Carbohydrates in Haemolymph and the Body of the Oriental Corn Borer, *Ostrinia furnacalis* (Guenée), during Larval Development

JONG-JIN LEE AND TAE-HEUNG KIM

李鍾鎭·金泰興 : 조명나방의 幼虫成長에 따른 혈림프와 虫體내 炭水化物에 관한 硏究

Korean J. Plant Prot. 25(4) : 215~220(1986)

ABSTRACT Glucose, trehalose, and glycogen content were measured in haemolymph and the body during larval development of the oriental corn borer, *Ostrinia furnacalis* (Guenée).

Glucose content varied in haemolymph, but in the body it gradually decreased at all larval stages. As the larva proceeds growth, the initial high level of trehalose increased further in haemolymph whereas the low level trehalose gradually decreased in the body. Glycogen levels in haemolymph rose gradually during larval growth whereas those in the body increased sharply at the two latter instar larval stages.

It is apparent that the existence of a homeostasis in carbohydrate levels between haemolymph and the body.

INTRODUCTION

During metamorphosis of an insect, energy-requiring process such as development, moulting, reproductive growth, vitellogenesis, and muscular activity demand an increase in metabolic flux. Since carbohydrate is one of the major sources of energy, their concentrations are clearly important during the above mentioned physiological events (Lohr and Gäde, 1983).

An excellent summary of the carbohydrate content of the haemolymph of a wide variety of insects is given by Wyatt (1961). Wyatt and Kalf (1957) reported that the major blood carbohydrate is trehalose, a non-reducing disaccharide, and that it is possibly an important reserve carbohydrate in insects. As this initial demonstration, trehalose has been shown to be present as the principal blood sugar in many other insects (Saito, 1963; Randall and Derr, 1965; Nettles, Jr. et al., 1971; Shimada et al., 1980; Lohr and Gäde, 1983; Kim et al., 1983).

As the major polymeric storage form of glucose in animals, glycogen is stored in large amounts in insects which use it as an immediate source of glucose units for growth and development, and is also stored in fat body, from whence glucose is provided to other tissues after transformation into the circulation blood sugar, trehalose (Friedman, 1985).

In a number of insects, fluctuations in the haemolymph trehalose concentration and/or stored glycogen in the fat body are thought to be under hormonal control by a factor from the corpus cardiacum (Steele, 1981; Gäde and Lohr, 1982).

The present investigation of glucose, trehalose, and glycogen content in the oriental corn borer, *Ostrinia furnacalis* (Guenée), was attempted to gain more insight into the changes in some important parameters of carbohydrate metabolism during larval development and to investigate a homeostasis of haemolymph and body carbohydrates.

MATERIALS AND METHODS

The second brood larvae of the oriental corn borer, *O. furnacalis*, were collected from a field of corn in 1986 and reared in the labo-

全北大學校 農生物學科(Dept. of Agricultural Biology, Chonbuk National University, Chonju 520, Korea)

ratory at room temperature.

Both sexes of the larvae were used throughout the experiment, and the five developmental stages of third, fourth, fifth, sixth, and last instars were determined after Lee et al. (1980).

Qualitative and quantitative determination of haemolymph and body sugars

Haemolymph and the body sugars were analysed qualitatively by thin-layer chromatography. Haemolymph from the larvae were collected from the prothorax by means of micro-capillaries and the bodies were washed thoroughly in cold physiological saline(0.85%) to remove haemolymph. The samples were homogenized in a glass homogenizer with 3ml of cold 14% trichloroacetic acid and centrifuged at 3000rpm for 10 min. Fifty microliters of the supernatant were spotted on a silica gel plate (Art. 7731, Merck). The solvent system consisted of ethyl acetate, acetic acid, methyl alcohol, and distilled water(60 : 15 : 15 : 10V/V). The sugars were detected by spraying the plate with a solution of anisaldehyde/95% H_2SO_4/95% ethyl alcohol(1 : 1 : 18 V/V) and the resulting colors developed for 20 min at 110°C. After confirmed, the spots were scraped up and centrifuged, and the optical density of the supernatant was measured at 500nm by Pye Unicam DU 8610 UV/VIS Kinetics Spectrophotometer (Philips, England).

Quantitative determination of glycogen

The cold trichloroacetic acid method of Stetten et al.(1956) was adopted for the preparation of glycogen from haemolymph and the bodies. As described above, the samples were homogenized with 3ml of 10% TCA. After centrifuging at 3000rpm for 10min, the supernatant was decanted. To the precipitate was added 3ml cold 5% TCA. Homogenization and centrifugation were repeated twice. To the combined supernatant, an equal volume of 95% ethanol was added. After standing in an ice-bath for 30min, the precipitate was collected by centrifuging at 3000rpm for 10min. The precipitated glycogen was taken up in 3ml water. Gycogen was precipitated by adding 5ml 95% ethanol. The solubilization and precipitation were repeated twice, and finally precipitated glycogen was dissolved in 5ml water. The solution was centrifuged at 3000 rpm for 5 min. Glycogen in the supernatant was determined by the anthrone method (Mokrasch, 1954).

RESULTS

By means of thin-layer chromatography, trehalose was identified as the predominant haemolymph sugar, and glucose, although in less quantity, could also be identified during larval development in *O. furnacalis*, respectively. There were also some other weak spots detectable, which were not analysed further in this study. Glycogen content was detected by spectrophotometric method with anthrone reagents.

Glucose content showed high level in haemolymph of the third instar larvae and decreased rapidly at the fourth instar larvae, and then it increased gradually again until the last instar larval stage(Fig. 1) whereas in the body glucose concentration gradually decreased from the third instar larvae to the last instar larval stage(Fig. 2).

Trehalose was present in large amounts in haemolymph, whereas in the body it was in small (Fig. 1 and 2).

Glycogen concentration in haemolymph and the body (Fig. 1 and 2) was gradually increased during larval development, and showed a maximum at the last instar larval stage.

Trehalose in haemolymph and glycogen in the body were present in large amounts, and appeared to function as the form in which

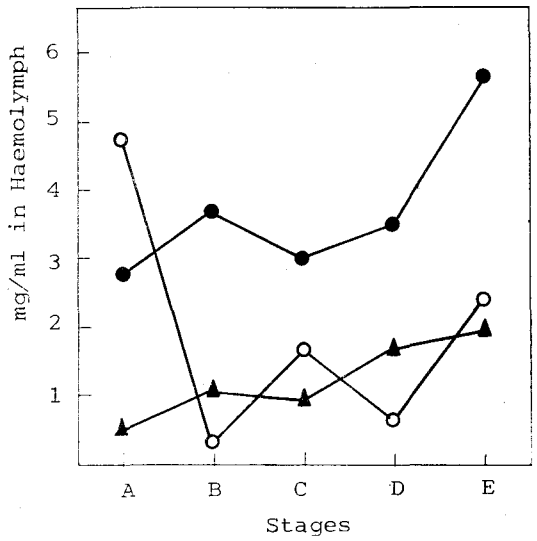

Fig. 1. Changes in glucose (○—○), trehalose (●—●), and glycogen (▲—▲) content in haemolymph of the oriental corn borer, *O. furnacalis*, during larval development.
Stages ; A : third instar larvae
B : fourth instar larvae
C : fifth instar larvae
D : sixth instar larvae
E : last instar larvae

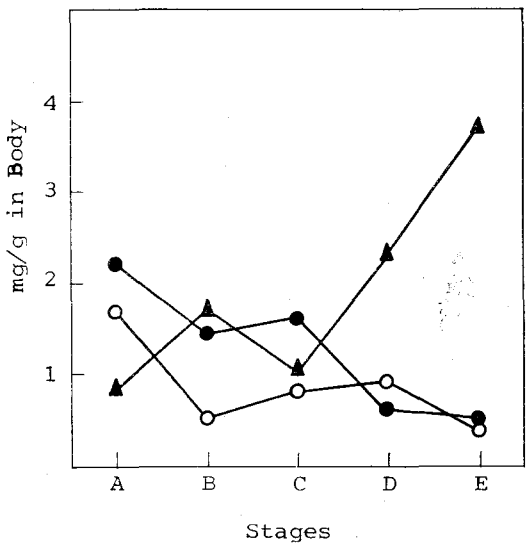

Fig. 2. Changes in glucose(○—○), trehalose (●—●), and glycogen (▲—▲) content in the body of the oriental corn borer, *O. furnacalis*, during larval development.
Stages as in Fig. 1.

carbohydrate is transported and synthesized through the blood from storage sites to sites of utilization.

DISCUSSION

Glucose and glycogen are generally present in haemolymph (Wyatt and Kalf, 1957), and, like trehalose, the levels of these sugars vary greatly among insect species.

As represented in Fig. 1 and 2, glucose content showed high level in haemolymph of the third instar larvae and decreased rapidly at the fourth instar larvae and then it increased again until the last instar larval stage while in the body glucose level decreased gradually during larval stage. On the other hand, trehalose concentration increased in haemolymph, but decreased in the body during larval development.

That quantity of haemolymph trehalose showed maximal in the latter instar larvae has been observed in *Pieris brassicae* (Moreau, 1969) and *Anthonomus grandis* (Nettles et al., 1971).

Treherne (1963) reported that the percentage absorption of glucose from solutions injected into the midgut could be related to the extent of conversion to trehalose in the haemolymph.

It is relevant to suggest that blood sugar is maintained at a constant level by a dynamic equilibrium between two processes, release of trehalose into body fluid and removal of the sugar from body fluid. Of the former process, two possibilities should be mentioned. The first possibility is that trehalose is formed directly from dietary carbohydrates and is released into the body fluid. The second is that the sugar is supplied from carbohydrate-rich tissues such as fat body and midgut. Although no data are available at present as to the time which is required for the digestion of diet and absorption of the resulting digested sugars, the first

possibility may be likely in the case of the present work, since five larval stages used were under the feeding conditions.

In some insect species glycogen is converted to sugar alcohols such as sorbitol and glycerol (Chino, 1958; Asahina, 1969) whereas in other species there is no appreciable accumulation of sugar alcohols but instead large amounts of trehalose are formed(Tanno, 1965; Hayakawa and Chino, 1981).

Quickenden(1970) reported that trehalose levels declined slightly during blastokinesis but this decrease is countbalanced by a net gain of glycogen. Kim et al. (1983) reported that glycogen was present in very small quantity in haemolymph but large in fat body with the peak at the last instar larvae in *Bombyx mori*.

The data presented in this report glycogen levels increased gradually in haemolymph during larval growth as trehalose changed, but glycogen in the body, showed large amounts, increased rapidly at the last instar larval stage whereas changes in trehalose content showed inversely. Thus, it is safe to conclude that the accumulation of glycogen as storage energy during the last instar larvae is necessary for both of larval-pupal transformation and the histogenesis of adult organs.

It is known that removal of the neurosecretory cells in *Calliphora* (Thomsen, 1954) or the corpora allata from *Dixippus* (L'Hélias, 1953) caused accumulation of glycogen. The removal of the brain from pupae of the silkworm prevented the fall in glycogen associated with pupal development (Kobayashi, 1957). The rate of blood trehalose synthesis at the expense of fat body glycogen could be controlled by a hyperglycaemic factor in the stick insect corpus cardiacum and was shown to activate fat body glycogen phosphorylase(Gäde and Lohr, 1982; Lohr and Gäde, 1983). These observations suggested that the presence of a mechanism of hormonal control of carbohydrate metabolism in insects. Although it has not been shown here, it is likely that the level of blood sugar is under the hormonal influence.

摘 要

조명나방의 幼虫成長에 따른 炭水化物代謝의 變化 및 혈림프와 虫體내 恒常性을 究明키 위해 Glucose, Trehalose 그리고 Glycogen을 측정한 결과는 다음과 같다.

혈림프내 Glucose는 3령유충에서 最高含量을 나타냈으며 4령유충에서 급격히 減少하다가 종령유충으로 成長하면서 다시 增加하였고, 虫體에서는 幼虫期 성장과정동안 서서히 減少하는 現象을 나타냈다.

Trehalose는 혈림프에서 유충기의 성장과 더불어 증가하면서 높은 함량을 나타낸 반면, 虫體에서는 낮은 함량을 보였다 .

Glycogen은 혈림프에서 Trehalose와 같은 패턴으로 증가하였으나, 虫體내에서는 Trehalose와 反對現象을 나타내면서 증가하였다.

LITERATURES CITED

1. Asahina, E. 1969. Frost resistance in insects. Adv. Insect Physiol. 6 : 26~34.
2. Chino, H. 1958. Carbohydrate metabolism in diapause eggs of the silkworm, *Bombyx mori*. II. Conversion of glycogen into sorbitol and glycerol. J. Insect Physiol. 2 : 1~12.
3. Friedman, S. 1985. Carbohydrate metabolism. In Comprehensive Insect Physiology Biochemistry and Pharmacology (Ed. by G.A. Kerkut and L.I. Gilbert). Vol. 10, Biochemistry pp. 43~76, Pergamon Press, New York.
4. Gäde, G. and P. Lohr. 1982. Restricted specificity of a hyperglycaemic factor from the corpus cardiacum of the stick insect, *Carausius morosus*. J. Insect Physiol.

28 : 805~811.

5. Hayakawa, Y. and H. Chino. 1981. Temperature-dependent interconversion between glycogen and trehalose in diapausing pupae of *Philosamia cynthia ricini* and *Pryeri*. Insect Biochem. 11 : 43~47.
6. Kim, H.R., S.M. Yoe, J.H. Yu and K.M. Kim. 1983. Carbohydrates and inorganic ions in haemolymph and fat body during metamorphosis of silkworm, *Bombyx mori* L. Kor. J. Entom. 13(2) : 55~59.
7. Kobayashi, M. 1957. Studies on the neurosecretion in the silkworm, *Bombyx mori*. Bull. Seric. Exp. Sta. Japan. 15 : 181~263.
8. Lee, Y.B., C.Y. Hwang, K.M. Choi and J.Y. Shim. 1980. Studies on the bionomics of the oreintal corn borer, *Ostrinia furnacalis*(Guenée). Korean J. Plant Prot. 19(4) : 187~192.
9. L'Hélias, C. 1953. Rôle des corpora allata dans le métabolisme des glucides, de l'azote et des lipids chez le phasmé *Dixippus morosus*. C.R. Acad. Sci., Paris. 236 : 2164 ~2166.
10. Lohr, P. and G. Gäde. 1983. Carbohydrate metabolism in the stick insect, *Carausius morosus*. J. Insect Physiol. 29(3) : 287~ 293.
11. Mokrasch, L.C. 1954. Analysis of hexosephoshate and sugar mixtures with the anthrone reagent. J. Biol. Chem. 208 : 55~ 59.
12. Moreau, R. 1969. Observations sur les variations du taux de tréhalose au cours du développement direct et de la diapause, chez *Pieris brassicae* L. C.R. Acad. Sci., Paris. 268 : 1441~1444.
13. Nettles, Jr., W.C., B. Parro, C. Sharbaugh and C.L. Mangum. 1971. Trehalose and other carbohydrates in *Anthonomus grandis*, *Heliothis zea*, and *Heliothis virescens* during growth and development. J. Insect Physiol. 17 : 657~675.
14. Quickenden, K.L. 1970. Carbohydrates in eggs of the grasshopper, *Aulocara elliotti*, during development. J. Insect Physiol. 16 : 171~183.
15. Randall, D.D. and R.F. Derr. 1965. Trehalose: Occurrence and relation to egg diapause and active transport in the differential grasshopper, *Melanoplus differentialis*. J. Insect Physiol. 11 : 329~335.
16. Saito, S. 1963. Trehalose in the body fluid of the silkworm, *Bombyx mori* L. J. Insect Physiol. 9 : 509~519.
17. Shimada, S., M. Kido, A. Kamada and S. Asano. 1980. Trehalose in the silkglands of the silkworm, *Bombyx mori*. Insect Biochem. 10 : 175~177.
18. Steele, J.E. 1981. The role of carbohydrate metabolism in physiological function. In Energy Metabolism in Insects (Ed. by Downer, R.G.H.) pp. 101~133, Plenum Press, New York.
19. Stetten, M.R., H.M. Katzen and D. Stetten. 1956. Metabolic inhomogeneity of glycogen as a function of molecular weight. J. Biol. Chem. 222 : 587~599.
20. Tanno, K. 1965. Frost resistance in the popular sawfly, *Trichiocampus populi* Okamoto. II. Extracellular and intracellular freezing in fat-cells. Low. Temp. Sci. Ser. 23 : 47~53.
21. Thomsen, E. 1952. Functional significance of the neurosecretory brain-cells and the corpora cardiacum in the female blow-fly, *Calliphora erythrocephala* (Meig.). J. Exp. Zool. 29 : 137~172.
22. Treherne, J.E. 1963. The absorption and metabolism of some sugar in the locust, *Schistocerca gregaria* (Forsk). J. Exp. Biol. 35 : 611~625.
23. Wyatt, G.R. 1961. The biochemistry of insect hemolymph. Ann. Rev. Ent. 6 : 75

~102.

24. Wyatt, G.R. and G.F. Kalf. 1957. The chemistry of insect hemolymph. II. Trehalose and other carbohydrates. J. Gen. Physiol. 40 : 833~847.