Electrophoretic Study of the Soluble Proteins of the Developing Chick Neural Retina and Brain

In recent years there has been great interest in the characterization of the proteins of the nervous system[1,2]. However, no attention has been given to the neural retina as a source of nervous tissue. In our laboratory we have undertaken the task of fractionating and characterizing the soluble proteins of the neural retina. As a starting point, the developmental pattern of soluble proteins found in the chick neural retina was compared to that of the developing chick brain.

Neural retina and brain tissue were collected from chick embryos at various stages up to hatching. In addition, neural retina and brain were taken from chicks at 7 days post-hatching and from adult chickens. The wet tissue was suspended in cold (4 °C) phosphate buffered saline to give a 33% w/v dilution and homogenized. The homogenate was centrifuged at 27,000 *g* for 15 min and the supernatant was frozen until used. Polyacrylamide gel electrophoresis of supernatant samples (30 µl stablized with 8 µl 40% sucrose) was done at room temperature according to the method of DAVIS[3] and ORNSTEIN[4] using only a 7.5% separating gel in the presence of 10 m*M* mercaptoethanol. The gels were stained with 0.05% amido black.

Figure 1 shows the gels of neural retina and brain of chicks at various stages of development. Although these do not show all the stages studied they are representative. Figure 2 is a composite drawing to represent the gels of both series. There are 15 discernible bands in the two series for which we have calculated the mobilities relative to the fastest migrating band which migrates with the front. Where two or more bands are very close together a single mobility, based on the average migration of the group, is indicated.

While there is a great similarity between the banding patterns of soluble proteins of neural retina and brain it can readily be seen that there are major differences. Bands designated 0.15, 0.68, 0.75, 0.82, and 1.0 are quite similar in neural retina and brain. They are present in all stages studied and they show similar staining intensities. Band 0.15 shows an increase in concentration with increasing age, and band 0.68 decreases in concentration with increasing age. However, bands 0.75, 0.82, and 1.0 remain fairly constant throughout development. Of course this does not preclude differences in molecular weights, number of actual proteins present in each band or the number of subunits in each protein, since a given band may represent different proteins with similar electrophoretic mobilities.

Bands 0.21, 0.30, 0.40, 0.47, 0.62, and 0.90 differ between brain and neural retina either in their time of appearance during development or whether they appear at all. Only the slower migrating portion of the two bands numbered 0.30 is present in brain, and it increases in concentration with age as do both parts in the neural retina. Band 0.47 is present in all stages of the neural retina and increases dramatically after hatching, but it does not appear in the brain until hatching and the concentration remains low. Band 0.62 is present only in the neural retina after hatching. Band 0.90 shows a difference in time of appearance during development. It is present in all stages studied in the brain, and the concentration remains constant while it does not appear in neural retinal until hatching and then increases in concentration. The other bands, 0.21 and 0.40, are different in the two tissues by not being present in one or the other. Band 0.40 is present only in the early stages of the brain, and band 0.21 is present only in the neural retina (all stages) and it increases with age.

On the basis of its mobility, band 1.0 probably contains S-100 protein[5]. Band 1.0 is found in neural retina in comparable concentration as is found in the brain. Albumin has a relative mobility of 0.68 in our system so it could be responsible, at least in part, for band 0.68. We have also shown immunologically that band 0.15 contains microtubule protein, but we have not distinguished which of the two components is giving the reaction[6].

We are now in the process of using more drastic methods to further fractionate the soluble proteins and to compare the neural retina proteins of several species to determine if there are proteins common to neural retina of all species.

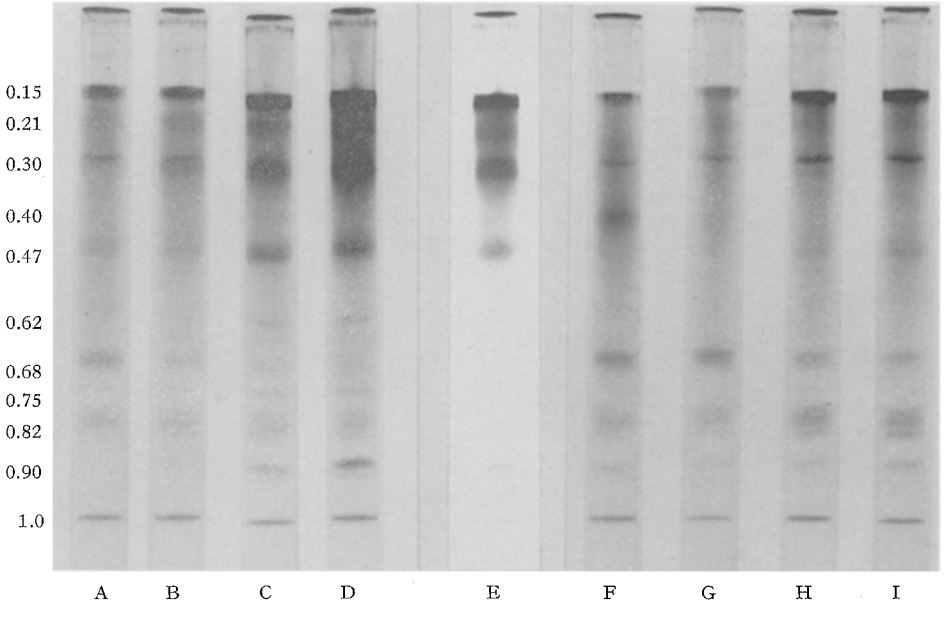

Fig. 1. Disc electrophoretic separation in 7.5% polyacrylamide gels of water soluble proteins from: A) 11-day chick embryo neural retina. B) 17-day chick embryo neural retina. C) 7-day post-hatching chick neural retina. D) adult chicken neural retina. E) adult chicken neural retina gel printed lighter to better illustrate the slow migrating bands. F) 11-day chick embryo brain. G) 15-day chick embryo brain. H) 7-day post-hatching chick brain. I) adult chick brain. Relative mobilities are indicated along the left edge.

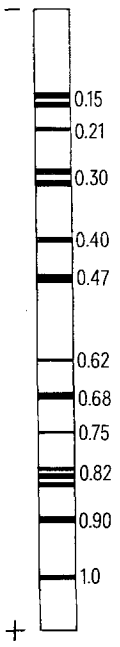

Fig. 2. A composite drawing of the neural retina and brain tissue bands in Figure 1 with mobilities indicated. Band 1.0 runs with the front and albumin has a mobility of 0.68 in this system.

Résumé. En examinant par électrophorèse en gel de polyacrylamide les protéines solubles de la rétine neurale et du cerveau du poulet au cours de sa croissance, nous avons comparé 15 bandes nettement visibles dans la série de ces deux tissus; 8 furent très semblables et 7 notablement différentes.

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[1] A. Lajtha, *Protein Metabolism of the Nervous System* (Plenum Press, New York 1970).
[2] E. M. Shooter and E. R. Einstein, A. Rev. Biochem. *40*, 635 (1971).
[3] B. J. Davis, Ann. N.Y. Acad. Sci. *121*, 404 (1964).
[4] L. Ornstein, Ann. N.Y. Acad. Sci. *121*, 321 (1964).
[5] B. S. McEwen and H. Hyden, J. Neurochem. *13*, 823 (1966).
[6] J. Alcala and A. LaVelle, Abstr. Am. Soc. Cell Biol. *11*, 6 (1971).
[7] This work was supported by a General Support Grant No. RR-5384-12 to Wayne State University School of Medicine from the National Institutes of Health.

Sulphite Oxidase from Pacific Hake (*Merluccius productus*)

Sulphite oxidase (sulphite:oxygen oxidoreductase E.C. 1.8.3.1) catalyzes the oxidation of sulphite to sulphate in mammals[1–5], plants[6] and bacteria[7]. With ferricytochrome c as the electron acceptor, the enzymic reaction may be depicted as: 2 ferricytochrome c^{3+} + SO_3^{2-} + H_2O = SO_4^{2-} + 2 ferrocytochrome c^{2+} + $2H^+$. The enzyme system can also transfer electrons from sulphite to a variety of other acceptors, e. g., molecular oxygen, methylene blue, ferricyanide, and 2,6-dichloroindophenol[1]. A clinical case of deficiency of the enzyme has been observed in an infant who excreted abnormally large quantities of sulphite and thiosulphate in the urine. The patient at 9 months showed severe neurological signs, mental retardation, and dislocated lenses[2].

Sulphite waste liquors are associated with activities of the paper and pulp industry. Because of possible effects of these spent liquors on fish, we decided to assay for sulphite oxidase in the Pacific hake (*Merluccius productus*), a commercially important fish of local waters. Buffer extracts (0.05 *M* K phosphate, pH 8.00) of acetone powder (75 mg/ml) prepared from hake liver were assayed spectrophotometrically by observing the oxidation of sulphite by ferricyanide through decrease in absorption of the latter at 420 nm. The reaction mixture

[1] R. M. MacLeod, W. Farkas, I. Fridovich and P. Handler, J. biol. Chem. *236*, 1841 (1961).
[2] L. Laster, F. Irreverre, S. H. Mudd and W. D. Heizer, J. clin. Invest. *46*, 1082 (1967). – S. H. Mudd, F. Irreverre and L. Laster, Science *156*, 1599 (1967).
[3] H. J. Cohen, I. Fridovich and K. V. Rajagopalan, J. biol. Chem. *246*, 374 (1971).
[4] H. J. Cohen and I. Fridovich, J. biol. Chem. *246*, 359, 367 (1971).
[5] D. L. Kessler, J. L. Johnson, H. J. Cohen and K. V. Rajagopalan, Biochim. biophys. Acta *334*, 86 (1974).
[6] P. Fromageot, R. Vaillant and H. Perez-Millan, Biochim. biophys. Acta *44*, 77 (1960).
[7] R. M. Lyric and I. Suzuki, Can. J. Biochem. *48*, 334 (1970).

Characteristics of sulphite oxidase from various sources

Characteristics	Sulphite oxidase		
	Fish (hake liver)	Mammal[4] (ox liver)	Bacterium[7] (*Thiobacillus thioparus*)
Type of protein	haemoprotein	haemoprotein	non-haemoprotein
Molecular weight	—	115,000	54,000
Absorption maximum (nm)	286, 410	280, 413	270
Optimum pH	8.7	8.6	8.0
Substrate	sulphite	sulphite	sulphite
Electron acceptor used	cytochrome c	cytochrome c	cytochrome c
Km for sulphite (*M*)	1.5×10^{-4}	1.4×10^{-4}	8.8×10^{-5}