

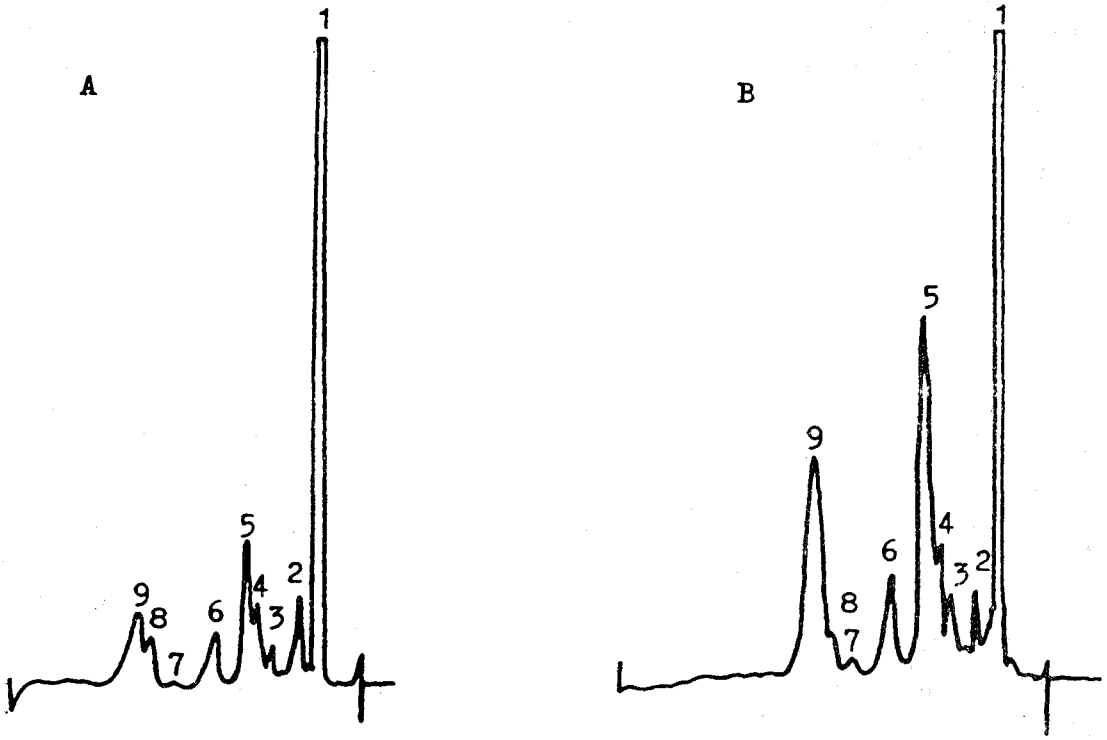

Fig. 1. High performance liquid chromatograms from naturally induced diapausing pupae kept for about 2 months either at room temperature (non-heated) (A) or at −5±1°C (1 : solvent; 2 : glycerol; 3 : xylose; 4 : fructose; 5 : sorbitol (and glucose); 6 : unknown; 7 : sucrose; 8 : inositol; 9 : trehalose).

Osmotic pressure of haemolymph in diapause-bound 6th instar larvae (DL) and diapausing pupae (DP1 and DP2) were higher than that in their normal counterparts (Table 3). Moreover, the osmotic pressure of diapausing pupae kept at −5±1°C was higher than that of those kept at room temperature.

Oxygen consumpion rate of non-diapausig pupae was much higher, up to about 6.5 times than that of naturally induced diapausing pupae (DP2) (Table 4). Also in the artificially induced diapausing pupae kept for about 1 month at 25±1°C, their oxygen consumption rate was higher than that of the naturally induced diapausing pupae left for about 1 month (during October) out-doors.

Table 2. Changes in carbohydrate (mg/10 individuals) contents in naturally induced diapausing pupae of *Hyphantria cunea* during storage at −5±1°C

Carbohydrates	Storage period(month)				
	1	2	3	4	5
Glycogen	14.9±0.7	14.7±1.6	12.2±2.8	11.4±0.1	11.0±1.1
Trehalose	5.1	5.7±0.4	4.1±0.4	5.0±0.2	4.3±0.5
Sorbitol(+glucose)	4.9±0.9	6.2±0.3	4.5±0.3	5.2±0.7	3.0±0.3
Others[a]	5.6±0.4	3.1±0.1	2.4±0.2	2.0±0.6	1.5±0.3
Total	30 ±0.6	29.7±0.6	23.2±0.9	23.6±0.4	19.8±0.5

[a] Others=fructose+glycerol+inositol+xylose+unknown

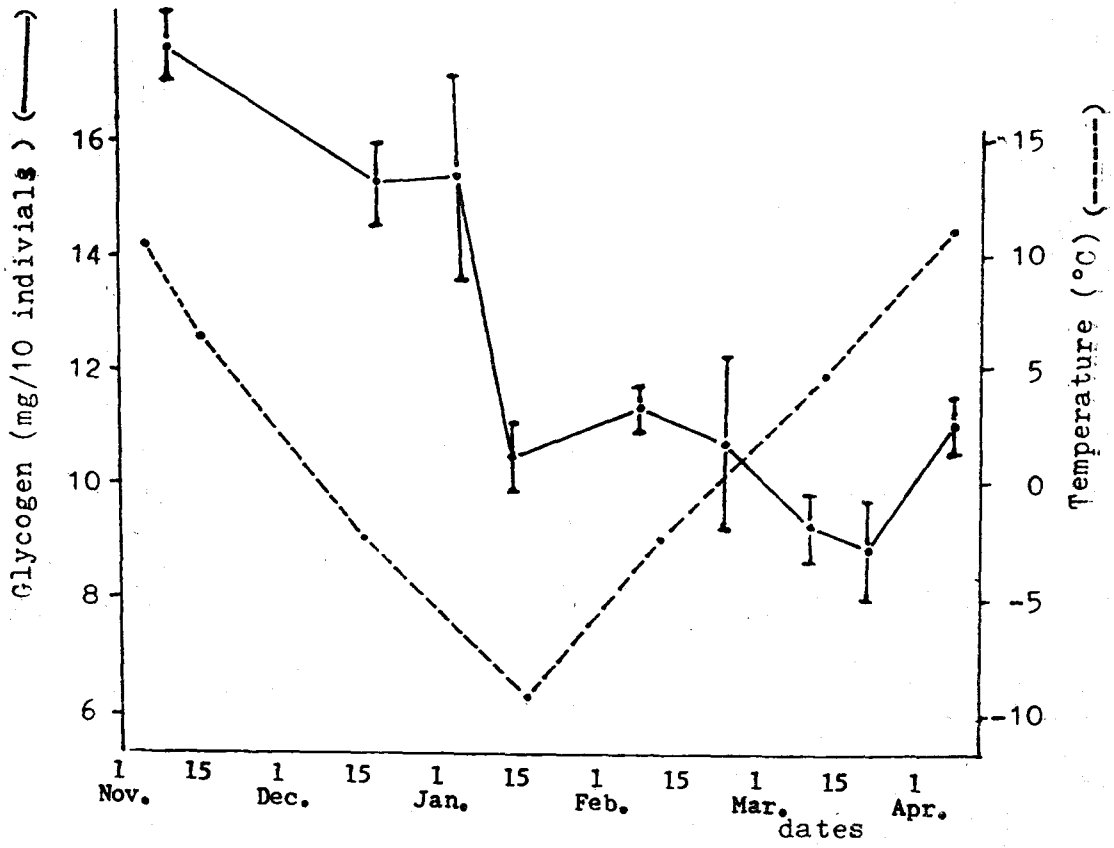

Fig. 2. Relation between monthly mean temperature and glycogen contents of diapausing pupae in *Hyphantria cunea* kept outdoors in Suweon (1976—1977).

DISCUSSION

Contents of major carbohydrates in diapausing pupae slowly decreased during storage at low temperature. This low metabolism is accompanied by low oxygen consumption rate in diapausing pupae. This rate is much lower, being only about one fourth to one seventh, than that of normal pupae, even though diapausing pupae have higher contents of carbohydrate, in terms of either concentration (1.9% vs. 1.6%) or absolute amount (3.31mg vs. 1.89mg per pupa).

Two distinct patterns of carbohydrate metabolism have been reported in diapausing insects. In some diapausing species, glycogen is

Table 3. Osmotic pressures of larval and pupal haemolymph in *Hyphnatria cunea*.

Sample[a]	No. of insects[b]	Mean(mosm/kg)
DL	15	504±25.58
DP 1	15	486±38.80
DP 2	30	515±42.74
NL	25	284±34.14
NP	15	367±40.39

[a] DL: diapause-bound 6th instar larvae; DP 1: diapausing pupae left for about 1 month at room temperature (non-heated); DP 2: diapausing pupae kept for about 1 month at −5±1°C; NL: 6th instar larvae bound for normal (non-diapause) development; NP: 3-day old non-diapausing pupae.

[b] haemolymph from 3~5 individuals were pooled for a single measurement.

Table 4. Oxygen consumption rate in *Hyphantria cunea* pupae at 25+1°C.

Pupae[a]	No. of pupae	Oxygen consumption (mm^3/hr/pupa)
NP	20	177±19.5
DP 1	10	44± 7.4
DP 2	10	27±10.0

[a] NP: non-diapausing 3-day old pupae, DP 1: artificially induced diapausing pupae; DP 2: naturally induced diapausing pupae.

converted to sugar alcohols such as glycerol, sorbitol and inositol (3, 4, 16, 17), and in others the large amount of trehalose are formed (9, 10). The present study indicates that the diapausing pupae of *H. cunea* have almost equal amounts of both, trehalose and sorbitol (+glucose) derived from tissue glycogen(Fig. 1, Table 1). The separation of sorbitol and glucose was not distinct in this analysis. But the retention time of two monosaccharides was different slightly in the separation procedure, and so it is most probable that the increased monosaccharide is sorbitol. The data reported here also demonstrate that this conversion is temperature-dependent: the conversion of glycogen to trehalose and sorbitol induced by exposure of diapausing pupae to low temperature (−5±1°C) (Table 1). But the significance remains to be seen.

적 요

흰불나방의 휴면용(休眠蛹)과 비휴면용에서 탄수화물 함량을 비교하였다.

1. 저온상태(−5±1°C)에 보관한 휴면용은 실온에 둔 것 보다 글리코겐(glycogen)함량은 적었지만 트레할로스(trehalose)와 솔비톨(sorbitol)의 양은 많았다.

2. 휴면용에서 혈액의 삼투압은 비휴면용의 것 보다 높게 나타났다.

3. 휴면용의 평균 산소 소비량은 비휴면용 보다 4~6.7배 적었다.

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