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# The Comparison of Protein Patterns of Several Species in Bivalvia by SDS Polyacrylamide Gel Electrophoresis

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# SDS Polyacrylamide Gel 電氣泳動에 依한 斧足綱數種의 蛋白質패턴의 比較

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## 요 약

斧足綱 數種의 蛋白質을 電氣泳動한 結果, 새꼬막과 피조개의 血漿의 蛋白質 패턴은 低分子 蛋白質에서 染色되는 强度가 약간 差異가 있으나 거의 同一하였고, 全血球의 蛋白質 패턴의 差異는 17,800 dalton의 band에서 나타났다.

그렇지만 위의 두 種의 筋肉의 蛋白質 패턴은 거의 비슷해서 10,000~100,000 dalton의 分子量 範圍內에서는 差異를 알아 내기가 매우 어려웠다. 그런데 대합과 비늘백합은 類緣關係가 있는 蛋白質 패턴이 많이 나타났으며 재첩은 대합이나 비늘백합과 같은 이들 두 種과 대칭이의 中間 程度에 位置하는 蛋白質 패턴을 나타내고 있었다.

본 연구에서는 Bivalvia가 함께 다 갖는 6개의 蛋白質 band들과, 꼬막과 새꼬막에서만 나타나는 4개의 特有한 蛋白質 band가 存在함이 發見되었으며 Eulamellibranchia目의 4종에 있어서 다 나타나는 2개의 protein band들 과, 대합과 비늘백합에서만 나타나는 23,000 dalton의 特有한 band도 發見되었다. 그리고 各 種마다 가지는 特有한 蛋白質 패턴의 分子量을 測定하여 比較하였다.

## INTRODUCTION

It is a unique characteristic that *Scapharca subcrenata* and *Scapharca broughtonii* of Filibranchia in Bivalvia contain the red color of blood, but most of the other species of

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Filibranchia do not.

Park et al (1974a) attempted to explain the relationship among species, distinguishing ten species of Bivalvia by using the cellulose acetate strip electrophoresis. Lactate dehydrogenase and esterase of *Carassius carassius* (Lee and Choo, 1973) and hemoglobin of anura (Park et al, 1974b) were also reported. Especially, Singh and Coulthart (1981) reported on the pattern of soluble protein of *Drosophila* by polyacrylamide gel electrophoresis. To investigate how much the same molecular weights exist in protein patterns by observing the patterns of protein in SDS polyacrylamide gel electrophoresis, the proteins in muscles and blood of *S. subcrenaia* and *S. broughtonii* of Filibranchia and the proteins in muscles of several species in Eulamellibranchia (molecular weights ranging between 10,000 and 100,000 daltons) were studied.

## MATERIALS AND METHODS

*Scapharca subcrenata* (Lischke) and *Scapharca broughtonii* (Schrenck) in Filibranchia, and the *Cristaria plicata* (Leach), *Corbicula fluminea producta* (V. Martens), *Meretrix lusoria* (Roding) and *Mercenaria stimpsona* (Gould) in Eulamellibranchia were purchased from Kwang-An-Ri Beach Market, Pusan, Korea. The blood samples from the hearts of *S. subcrenata* and *S. broughtonii* were obtained by using syringe and centrifuged for 20 min at 10,000 rpm (Hitachi-20 PR-5, Japan). All procedures were performed at 4°C. One volume of the supernatant was added to the 9 vol of 62.5 mM Tris-HCl buffer (pH 6.8) containing 2% SDS and 5% 2-mercaptoethanol. The mixture was heated to 100°C for 2 min and then cooled. A small amount of sucrose was added to the mixture and then bromophenol blue for tracking. The prepared samples were applied directly to the gels. To determine the protein patterns of muscles, the muscles of the samples were put in mortar dish containing the Tris-HCl buffer on dry ice and ground with sea sand. After the grinding, the samples were filtered through Whatman No. 1 filter paper and dialyzed by a dialysis membrane (250~7 U, Sigma) at 4°C, and then the samples were heated and cooled as the above mentioned methods.

The samples were subjected to SDS polyacrylamide (T=10) on a vertical slab gel apparatus (LKB), and all buffer systems employed in this study were followed from Laemmli (1970) method. Electrophoresis was conducted at 4°C at 100 V for 5 hours. To determine the molecular weights of the samples, the following known proteins were employed: Lysozyme (14,300 dalton), β-lactoglobulin (18,400 dalton), trypsinogen (24,000 dalton), egg albumin (45,000 dalton), and bovine albumin (66,000 dalton). And the molecular weights were measured by the molecular weight analysis methods (Banker and Cotman, 1972; Neville, 1971; Swank and Munkres, 1971). After the electrophoresis, the gels were stained with Coomassie Brilliant Blue R 250 (Sigma).

The stained gels were scanned at 570nm with a Toyo DMU-33C Densitometer (Japan).

## RESULTS

The protein patterns of whole blood obtained from the hearts of *Scapharca subcrenata* and *Scapharca broughtonii* are shown in Figures 1 and 3. And also the protein patterns of the plasma are shown in Figures 2 and 4. The molecular weights of the representative protein bands of blood corpuscles between 10,000 and 100,000 daltons of *S. subcrenata* and *S. broughtonii* are the bands of a (12,200 dalton), c (17,800 dalton), d (35,000 dalton), e (44,000 dalton) and f (67,000 dalton) (Figs. 1 and 3). On the other hand, the plasma contains the protein bands of b (13,800 dalton), g (73,600 dalton), and h (78,400 dalton) as shown in Figures 2 and 4. It is hardly distinguished the protein patterns of plasma (molecular weights between 10,000 and 100,000 daltons) in *S. subcrenata* and *S. broughtonii*. The band c (17,800 dalton) (Fig. 1 and Table 1) of the blood corpuscles was found in *S. subcrenata*, but the same band was not occurred in *S. broughtonii* (Fig. 3). Although the bands of d (35,000 dalton), e (44,000 dalton), and f (67,000 dalton) appeared in all two species (Figs. 1 and 3), the bands of *S. broughtonii* were rather dense in color than in those of *S. subcrenata* (Table 1). This fact indicates that the larger amounts of the proteins existed in the bands, d and f, of *S. broughtonii* than those of *S. subcrenata*.

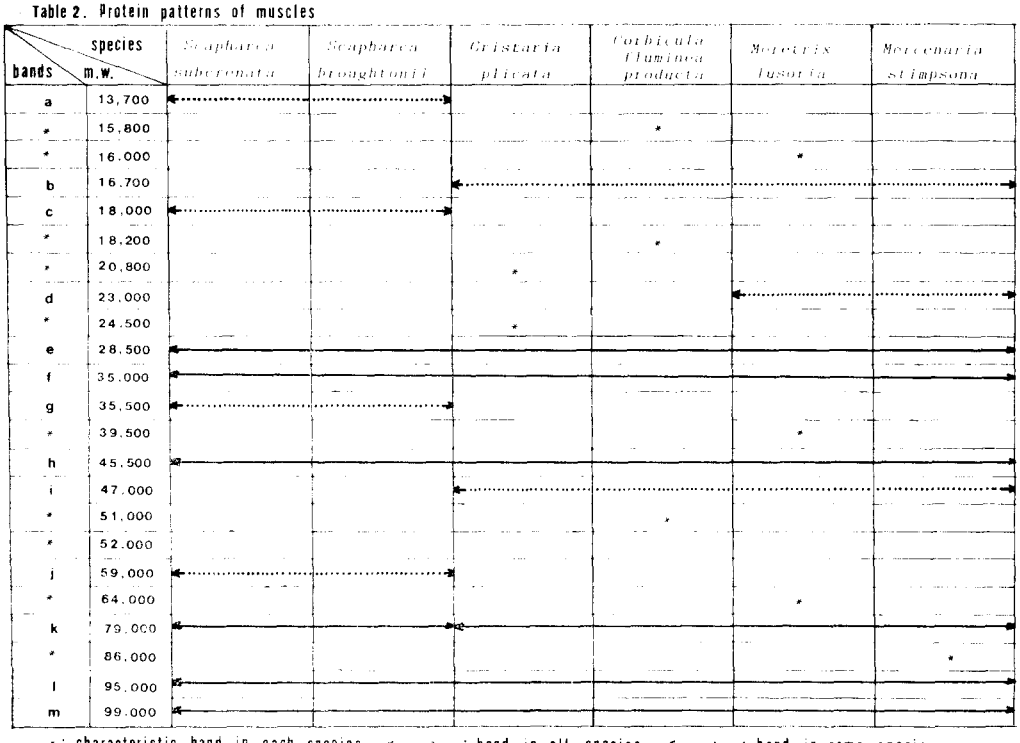
The six representative protein bands, which are bands of e (28,500 dalton), f (35,000 dalton), h (45,000 dalton), k (79,000 dalton), l (95,000 dalton) and m (99,000 dalton), were occurred in muscles of all species of *S. subcrenata*, *S. broughtonii*, *Cristaria plicata*, *Corbicula fluminea producta*, *Meretrix lusoria* and *Mercenaria stimpsona* (Figs. 5-10, Table 2). The protein patterns obtained from muscles of *S. subcrenata* and *S. broughtonii* which are same genus in Filibranchia were almost similar to each other (Figs. 5-6) with the exception of only one band (46,500 dalton) which clearly existed in *S. subcrenata* (Fig. 5 and Table 2). The band b (16,700 dalton) of the protein patterns is shown in all four

**Table 1.** The protein patterns of blood corpuscles (BC) and plasma (P)

| bands | mol. wt. | *Scapharca sucrenata* | *Scapharca broughtonii* |
|---|---|---|---|
| a | 12,200 | BC | BC |
| b | 13,800 | P | BC |
| c | 17,800 | BC | none |
| d | 35,000 | BC | BC/very intensive |
| e | 44,000 | BC | BC/very intensive |
| f | 67,000 | BC | BC/very intensive |
| g | 73,600 | P | P |
| h | 78,400 | P | P |

mol. wt.: molecular weight. Numerals are indicated in daltons.

Table 2. Protein patterns of muscles




*: characteristic band in each species. ⟷ : band in all species. ⟷ : band in some species.
m.w.: molecular weight. numerals mean daltons

speies (*C. plicata*, *C. fluminea producta*, *M. lusoria* and *M. stimpsona*) of Eulamellibranchia (Figs. 7-10). However, the band f is occurred in all muscles of six species of the samples employed in this study, and especially, the band was very intensively stained in the four species of Eulamellibranchia (Figs. 7-10).

The differences of the reprsentative bands among the species of Eulamellibranchia were occurred as follows: in *Cristaria plicata*, two bands of molecular weights 20,800 and 24,500 daltons appeared in unique (Fig. 7). Three bands (15,800, 18,000 and 51,000 daltons) of *Corbicula flumina producta* (Fig. 8) and three bands (16,000, 39,500 and 64,000 daltons) of *Meretrix lusoria* (Fig. 9) were occurred in unique. And also, the band (86,000 dalton) appeared in *Mercenaria stimpsona* (Fig. 10). These are the characteristic bands in each species only.

## DISCUSSION

Recently, many investigators attempted to study on the relationships among the species in animals or plants by electrophoresis method. Most studies in this field were mainly concerned with enzymes (Bonavita and Guaneri, 1973; Chen, 1968; Hogan, 1971; Kidder,

1983; Markert and Faulhaber, 1965; Massaro, 1972; Offermann *et al.*, 1983; Rim, 1970; Takayama *et al.*, 1966; Wright and Richard, 1982). But this study deals with the molecular weight measurement of protein patterns in muscles and blood. The protein patterns of the same genus animals are, in general, appeared very similar each other. In the comparison of protein patterns of muscles, it was interested in attempting to compare the proteins which have relatively lower molecular weights. The protein patterns of the muscles of *Cristaria plicata* in Eulamellibranchia are the most complicated, and the proteins which were over 60,000 daltons in molecular weight were similar to *S. subcrenata* and *S. broughtonii* of Filibranchia. It suggests that *Cristaria plicata* among four species of Eulamellibranchia has more close relationship with the species of Filibranchia.

The 23,000 dalton band of *Mercenaria stimpsona* and *Meretrix lusoria*, does not appear in any other species. Therefore, these two species have more close relationship each other among four species which are belonged to Eulamellibranchia. It is resasonable decision that they are belong to the same family of Veneridae, which are in the same case when the species is classified by systematical study.

*Corbicula fluminea producta* has the similar protein bands of muscles, respectively, which are contained in *Meretrix lusoria* and *Mercenaria stimpsona*, and *Cristaria plicata; Corbicula fluminea producta* in observing the protein patterns of muscles probably has position between *Meretrix lusoria* and *Mercenaria stimpsona* of Veneridae, and *Cristaria plicata* of Uninidae. Thus, the bands of protein, which were shown in the protein patterns of whole muscles in the species of Bivalvia used in this study, were 6. Their molecular weights are 28,500, 35,000, 45,000, 79,000, 95,000 and 99,000 daltons. As it was explained in the results, the protein patterns, which have relationship one another and the characteristic protein patterns in the species, can be observed with ease. From this fact, it can be attempted to study about the relationship of the differentiation in the species with the protein patterns of muscles.

Moreover, it is worthwhile to compare the protein patterns of the blood corpuscles in *S. subcrenata* and *S. broughtonii.* The protein patterns of the blood corpuscles could be compared easily in *S. subcrenata* and *S. broughtonii* although it hardly distinguish the species by observing the protein patterns obtained from the plasma. It is magnificient event in the above fact the protein patterns of plasma are same despite their species are different. The reason is that the protein patterns of corpuscle in man and woman are same, but the protein patterns of plasma are different, and especially in women who are in disease or in pregnancy the protein patterns of plasma are different. It is more needed on the comparative studies for the protein patterns of the plasma and blood corpuscles by electrophoresis, and the studies on the characteristics and amino acid composition of the proteins or physiological differences between the blood corpuscles which contain nuclei and plasma, are demanded.

## ABSTRACT

SDS-polyacrylamide gel electrophoresis for the proteins obtained from the plasma, *Scapharca subcrenata* and *suapharca broughtonii*, and for the proteins of muscles of several species in Bivalvia was performed. The protein patterns of plasma showed little difference between *S. subcrenata* and *S. broughtonii* in lower molecular weight weight proteins. However, the protein patterns of muscles of other species, which were used in this study, were more shown in the lower molecular weights than the higher molecular weights in difference. Thus it is thought be an interesting fact. The protein band of blood corpuscles, 17,800 dalton, was not appeared in *S. broughtonii*, but this band was appeared in *S. subcrenata*. Henceforth this is the significantly important difference in these two species. But the protein patterns obtained from muscles of the species did not show a difference in a range of molecular weights between 10,000 and 100,000 daltons. Meanwhile, several protein bands obtained from *Meretrix lusoria* were similar to those of *Mercenaria stimpsona*. Hence, in this study, 6 protein bands which exist all species in Bivalvia and 4 characteristic protein bands in *S. subcrenata* and *S. broughtoniionly*, were investigated. And in four species of Eulamellibranchia, two protein bands in common and the charateristic band of 23,000 dalton which is belong to *Meretrix lusoria* and *Mercenaria stimpsona*, were found. The molecular weights of the characteristic proten patterns, which are contained in each species, were measured and compared.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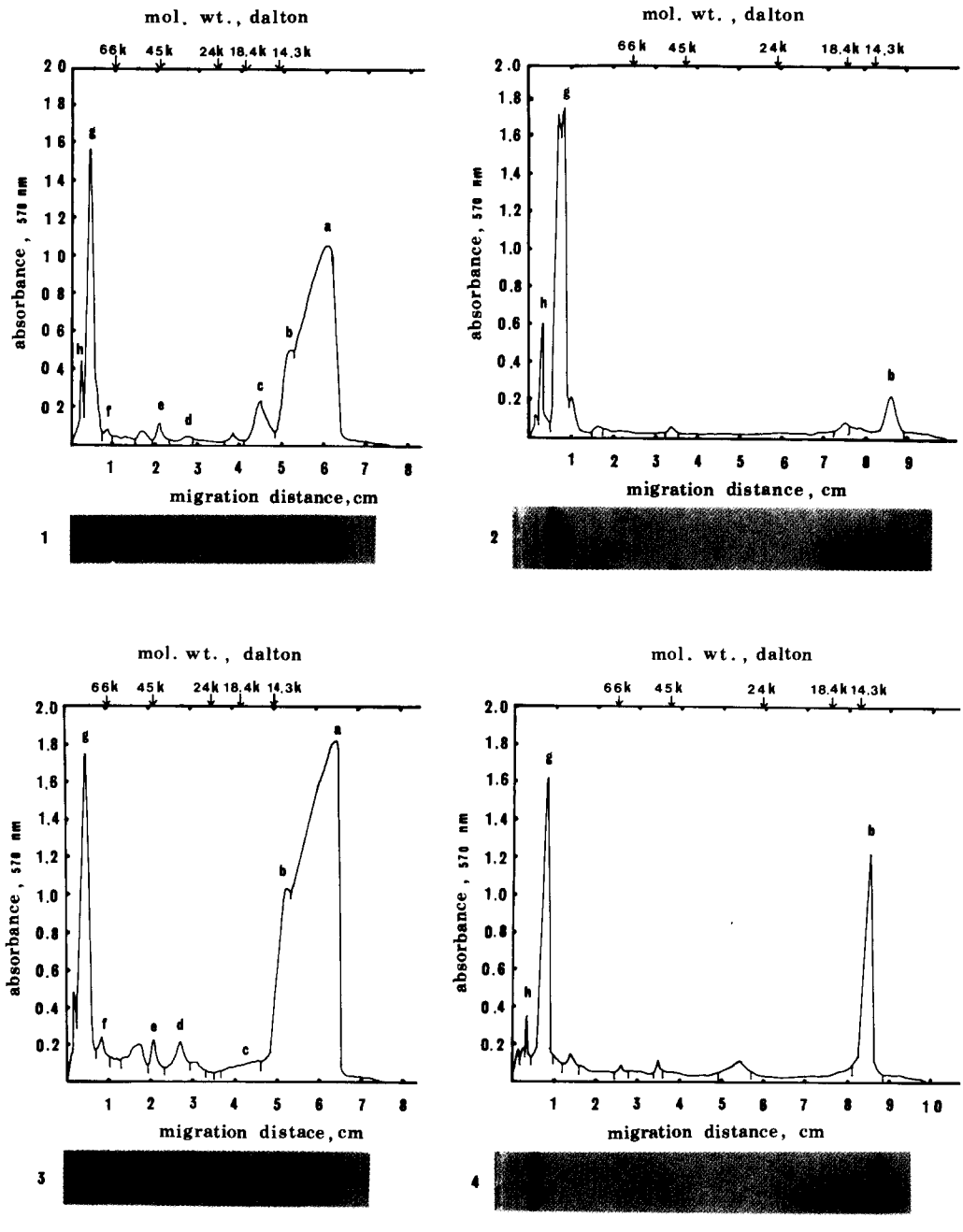
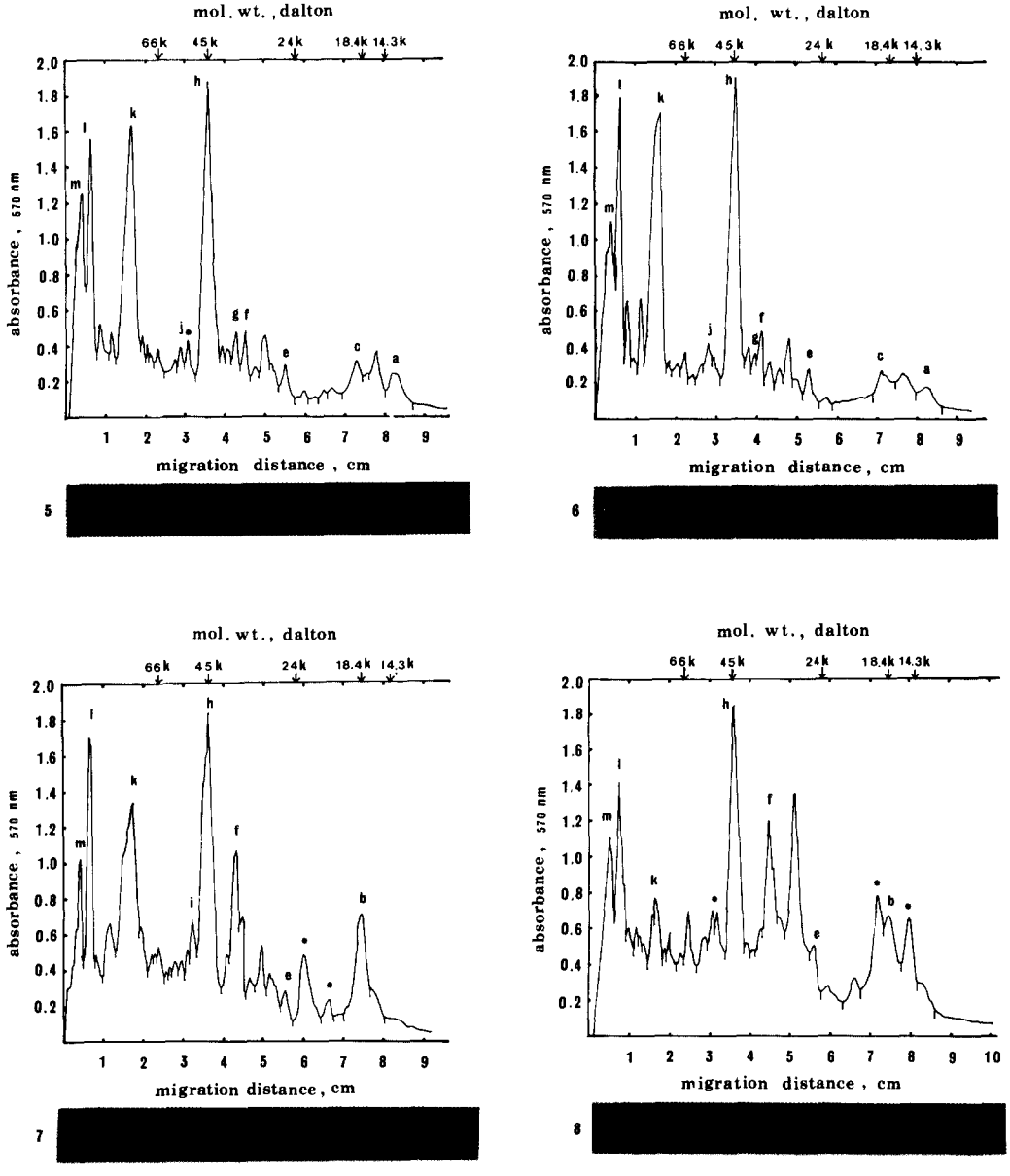



**Figs.** 1-2. Protein patterns of *Scapharca subcrenata*. 1) whole blood, 2) plasma.
**Figs.** 3-4. Protein patterns of *Scapharca broughtonii*. 1) whole blood, 2) plasma.



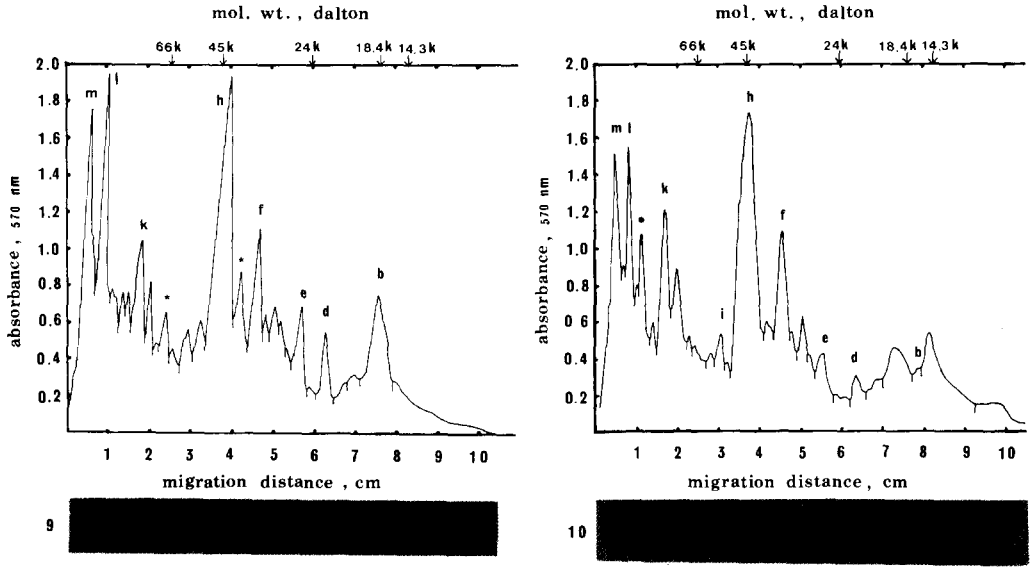

**Figs.** 5-8. Protein patterns of muscles.

5) *S. subcrenata*, note the characteristic band (*) is occurred between bands h and j.

6) *S. broughtonii*, note the characteracteristic band (*) is not existed between bands h and j.

7) *Cristaria plicata*. note two characteristic bands (*) are occurred between bands b and e.

8) *Corbicula fluminea producta*. note the characteristic bands (*) appeared.




**Figs. 9-10.** Protein patterns of muscles.

9) *Meretrix lusoria*, note two characteristic bands (star marks) appeared.

10) *Mercenaria stimpsona*, note one band is occurred between bands k and l.