

## INTERCONVERSION BETWEEN GLYCOGEN AND INOSITOL IN HIBERNATING ADULTS OF A PHYTOPHAGOUS LADYBEETLE, *EPILACHNA* *VIGINTIOCTOMACULATA*

KAZUO HOSHIKAWA

Zoological Section, Institute of Low Temperature Science, Hokkaido University, Sapporo 060, Japan

(Received 24 March 1986; accepted 21 May 1986)

**Abstract**—The accumulation of inositol in hibernating adults of a phytophagous ladybeetle, *Epilachna vigintioctomaculata*, was demonstrated by a gas-chromatography and mass-spectrometry system. Inositol began to increase at the beginning of hibernation in early October and attained its maximum level of about 30 mg/g of body weight in January. It was also demonstrated that the source of inositol accumulated during hibernation is stored glycogen, and that interconversion between inositol and glycogen took place at the beginning and the end of hibernation.

**Key Word Index:** *Epilachna vigintioctomaculata*, Coccinellidae, inositol, glycogen, hibernation

### INTRODUCTION

Since Chino (1957), and Wyatt and Meyer (1959) demonstrated the conversion of glycogen into glycerol and/or sorbitol in diapausing eggs of *Bombyx mori* and diapausing pupae of *Hyalophora cecropia*, such sugar-alcohols have been found in many insect species (Sømme, 1982). That these compounds contribute to protect insects from cold injury is now widely accepted (Salt, 1959; Asahina, 1969), and the compounds are regarded as protective substances or cryoprotectants. Asahina and Tanno (1964) found the accumulation of trehalose instead of sugar alcohol in overwintering prepupae of the poplar sawfly, *Trichiocampus populi*. Miller and Smith (1975) found threitol and sorbitol accumulated in adults of a tenebrionid beetle, *Upis ceramboides*. Furthermore, several insects accumulate mannitol together with glycerol (Sømme, 1982), and ethylene glycol was found in a beetle *Ips acuminatus* in addition to trehalose (Gehrken, 1984). Thus, all such compounds hitherto reported for hibernating insects belong to sugar-alcohol or disaccharide in terms of chemical structure. Among them, glycerol is most widely distributed, and the accumulation of trehalose has also been reported for many insect species (Rains and Dimock, 1978; Hayakawa and Chino, 1981; Moreau *et al.*, 1981; Shimada *et al.*, 1981; Hoshikawa, 1981b), whereas threitol is found in only one species (*op. cit.*).

Here I report the accumulation of inositol in hibernating adults of a phytophagous ladybeetle. This compound has a hydroaromatic molecular structure differing from normal sugar-alcohols. I have previously reported that some beetles accumulate a compound which may be identified with inositol on gas chromatography (Hoshikawa, 1981a). However, the evidence presented was insufficient to conclude that the compound is truly inositol. In this paper, several lines of evidence will be presented that hibernating adults of a phytophagous ladybeetle ac-

cumulate inositol, and that this compound is derived from stored glycogen.

### MATERIALS AND METHODS

#### Animals

A phytophagous ladybeetle, *Epilachna vigintioctomaculata* Motschulsky, used in the present study has a univoltine life cycle under natural condition in Sapporo area. The female adults lay the eggs from mid June to mid July, and the hatched larvae continuously feed on host plant, usually on potato, from June to August. The adults emerge in August or early September.

About 400 newly emerged adults were collected from potato fields near Sapporo in early September. They were allowed to feed on another host plant, *Solanum nigrum*, which was covered by an outdoor net cage. Most of the beetles entered into litter layer and began to hibernate in late September or early October. The hibernating site was exposed by removing the net cage during snow-covered period, from early December to mid April, to simulate natural conditions. About 30 individuals were collected monthly from the layer and subjected to analyses. An additional sample was collected in mid June from the same potato fields, and was analyzed immediately.

#### Determination of sugars and glycogen

After measuring the body weight, each individual was homogenized with 2 ml of 80% (v/v) ethanol and 0.03 ml of mesoerythritol solution (100 mg/10 ml) as an internal standard. After centrifuging at 3000 g for 10 min, the supernatant was used for sugar determination and the sediment for glycogen determination.

The supernatant was evaporated to dryness under vacuum at 55°C. To the residue, 0.1 ml TMSI-C (trimethylsilylating reagent, Gasukuro Kogyo Co.) was added and the solution was heated to 65°C for 45 min. The resulting TMS-derivative was applied to a gas chromatograph (Shimadzu, GC-4CMPF) using a glass column (3 mm × 3 m) with 1.5% (w/w) OV-1 on Chromosorb W. The temperature was programmed from 130 to 270°C at 5°C/min and held at 270°C for 7 min. The elution profile was followed using a flame ionization detector. As reference,

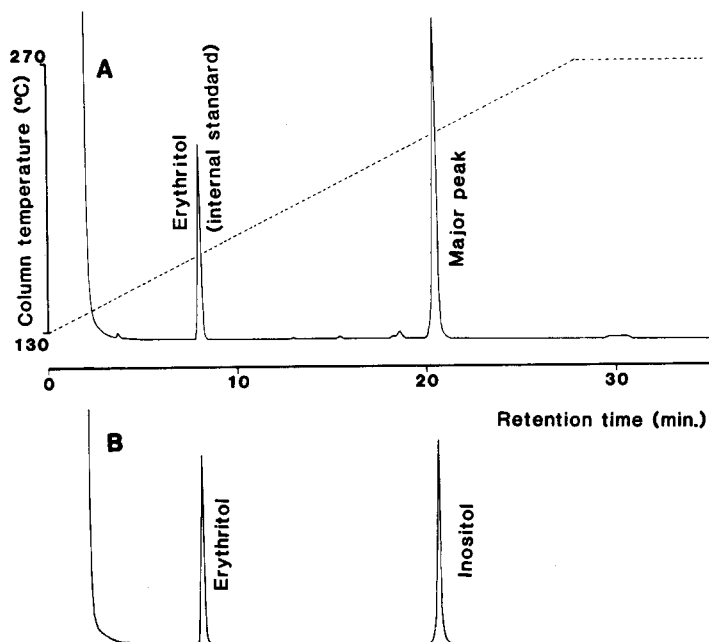


Fig. 1. Gas chromatogram of trimethylsilylated derivatives from the extract of hibernating adults of *Epilachna vigintioctomaculata* (A), and *myo*-inositol as reference (B). Detailed conditions are given in Materials and Methods section.

aliquots of authentic sugar or sugar-alcohol solutions together with the internal standard were subjected to the same process for trimethylsilylation and gas chromatography.

For glycogen determination, the above sediment was washed with 2 ml 80% ethanol and suspended with 2 ml 10% (w/v) trichloroacetic acid. The suspension was boiled at 100°C for 15 min, then centrifuged at 3000 *g* for 10 min. The glycogen content in the aliquot of the supernatant was determined by the anthrone/sulphuric acid method (Trevelyan and Harrison, 1952).

#### Mass spectrometry

A major peak having a retention time identical with that of inositol was observed when the TMS-derivative from the extract of beetle was applied to the gas chromatography (Fig. 1). The peak fraction was further analyzed using mass spectrometry in which authentic *myo*-inositol (Nakarai Chem. Ltd) was used as a reference. The mass spectra were recorded with a combined gas chromatograph-mass spectrometer (JEOL, JMS-D300). A 2 m glass column packed with 10% SE30 was used for chromatography, for which the temperature was programmed from 200°C at 8°C/min. A helium separator was maintained at 180°C, and all spectra were obtained at 23 eV ionizing potential, and scanned for 3 sec.

## RESULTS AND DISCUSSION

### Identification of inositol

The gas chromatogram of the TMS-derivative from the extract of hibernating beetles is illustrated in Fig. 1A and demonstrates a distinct major peak of which the retention time is identical with that of *myo*-inositol (Fig. 1B). The peak fraction was further analyzed by mass spectrometry. The mass spectrum of the peak fraction is given in Fig. 2A and demonstrates close similarity to that of *myo*-inositol (Fig. 2B).

Inositol has eight diastereoisomers, and three of them [*myo*-, (+)-*chillo*-, *scyllo*-inositol] usually occur in nature. Each TMS-derivative of the eight isomers possesses a respective unique spectrum consisting of the same ions with remarkable variation in intensity (Sherman *et al.*, 1970). Comparing the spectrum of the peak fraction with the spectra of the eight isomers reported by Sherman *et al.* (1970), revealed that the spectra illustrated in Fig. 2 are essentially similar to that reported for *myo*-inositol with higher intensities of *m/e* 217 and *m/e* 305 ions, and a lower intensity of *m/e* 318 ion.

Based on the above data, it is concluded that the major peak fraction observed on the gas chromatography can be identified with inositol, very probably with *myo*-inositol.

### Changes in inositol and glycogen contents

It seems most likely that inositol found in hibernating beetles is derived from glycogen stored in the fat body. Seasonal changes of both inositol and glycogen were determined to test this assumption. The results are given in Fig. 3. Although traces of monosaccharides (mainly glucose), disaccharides (mainly trehalose) and glycerol were detected occasionally by the gas chromatograph (Fig. 1A), no consistent seasonal change was found in their contents.

In the adults just after emergence in early September, both glycogen and inositol were found in only small amounts, 2.2 and 1.6 mg/g of fresh body weight, respectively. The level of glycogen increased rapidly in September throughout the post-emergence feeding stage and attained 34.0 mg/g in October. The amount of inositol did not change during this period and remained at 2.0 mg/g only in early October. After

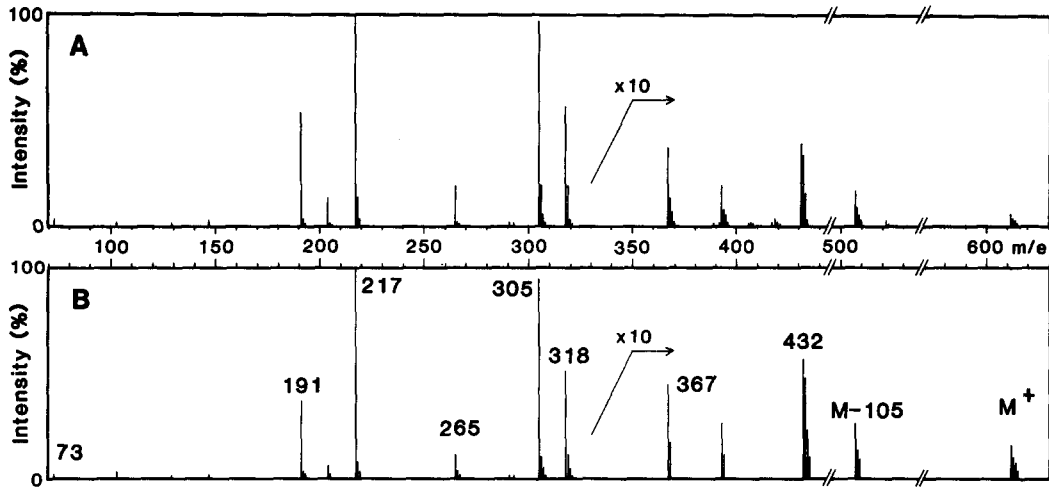


Fig. 2. Mass spectra of the peak fraction (Fig. 1A) and TMS derivative of *myo*-inositol. (A) peak fraction; (B) *myo*-inositol.

the beginning of hibernation, glycogen fell sharply with concomitant increase of inositol; glycogen dropped to the minimum value, 2.3 mg/g and inositol attained the maximum value, 30.3 mg/g, in January. Inositol began to decrease in March with concomitant increase of glycogen. After glycogen reached a maximum value (12.8 mg/g) at the end of April, it fell again throughout May and June. Inositol dropped to a minimum level (2.1 mg/g) at the end of April and the lowest level persists throughout May and June. The levels of both compounds in June were almost equivalent to those observed just after emergence.

An increasing amount of glycogen observed during March and April does not correspond with decreasing amount of inositol during the same period; the former is much less than the latter (Fig. 3). This suggests that glycogen and/or inositol may be utilized

as a metabolic fuel in this period. Indeed, as shown in Fig. 3, the total amount of carbohydrate decreases rapidly in April when relatively high temperature, sometimes above 15°C, is observed in the hibernating site among the litter layer (Hoshikawa, 1981b).

All data presented in this report lead to the conclusion that the adults of a phytophagous ladybeetle, *Epilachna vigintioctomaculata*, accumulate considerable amounts of inositol during hibernation, which is derived from stored glycogen. The possible function of inositol as a cryoprotectant in this species is currently under investigation in this laboratory.

*Acknowledgements*—I wish to thank Professor H. Chino for his reading of the manuscript and available criticism. This paper is contribution No. 2921 from the Institute of Low Temperature Science, Hokkaido University.

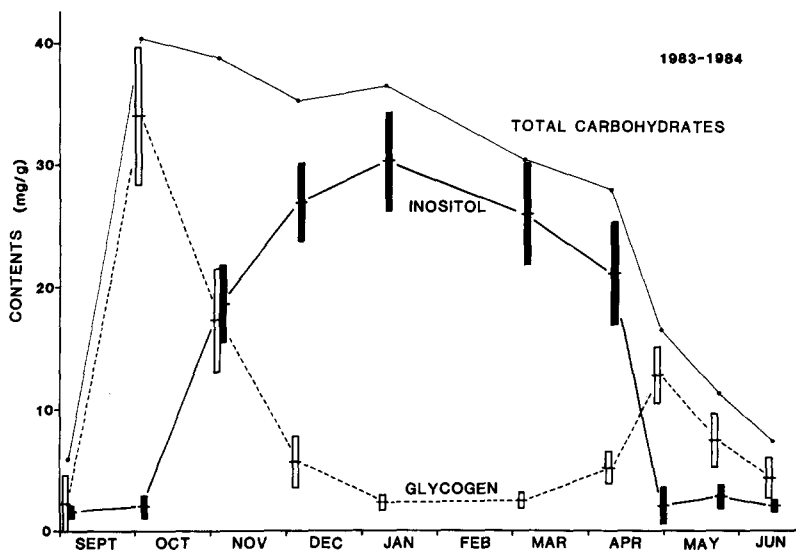


Fig. 3. Seasonal changes in glycogen and inositol contents in adult *Epilachna vigintioctomaculata*. The contents are expressed as mean  $\pm$  SD ( $n > 10$ ). The total amounts of carbohydrates represent the sum of inositol and glycogen with traces or small amounts of monosaccharides, disaccharides, and glycerol.

## REFERENCES

- Asahina E. (1969) Frost resistance in insects. *Adv. Insect Physiol.* **6**, 1-49.
- Asahina E. and Tanno K. (1964) A large amount of trehalose in a frost resistant insect. *Nature, Lond.* **204**, 1222.
- Chino H. (1957) Conversion of glycogen to sorbitol and glycerol in the diapause egg of *Bombyx* silkworm. *Nature, Lond.* **180**, 606-607.
- Gehrken U. (1984) Winter survival of an adult bark beetle *Ips acuminatus* Gyll. *J. Insect Physiol.* **30**, 421-429.
- Hayakawa Y. and Chino H. (1981) Temperature-dependent interconversion between glycogen and trehalose in diapausing pupae of *Phylosamia cynthia ricini* and *pryeri*. *Insect Biochem.* **11**, 43-47.
- Hoshikawa K. (1981a) Accumulation of inositol by hibernating adults of coccinellid and chrysomelid beetles. *Low Temp. Sci. Ser. B* **39**, 45-48.
- Hoshikawa K. (1981b) Notes on adult hibernation of *Epilachna admirabilis*, with special reference to comparison with larval hibernation (Coleoptera: Coccinellidae). *Low Temp. Sci. Ser. B* **39**, 9-20.
- Miller L. K. and Smith J. S. (1975) Production of threitol and sorbitol by an adult insect: association with freezing tolerance. *Nature, Lond.* **258**, 519-520.
- Moreau R., Olivier D., Gourdox L. and Dutrieu J. (1981) Carbohydrate metabolism in *Pieris brassicae* L. (Lepidoptera); variations during normal and diapause development. *Comp. Biochem. Physiol.* **68B**, 95-99.
- Rains T. D. and Dimock R. V. Jr (1978) Seasonal variation in cold hardiness of the beetle *Popilius disjunctus*. *J. Insect Physiol.* **24**, 551-554.
- Salt R. W. (1959) Role of glycerol in the cold-hardening of *Bracon cephi* (Gahan.). *Can. J. Zool.* **37**, 59-69.
- Sherman W. R., Eilers N. C. and Goodwin S. L. (1970) Combined gas chromatograph-mass spectrometry of the inositol trimethylsilyl ethers and acetate esters. *Organic Mass Spectrom.* **3**, 829-840.
- Shimada K., Sakagami S. F., Honma K. and Tsutsui H. (1981) Seasonal changes of glycogen/trehalose contents, supercooling points and survival rate in mature larvae of the overwintering soybean pod borer *Leguminivora glycinivorella*. *J. Insect Physiol.* **30**, 369-373.
- Sømme L. (1982) Supercooling and winter survival in terrestrial arthropods. *Comp. Biochem. Physiol.* **73A**, 519-543.
- Trevelyan W. E. and Harrison J. S. (1952) Studies on yeast metabolism. I. Fractionation and microdetermination of cell carbohydrates. *Biochem. J.* **50**, 298-303.
- Wyatt G. R. and Meyer W. L. (1959) The chemistry of insect hemolymph. III. Glycerol. *J. gen. Physiol.* **42**, 1005-1011.