Korean J. Zool. 32:337-350, 1989

Protein Patterns of Ovary and Hemolymph in *Gerris paludum*

Myung Sil Hwang and Won Chul Choi

Department of Biology, College of Natural Sciences,
Pusan National University

In this study, we compared patterns of hemolymph with ovarian proteins during the yolk formation of *Gerris paludum* by using SDS/polyacrylamide gel electrophoresis and two dimentional electrophoresis. In addition, we examined patterns of glycoprotein which were composed of yolk substances. The results were as follows: The protein patterns in ovary of the 5th instar without eggs were similar to those of adult after ovulation. Protein amounts of the ovary without developing eggs were less than those of the ovary containing oocytes or matured eggs at the molecular weights range from 66,000 to 110,000 daltons. No glycoproteins were observed in ovary without eggs. But the glycoproteins were gradually increased according to development of eggs in the 5th instar. After the ovulated ovary of adult, no glycoprotein bands were occurred as the bands of the ovary without eggs in the 5th instar. Also, amounts of hemolymph protein between 66,000 and 110,000 in molecular weight were increased during yolk formation of the 5th instar. The results suggest that ovarian protein substances may originate from hemolymph. In the 5th instar, the amounts of glycoprotein of developing eggs was gradually increased in the hemolymph.

The band of M.W. 29,500 was occurred in the hemolymph of the 5th instar and adult without eggs. This protein may be precursor of glycoprotein in the high molecular weight area in the ovary of more developed eggs. The number of spots and the amounts of protein in ovary without eggs were less than those of ovary containing eggs by two dimensional electrophoresis. The protein bands between 45,000 and 110,000 almost appeared in acidic field of two dimensional gel. Especially, the band, M.W. 109,000, was separated 3 spots, a_1, a_2, and a_3. The band, M.W. 102,000, has spots which designated b_1, b_2, b_3, and b_4. Besides, proteins below M.W. 24,000 were occurred less spots in the basic field than those of acidic field. The mechanism of intracellular organs (= cell organells) was related to the yolk protein synthesis of oocyte in the process of yolk protein formation of *Gerris paludum*.

KEY WORDS: **hemolymph, follicle cell, yolk protein, oocyte**

The ovaries of the insects have been established three types as panoistic, polytrophic meroistic, and telotrophic meroistic ovaries. The telotrophic ovaries have been studied in Heteroptera *Pyrrhocoris* (Mays, 1972), Coleoptera *Tenebrio* (Ulmann, 1963), and Hemiptera (Vanderberg, 1963). Especially Choi and Nagl (1976) have been reported about the differentiational and developmental process of trophocytes and oocytes, the structure of germarium and the patterns of DNA and RNA synthesis during the development of ovarian nurse cell in *Gerris najas*.

Besides, the research on the various patterns of ovarian protein and hemolymph protein in the developmental process of the ovary of *Drosophila* has been carried out by Park and Lee (1986). On the other hand, it has known that yolk protein transfer into oocyte via follicle cells through hemolymph after yolk substances are synthesized in either fatty body or ovary (Garabedian et al.,

1985; Garabedian et al., 1986). In present study, we compared hemolymph with ovarian total protein, and examined patterns of glycoprotein during the yolk formation of *Gerris paludum*.

Materials and Methods

Chemicals

Acryamide and N, N'-methylenebisacrylamide were re-crystalized in chloroform and aceton respectively before they were used. And also used were TEMED, urea, coomassie brilliant blue R 250, 2-mercaptoethanol (Junsei Chemical Co., Japan), and ammonium persulfate. In order to glycoprotein staining, tetrachloroacetate (TCA), periodic acid, Schiff's reagent and sodium metabisulfate were used.

Preparation of samples

The ovaries were isolated from *Gerris paludum* after they were gathered in the upper waters of DONG CHON river in Pusan, Korea. In the 5th instar, these ovaries were divided into individuals without eggs and containing eggs. And, 5th instar containing eggs were divided into four stages by their oocyte numbers as follows: stage I: 1-6 containing oocytes, stage II: 7-15 oocytes, stage III: 16-23 oocytes, and stage IV: 24 oocytes and more. In the case of the adult, two different periods were used, one of which had well-developed and filled with oocytes in vitellarium, the other of which had only germarium and empty-vitellarium existed after complete ovulation.

Hemolymph was gathered according through a capillary tube after the legs of *Gerris paludum* were cut.

The measurement of protein and SDS/polyacrylamide gel electrophoresis.

Protein was measured by the method of Lowry *et al.* (1951). For SDS/polyacrylamide gel electrophoresis, each ovary and hemolymph was homogenized in 0.0625 M Tris/HCl buffer (pH 6.8) containing 2 % SDS, 5 % 2-mercaptoethanol and 10 % sucrose, and were centrifuged for 20 min at 10,000 rpm. The supernatant of 800 μg per ml was heated to 100°C for 2 min and then cooled. A small amounts of bromophenol blue was added to the sample for tracking.

Resolving gel of 10 % and stacking gel of 5 % were used and buffer system was based on the method of Laemmli (1970). After the electrophoresis, the gels were stained with 0.1 % coomassie brilliant blue R 250 solution and then destained for 24 hours in the solution of 45 % isopropanol and 10 % acetic acid.

The stained gels were scanned at 570 nm with Toyo DMU-33c Densitometer (Japan), and the maximal range of optimal density was adjust 3.0.

The preparation for Glycoprotein

The ovaries and hemolymph were homogenized with homogenizing buffer, which had 2 % SDS, 0.0625 M Tris/HCl (pH 6.8), and then they were centrifuged. Each supernatant was heated to 100 °C for 2 min and then cooled and urea was added to it. Electrophoresis was conducted at 4°C at 150 V for 6 hours. The gels were stained with Periodic acid-Dansyle Hydrazing procedure after its electrophoresis.

Gel after its running, it was put to scanning by Densitometer under UV 750 nm, density range 1.0.

Molecular weights estimation of protein bands

In order to estimate the molecular weight of each protein band, the following known proteins were employed: Lysozyme (M.W. 14,300), β-lactoglobulin (M.W. 18,400), trypsinogen (M.W. 24,000), pepsin (M.W. 34,000), and bovine albumin (M.W. 66,000). These marker proteins were electrophoresized under the same treatment as the above mentioned samples and were calculated the molecular weight according to the computer program by Choi (1987).

Two-dimensional electrophoresis

(1) First dimension: The gel for isoelectric focusing employed 4 % (W/V) polyacrylamide which contained 9 M urea, 2 % (V/V) Nonidet P-40, 2 % (V/V) Ampholine (pH range 5-8 and 3-10). For the isoelectric focusing, the glass tube which was 130 mm in height and 22.5 mm in the inner diameter was applied. After the bottom of glass tube was sealed with parafilm it was filled with first

Table 1. Molecular weights of ovarian and hemolymph protein.

band	molecular weight	band	molecular weight
1	16,500	15	53,000
2	17,000	16	55,000
3	19,000	17	58,000
4	20,500	18	60,000
5	22,000	19	65,000
6	23,000	20	68,000
7	24,500	21	70,000
8	25,000	22	75,000
9	25,200	23	82,000
10	29,500	24	89,000
11	34,000	25	93,000
12	39,000	26	102,000
13	46,000	27	109,000
14	47,500	a*	34,900
		b*	52,400

a* and b*: these protein bands appeared in hemolymph only.

dimensional separation gel to 120 mm in height and the rest of the volume filled 20 μl of 8 M urea solution.

After the gel had been polymerized completely it was prerun by the method of O'Farrell (1975) for 2 hours. And then 70 μl of protein was injected into the gel. Running was performed at 400 V for 12 hours, followed by a run at 800 V for an hour.

(2) Second dimension: Immediately after first dimensional isoelectric focusing gel had been soaked in 0.0625 M Tris/HCl buffer (pH 6.8) (containing 2 % SDS, 5 % 2-mercaptoethanol and 10% sucrose), second dimensional electrophoresis was performed; otherwise, it was preserved at −70°C until used.

Second dimensional electrophoresis was performed in the same method as SDS/polyacrylamide gel electrophoresis. Running was carried out at 150 V for 5 hours at 4°C.

Results

In the 5th instar, the protein patterns of the ovary containing eggs and the ovary without eggs are shown Fig. 1, and those of the hemolymph

Table 2. Optimal densities of ovarian and hemolymph proteins in the 5th instar

band	Ovarian protein w/o	Ovarian protein w/ I	II	III	IV	Hemolymph protein w/o	Hemolymph protein w/ I	II	III	IV
1	2.50	2.55	2.50	2.70	2.70	2.32	2.30	2.15	2.20	2.20
2	0.80	1.10	0.53	0.62	0.60	2.32	2.30	2.15	2.20	2.20
3	0.50	1.55	0.64	0.50	1.10	1.30	0.92	1.15	1.29	1.31
4	0.59	0.60	0.72	0.52	0.46	2.10	0.86	1.00	1.08	1.12
5	0.42	0.53	0.60	0.54	0.41	0.60	0.55	0.56	0.51	0.61
6	0.61	0.55	0.60	0.52	0.43	0.43	0.49	0.42	0.43	0.48
7	0.72	0.71	0.60	0.55	0.43	0.23	0.28	0.30	0.30	0.33
8	0.73	0.70	0.61	0.55	0.45	0.23	0.32	0.32	0.33	0.30
9	1.11	0.72	0.70	0.68	0.50	0.20	0.36	0.27	0.25	0.25
10	0.45	0.49	0.78	0.80	0.80	0.19	0.27	0.25	0.29	0.20
11	0.42	0.85	0.91	2.48	2.43	0.24	0.23	0.27	0.29	0.30
12	0.41	0.48	1.20	1.40	1.58	0.38	0.30	0.30	0.35	0.35
13	0.42	1.10	1.08	0.95	0.80	0.62	0.59	0.67	0.75	0.78
14	1.20	0.65	1.41	1.20	0.90	0.34	0.40	0.45	0.45	0.45
15	1.00	0.58	1.00	1.00	0.90	0.30	0.33	0.39	0.40	0.41
16	0.92	0.50	0.73	0.78	0.82	0.43	0.58	0.70	0.67	0.74
17	0.58	0.40	0.64	0.90	1.20	0.29	0.45	0.53	0.49	0.55
18	0.62	0.32	0.70	0.68	0.80	1.22	0.58	0.72	0.64	0.88
19	0.47	0.60	1.50	1.78	2.10	1.50	0.41	1.63	1.75	2.03
20	0.82	0.30	0.75	0.70	0.83	0.65	1.50	1.16	1.13	1.20
21	0.52	0.30	0.62	0.55	0.60	0.65	0.90	1.01	1.00	1.00
22	0.65	0.52	1.40	1.80	1.99	0.34	0.40	0.45	0.40	0.45
23	0.65	0.42	0.98	0.90	1.00	0.27	0.30	0.38	0.36	0.39
24	0.42	0.15	0.61	0.63	0.90	0.35	1.08	1.28	1.17	1.33
25	0.49	0.20	0.83	1.02	1.40	0.30	0.35	0.45	0.47	0.48
26	0.30	0.43	1.70	1.20	0.95	0.80	1.15	1.38	1.30	1.43
27	0.39	0.50	1.70	2.23	2.37	1.88	1.38	1.77	2.10	2.03

w/o: ovary without oocytes in vitellarium
w/ :ovary containing oocytes in the vitellarium.
I : stage of the 5th instar containing 1-6 oocytes.
II : stage of the 5th instar containing 7-15 oocytes.
III: stage of the 5th instar containing 16-23 oocytes.
IV : stage of the 5th instar containing over 24 oocytes.

are as in Fig. 2. In the adult, the patterns of the ovary filled with oocytes and the ovary of the completed ovulation were compared (Fig. 3.), and those of the hemolymph are shown in Fig. 4. The molecular weights of the ovarian and hemolymph protein are as in Table 1.

(1) The 5th instar: The protein patterns of the ovary without eggs and the ovary containing eggs in the 5th instar were studied (Fig. 1). The ovary containing eggs was divided into four stages as

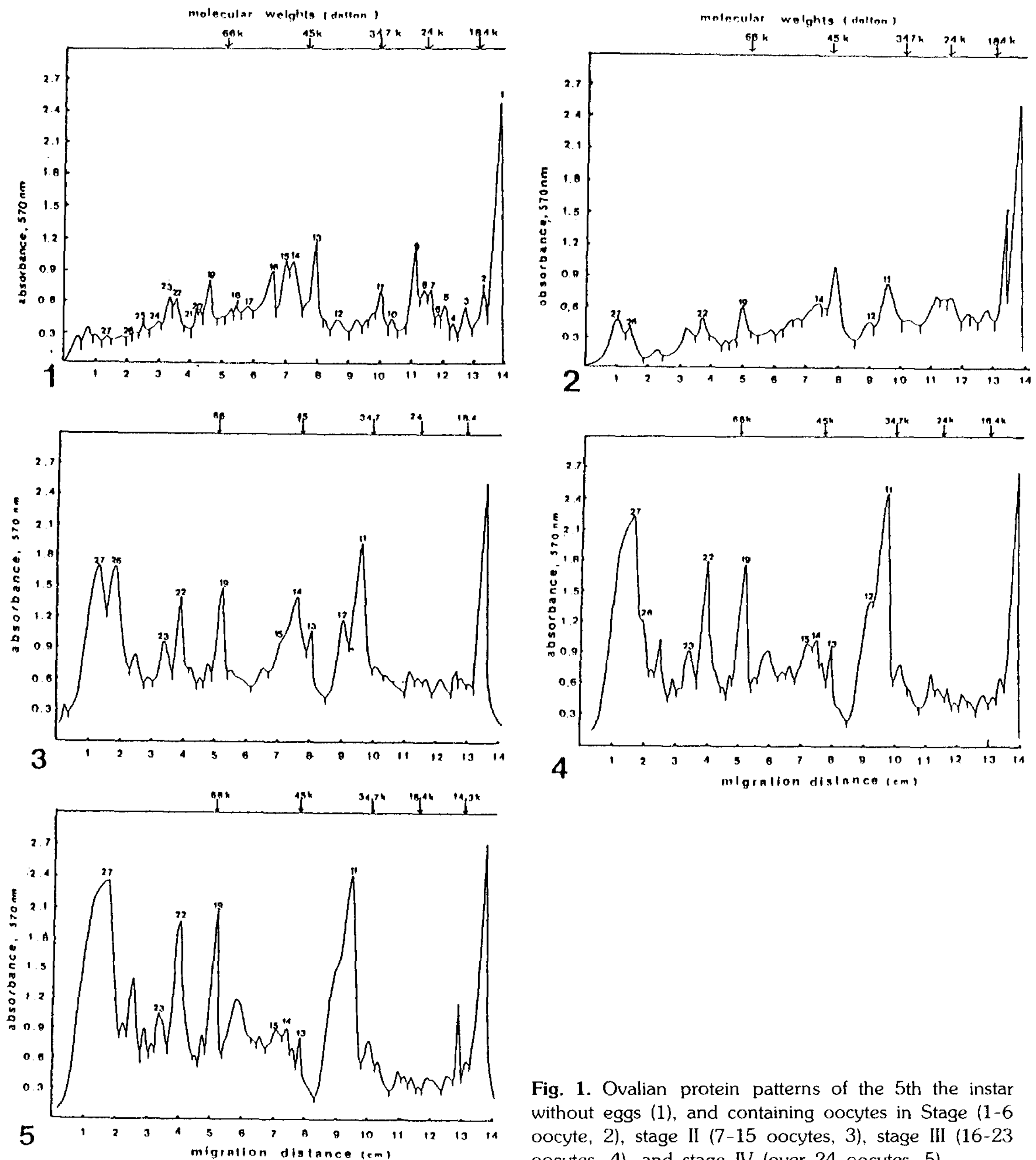

Fig. 1. Ovalian protein patterns of the 5th the instar without eggs (1), and containing oocytes in Stage (1-6 oocyte, 2), stage II (7-15 oocytes, 3), stage III (16-23 oocytes, 4), and stage IV (over 24 oocytes, 5).

follows: stage I (1-6 oocytes), stage II (7-15 oocytes), stage III (16-23 oocytes), and stage IV (24 oocytes and more). Also, the optimal densities of ovarian and hemolymph protein in each stage are as in Table 2.

Among the protein bands that have more than M.W. 34,700, the amounts of protein with bands 11, 12, 14, 19, 22, and 27 are particularly abundant. However, the protein of band 26 (O.D.: 0.43) is almost the same as that of band 27 (O.D.:

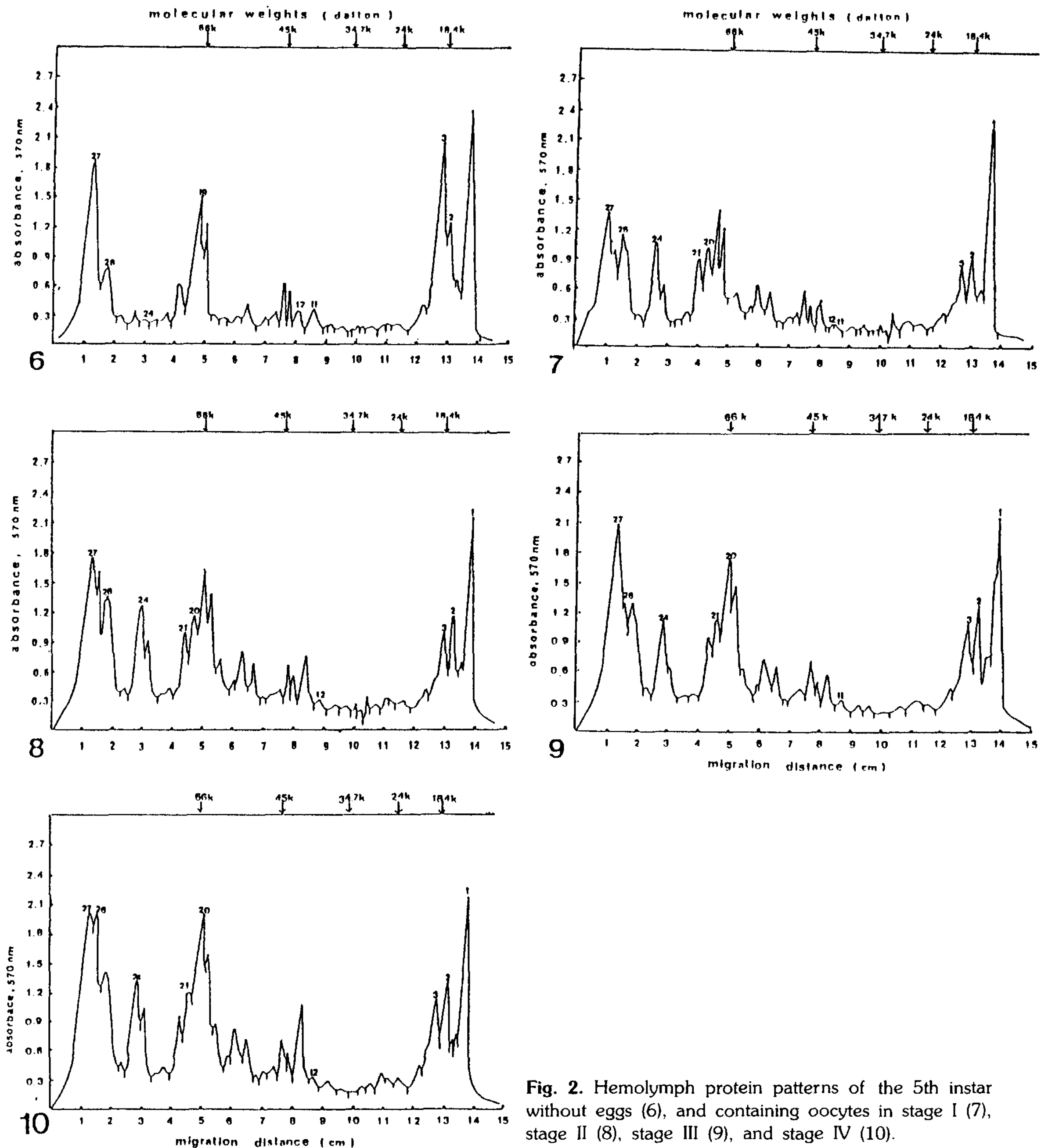

Fig. 2. Hemolymph protein patterns of the 5th instar without eggs (6), and containing oocytes in stage I (7), stage II (8), stage III (9), and stage IV (10).

0.50) at the stage I but as the stage varies from the second to the third to the fourth, the protein of band 27 was increased (Table 2).

On the contrary, the amounts of protein of bands 13, 14, 15 and 16 were increased at the stage I and stage II but decreased at the stages III and IV. In the case that the stages varies from I to IV, the amounts of protein in bands 20, 23, and below the M.W. 34,700 were less than those of the bands of more than M.W. 34,700, and there

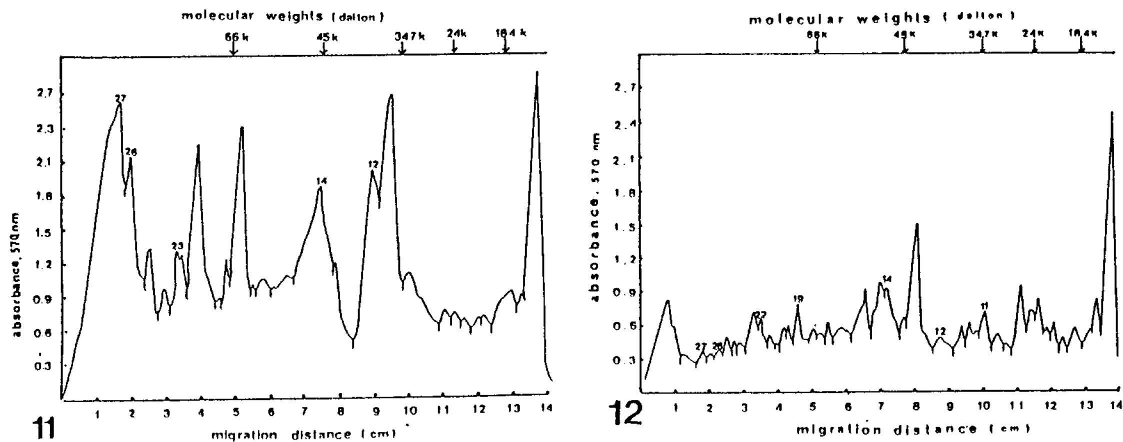

Fig. 3. Ovanian protein patterns of the adult containing mature eggs (11) and after ovulation (12).

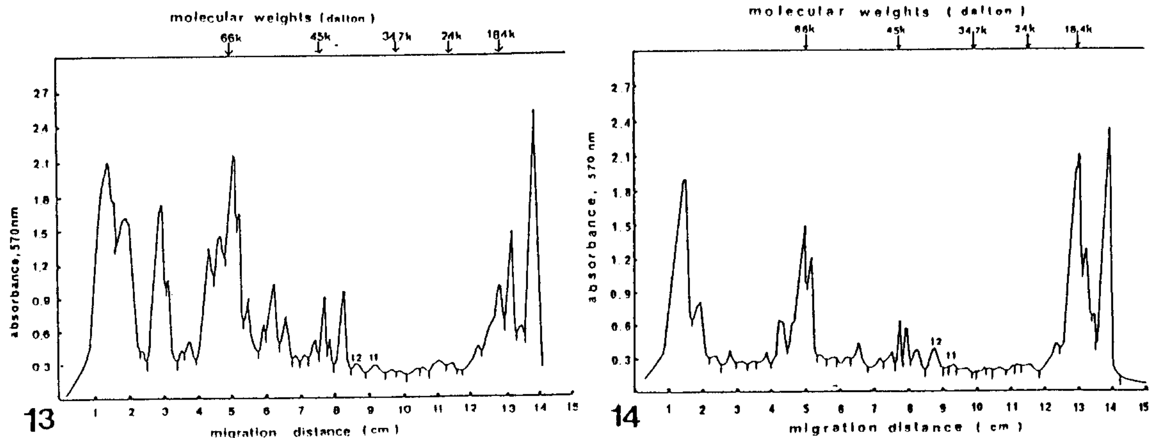

Fig. 4. Hemolymph protein patterns of the adult containing mature eggs (13) and after ovulation (14).

was little change in quantity.

When the hemolymph protein was compared the ovary containing eggs with the ovary without eggs, the proteins between M.W. 66,000 and 110,000 were increased, and also the amounts of hemolymph protein was increased in the same way as the change in quantity of the protein of ovary containing eggs according to the stages in the 5th instar. The bands 19, 20, 21, 24, 26, and 27 of hemolymph protein had more than optimal density 0.35 in the whole stage, and these of bands 26 and 27 were increased the stage I through the stages III, but decreased at the stage IV.

In the protein amounts of the ovary containing eggs, the band 26 was decreased than the band 27 in whole stages. But that of the band 26 in the hemolymph protein was similar with the band 27. And the band 1, 2, and 3 of hemplymph had more protein than the ovaries containing eggs (Fig. 2). The amounts of the protein of the band 11 and 12 which abundantly existed in the ovary containing eggs were not observed in hemolymph protein in this instar.

Glycoprotein was not observed in ovaries without eggs but in the ovaries containing eggs it was appeared between M.W. 66,000 and 110,000 in the 5th instar, and in addition, the increase in quantity was appeared throughout the whole stages (Fig. 5). The glycoprotein of hemolymph in the ovary without eggs had only band A (M.W. 29,500) (Fig. 6), and band A in glycoprotein of

Table 3. Optimal densities of ovarian and hemolymph proteins of the adult.

band	Ovarian Protein		Hemolymph protein	
	c/e	ovu	c/e	ovu
1	2.85	2.49	2.55	2.32
2	0.89	0.89	0.63	0.75
3	0.90	0.55	1.51	1.25
4	0.83	0.56	1.01	2.01
5	0.71	0.45	0.66	0.45
6	0.70	0.58	0.44	0.22
7	0.70	0.80	0.30	0.22
8	0.73	0.70	0.32	0.20
9	0.75	0.92	0.25	0.20
10	1.10	0.72	0.25	0.24
11	2.68	0.62	0.30	0.40
12	2.02	0.53	0.30	0.40
13	1.20	1.50	0.90	0.63
14	1.89	0.92	0.52	0.33
15	1.23	1.00	0.40	0.30
16	1.08	0.68	0.74	0.44
17	1.05	0.59	0.70	0.30
18	1.00	0.60	0.91	0.35
19	2.44	0.42	2.17	1.50
20	1.26	0.52	1.46	0.65
21	0.89	0.72	1.35	0.65
22	2.28	0.55	0.52	0.35
23	1.32	0.76	0.45	0.27
24	1.00	0.50	1.72	0.40
25	1.35	0.52	1.42	0.33
26	2.0	0.40	1.61	0.82
27	2.62	0.89	2.10	1.90

c/e: ovary containing eggs in vitellarium.
ovu: ovary after ovulation.

hemolymph of the ovary containing eggs more rapidly was decreased than in the ovary without eggs. In particular, bands (M.W. 109,000, 93,000, 89,000, 75,000, and 65,000), which were not observed in the ovary containing eggs, existed between M.W. 65,000 and 110,000, and the glycoprotein of hemolymph in the ovary containing eggs was increased gradually (Fig. 6).

(2) The adult: The amounts of bands 12, 14, 23, and 26 were greatly increased in the protein of the ovary containing the well-developed eggs right before ovulation. On the other hand, the band 27 was decreased (Table 3).

But the protein patterns of adult having only germarium after ovulation were quite different from those patterns before ovulation (Fig. 3), they were rather similar to those in the ovary before the egg formation of the 5th instar. The amounts of protein between M.W. 45,000 to 110,000 dramatically decreased (Tabel 3).

Nevertheless, there was no increased in quantity between the hemolymph protein of the ovary containing eggs of the 5th instar and that of the adults right before ovulation. The protein of the band 23 in the hemolymph of the ovary containing eggs of the 5th instar and that of the a great deal in that of the adult.

But, the bands 11 and 12 which showed the high optimal density in the adult ovary containing eggs, were hardly occurred in the hemolymph. The amounts of the bands 3 and 4 of hemolymph were higher appeared than those of ovaries (Table 3). In the glycoprotein patterns, proteins between M.W. 66,000 and 110,000 in the adult ovary protein containing eggs (Fig. 5) were more increased than in the containing eggs in the 5th instar. On the contrary, the glycoprotein was not occurred in the ovary after ovulation in the adult. This phenomenon was similar to the ovary without eggs in the 5th instar.

The glycoprotein of adult hemolymph of the ovary containing eggs was found less than that of the ovary containing eggs in the adult. In the case of adult hemolymph after ovulation, the glycoproteins between M.W. 66,000 to 110,000 were hardly observed except for the glycoprotein band of M.W. 25,000 (Fig. 7).

(3) Two-dimensional electrophoresis: The ovarian protein patterns of the 5th instar and the adult using by two-dimensional electrophoresis are as in Fig. 8. The ovarian proteins between M.W. 18,400 and 110,000 of the ovary without eggs in the 5th instar appeared 14 spots and they had little amounts of the protein. The protein patterns of the ovary containing eggs in the stage I and II of the 5th instar were isolated in many bands and much more the protein quantity than that of the ovary without eggs between M.W. 66,000 to 110,000. The patterns of the ovary without eggs and the ovary containing eggs in the stage I through stage IV were nearly constant without an increase in quantity of spots 6, 7, and 8.

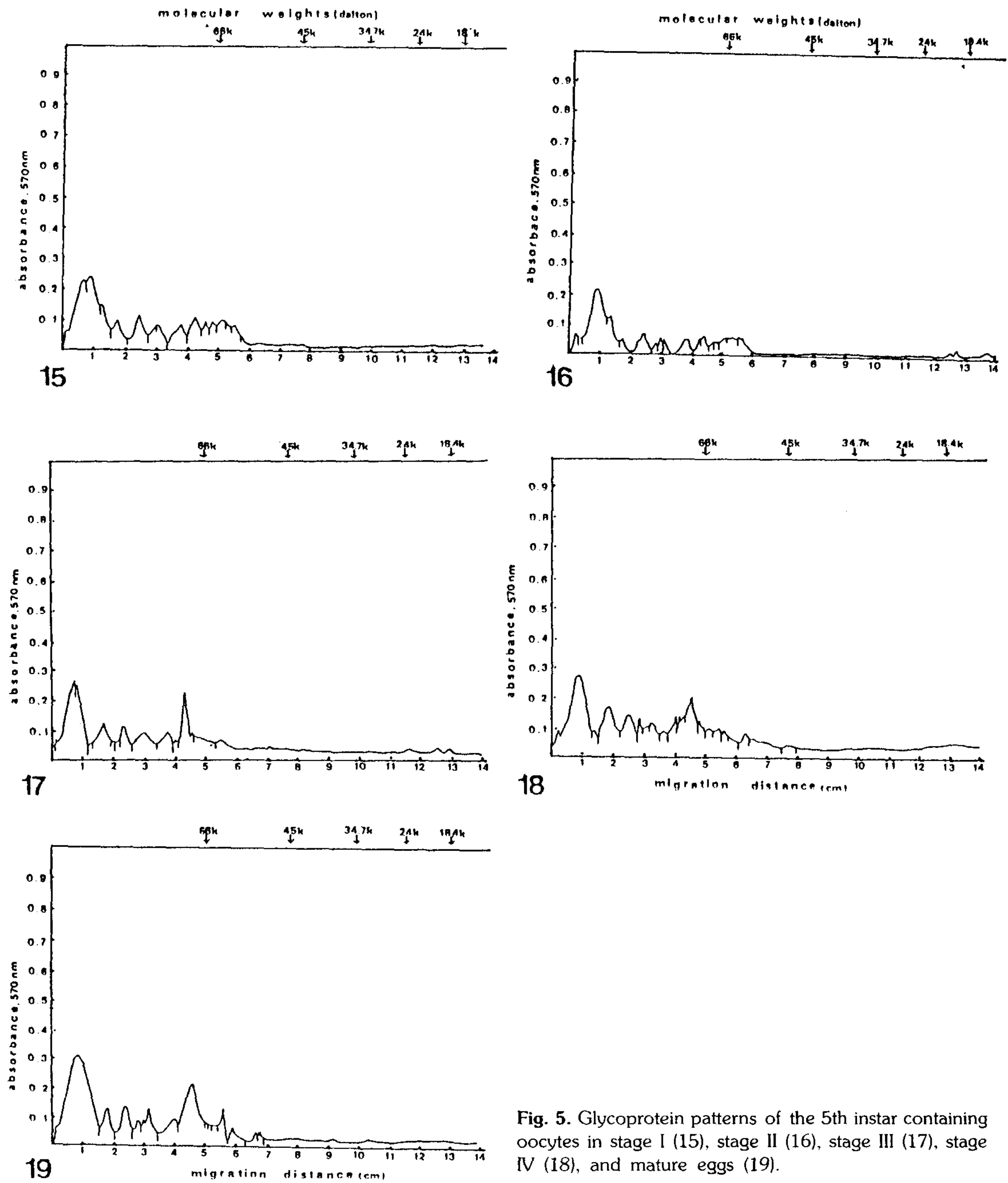

Fig. 5. Glycoprotein patterns of the 5th instar containing oocytes in stage I (15), stage II (16), stage III (17), stage IV (18), and mature eggs (19).

In the stage I and the stage II (Fig. 8), the band of M.W. 109,300 was divided into three different spots, a_1, a_2, and a_3 in terms of IP (isoelectric point). Protein spots a_1 and a_2 were occurred at the stage III and IV by two dimensional electrophoresis, but spot a_3 was disappeared at these stages. Also the band of M.W. 102,000 had four different spots of b_1, b_2, b_3, and b_4 but b_1 and b_2 were not found on the part of stage III and stage IV. The protein amounts of b_4 in the stage III and

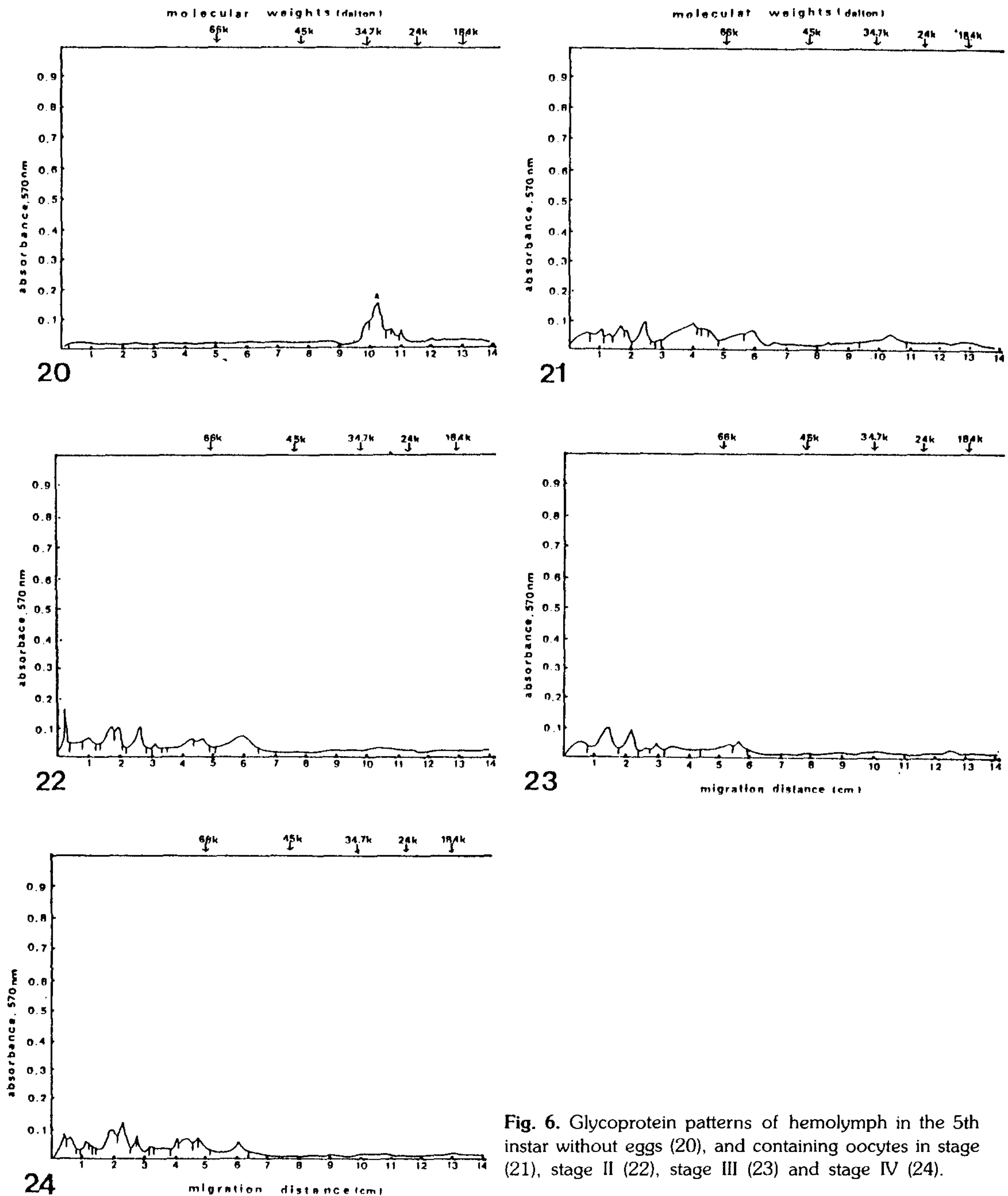

Fig. 6. Glycoprotein patterns of hemolymph in the 5th instar without eggs (20), and containing oocytes in stage (21), stage II (22), stage III (23) and stage IV (24).

IV were more increased than the stage I and II.

The protein bands which are more larger than M.W. 34,000 in the developing eggs, were separated on the acidic field (pH 3-6) of the two dimensional electrophoretic gel. The protein that has less than M.W. 34,700 was distributed throughout the entire field of pH 3 to pH 10. The protein patterns of the ovary right before ovulation and of the developing eggs in the 5th instar were almost similar patterns, and those after

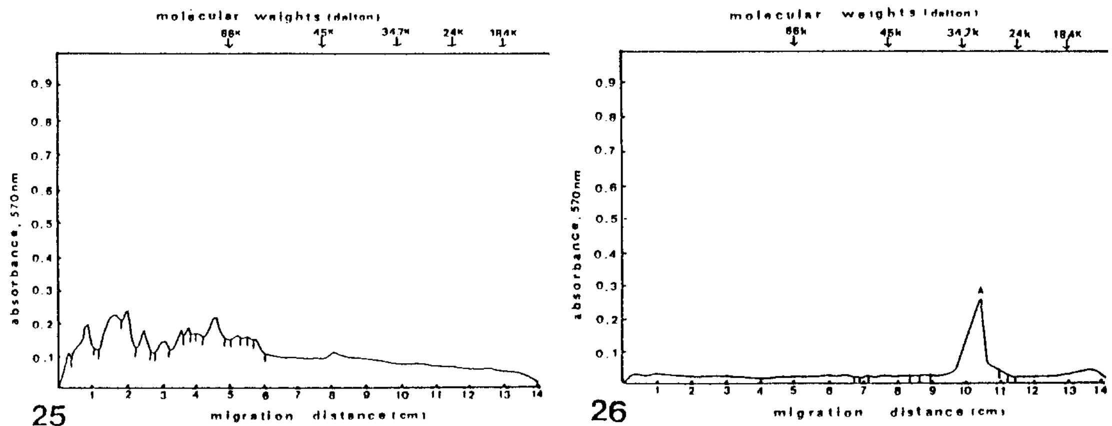

Fig. 7. Glycoprotein patterns of hemolymph in the adult containing mature eggs (25) and after ovulation (26).

Fig. 8. Two-demensional electrophoretic analysis of ovarian proteins patterns of in the 5th instar without eggs (27), and containing oocytes in stage I (28), stage II (29), stage III (30) and stage IV (31). Ovarian protein patterns of the adult containing mature eggs (32) and after ovulation (33).

ovulation were the same as the 5th instar without eggs.

Discussion

Gerris paludum (Heteroptera) belongs to telotrophic meriostic ovary. The nutritive substances, protein or ribonucleic protein (RNP), which are essential to the development of oocyte, are made in trophocyte and accumulated into trophic core and then transferred into oocyte via the nutritive cord. Choi and Nagl (1977) established that synthesized RNP is transferred to oocyte from the trophic core. It was suggested that the volume of oocyte in the 5th instar dramatically increase than that of the 4th instar in the increased rate of protein synthesis. Although the adult have lower RNA synthesis than the 5th instar, trophocytes nuclei of all region of germarium were synthesized RNA (Choi and Nagl, 1977). This phenomenon was provided sufficient protein into ooplasm. Some authors reported that the precusor of yolk protein was found in hemolymph proteins reach to the surface of oocyte by intercellular route (Chen and Levenbook, 1966; Chippendale and Beck, 1966; Kim and Seo, 1981; Park and Lee, 1987; Patel and Schneiderman, 1969; Telfer, 1961; Tobe and Loughton, 1969). This hypothesis was proved that many proteins of in the yolk forming the oocyte are immunologically indistinguishable from proteins in hemolymph, and oocytes (Telfer, 1961; Pelherr, 1975). In this realtionship between the yolk formation of oocyte and hemolymph protein, it suggested that the oocytes take up proteins from the hemolymph during their period of yolk formation. Therefore, the important metabolic pathway that the protein of hemolymph is arrived at oocyte through intercellular route and is formed the yolk, has been described in *moth* by Telfer (1961). The relationship between the yolk protein and the hemolymph protein is still under much research (Chen and Levenbook, 1966; Chippendale and Beck, 1966; Telfer, 1961; Tobe and Loughton, 1969). But many reports have not been found on the distribution of the hemolymph in ovarian cells for the yolk formation. The hemolymph protein between M.W. 66,000 and 110,000 also increase similarly as the amounts of ovarian protein increase gradually in *Gerris paludum*. Hence the proteins with such molecular weights are the ones believed to be transported through the hemolymph for the yolk development. The bands 2, 3, 4, and 5 seem to be the protein of hemolymph that they were composed of large amounts of protein in the hemolymph, but they were not observed in the ovarian protein.

On the other hand, some authors reported that the hemolymph protein become associated with a brush border of the oocyte surface in *Saturniid moth* (polytrophic ovary type), and that they are accumulated within the protein yolk spheres (Telfer, 1961; Bast and Telfer, 1976). But there is not known a mechanism that the hemolymph protein may pass directly from the surface of the oocyte into the growing yolk spheres. However the oocyte of *Saturniid moth* and the pigments of hemolymph were the protein binding pigments. The hemolymph proteins is related with the yolk formation can be confirmed on the ground that carotenoid protein in the hemolymph and the yolk, and the female protein which appeared in the ovary only are moved to these pigments within the oocyte (Telfer, 1961). This fact is different from *Gerris paludum*. We believed that it is not always true that the whole proteins of the oocyte are come from hemolymph protein, and the yolk protein formation from the hemolymph is quite different way in the *Gerris paludum*, that the hemolymph of *Gerris paludum* may drive to follicle cells. And the follicle cells uptake the protein from hemolymph.

Choi and Nagl (1977) demonstrated that the ribonucleic protein synthesized in the follicle cells, and then gathered together in the trophic core, and then were moved into the growing oocyte via nutritive cord in *Gerris najas*. Therefore, the transport mechanism of protein the hemolymph is neither the using by intercellular route or the hemolymph protein passing to the surface of the oocyte directly.

The protein in the bands 11 (M.W. 39,000), and 12 (M.W. 46,000) in the hemolymph were little amounts in the ovary without eggs and the ovary containing eggs, but they existed abundantly in the ovary during the process of egg development. It seems to be that the precursor of the protein in hemolymph are dramatically synthesized after they

come into the follicle cells and migrated through the trophic core and the nutritive cord into the oocyte. Hence, although the protein of hemolymph was appeared a little amounts, the little amounts of hemolymph is essential enough to the development of the eggs. The protein bands, M.W. 18,400 and 24,000, are found very little in the both, the ovary without eggs and the ovary containing the developing eggs, and they are simply thought to be the structural protein of the ovary.

As it grow to adult, the amino acid composition of hemolymph exists much threonine, serine more acidic than isoluecine and leucine at a constant rate (Levenbook and Dinamarca, 1966).

For this reason according as the most proteins from M.W. 66,000 to 110,000 are distributed on the acidic field in the two dimensional electrophoresis, the components of the protein in the development of yolk substances are mainly acidic protein. The bands, M.W. 34,000, 39,000, and 46,000, are kept constant irrespectively of the egg development, and these same bands are divided into spots 6, 7, and 8 on the basic field of the two dimensional electrophoresis. So, as the molecular weights get higher, the proteins are situated in acidic field and some protein containing low molecular weights are occurred in the basic field.

Glycoprotein which binds to the protein of the relative high molecular weight is increased in quantity according to the egg development in the 5th instar. It shows that the glycoprotein is necessary component of high molecular weight yolk substances. The phenomenon that there is no glycoprotein in ovary after completed ovulation corresponds to another phonemonon that there is also no glycoprotein may be composed of the yolk protein of the high molecular weight in matured eggs. On the other hand, glycoprotein is stored in golgi complex in which it is transformed after its core sugar is acquired from dolichol donor in endoplasmic reticulum (Stryer, 1981). Hence glycoprotein was synthesized in ER of oocyte in the process of maturation, then combined with yolk protein and was constituted one part of the mature yolk protein. Choi (1977) established that mitochondria of cell orgnelles are related to the yolk formation of *Gerris*. When the egg eventually grows fully mature, mitochondria starts to function in order to form the mature yolk droplet and yolk granule gets bigger and so accumulated to turn into complete yolk protein by the mitochondria (Anderson, 1964; Choi, 1977).

The glycoproteins of hemolymph appeared one band (M.W. 29,500) in the ovary without eggs in the 5th instar and the ovary after ovulation in the adult, but this band is not observed in the ovary containing of the developing eggs. As the egg development started, the glycoprotein bands were found in the area of the high molecular weight. This phenomenon seems that the substances of high molecular weights in hemolymph are used to constitute the yolk protein fromation through the follicle cells.

The change of hemolymph protein according to the egg development is sometimes controlled by the hormone out of neurosecretory cell of insects brain (Chippendale and Beck, 1966; Tomsin and Moller, 1963; Park and Lee, 1980), exspecially that can be stimulated while it is treated with Junevile hormone analogue when yolk polypeptide out of puparium is synthesized within female *Drosophila melanogaster* (Jowett and Postlethwait, 1980). It was believed that hormone is related to its translation or transcription, but clear evidences have not been reported yet. Also, it is not clear whether the synthesis of yolk protein in ovary is purely stimulated by Juvenile hormone analogue or it results from the inter-reaction of other factors.

Futher research should be carried out to study the hormone factors according to yolk formation and development synthesis mechanism of hemolymph and the inter-reaction between hemolymph protein and yolk protein in follicle cells.

References

Anderson, E., 1964. Oocyte differentiation and vitellogenesis in the roach *Periplaneta americana*. *J. Cell Biol.* **20**:131-155.

Barnett, T., and P. Wensink, 1981. Transcription and translation of yolk protein mRNA in the fat bodies of *Drosophil. ICN-UCLA Symposia on Molecular and Cellular Biol.* **103**:97-116.

Bast, R. E., and W› H. Telfer, 1976. Follicle cell protein synthesis and its contribution to the yolk of the *Cercropia moth oocyte. Dev Biol.* **52:**83-97.

Chippendale, G. M., and S. D. Beck, 1966. Haemolymph proteins of *Ostrinia nubilalis* during diapause and prepupal. *J. Insect Pysiol.* **12:**1629-1638.

Chen, P. S., and L. Levenbook, 1966. Studies on the hemolymph proteins of the blowfly *Phormia regina*-I. Changes in ontogenetic pattern. *J. Insect Physiol.* **12:**1595-1609.

Choi, W. C., 1977. Einige ultrastruktulle aspekte der dotterbildung der telotrophischen ovarien bei *Gerris najas* DEG. *These Coll. Korea soc. Work Coll.* **7:**227-238.

Choi, W. C., 1987. Computer program for estimation of molecular weight by electrophoretic method. *Nature and Life* **17:**29-34.

Chio, W. C., and W. Nagl, 1976. Electron microscopic study of the differentiation and development of trophocytes and oocytes in *Gerris najas* (Heteroptera). *Cytobios* **17:**47-62.

Choi, W. C., and X. Nagl, 1977. Patterns of DNA and RNA synthesis during the development of ovarian nurse cells in *Gerris najas* (Heteroptera). *Dev. Biol.* **61:**262-272.

Eckhart, A. E., C. E. Hayes, and I. J. Goldstein, 1976. A Sensitive method for detection of glycoproteins in polyacrylamide gels. *Anal. Biochem.* **73:**191-197.

Garabedian, M. J., Hung, M. C. and Wensink, P. C., 1985. Independent control elements that determine yolk protein gene expression in alternative *Drosophila* tissues. *Proc. Natl. Acad. Sci. USA* **82:**1369-1400.

Garabedian, M. J., Shepherd, B. M., and wensink, P. C., 1986. A tissue-specific transcription enhancer from the *Drosophila* yolk protein 1 gene. *Cell* **45:**859-867.

Gelti-Douka, H., T. R. gingeras, and M. P. Kambyselli, 1974. Yolk protein in *Drosophila* :Identification and site of synthesis. *J. Exp. Zool.* **187:**167-172.

Giorgi, F., and J. Jacob, 1977. Recent findings in oogenesis of *Drosophila melanogaster.* I. Ultrastructural observation on developing ooplasm. *J. Embryo. Exp. Morph.* **38:**115-124.

Handler, A. M., and J. H. Postlethwait, 1977. Endocrine control of vitellogenesis in *Drosophila melanogaster*: Effects of the brain and corpus allatum. *J. Exp. Zool.* **202:**389-402.

Hill, L., 1962. Neurosecretory control of haemolymph protein concentration during ovarian development in the desert locust. *J. Insect Physiol.* **8:**609-619.

Jowett, T., and J. H. Postlethwait, 1980. The regulation of yolk polypeptides synthesis in *Drosophila* ovaries and fat body by 20-hydroxyecdysone and a juvenile hormone analog. *Dev. Biol.* **80:**225-234.

Kim. H. R., and E. W. Seo, 1981. A change of haemolymph proteins during metamorphosis of *Pieris rapae L. Korean J. Entomol.* **11:**33-41.

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:**680-685.

Levenbook L., and M. L. Dinmarca, 1966. Free amino acids and related compounds during metamorphosis of the blowfly *Phormia reginal. J. Insect Physiol.* **12:**1343-1362.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:**2654-275.

Mays, U., 1972. Stofftransport in Ovar von *Pyrrhocoris aoteurs* L. Autoradiographische untersuchungen zum Stofftransport von de Naarzellen zur oocyte der Feuerwanze *Phyrrhocoris apterus* L. (Heteroptera). *Z. Zellforsch.* **123:**395-410.

Nakanish, Y., and A. Garen, 1984. Selective gene expression induced by ecdysterone in cultrued fat bodies of *Drosophila. Proc. Natl. Aca. Sci.* USA. **80:**2971-2975.

O'Farrell, P. H., 1975. High resolution two-dimensional electrophoresis of protein. *J. Biol. Chem.* **250:**4007-4021.

Park, S. S., and Y. L. Lee, 1987. Changes of protein sythesis during oogenesis of *Drosophila melanogaster. Korean J. Zool.* **30:**10-28.

Patel, N. G., and H. A. Schneiderman, 1969. The effects of perfusion on the synthesis and release of blood proteins by diapausing pupal of the *Cecropia silkworm. J. Insect Physiol.* **15:**643-660.

Petri, W› H., A. R. Myman, and F. C. Kafatos, 1976. Specific protein synthesis in cellular differentiation. *Dev. Biol.* **49:**185-199.

Postlethwait, J. H., and P. Gray, 1975. Regulation of acid phosphatase activity in the ovary of *Drosophila melanogaster. Dev. biol.* **47:**196-205.

Postlethwait, J. H., M. Bownes, and T. Jowet, 1980. Sexual phenotype and vetellogenin synthesis in *Drosophila melanogaster. Dev. Biol.* **79:**379-387.

Rubenstein, E. C., T. J. Kelly, M. B. Schwartz, and C. W. Wood, 1982. In vitro synthesis and secretion of ecdysteroids by *Drosophila melanogaster* ovareis. *J. Exp. Zool.* **223:**305-308.

Shirk, P. D., P. Minoo, and J. H. Postlethwait, 1983. 20-hydroxyecdysone stimulates of translatable yolk polypeptide gene transcript in adult male *Drosophila melanogaster. Proc. Natl. Acad. Sci. USA.* **80:**186-190.

Stryer, L., 1981. Biochemistry. 2nd Ed. W. H. Freeman and company, San Francisco 714-719.

Telfer, W. H., 1961. The route of entry and localization of blood proteins in the oocytes of *Saturniid moths. J.*

Biophy. biochem. Cytol. **9**:747-759.

Thomsen, E., and I. Moller, 1963. Influence of neurosecretory cells and of corpus allatum on intestinal protease activity in the adult *Calliphora erythrocephala* Meig. *J. Exp. Biol.* **40**:301-321.

Tobe, S. S., and B. G. Loughton, 1969. An autoradiographic study of haemolymph protein uptake by the tissue of the fifth instar locust. *J. Insect Physiol.* **15**:1331-1346.

Ulmann, s., 1973. Oogenesis in *Tenebrio molitor*: Histological and autoradiographical observations on pupal and adult ovaries. *J. Embryo. exp. Morph.* **30**:179-217.

Vanderberg, J. P., 1963. Synthesis and transfer of DNA, RNA and protein during vitellogenesis in *rhodnius prollixus* (Hemiptera). *Biol. Bull.* **125**:556-575.

(Accepted August 15, 1989)

---

*Gerris paludum*의 난소와 혈림프에서 단백질 양상

황명실 · 최원철(부산대학교 자연대 생물학과)

본 연구에서는 SDS/polyacrylamide젤 전기영동과 2차원 전기영동을 사용하여 *Gerris paludum*(소금쟁이)의 난 형성 동안 난소 단백질과 혈림프 단백질을 비교하였다. 또한 난물질의 구성성분인 glycoprotein의 양상을 살펴 보았다.

결과는 다음과 같다.

난을 갖지 않는 5령의 난소 단백질의 양상을 성체의 산란 후 난소 단백질 패턴과 유사하였다. 난 발달동안의 난소의 단백질중 M.W. 66,000-110,000 사이의 단백질이 난을 갖지 않은 난소 단백질에 비해 많은 양이 존재하였다. 난을 갖지 않은 난소에서는 glycoprotein은 나타나지 않았다. glycoprotein은 5령의 난 발달이 진행됨에 따라 점차적으로 증가하였다. 산란후 성체의 난소에서는 5령의 난을 갖지 않는 난소에서처럼 glycoprotein은 나타나지 않았다.

또한, M.W. 66,000-110,000 사이의 혈림프 단백질은 5령의 난 형성 동안 양적으로 증가하였다. 이러한 결과는 난소 단백질 중 일부를 혈림프로부터 얻을 것이라는 사실을 제시하는 것이다. 발달하고 있는 난의 glycoprotein은 이 단계의 혈림프에서도 점차 증가하였다. 낮은 분자 영역에서 하나의 band (M.W. 9,500)가 난을 갖지 않는 5령과 성체의 혈림프에서 분리되었다. 이 단백질은 고분자량의 영역(M.W. 66,000-110,000)에서 나타나는 glycoprotein 전구체라고 생각된다.

2차원 전기영동에서는 난을 갖지 않은 난소 단백질이 난을 가지고 있는 난소 단백질에 비해 적은 spots로 분리되었고 그 양도 적었다. M.W. 45,000-110,000 사이의 band들은 2차원 전기영동에서 산성쪽으로 분리되어 나타났다. 특히, M.W. 102,000의 분자량을 가지는 band는 4개의 spots b_1, b_2, b_3 및 b_4로 분리되었다. 반면에 M.W. 24,000이하의 단백질들은 산성쪽의 단백질보다 염기성쪽으로 분리되었다.

*Gerris paludum*의 난황 형성과정에 있어서 세포내 기관(= 세포 소기관)의 기작이 난세포의 난황단백질 합성과 관계를 가지고 있었다.