Changes in Hemolymph Protein Concentration and Oxygen Consumption during the Metamorphosis in *Pieris rapae* L.

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배추흰나비의 變態에 따른 血蛋白質과 酸素消費量의 變化

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(Received July 11, 1969)

摘　　要

배추흰나비(*Pieris rapae* Linne)의 變態에 따른 hemolymph protein의 濃度變化와 酸素消費量 및 5種의 呼吸基質에 對한 活性度를 各各 Biuret method와 Warburg manometric method로 測定하여 形態的 變化와 比較하였다.

1. Hemolymph protein의 濃度는 終齡幼蟲에서 가장 높으며 變態에 따라 감소하여 蛹期末에서 다시 增加한다.
2. Endogenous respiration은 前蛹期에서 가장 높은 酸素消費量을 보이며 變態에 따라 감소하여 蛹期末에서 다시 增加한다.
3. Glucose는 全變態期를 通하여 다른 呼吸基質보다도 가장 큰 活性을 보이며 前蛹과 蛹期末에서 현저하게 영향을 미친다.
4. Hemolymph protein의 濃度變化와 酸素消費量은 全變態期를 通하여 밀접한 연관을 가지며 幼蟲器官의 解消와 成蟲器官의 新生에 따라 U字型을 나타낸다.

INTRODUCTION

The rate of oxygen consumption of insects exhibits an elusive value that varies with many internal and external factors and the level of oxygen consumption changes strikingly during the life cycle of the insects.

The hemolymph proteins of insects are found to serve as an important internal environmental factor for the metabolism, especially having a close relation with oxygen consumption during the growth and metamorphosis.

The content of protein of insect hemolymph fluctuates much more widely than that of free amino acid and other non-protein nitrogen. Taken together with the decrease in volume of hemolymph during adult development this phenomenon suggests that the hemolymph protein serves as an important source for protein synthesis of the adult organ.

The hemolymph proteins of various insects had been investigated on various view points by many workers (Oda, 1957; Stephen, 1958; Steinhauser, 1959; Laufer, 1960; Loughton, 1965; McCormick, 1966; Nowosielski, 1965). However, there were few studies on hemolymph protein concentration and oxygen consumption through the life cycle.

Recently Terando *et al.* (1966) measured the hem-

olymph protein concentration of the *Oncopeltus fasciatus* by the Lowry method (Lowry *et al.*, 1951) and Geest (1968) studied the effect of diets on the hemolymph protein of larvae of *Pieris brassicae*.

Krogh (1924) described that oxygen consumption of holometabolus insects follows a U-shaped curve during metamorphosis. Thus, during the pupal stage of exopterygote insects, oxygen uptake follows a U-shaped curve, which has been correlated with histolytic and histogenic processes during the pupal-adult transformation. Wigglesworth (1953) studied the histogenesis over before respiration reached its minimum, and the adult organs were anatomically completed before oxygen consumption started to rise.

It was shown by Lee (1967, 1969) that the relationship between histolytic and histogenic processes during metamorphosis of chestnut gall wasp, *Dryocosmus kuriphilus* could be clarified in part by measurement of Qo_2.

The present work concerned primarily with the changes of hemolymph protein concentration and oxygen consumption during the metamorphosis in *Pieris rapae* Linné.

MATERIALS AND METHODS

The cabbage worm larvae were obtained from the Duck Sum cabbage fields near Seoul city and maintained in breeding box at temperature of 28°C until used in the experiment. The healthy materials at each developmental stage, last instar larva, newly molted pupa, pupae of 2, 4, 6 and 8 days as newly emerged adult and adult were chosen as samples.

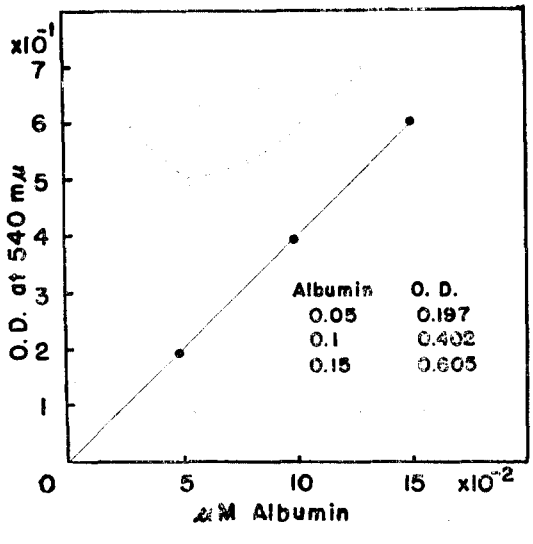

Fig. 1. The standard curve of protein concentration by biuret reaction.

1. Determination of hemolymph protein concentration.

Hemolymph samples of *Pieris rapae* employed in the present study were taken from the larvae and pupae at two day intervals after newly molted. For collection of hemolymph, prolegs for larvae, top parts of the body for pupae were cut off at various time intervals and then, the exuded hemolymph was aspirated into a micropipette and collected in haematocrit centrifuge tube chilled in an ice-box.

Hemolymph was removed by centrifugation at 4,000 r.p.m. for 20 min. The pooled samples were kept in an ice-box in order to prevent melanization as no tyrosinase inhibitors were used because they interfered with protein determination.

The hemolymph protein concentration of the various stages was determined by biuret method (Gornall *et al.*, 1949). The pooled and filtered samples were mixed with nine-fold Neisenheiner's insect Ringer solution to keep within a convenient range on the albumin standard curve. And then, 4 m*l* of biuret reagent were added to 1 m*l* of the diluted hemolymph serum. The mixture was kept in water bath at 18-20°C about 30 min for color development. The optical density (O.D.) of diluted hemolymph was read at 540 mμ in a photoelectric photometer Type N5 and compared with a standard curve prepared from bovine serum albumin shown in Fig. 1. for quantity.

2. Estimation of respiratory activity.

The oxygen consumption of insects kept individually in the respirometer was measured using the conventional Warburg manometers. Changes in respiratory activity according to the respiratory substrates were compared with normal endogenous respiration at various developmental stages.

Oxygen uptake of the homogenized cell suspension was measured at 30°C with the Warburg manometers shaken at the rate of 120 strokes per minute. The flask contained 3.0 m*l* cell suspension in phosphate buffer (1/15 M, pH 6.8) and in the central well, 0.2 m*l* of 20% KOH.

Using air as a gas phase, oxygen uptake was measured for 120 min after 20 min of temperature equilibration.

Oxidation of various respiratory substrates (glucose, citrate, succinate, α-ketoglutarate, malate) by cell-free exracts was also measured with the respirometer. To the reaction mixture containing 2.0 m*l* of cell suspension, 1.0 m*l* of substrate solution was poured from the side arm after 20 minute of equilibration. The control without added substrate was also measured simultaneously.

RESULTS

Table 1 shows the values for hemolymph protein concentration of the filtered and pooled samples during metamorphosis in *Pieris rapae*. The ordinate indicates mg protein/m*l* of hemolymph and the vertical number represents the fifth larval instar stage and the pupal stages at two day intervals after newly molted respectively. As revealed in Table 1 the fifth larval instar had the highest protein concentration of 46.6 mg protein/m*l* hemolymph. At the newly molted pupal stage the value was decreased to 32.8 mg protein/m*l* hemolymph. The two day pupa had a protein concentration of 27.6 mg protein/m*l* hemolymph.

The concentration kept decreasing until at least the six-day pupa. Values for the four-day and the six-day pupae were 23.3 and 21.6 mg protein/m*l* hemolymph respectively.

Table 1. Values for the hemolymph protein concentration during the metamorphosis in *Pieris rapae* L.

No.	Stage	O.D.	μM/0.1 m*l*	mg/m*l*
1	Last larva	0.215	0.0675	46.6
2	Pupa, newly molted	0.153	0.0475	32.8
3	Pupa, 2 days	0.130	0.0400	27.6
4	Pupa, 4 days	0.115	0.0338	23.3
5	Pupa, 6 days	0.108	0.0313	21.6
6	Pupa, 8 days	0.135	0.0393	27.1
7	Adult, newly emerged			

However, the concentration started to increase the six-day pupa and the value for the eight-day pupa showed an increase to 27.1 mg protein/m*l* hemolymph.

In general, the enormous increase in the concentration of hemolymph protein during larval development was considered to be particularly impressive.

Also, the values for the fifth larval instar and the above mentioned pupal stages were depicted diagrammatically in Fig. 2.

As is evident from Fig. 2 the concentration was maximum during the last larval stage, with general decreasing patterns through the pupal stages. The value for larva was about two times the value for the four-day pupa midway through the pupal stage.

The rates of oxygen consumption with and without added respiratory substrate during the metamorphosis in *Pieris rapae* as determind by means of Warburg manometric method were represented in Table 2 and in Fig. 3.

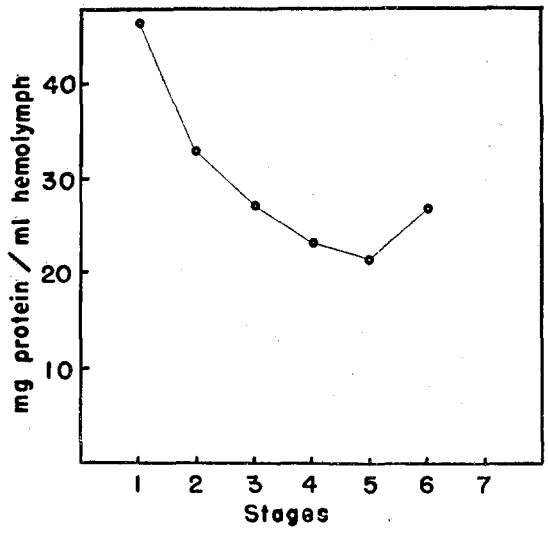

Fig. 2. Protein concentration of hemolymph during the metamorphosis in *Pieris rapae*.
1. last larva 2. pupa, newly molted 3. 2-day pupa
4. 4-day pupa 5. 6-day pupa 6. 8-day pupa
7. adult, newly emerged

Table 2. Average oxygen consumption during the metamorphosis of *Pieris rapae* L.

No.	Stage	Endogenous respiration	Q_{O_2} Substrate				
			Glucose	Citrate	Succinate	α-Ketoglutarate	Malate
1	Last larva	0.806	0.879	0.839	0.726	0.049	0.049
2	Pupa, newly molted	1.046	0.848	0.793	0.714	0.048	0.047
3	Pupa, 2 days	0.801	0.709	0.601	0.633	0.064	0.021
4	Pupa, 4 days	0.505	0.674	0.289	0.381	0.035	0.053
5	Pupa, 6 days	0.566	1.643	0.392	0.452	0.095	0.075
6	Pupa, 8 days	0.707	1.687	0.684	0.787	0.240	0.257
7	Adult, newly emerged	0.955	1.412	0.703	0.995	0.521	0.298

The comparison of the oxidation of glucose, citrate, succinate, α-ketoglutarate and malate of the homogenized total organ with endogenous respiration during the metamorphosis in *Pieris rapae* was as follows:

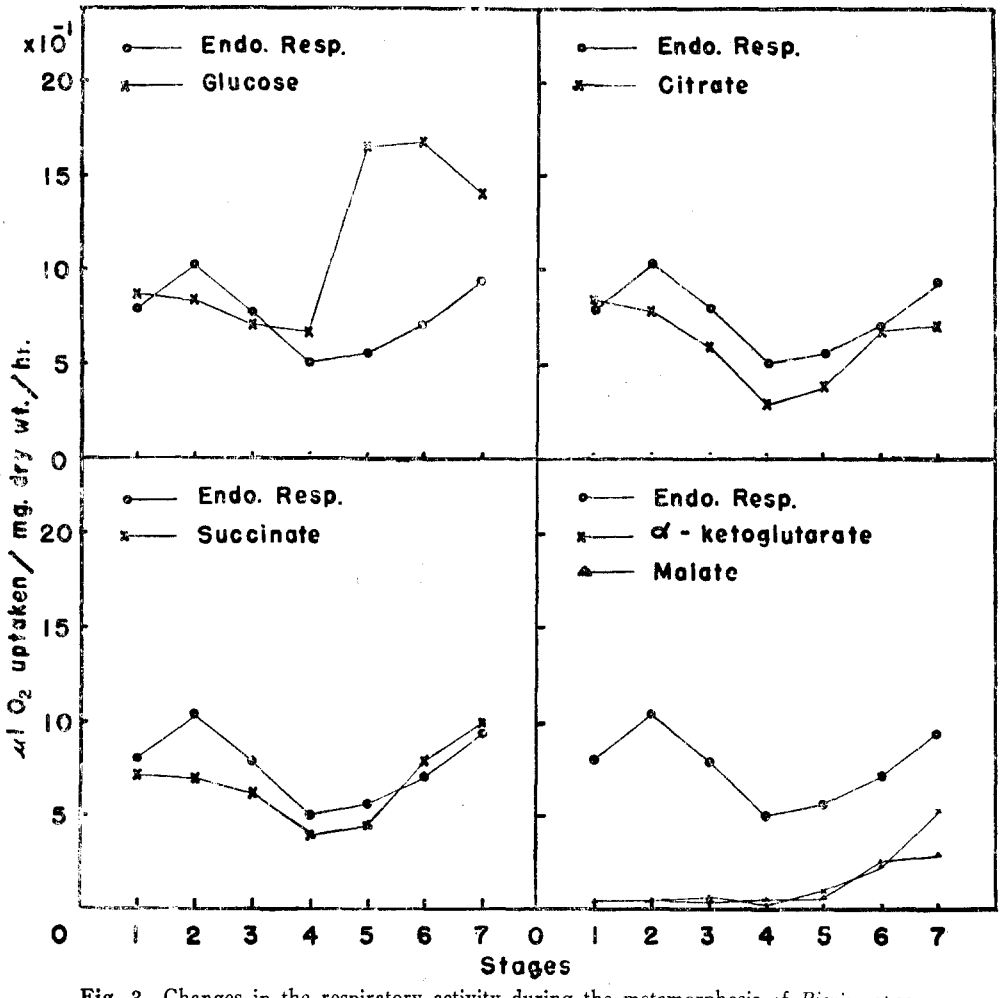

Fig. 3. Changes in the respiratory activity during the metamorphosis of *Pieris rapae*.

1. Endogenous respiration was very high at the prepupal stage, decreased at 2-day pupa and increased again at the late pupal stage. These changes were in agreement with the morphological characters during the metamorphosis.

2. Glucose showed a marked activity throughout all stages and had a striking influence on both the prepupal and adult stages. However, the oxygen consumption started to increase from the six-day pupa and value for the eight-day pupa was 1.687 μl O_2/mg/hr.

3. Citrate exhibited a striking activity after the last larval stage. The respiration decreased from the two-day pupa and increased again from the six-day pupa.

4. Succinate made a remarkable decrease in oxygen consumption throughout prepupal stages and increased again from the late pupal stages, and showing a point of similarity as compared to the endogenous respiration, especially in the histogenesis of the adult organ at eight-day pupa.

5. The activities of fumarate, malate and α-ketoglutarate throughout all stages were found to be very low compared with endogenous respiration.

DISCUSSION

The hemolymph proteins of the insect are found to serve as an important internal environmental factor for the metabolism and especially have a close relation with oxygen consumption during the growth and metamorphosis.

The changes in protein concentration in the hemolymph during metamorphosis *Pieris rapae* was essentially similar to that already found for *Deilephila euphorbial* (Heller and Moklowska, 1930), *Hyalophora cecropia* (Chefurka, 1953), *Popillia japonica*(Ludwig, 1953) and *Bombix mori*(Wyatt *et al.*, 1956).

The concentration rises rapidly in the growing larva and decreases gradually during the pupal stages.

Siakotos (1960) reported that the protein concentration on the hemolymph of the American cockroach, *Pieriplaneta americana* Linne, decreased by two-thirds on the fourth day after the last nymphal molt, but by the eighth day it was back to the initial level.

Also, Laufer (1960) and Williams (1953) indicated that the protein of *Hyalophora cecropia* increased in the last larval instar during the time of increased prothoracic hormone activity and as the adult developed the protein concentration decreased.

The data on the cabbage worm, *Pieris rapae* indicated the highest value in protein concentration during the fifth larval instar, followed by a gradual decrease until the sixth day pupa. These results were in good agreement with those mentioned above. This seemed to be caused by the fact that the cabbage worm stop feeding after newly molted.

In the recent reports by Orr (1964) and Chen (1966) who also worked on *Phormia regina* corresponding values of 20.8 to 36.5μg/μl have been obtained for adult flies fed on a complex medium. In the house cricket *Acheta domesticus*, Patton (1965) found that the hemolymph protein concentration decreased gradually during the course of adult life. Similar changes took place in *Pieris rapae*. Taking into consideration together with decrease in volume of hemolymph during adult development, this phenomenon is suggestive of Chen's observation (1966) that the hemolymph protein serves as an important source for the synthesis of protein of the adult.

There seems little doubt that culturing conditions, nutritional state, hormonal secretion as well as age can exert a large influence on the protein content.

And then, according to Siakotos (1960), blood proteins of the American cockroach *Periplaneta* might be involved in the transport of nutrient such as lipids and carbohydrates which are joined to proteins as conjugated groups. Probably, the hemolymph protein might serve as an important source for the synthesis of adult tissue proteins. Several studies suggest that insect hemolymph, being the only extracellular fluid, might be a good indicator of metabolic changes.

Oxygen consumption during development was presented in Table 2 and Fig. 3. The general tendency appears to be of a high oxygen consumption at 2-day pupa followed by an increase at the late pupal stage.

Wigglesworth (1953) studied the histogenesis until before respiration reached its minimum, and found that the adult organs were anatomically completed before oxygen consumption started to rise. These results were similar to those in *Pieris rapae* Linne. As shown above,

the values of oxygen was shown to be similar rate to those of each one preceeding stage in hemolymph protein of the same materials.

This fact indicates that the rate of oxygen consumption and the hemolymph protein were very closely correlated during metamorphosis. Some factors that increase activity or metabolic rate also increase the oxygen consumption.

The experiment described in the present work clearly showed the high level of general metabolism associated with growth and development and the low level when these processes ceased. Therefore, they seemed to play an important role in the intermediary metabolism of living insect cells taking part in the chain reaction associated with the cytological changes according to histogenesis of the adult organ and besides, histolysis of the larval organ during the metamorphosis.

SUMMARY

Changes in the protein concentration in the hemolymph and the oxidation of respiratory substrates at various developmental stages in cabbage worm, *Pieris rapae* Linne were measured using Biuret method and Warburg manometric method, respectively.

The results are summarized as follows:

1. The hemolymph protein contentration decreased gradually until the six-day pupa and increased thereafter. In the fifth larval instar the concentration reached a maximum and was about two times the value for the six-day pupa.

2. Endogenous respiration was very high at the prepupal stage, decreasing at two-day pupa, followed by an increase at the late pupal stage.

3. Glucose showed the marked activity throughout the whole stages, and had the striking influence on both the prepupal and adult stages.

4. The changes in protein concentration and the rate of oxygen consumption exhibited a general pattern of U-shaped curve through the process of life cycle according to histogenesis of the adult organ and histolysis of the larval organ.

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彙　　報

"과학의 날" 기념학술강연회

일시 : 1969 년 4 월 22 일
장소 : 서울대학교 문리과대학

연제 및 연사

산업개발과 생물학	고 대·이공대	김 창 환
생물의 생산성과 인류의 복지	서울대·사 대	최 기 철
출산력 조절과 생물학	서울대·문리대	조 완 규

한국 동물학회 1969 년도 춘계연구 발표회

일시 : 1969 년 5 월 27 일
장소 : 동국대학교

특별강연

Problems in the Endocrinology of the Chickens

美國 Illinois 大學 Dr. A.V. Nalvandov

哺乳類의 卵巢의 分化 및 機能과 腦下垂體호르몬과의 相互關係에 대한 硏究는 오늘날까지 널리 행하여져 왔다. 그러나 이 호르몬이 家禽 특히 닭의 卵巢나 生殖輸管에 미치는 영향에 대해서는 그다지 연구가 많지 않다. 哺乳類 중 양이나 돼지의 腦下垂體에서 추출한 濾胞刺戟호르몬(FSH) 및 黃體形成호르몬(LH) 그리고 닭의 腦下垂體分泌物이 닭의 卵巢나 生殖輸管에 미치는 영향에 대해 고찰해 보고자 한다.

우선 成熟한 닭에서 腦下垂體를 除去하면 卵巢는 약 2 週日 이내에 萎縮되고 거의 退化한다. 또한 輸卵管이나 子宮도 그 크기나 굵기가 극도로 줄어 든다. 이러한 닭에다 FSH 를 注射해 주면 점차로 卵巢의 濾胞가 生長하게 되고 卵黃의 크기도 는다. 계속하여 FSH 로 卵巢를 刺戟주고 LH 를 處理하면 하루에 한 개 씩의 鷄卵을 産卵하는 것이 아니라 異例的으로 3 개 내지 4 개씩 産卵하는 경우를 볼 수 있다. 哺乳類에서 얻은 腦下垂體호르몬의 作用이 크긴 하지만 닭의 腦下垂體에서 얻은 分泌物로 處理하면 卵巢의 過大分化의 경우가 더 두렷하게 나타난다.

그러한 處理를 받았을 경우 卵巢만이 아니라 輸卵管이나 子宮의 機能回復도 아울러 일어나고 있다. FSH 나 LH 와 같은 호르몬의 機能에 대해서는 오늘날까지 여러가지 實驗에 의하여 확인되고 있으나 그의 機作에 대해서는 아직 모호한 점이 많다.

연구발표논문 및 요지

초파리에서 바이러스에 의한 돌연변이

延世大 醫大　尹鍾浣·金元中

초파리(*D. melanogaster*)에서 誘癌바이러스의 1 종인 Rous—sarcoma virus 에 의한 遺傳的影響을 硏究하였다. 숫초파리의 ($sc^8 \cdot Y \cdot B^s/y^2w^1c^tf^1$)를 幼蟲에서 成蟲時까지 同 바이러스를 먹여 기른 後 그 生殖細胞에서 測定된 遺傳的 被害는 劣性致死 突然 變異率이 2.1%를 보였으며 染色體의 非分離, Y 染色體의 缺失 및 可視突然變異가 나타났다. gynandromorph 및 mosaic 의 出現은 生殖細胞의 突然變異와 함께 體細胞에서도 變異가 誘發됨을 暗示하고 있다.