家蚕の第4眠期における呼吸活性と諸物質含量の変化

誌名	日本蠶絲學雜誌
ISSN	00372455
著者	中井沢, 健二 諸橋, 征雄 中島, 誠
巻/号	48巻1号
掲載ページ	p. 24-30
発行年月	1979年2月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

AgriKnowledge

日蚕雑. 48(1), 24～30, 1979
(J. Sericult. Sci. Japan)

Changes in Respiratory Activity and Utilization of Reserves During the Pharate Fifth Larval Instar of the Silkworm, *Bombyx mori* L.

Kenji NAKAIZAWA, Yukio MOROHASHI and Makoto NAKAJIMA

Department of Sericulture, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, 〒183

(Received August 14, 1978)

It has been shown that during the pharate larval instar respiratory activity of the silkworm, ***Bombyx mori***, first falls to a low level and then increases rapidly (ITAYA, 1940; KAMIOKA, 1950; ITO and SEKI, 1954). However, available informations concerning the mechanisms on changes in respiratory activity during the pharate larval instar are very limited and respiratory metabolism occurring during the instar are not elucidated well.

In the present study, as the first step to investigate the mechanisms on the changes in respiratory activity and the metabolic relations among the tissues during the pharate fifth larval instar, changes in respiratory activity of the silkworm and in the contents of various components in the tissues were followed.

MATERIALS and METHODS

Insects

Larvae of the silkworm, ***Bombyx mori*** L., of F_1 hybrid, Ryuhaku X Shuko, were reared at 26℃ on artificial diet, which was provided by Nihon Nosan Kogyo Co. Ltd., Yokohama.

The time of the larval-larval fourth apolysis was determined on the basis of changes in morphological appearances of the fifth instar spiracles, which were shown to correspond to histological changes of the cuticle by KIGUCHI (1976).

Tissue preparation

The midgut, midgut content, perivisceral fat body (pv. fat body) and body wall of a larva were dissected in cold 0.9% NaCl solution. Hemolymph was collected in cold test tube by cutting the abdominal legs.

Oxygen uptake

The rate of O_2 uptake of a larva was determined by warburg manometric technique at 26℃ under the condition where the larva was placed in a tight holder so that it could not move.

Analytical methods

Lipid Lipids of various tissues were extracted with 20 vol. of chloroform-methanol (2 : 1 by volume) mixture at 60℃. The lipid fraction was evaporated to dryness and lipids were extracted again with the same solution at room temperature. This lipid fraction was weighed after being dried at 90℃.

Carbohydrate Carbohydrates of various tissues except the hemolymph were extracted with hot 5% (w/v) trichloroacetic acid (TCA) containing 0.1% (w/v) Ag_2SO_4 (KEMP and KITS van HEIJNINGEN, 1954). Carbohydrates in the hemolymph were extracted with the same reagent at room temperature. Assays of carbohydrate contents were carried out by the method of MENDEL *et al.* (1954). KIMURA's method (1973) was used for the preparation and assay of chitin.

Nitrogen The determination of total nitrogen was carried out by the ordinary micro-Kjeldahl method.

Amino sugar Amino sugars in the exuviae were extracted with water and those on the body surface were collected by washing with water. Amino sugars were determined by the method of AMINOFF *et al.* (1952).

RESULTS

Course of respiration

The changes in respiratory activity of a larva during the pharate fifth larval instar are shown in Fig. 1. It could be resolved into four successive phases. For the first 12 h (provisionally referred to as Phase a) the respiration rate gradually increased. After this phase there was a phase of 12 h during which the respiration rate sharply decreased (Phase b). Then followed a phase of 12 h during which the respiration rate gradually increased (Phase c). In the following phase of 12 h, the respiration rate sharply increased (Phase d).

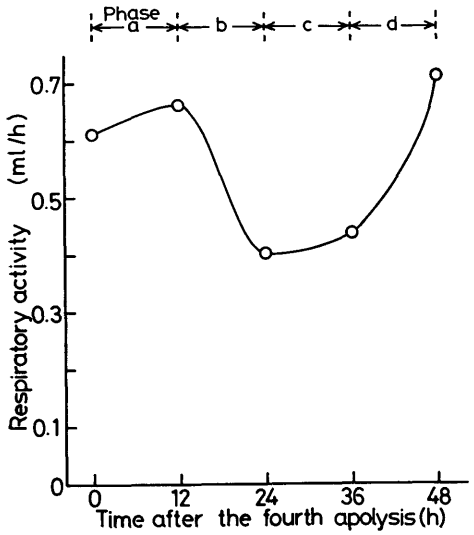

Fig. 1. A changes in respiratory activity of a larva during the pharate fifth larval instar.

Lipid content

Fig. 2 shows the changes in the lipid contents of the tissues during the pharate fifth instar. In Phase b, the lipid contents of the pv. fat body, midgut and hemolymph increa sed. In Phase d, the lipid contents of the body wall, pv. fat body and hemolymph decreased, while that of the midgut increased.

Carbohydrate content

As shown in Fig. 3, the contents of TCA soluble carbohydrate in the pv. fat

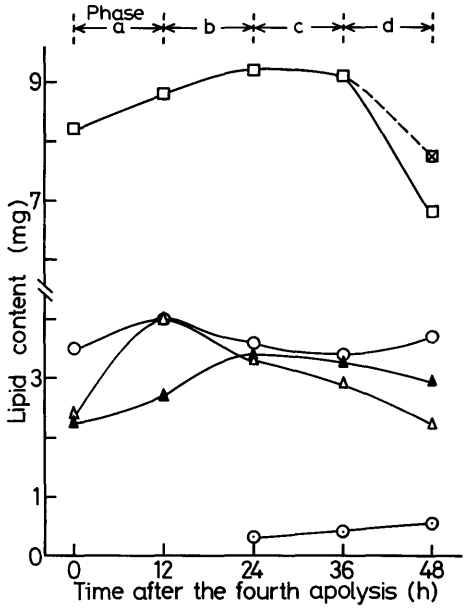

Fig. 2. Changes in the amounts of lipids in the tissues of a larva during the pharate fifth larval instar. (□), body wall; (⊠), bodywall and exuvium; (○), midgut; (△), pv. fat body; (▲), hemolymph; (⊙), midgut content.

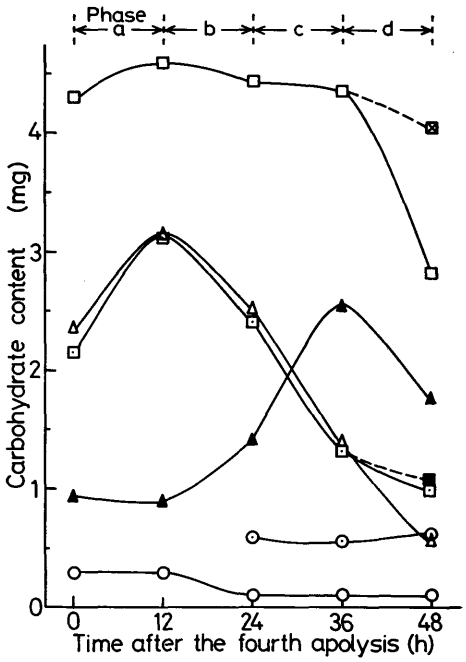

Fig. 3. Changes in the amounts of carbohydrates in the tissues of a larva during the pharate fifth larval instar. (□),chitin in the body wall; (⊠), chitin in the body wall and exuvium; (▣), TCA sol. carbohydrate in the body wall; (■), TCA sol. carbohydrate in the body wall and exuvium. Other symbols as in Fig. 2.

body and body wall decreased markedly in Phase b and c, while that in the hemolymph increased. The chitin content decreased through Phase b to d.

Total nitrogen content

As seen from Fig. 4, in Phase b the nitrogen content of the body wall increased, while those of the hemolymph and midgut slightly decreased. In Phase d, the nitrogen content of the body wall decreased, while that of the midgut increased.

Amino sugar

The amounts of amino sugars, decomposition products of chitin, present in the exuvium and on the body surface were 97.2μg and 29.8μg per a larva, respectively.

DISCUSSION

The course of respiration of a larva during the pharate fifth larval instar can be conveniently divided into four phases. Similar course of changes in the respiration during the instar of ***Bombyx mori*** was reported by KAMIOKA (1950).

For the first 12 h after the fourth apolysis (Phase a), the contents of various components in the tissues increased. In fact, the fresh weight of a larva increased from

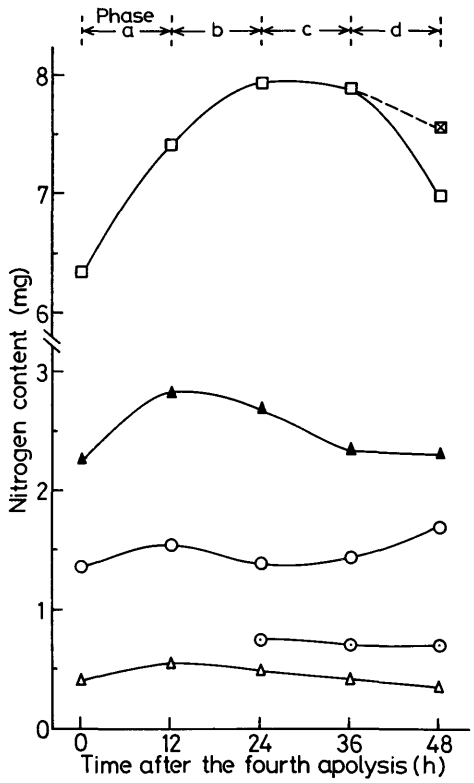

Fig. 4. Changes in nitrogen contents in the tissues of a larva during the pharate fifth larval instar. Symbols as in Fig. 2.

850mg to 1025mg in Phase a. Therefore, somatic growth was supposed to actively occur during this phase. The increase in respiration rate in Phase a might be related to the high synthetic activities of the tissues. SLAMA (1959) showed with the saw fly that the more intensive was body growth, the higher was respiration. In Phase b, as the chitin content of the body wall decreased, the breakdown of the old cuticle seemed to occur. KIMURA's report (1974) that chitinase activity in the body wall of ***Bombyx mori*** increased in the stage roughly corresponding to Phase b, supports this view. Furthermore, the lipid and nitrogen contents of the midgut decreased in Phase b. So it is supposed that the degeneration of the midgut cells also occur in this Phase. TSUJITA (1943) reported that the degeneration of the midgut cells occurred during the pharate larval instar of ***Bombyx mori.*** In Phase d, on the contrary, the regeneration of the midgut cells seems to occur, since the lipid and nitrogen contents of the tissue increased in this phase. According to AKAL (1970), during the pharate larval instar of ***Bombyx mori*** the regeneration of the midgut cells occurs. The chitin content of the body wall, on the other hand, decreased through Phase c to d. This result suggests that the breakdown of the old cuticle still continued to occur in these phases. However, KIGUCHI (1976) observed histologically that the new cuticle was formed in the stage corresponding to Phase c and d. Presumably, the breakdown and the formation of the cuticle simultaneously occur in Phase c and d. Anyway, in Phase d the biosynthetic activities are supposed to be high in the midgut and epidermis. From these results, it seems that Phase b might be a stage of the degeneration of tissues of the fourth instar larva and that Phase d might be a stage of the formation of new tissues of the fifth instar larva. Presumably, the decrease in the respiration rate in Phase b and the rapid increase in Phase d reflects these situations.

As larvae took no food in Phase c and d, it is important to examine which reserves were used for respiration. The theoretical amount of oxygen to be consumed in these phases, which was estimated from the decrease in the contents of reserves,

Table 1 Oxygen consumption of a larva and that estimated from the decreases in the amounts of reserves in Phase c and d of the pharate fifth larval instar

Phase	Component	Reduction in weight of component (mg)	Oxygen consumption (m*l*)		
			Estimated*		Observed
c	Lipid	0.75	1.52	5.10	4.82
	Carbohydrate	1.18	0.97		
	Nitrogen**	0.43	2.61		
d	Lipid	1.92	3.90	7.13	6.68
	Carbohydrate	1.94	1.59		
	Nitrogen**	0.27	1.64		

* The amount of oxygen consumed for lipid, carbohydrate or protein oxidation was calculated on the basis of the values of Prosser and Brown (1965) as follows: 0.82 m*l* O_2/mg carbohydrate, 2.03 m*l* O_2/mg lipid, 6.06 m*l* O_2/mg protein nitrogen.

** assumed to be protein nitrogen.

is shown in Table 1. As seen in Table 1, the calculated amount of oxygen was in accord with the observed one. This reflects the fact that larvae fed on no food in Phase c and d. The main fuels of respiration in Phase c are nitrogen compounds contained in the hemolymph and lipids in the pv. fat body. On the other hand, in Phase d lipids contained in the body wall and pv. fat body were the main fuels. Utilization of lipids during the pharate larval instar was also demonstrated with the whole body of ***Bombyx mori*** by NIEMIERKO ***et al.*** (1956).

As a small amount of the chitin or its decomposition products, amino sugars, was found in the exuvium or on the body surface, it is clear that considerable amount of the chitin of the fourth instar larva remained in the body without being cast away. This results does not agree with that of KIMURA (1974), who suggested that in ***Bombyx mori*** the chitin or its decomposition products of the old cuticle was cast away in the exuvium and on the body surface of the newly ecdysed larva. It has been shown with ***Hyarophora cecropia*** (POSSONEAU and WILLIAMS, 1955; BADE and WYATT, 1962) and ***Locusta migratoria*** (HILL and GOLDSWORTHY, 1968) that the compounds of the new cuticle were derived from the old one. The amounts of the chitin of the old cuticle which remained in the body were sufficient to supply that of the new fifth instar cuticle. Therefore, it seems possible that in ***Bombyx mori*** also, materials for the formation of the new cuticle were mostly derived from the old one.

SUMMARY

The course of respiration of a larva during the pharate fifth larval instar can be conveniently divided into four phases. The increase in the respiration rate in the first 12 h after the fourth apolysis (Phase a) may be related to the biosynthetic activities. The decrease in the respiration rate from the 12 th h to the 24 th h after

the fourth apolysis (Phase b) might reflect the degradation of tissues of the fourth instar larva. The increase in the respiration rate from the 36 th h to the 48 th h (Phase d) might be related to the formation of new tissues of the fifth instar larva. Larvae fed on no food from the 24 th h to the 48 th h after the fourth apolysis (Phase c and d). The main fuels of respiration in Phase c were nitrogen compounds contained in the hemolymph and lipids in the perivisceral fat body. On the other hand, in Phase d lipids contained in the body wall and perivisceral fat body were the main fuels. The chitin content of the old cuticle remained in the body was sufficient to supply that of the new fifth instar cuticle.

ACKNOWLEDGEMENTS

We wish to thank the members of the Sericultural Laboratory, Central Research Institute, Nihon Nosan Kogyo, Yokohama, for supplying the artificial diet. Thanks are also due to Dr. M. NAGATA, Tokyo University, for providing information on the hemolymph volume of the silkworm and Dr. K. KIGUCHI and Dr. S. KIMURA, Sericultural Experiment Station, for their kind advice. The assistance of Mr. T. KUBOTA for the determination of carbohydrates was highly appreciated.

REFERENCES

AKAI, H. (1970): Bull. Sericul. Exp. Sta. Jap., 24, 303—335.

AMINOFF, D., W.T.J. MORGAN and W.M. WATKINS (1952): Biochem. J., 51, 379—389.

BADE, M. L. and G. R. WYATT (1962): Biochem. J., 83, 470—480.

HILL, L. and G. J. GOLDSWORTHY (1968): J. Insect Physiol., 14, 1085—1098.

ITAYA, K. (1940): J. Sericult. Sci. Jap., 11, 113—140.

ITO, T. and M. SEKI (1954): Acta Sericologica, 9, 1—4.

KAMIOKA, S. (1950): Oyo Konchu, 6, 144—149.

KEMP, A. and A. J. M. KITS van HEIJNINGEN (1954): Biochem. J., 56, 646—648.

KIGUCHI, K. (1976): Agr. D. Thesis, University of Tokyo.

KIMURA, S. (1973): J. Insect Physiol., 19, 2177—2181.

KIMURA, S. (1974): Jap. J. Appl. Ent. Zool., 18, 183—188.

MENDEL, B., A. KEMP and D. K. MYERS (1954): Biochem. J., 56, 639—646.

NIEMIERKO, S., P. WLODAWER and A. F. WOJTCZAK (1956): Acta Biol. Exper., 17, 255—276.

PASSONNEAU, J. V. and C. M. WILLIAMS (1955): J. Exp. Biol., 30, 545—560.

PROSER, C. L. and F. A. BROWN Jr. (1965): "Comparative animal physiology". (2 nd ed.), 688 pp., W. B. Saunders Company, Philadelpia and London.

SLAMA, K. (1959): Acta Soc. Entomol, Bohem., 56, 113—125.

TSUJITA, M. (1943): Bull. Sericul. Exp. Sta. Jap., 11, 211—293.

摘　　要

家蚕の第4眠期における呼吸活性と諸物質含量の変化

中井沢健二・諸橋征雄・中島　誠
（府中市・東京農工大学農学部　〒183）

第4眠期（48時間）の呼吸活性は最初の12時間（Phase a）で増加し，次の12時間（Phase b）で急減するが，最後の12時間（Phase d）で再び増加するという変化を示した。Phase aでは諸物質量が増加し体重も増加することからPhase aの呼吸活性の増加は，生合成活性の増加と関連しているように思われた。Phase bではキチン量および中腸内の脂質および窒素量が減少した。これは第4齢幼虫の諸組織の分解の結果と考えられ，このPhaseにおける呼吸活性の急減もこれらの事を反映しているように思われる。一方，Phase dでは中腸内の脂質および窒素量の増加が観察された。この時期は新外皮形成期に相当しているという事を考えあわせると，Phase dは第5齢幼虫の諸組織の形成が行なわれている時期に相当していると考えられ，Phase dの呼吸活性の増加はこの観点から理解することができる。

Phase cとdでは幼虫は無摂食であったので，この時期の呼吸基質を調べた所，Phase cでは体液の窒素化合物および体腔側脂肪体の脂質が主に利用されており，Phase dでは体壁内および体腔側脂肪体に含まれる脂質が主に利用されていた。

旧クチクラのキチンはその一部が脱皮殻，あるいは起蚕の体表面に捨てられているにすぎなかった事から，そのほとんどは回収されて再利用されているものと考えられた。