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韓應昆誌 27(1) : 6—13 (1988)
Korean J. Appl. Entomol.

Diapause-initiation Stage and Changes in Proteins of the Fall Webworm (*Hyphantria cunea* Drury) Pupae

흰불나방의 용휴면이 결정되는 시기와 용 체내단백질의 변화

No Joong Park and Kyung Saeng Boo
박 노 중 · 부 경 쟁

ABSTRACT Studies were carried out to investigate the diapause-sensitive stage, the effect of 20-hydroxyecdysone on diapausing pupae and the changes in pupal proteins of the fall webworm, *Hyphantria cunea* Drury. The stage sensitive to diapause-inducing photoperiod lies between 5th and 15th day of the larval development. And the oxygen consumption rate of diapausebound pupae decreased to a mean level of 30μl O_2/g/hr. The 20-hydroxyecdysone was effective in raising the respiratory acivity of 2.5% homogenate of the diapausing fall webworm pupae and in causing a normal adult development of the diapause-bound pupae. The soluble protein content rapidly declined in normally-developing pupae, but in diapause-bound pupae it was more or less static with much higher quantity. And protein bands separated from fat body of diapause-bound pupae were different from those of nondiapausing pupae.

Key words fall webworm, *Hyphantria cunea*, diapause initiation, protein content, 20-hydroxyecdysone, oxygen consumption rate.

抄 錄 흰불나방(*Hyphantria cunea* Drury)의 용휴면이 결정되는 시기, 휴면용에서의 20-hydroxyecdysone의 영향과 전기영동 방법으로 측정된 용단백질의 변화는 다음과 같았다. 용휴면의 결정은 유충발육기간중 초기 5~15일에 이루어졌다. 산소소비율의 변화는 정상발육용에서 U자 모양의 곡선을 나타내었고, 휴면용에서는 30μl O_2/g/hr 수준으로 감소되어 유지되었다. 휴면용에서 추출된 2.5% 균질액은 20-hydroxyecdysone의 처리로 호흡활성이 증가하였고, 휴면예정용은 직접 주사로 우화하였다. 수용성단백질의 함량변화는 정상발육용에서는 급격하게 이루어졌으나, 휴면용에서는 완만하였고, 더 많은 양이 포함되어 있었다. 휴면예정용의 지방체에서 분리한 단백질의 종류는 비휴면용의 경우와 달랐다.

檢索語 흰불나방, 휴면결정, 단백질 함량, 20-hydroxyecdysone, 산소소비율

Environmental cues that signal future environmental changes are percieved by the insect, often long in advance of the diapausing stage itself. Phisiologically, the senstive stage is involved in the perception of environmental cues and the storage of information within the insect for later translation into neuroendocrine function(Tauber et al. 1986).

After diapause phenomenon has been initiated, metabolic activity slowley declines until it levels off at a very low rate, where it remains until diapause ends. Thus, during the course of pupal diapause, the oxygen consumption in almost all insect is considerably distinct from that of non-diapausing insects showing a characteristically U-shaped curve(Wigglesworth 1972).

The endocrinology of pupal diapause is not fully understood, but an absence of the molting hormone was correlated with the failure of the pupal brain to secrete the prothoracicotropic hormone required to initiate the secretion of molting hormone by the

서울대학교 농과대학 농생물학과(Dept. Agric. Biol., Coll. Agric., Seoul Nat'l. Univ. Suwon 440-747, Korea)

prothoracic glands (Bowen et al. 1985, Wyatt & Pan 1978).

During diapause the fat body serves as the principal storage center for lipids, glycogen and proteins to sustain insects (Brown 1980, Brown & Chippendale 1977). Protein storage in haemolymph and fat body is evidently a widespread or universal phenomenon in insects. A study of the proteins in the fat body and haemolymph was considered important since these tissues together probably play a central role in the synthesis, storage and translocation of proteins, although the exact knowledge of the exchange of proteins between them is limited (Locke & Collins 1968, Wyatt & Pan 1978).

This study was carried out in laboratory to investigate the diapause-sensitive stage, the effect of 20-hydroxyecdysone on diapausing pupae and changes in pupal proteins of the fall webworm, *Hyphantria cunea* Drury.

MATERIALS AND METHODS

Sensitive stage

Larvae were reared on *Morus alba* leaves at 25°C, and they were exposed to diapause-inducing photoperiod (13L : 11D) and diapause-free photoperiod (16L : 8D)(Choi & Boo 1987). Lavae growing at 16L : 8D were transfered to 13L : 11D, and those growing at 13L : 11D transfered to 16L : 8D in every five days during their development.

The resulting pupae were collected daily and placed at the same condition as for the second part of the larval growth. After 15 days the number of emerging adult was counted daily.

Determination of the oxygen consumption.

Oxygen consumption during pupal stage was directly measured with a Warburg manometer(13 positions, circular model, T-ownson & Mercer, Ltd.) at 25°C(Denlinger et al. 1972). The individulals reared at 16 L : 8D and 13L : 11D were measured during 2 or 3 days and then replaced with different pupae from the same treatment.

Effects of 20-hydroxyecdysone

The effects of 20-hydroxyecdysone to respiratory activity were measured with a polarographic method using dropping mercury electrode (Jung et al. 1985). Different amounts of 20-hydroxyecdysone (SIGMA Chemical Company) were added to 2.5% whole body homogenate of diapausing pupae kept at 0°C for 1 month, and then the mixtures were incubated at 25°C before measurement.

The moulting hormone was also injected into 12~15 day-old diapause-bound pupae following the method of Bodnaryk (1985). Each dose was carefully delivered from a 25μl Hamilton syringe through the membraneous cuticle between the 1st and 2nd abdominal segments.

Protein analysis

Collected haemolymph were centrifuged at 5,000g for 10 min. The supernatants were freezed at −30°C until use. Fat bodies were dissected and collected in the insect saline. Then they were homogenized before centrifugation at 5,000g for 10min.

Protein was determined from haemolymph and fat body extracts with Lowry method (Lowry et al. 1951). Haemolymph and fat body protein samples were subsequently analyzed by SDS-polyacrylamide vertical slab gel electrophoresis (Vertical Slab Unit 2,000, Vocam 2000-300-150, Shandon) using the method of Weber et al.(1972) with the marker proteins (M.W. Range 14,300—71,500

& 56,000—280,000 daltons, Shandon). The separating gel consists of a 10% polyacrylamide slab gel overlaid with a 5% stacking gel. After electrophoretic separation, the results were recorded with a densitometer (*Chello* 3, Shandon) with the red filter.

RESULTS

In *H. cunea*, diapause determination was very sensitive during 5th—15th day of larval development (Fig. 1). Diapause-bound pupae were obtained only when the larvae were exposed to the short period (13L : 11D) during 10 days of the period alone. Larvae developed into a normal (non-diapausing) pupae when they were only given the short photoperiod before or after the period. And the total larval period was significantly longer in diapausebound insects (about 31.4 days) than in diapause-free insects (about 21.5 days) (Fig. 1).

The U-shaped curve of metabolic activity through the pupal stage and during development of pharate adult at 25°C was recorded in Fig. 2. But adult development was not initiated in diapause-bound pupae, with a very low level of oxygen consumption (30μl O_2/g/hr)(Fig. 2).

Mixing of 20-hydroxyecdysone on 2.5% whole body homogenate of diapausing fall webworm pupae kept at 0°C for 1 month increased respiratory activity (Fig. 3). And the diapausebound pupae also developed into adults when injected with this hormone (Fig. 4). The response pattern was quite similar in both treatments in terms of the hormone concentration.

Fat body protein content increased almost twice from about 136mg/g to 222mg/g from the last instar larvae to prepupae, and then sharply decreased to about 110mg/g in the 6day-old pupae in non-diapauing pupae (Fig. 5). But the content of fat body protein of diapause-bound insects was rather static, fluctuating between 250—300mg/g(Fig. 5).

Scans of all anodally-migrating bands sep-

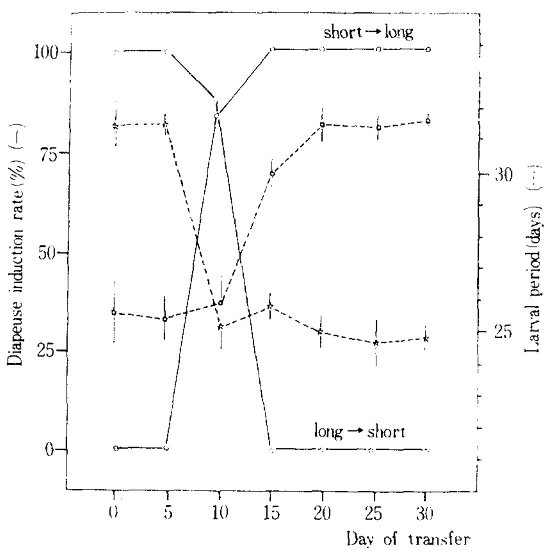

Fig. 1. Diapause induction rate(%) and larval developmental period(days) of the fall webworm reared first in long(*) or short photoperiod(◦), and then transfered to the other photoperiod every 5 days.

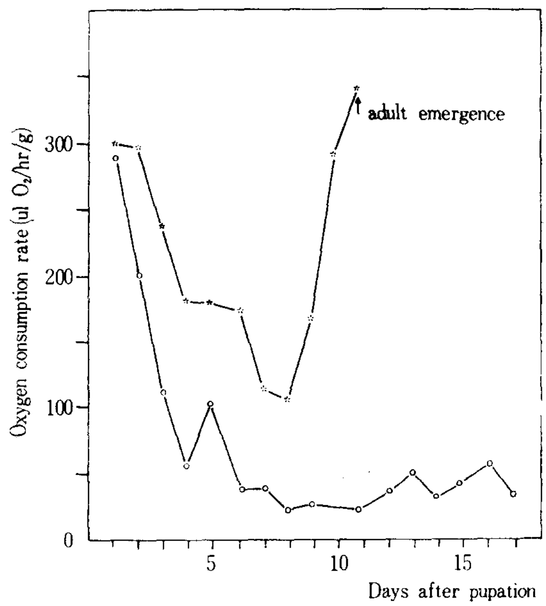

Fig. 2. Change in the oxygen consumption rate of diapause-bound(◦) and non-diapausing(*) fall webworm pupae after pupation.

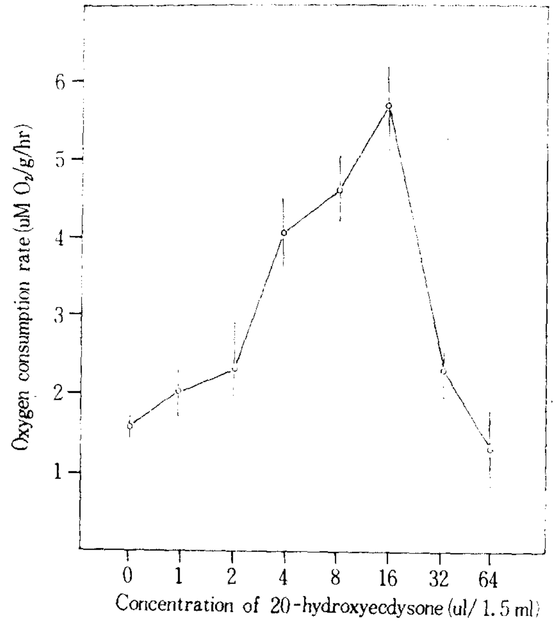

Fig. 3. Polarographically measured respiration rate of 2.5% whole body homogenate, with different concentration of 20-hydroxyecdysone, from the fall webworm. treated Samples were taken from diapausing pupae kept for about 1 month at 0°C and under continuous darkness after pupation.

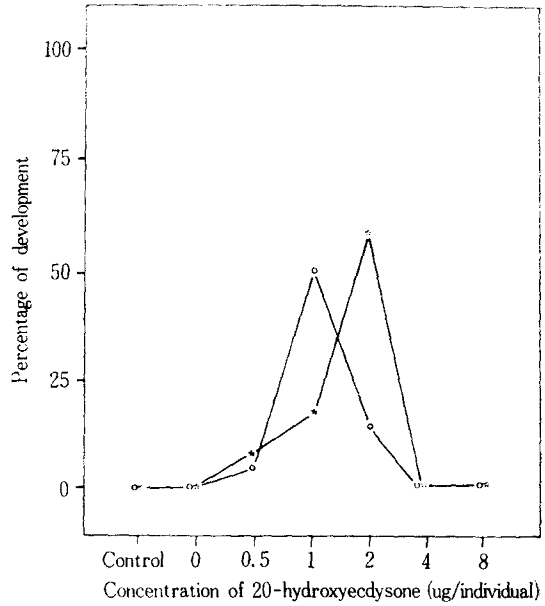

Fig. 4. Diapause termination rate of diapause-bound fall webworm after injection of different concentration of 20-hydroxyecdysone. The injection was performed during 12~15 days after pupation(o : emergence rate, * : overdevelopmental rate(metamorphological failure).

arated by SDS-electrophoretic run of haemolymph proteins at differental stages are presented in Fig. 6. In both cases only one major band of about 80 Kd protein was detected, except for the diapausing pupae stored at 0°C (Fig. 8), which showed several bands of very high molecular weight (more than 90Kd) proteins in large quantity.

It is observed that the pattern of fat body protein bands was very different between diapause-bound and diapause-free pupae(Fig. 7). At the prepupal and 3-day-old pupal stage the scans were almost same in both cases, but they were different from each other at or after the 6th day of the pupal stage. But the pattern of separated fat body proteins of separated fat body proteins of diapausing pupae under low temperature is similar to that of diapause-free 6-day-old pupae (Figs. 7 and 8).

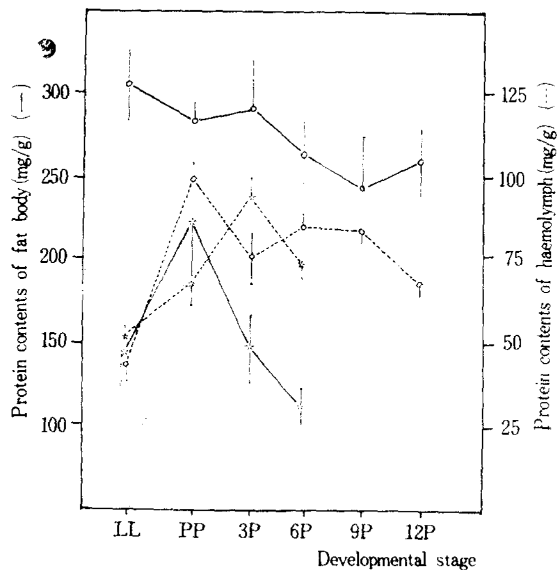

Fig. 5. Change in total soluble protein contents of fat body and haemolymph of diapause-bound(o) and non-diapausing(*) fall webworm(LL: last instar larvae, pp: prepupae, 3p: 3-day-old pupae, 6p: 6-day-old pupae, 9p: 9-day-old pupae, 12p: 12-day-old pupae).

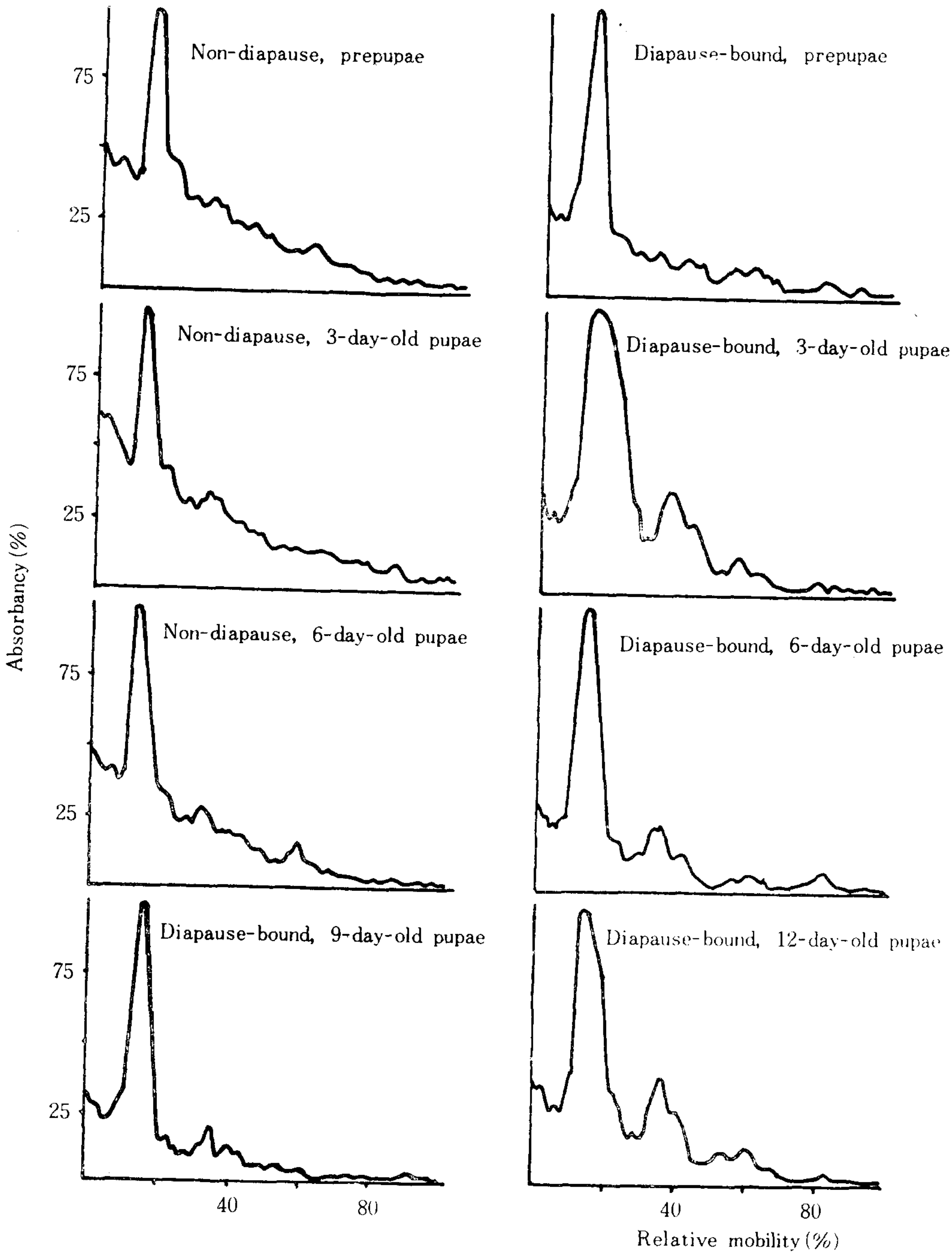

Fig. 6 Densitometric scans of hemolymph proteins in non-diapausing and diapause-bound fall webworm prepupae and pupae.

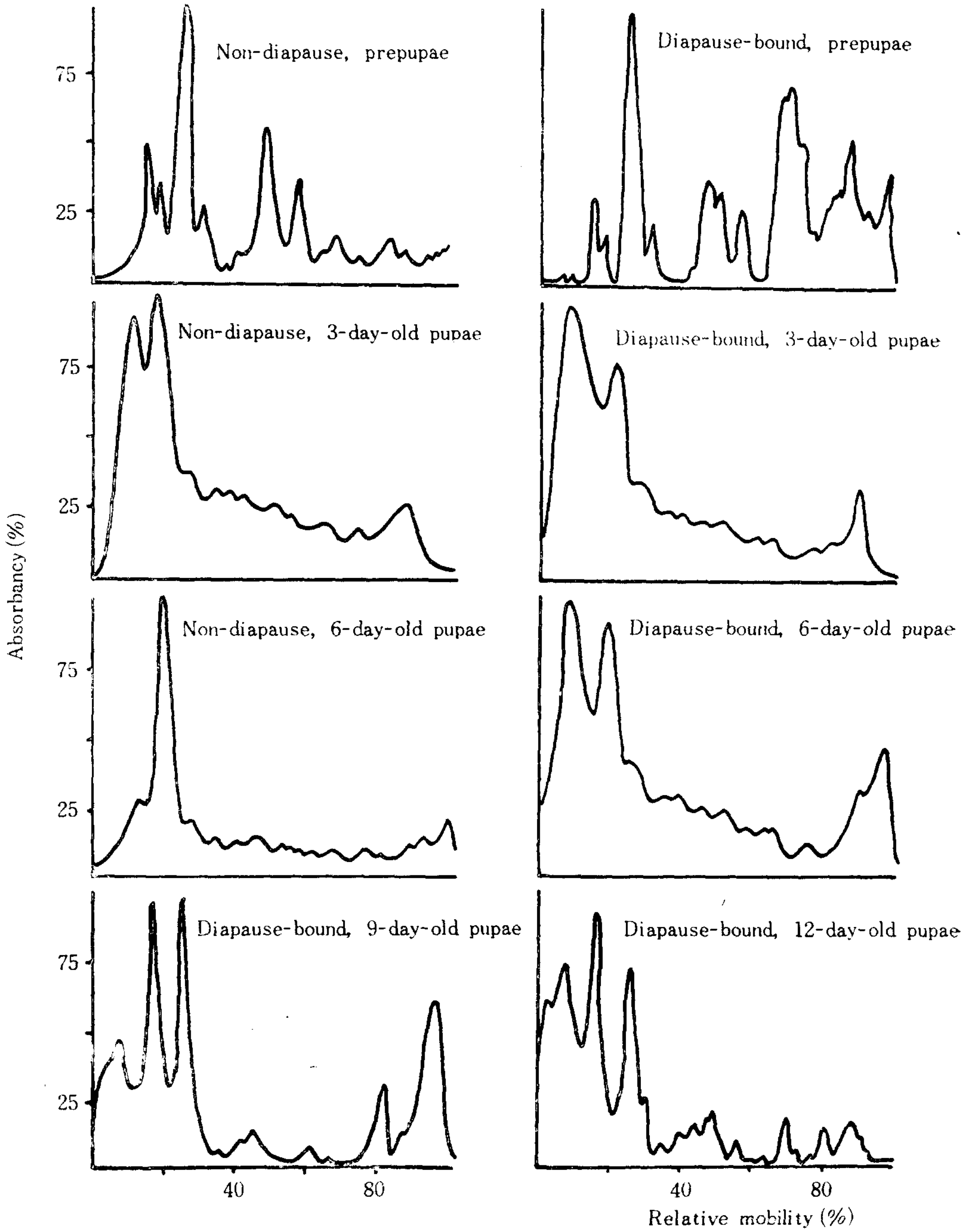

Fig. 7. Denstometric scans of fat body proteins in non-diapausing and diapause-bound fall webworm prepupae and pupae.

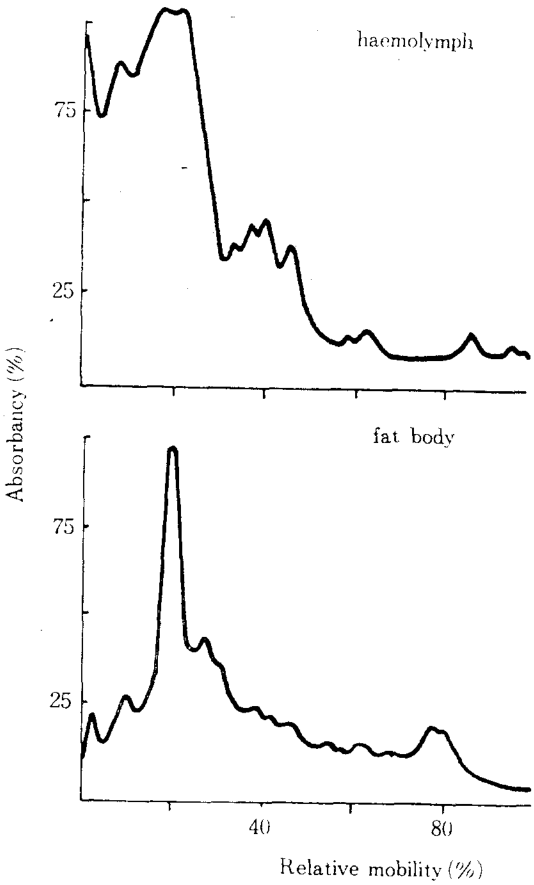

Fig. 8. Densitometric scans of fat body and haemolymph proteins in diapausing pupae of the fall webworm kept for 1 month at 0°C under continuous darkness.

DISCUSSION

The sensitive period for inducing pupal diapause definitely belong to the early part of the larval period in the fall webworm. The stimuli for diapause induction may be perceived during the period from 5th to 15 th day of larval development at 25°C under 13L : 11D. And the period of maximum sensitivity extends over 10 days, which is less than that of Masaki's experiment (about 15days) (Masaki 1977).

Pupal diapause is characterized by a strongly suppressed metabolic rate and cessation of adult differentiation. Metabolic rate, as measured in terms of oxygen consumption rate, in diapausing insects may be as low as 3% to that of non-diapausing insects (Denlinger et al. 1972, Duman & Horwath 1983). But the rate of oxygen consumption rate in the diapausing fall webworm pupae decreased to the range of 20—50μl O_2/g/hr (9% and 25% of the minimum and maximum of non-diapausing pupae).

The respiratory activity of whole body homogenate of the diapausing fall webworm pupae is low at the level of 1.6μM O_2/g/hr. When treated with the moulting hormone, however, it highly increased up to 5μM O_2/g/hr in spite of the less susceptibility to pharmacological agents (Sullivan et al. 1970). Interestingly, optimum concentration of 20-hydroxyecdyson, in activating respiration and adult development, was about 6.40μg/g and 6.36μg/g for the homogenate and intact pupe, respectively. But there are many physiological aspects involved, so much more detailed studies should be carried out to elucidate such a similarity.

The change of protein contents between fat body and haemolymph was rather smoothly proceeding in the diapause-bound fall webworm than in the diapause-free insects. The pattern of various fat body protein bands in prepupal stage during non-diapause and prediapause may be expressed as a result of an increasing protein synthesis. The retainment of the fat body protein was observed in the scan of electrophoretic separation after the diapause-bound 6-day-old pupal stage. But the retained proteins were released into the haemolymph after treatment of low temperature(0°C) for about 1 month.

The information about the properties of such proteins is still in insufficient in *H.*

cunea, but it is easily imagined that they may play an important role related to the diapause metabolism, such as the ability to lower the supercooling point implicated by Duman & Horwath(1983).

ACKNOWLEDGEMENT

This research was financially supported from the Korea Research Foundation.

REFERENCES CITED

Bodnaryk, R.P. 1985. Ecdysteroid levels during post-development and 20-hydroxyecdysone-induced development in male pupae of *Mamestra configurata* WLK. J. Insect Physiol. 31 : 53—58.

Bowen, M.F., R. Irish, L.R. Whisenton, N.A. Granger, L.I. Gilbert & W.E. Bollenbacher. 1985. Endocrine events during prediapause and non-diapause larval.-pupal development of the tobacco hornworm, *Manduca sexta*. J. Insect Physiol. 31 : 83—90.

Brown, J.J. 1980. Haemolymph protein reserves of diapausing and non-diapausing codling moth larvae, *Cydia pomonella*(L) (Lepidoptera: Totricidae). J. Insect Physiol. 26 : 487—492.

Brown, J.J. & G.M. Chippendale. 1977. Ultrastructure and respiration of the fat body of diapausing and non-diapausing larvae of the corn borer, *Diatraea grandiosella*. J. Insect Physiol. 23 : 1135—1142.

Choi, M.Y. & K.S. Boo. 1987. Initiation and termination of pupal diapause in the fall webworm, *Hyphantria cunea* Drury. Korean J. Plant Prot. 26 : 139—144.

Denlinger, D.L., J.H. Williams & G. Fraenkel. 1972. Rates and cycles of oxygen consumption during pupal diapause in *Sarcophaga* flesh flies. J. Insect Physiol. 18 : 871—882.

Duman, J.G. & K. Horwath. 1983. The role of haemolymph proteins in the cold tolerance of insects. Ann. Rev. Physiol. 45 : 261—270.

Jung, J., S.G. Park, S.K. Lee & S.H. Kim. 1985. Determination of respiratory activity of mitochondria and submitochondrial particles by using dropping mercury electrode. J. Kor. Agri. Chem. Soc. 28 : 271—277 (Korean).

Keeley, L.L. 1970. Diapause metabolism and rearing methods for the white-lined sphinx, *Celerio lineata*(Lepidoptera: Sphingidae). Ann. Entomol. Soc. Amer. 63 : 905—907.

Locke, M. & J.V. Collings. 1968. Protein uptake into multivesicular bodies and storage granules in the fat body of an insect. J. Cell. Biol. 36 : 453—483.

Lowry, O.H., N.J. Rosebrough, A.L. Harr & R.J. Randal. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 : 265—275.

Masaki, S. 1977. Life cycle programming. In Adaptation and Speciation in the Fall Webworm (Ed. by T. Hidaka), pp. 179. Kodansa, Tokyo.

Sullivan, W.N., B. Cawley, D.K. Hayes, J. Rosenthal & F. Halberg. 1970. Circadian rhythm in susceptibility of house flies and madeira cockroaches. J. Econ. Entomol. 63 : 159—163.

Tauber, M.J., C.A. Tauber & S. Masaki. 1986. Seasonal adaptations of insects, 411 pp. Oxford University Press, Oxford.

Weber, K., J.R. Prince & M. Osborn. 1972. Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. In Method in Enzymology (Ed. by Hirs, C.H.W. & S.N. Timasheff), Vol. 26, pp. 3—27.

Wigglesworth, V.B. 1972. The principles of insect physiology, 7th ed, 827 pp. Chapman & Hall, London.

Wyatt, G.R. & M.L. Pan. 1978. Insect plasma proteins. Ann. Rev. Biochem. 47 : 779~817.

Yin, C.M., I.S. Wang & W.D. Chaw. 1985. Brain neurosecretory cell and ecdysiotropin activity of the non-diapausing, pre-diapausing and diapausing southwestern corn borer, *Diatraea grandiosella* Dyar. J. Insect Physiol. 31 : 659—667.

(Received March 29, 1988)