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Studies on the Distribution of Soluble Proteins within the Central Nervous System during Embryogenesis of the Chick Embryo

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鷄胚 發生中 中樞神經系內의 可溶性 蛋白質 分布에 關한 硏究

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요 약

계배 중추신경계의 분화에 따른 단백질 합성 양상을 SDS-PAGE와 2차원 전기영동으로 분석하였다. 약 24~26개의 band가 densitometer에 의해 기록되었고, 주요 band들의 분자량은 90K에서 11K 범위에 분포하였다. 대뇌, 시엽 및 소뇌에서 가장 현저한 band는 분자량이 44.7K로 actin으로 사료되었다. 대뇌의 85.5K 및 47.9K, 시엽의 85.5K, 71.6K 및 34.9K 소뇌의 47.9K 및 34.9K band들은 부화전까지는 증가하다가 부화후 감소하였다. 기록된 약 300개의 polypeptide중 약 9%가 분화가 진행됨에 따라 그들의 합성율에 변화를 보였으며, 대뇌의 spot 19(Mr, 63K; pI, 6.95)와 소뇌의 spot 22(Mr, 43K; pI, 6.5)는 부화후 4일베에서 새로이 합성되었다.

INTRODUCTION

Differentiation is largely an intracellular process involved in the appearance of cells which have certain biochemically or cytologically recognizable characteristics through the differential activation of genes (Garrod, 1975). The understanding of complex biochemical phenomena such as growth regulation and the expression of tissue-specific functions a vast knowledge in proteins, which mediate and regulate these functions (Garrels, 1979).

Recent advances in molecular biology have greatly stimulated researches in differentiational and biochemical aspects of development. Recently many investigators took a growing interest in the study of the proteins as markers of development and differentiantion (Oppenheimer, 1985).

Accordingly, the changes of protein patterns during development and differentiation have

been frequently studied in chick embryo by many researchers. Ebert (1953) attempted to analyze the synthesis and distribution of the contractile protein, myosin, during the development of heart of chick embryo. Lerner *et al.* (1963) reported that developing chick embryos from the stage of primitive-streak formation made ribosomal (28S and 16S) RNA. The size and composition of the RNA appears to constant throughout the first 7days of development. Scott and Bell (1964) showed that polyribosomal protein synthesis in the brain of 9-day chick embryos was inhibited by actinomycin. The proteins of pectoral, gizzard-muscle and brain of chick embryo in various developmental stages were examined with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by some investigators. The results indicated that the protein patterns were far different among their tissues (Kim and Kim, 1982, Hah and Han, 1983). By using two-dimensional gel electrophoresis and computer analysis, the patterns of protein synthesis in the nerve cell line B103 and the glial cell line B9 derived from the BDIX strain of inbreed rats were analyzed and the relative rates of synthesis of approximately 300 proteins were compared (Garrels, 1979).

On the basis of previous results, we recently examined the protein patterns occured during differentiation in chick embryo. In this study, quantitative and qualitative analysis of soluble proteins in the central nervous system during chick embryogenesis was carried out using SDS-PAGE and 2-dimensional gel electrophoresis, and the results obtained are presented.

MATERIALS AND METHODS

Preparation of Soluble Proteins

White leghorn fertilized eggs (55~60g) were incubated in a forced-draft humidified incubation at 39°C to induce the embryogenesis.

Cerebrum, optic lobe, and cerebellum at various stages (Fig. 1) of chick embryo were removed and rinsed with sonication beffer (O'Farrell, 1975). Tissues were homogenized with Pyrex homogenizer [sample:buffer=1:3 (w/v)] and sonicated three strokes for 20-sec at 160 intensity on ice with Labline ultrasonic system (Model 9100) followed by 30-sec intervals for cooling. The sonicated suspension was centrifuged at 3,000g for 15 min, and the supernatant was again centrifuged at 15,000g for 45 min. Aliquots of supernatant was used for electrophoresis immediately, and the remaining portion was stored at 60°C. The protein concentration of the supernatant was determined by the method of Lowry *et al.* (1951).

SDS-PAGE

Soluble proteins of samples were analyzed by SDS-PAGE as described by Laemmli (1970) using 8~12% linear gradient slab gel (140×180×1.2mm). Aliquots (40 μl) containing 40 μg proteins were applied onto each well after boiling for 90 sec in a solution containing 1% SDS, 5% mercaptoethanol, 0.02% bromophenol blue, 2.5% sucrose, and 0.125M Tris-

glycine buffer (pH 6.8). Afetr filling both upper and lower tanks of the apparatus with 0.025M Tris-glycine buffer (pH 8.3) containing 0.1% SDS, electrophoresis was carried out with a current of 20 mA per slab until the tracking dye reached the bottom on the gel.

After the electrophoresis, the gel was stained with 0.2% Coomassie blue R-250 by the method of Fairbanks *et al.* (1971) and destained by destain I (25% ethanol, 10% acetic acid) for 1~2 hr and stored in a destain II (7% acetic acid, 2% glycerol). The intensity of protein bands was determined by their absorbance at 633 nm using 2202 Ultrascan Lazer Densitometer (LKB, Sweden). The molecular weights of major bands were calibrated by five marker proteins (Weber and Osborn, 1969, Hoefer Scientific Instruments, 1980).

2-Dimensional Gel Electrophoresis

Isoelectric focusing (IEF) combined with SDS electrophoresis was carried out as described by O'Farrell (1975) with the following modifications. IEF gels were prepared with ampholines (pH 5~8 and 2~10) and polymerized in glass tube (150×2.3 mm, inner diameter). About 600 μg of soluble proteins were applied to each gel. After IEF for 20 hr at 400 V and then for 2 hr at 550 V, the gels were gently shaken in SDS equilibration buffer for 30 min prior to runing on the slab gels. The remaining gels were placed in the same buffer (5 ml) and stored at −70°C for up to 1 week before runing in the second dimensional electrophoresis. Unstained, unequilibated IEF gels which were run without samples were cut into 0.5 cm pieces and soaked overnight in degassed distilled water (2 ml), and the pH of each segment was determined.

For the second dimensional electrophoresis, all conditions were same as SDS-PAGE except using 8~14% linear gradient gel. After electrophoresis, the gels were stained with stain I (containing 50% ethanol, 10% acetic acid, 10% trichloroacetic acid, and 0.005% Coomassie blue R-250) for 2 hr, and then with stain II (0.2% Coomassie blue R-250, 50% ethanol, 10% acetic acid) for 12 hr. The gels were destained with destain I of SDS-PAGE and stored in a mixture of 10% acetic acid and 2% glycerol (Weil and Mcilwain, 1981).

RESULTS

Numerical and Quantitative Analysis of Soluble Proteins

The one-dimensional electrophorograms of proteins from the central nervous system of chick embryos were shown in Figure 1. The molecular weights of the protein band were estimated from the standard curve. The range of molecular weights of major bands appeared to be in a wide scope from 90K (kilodalton) to 16K. For quantitative analysis, the gels were analyzed with the densitometric scanner (Fig. 2). About 24~26 bands were detected by densitometer.

The proteins synthesized in cerebrum were compared to those synthesized during differentiation. The amount of the a (85.5K) and b (47.9K) bands was gradually increased before hatch and then decreased after hatch. The c (44.7K; actin) band, the most predominant

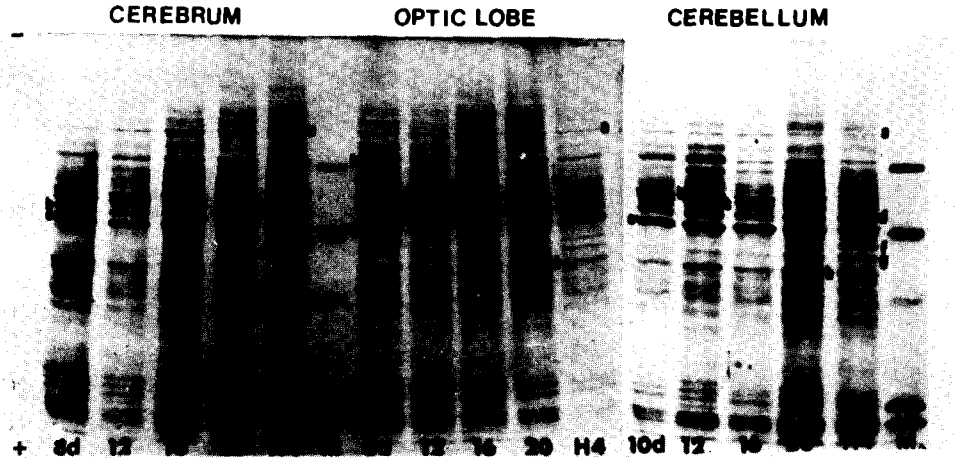

Fig. 1. The protein patterns according to differentiation of central nervous system in the chick embryo. The numbers indicate developing days (d, day; H4, 4-day after hatch). Lane M. is marker proteins (From upper: Bovine serum Albumin, 68K (K, kilodalton); Ovalbumin, 43K; Carbonic Anhydrase, 29K; Lysozyme, 14.3K; Ribonuclease, 13.7K).

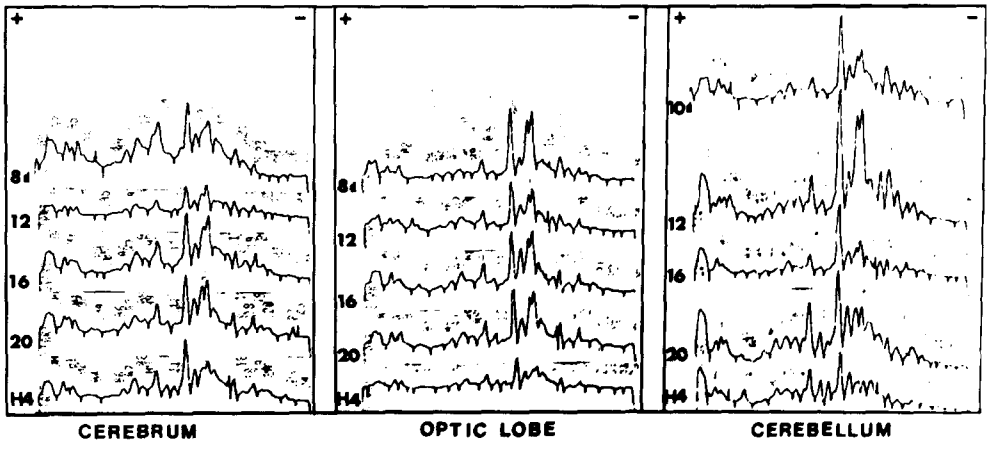

Fig. 2. Densitometric scanning of electrophoretic patterns of central nervous system in the chick embryo. For designation of numbers, see Fig. 1.

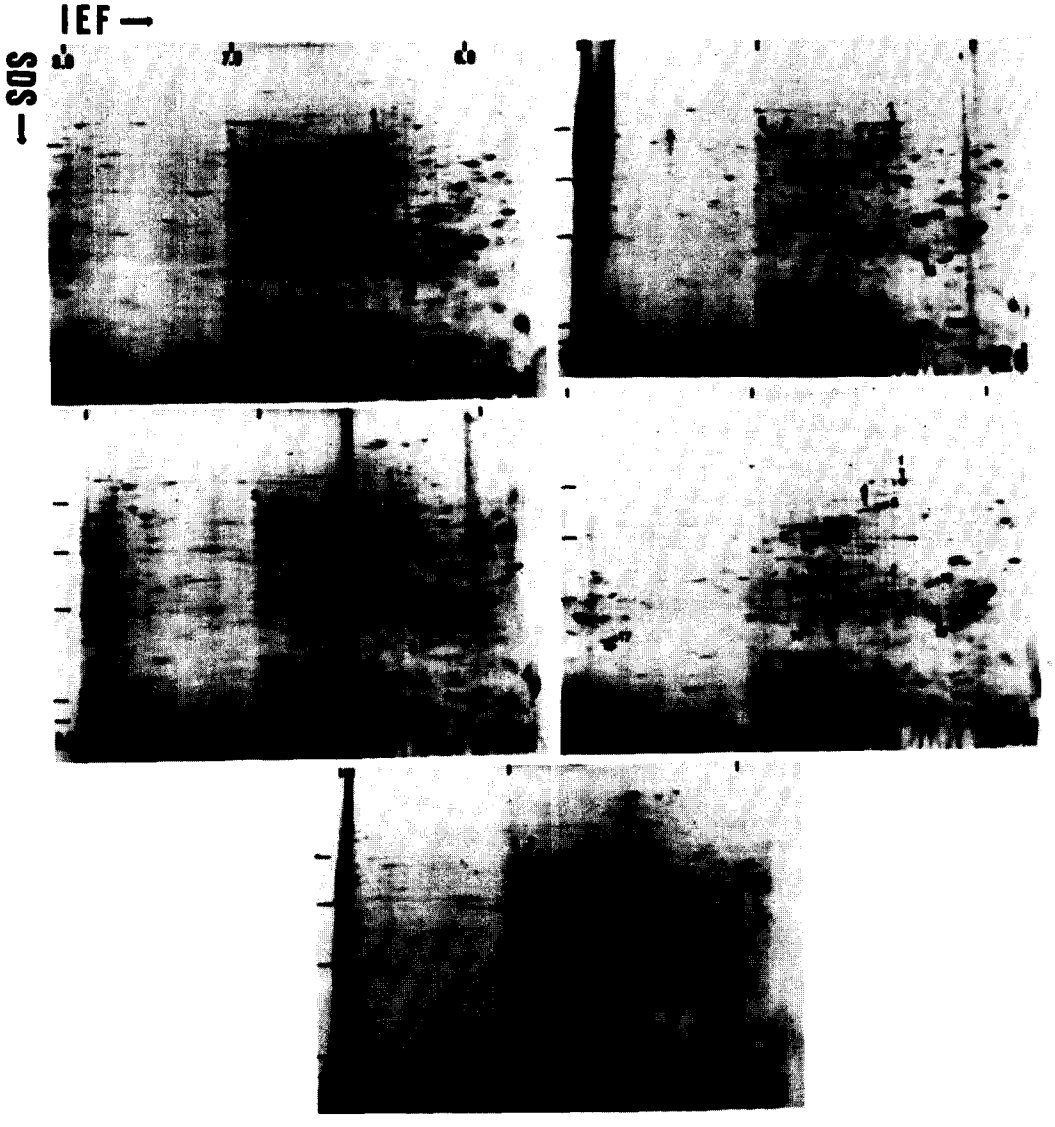

Fig. 3. Distribution of soluble protein of cerebrum in the chick embryo. For designation of numbers and marker proteins, see Fig. 1.

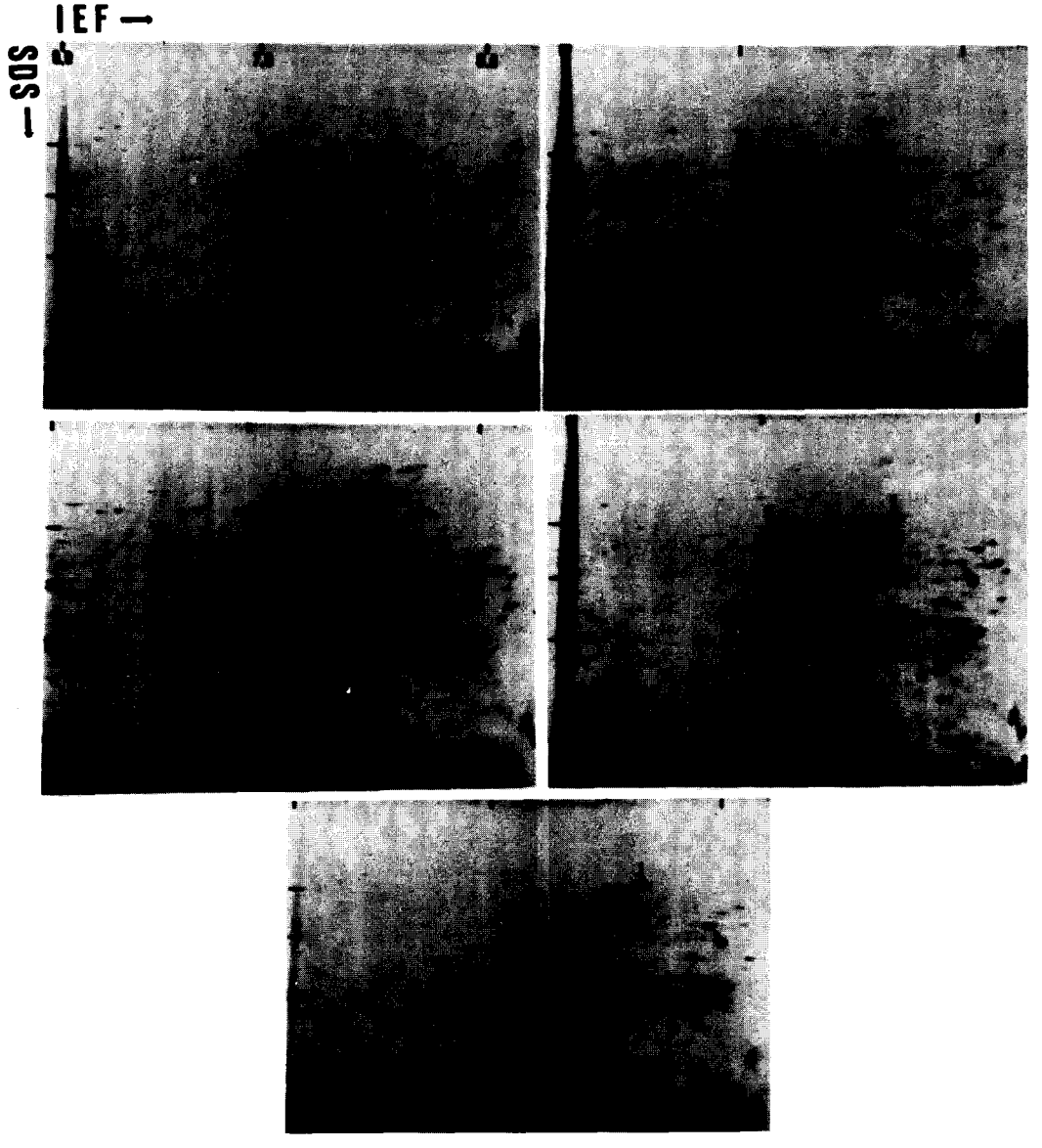

Fig. 4. Distribution of soluble protein of optic lobe in the chick embryo. For designation of numbers and marker proteins, see Fig. 1.

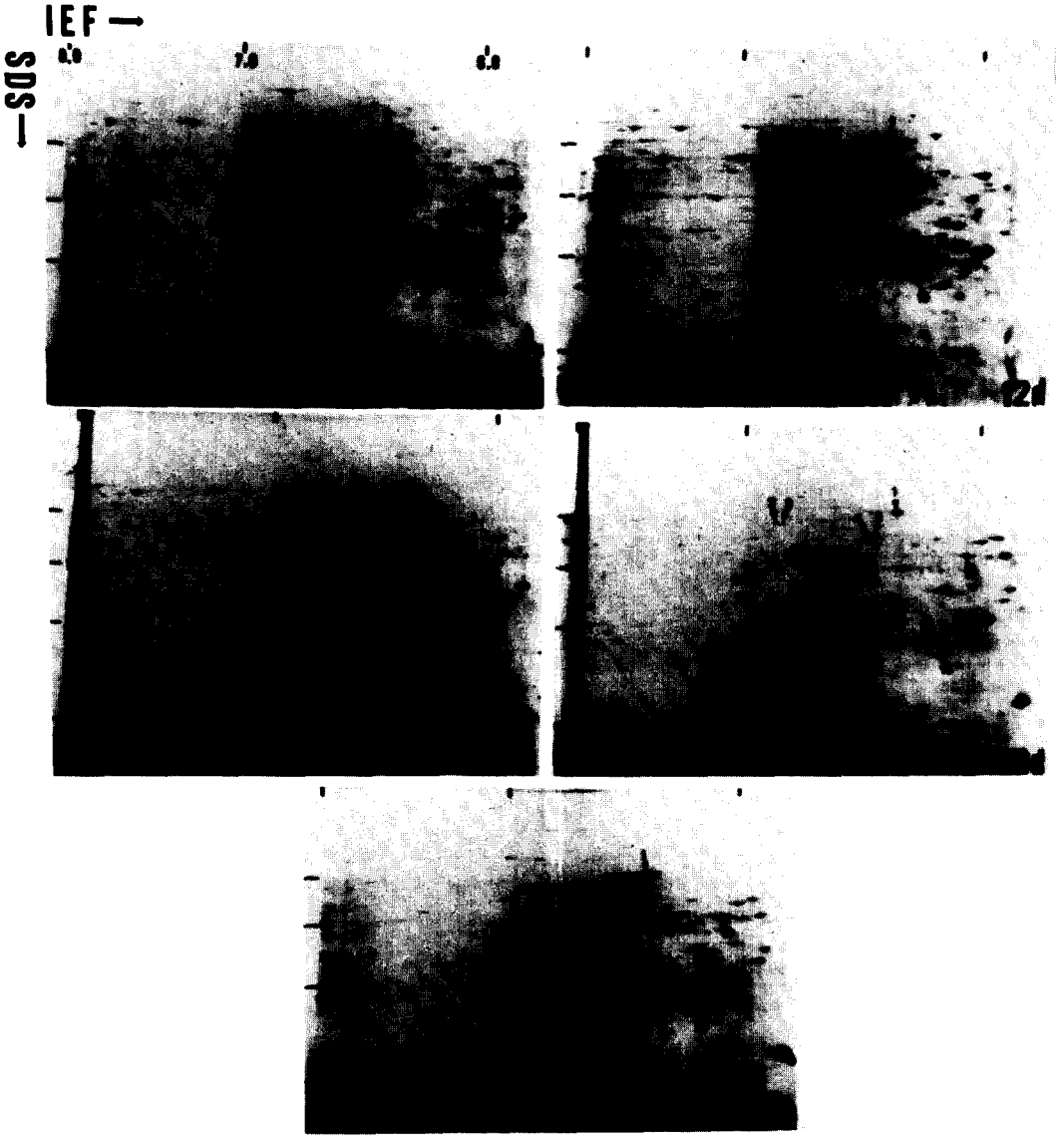

Fig. 5. Distribution of soluble protein of cerebellum in the chick embryo. For designation of numbers and marker proteins, see Fig. 1.

one of them, was increased throughout the experimental periods. The d (40.7K) and e (38.7K) bands were also increased except a decrease at 12-day embryo.

The amount of all protein bands in optic lobe was rapidly decreased after hatch. The a (85.5K), b (71.6K) and d (34.9K) bands in optic lobe, along with d (47,9K) and h (34.9K) bands in cerebellum were clearly appeared before hatch during differentiation. The difference of amount of the c band (actin) of optic lobe and e band of cerebellum was almost samed as c band of cerebrum. The e band (16.7K) of optic lobe was decreased as the differentiation proceeded.

The protein patterns in the cerebellum were very variant. The a band (85.5K) weakly appeared at embryo of 4-day after hatch in its relative amount during differentiation. The f (40.7K) bands were slightly increased during differentiation and the h (34.9K) band was particularly appeared at 20-day embryo.

Qualitative Analysis of Soluble Proteins

Proteins extracted from embryos were analyzed by two-dimensional electrophoresis. The proteins synthesized in cerebrum, optic lobe and cerebellum of chick embryo during differentiation were compared in Figures 3~5.

Two types of changes were observed in each organ. These appeared to be quantitative changes such as changes in the relative rates of synthesis of a particular polypeptides and qualitative changes such as barely detected synthesis of polypeptides at each stage. As described below, quantitative changes was more prominent than the qualitative changes.

Synthetic rate of the proteins marked with arabic number in cerebrum and cerebellum was greatly declined before hatch and increased after hatch. About 9% of the nearly 300 polypeptides showed changes in relative rates of synthesis during differentiation.

Clearly there were substantial changes in the pattern of protein synthesis during differentiation. In the relative rates of synthesis, only a few polypeptides increased during early embryonic development (Fig. 3~5, spots 15, 16, and 18). No major spots undergoing transient decreases in synthesis were detected.

The synthesis of several other polypeptides began *de novo* or increased greatly to become noticeable among the newly synthesized proteins of later stage. This was the case for polypeptides labeled 9, 14, and 19 in cerebrum; 19, and 20 in optic lobe; and 9, 14, 19, 22, 24, and 25 in cerebellum. Spot 19 (63.0K, pI=6.95) in cerebrum and spot 22 (43.0K, pI=6.5) in cerebellum were newly synthesized at 4-day after hatch.

DISCUSSION

Changing patterns of protein synthesis during chick embryogenesis were analyzed reproducibly by the method of SDS-PAGE and high resolution two dimensional gel electrophoresis.

In Figures 1 and 2, the patterns of protein showed that the nature of protein synthesis was unique for each stage. Whether the changes in protein pattern are due to the differences

in the types of protein synthesized at each stage, or due only to changes in the relative synthetic rates of the same group of proteins, cannot be determined from our data. Irrespective of which actually occurs, the overall pattern of protein synthesis changes significantly with developmental stage (Ecker and Smith, 1971).

Brandhorst (1976) reported that the increased rate of protein synthesis in the fertilized egg of sea urchin is due to increased translation of mRNA of the same species, not different species. The changes of protein patterns during early development are presumably due to selective utilization of maternal mRNAs derived from untranslated mRNA particles, early zygotic gene activity, or a combination of the two. Evidence exists for both types of temporal control (Berry, 1982). The fact that changes in the protein patterns of early *Drosophila* embryos can be reproduced by *in vitro* translation of mRNA from the same stage indicates that translational control of different mRNAs takes place during early development as reported by Trumbly and Jarry (1683). We suggest that the changes of protein pattern in this study are same as results reported by previous studies.

In current study, generally, the rates of protein study, generally, the rates of protein synthesis were highly evident at early embryonic stage. Santon and Pellegrini (1981) reported that the rate of protein synthesis in *Drosophila* was maximal at very early period during embryogenesis, i.e., between 1.5 and 2.5 hr after oviposition, but the rate was decreased after 2.5 hr. The reason for the decrease in protein synthesis observed at 12-day embryo of cerebrum is not clear. Perhaps, the lower rate of protein synthesis is due to a progressive changes taking place in the embryos, which affect the pool of amono acids available for protein synthesis.

Bamburg *et al.* (1973) suggested that nearly one-fourth of the total protein in 11 to 17-day old embryonic chick brain were neutrotubule proteins. A very sharp increase in neutrotubule protein concentration took place between 5 days of development and 11 days of development *in ovo*. A plateau level appeared to be maintained from 11 to 17 days of development, and then the neutrotubule protein concentration decreased gradually to the adult level. In this study, the concentration of a few proteins was largely increased at 8-day embryo and gradually decreased from 16-day to 20-day embryo in the cerebellum.

In our study, results suggested that proteins as well as mRNA could be stored during embryogenesis. Some of major proteins of the embryo gradually disappeared during differentiation. They are presumably processed to other biosynthetic products. Other embryo proteins may be stored for certain specialized uses during differntiaton.

ABSTRACT

Pattern of protein synthesis during differntiation of the central nervous system of chick embryo was analyzed by SDS-PAGE and two-dimensional electrophoresis.

About 24~26 bands were detected by densitometer, and molecular weights of major bands

were distributed in a wide range from 90K. to 16K. The brain actin appeared to have a molecular weight of 44.7K as determined by marker proteins, and was the most predominant in the cerebrum, optic lobe, and cerebellum.

The amount of the 85.5K and 47.9K bands in cerebrum; the 85.5K, 71.6K and 34.9K bands in optic lobe; and the 47.9K and 34.9K bands in cerebellum were slowly increased before hatch and decreased after hatch as the differentiation proceeded.

About 9% of the nearly 300 polypeptides showed changes in relative rates of synthesis during differentiation. The spot 19 (Mr, 63.0K; pI, 6.95) in cerebrum and spot 22 (Mr, 43.0K; pl, 6.5) in cerebellum were newly synthesized at embryo of 4-day after hatch.

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