

STUDY OF PROTEIN DENATURATION BY ELECTROPHORESIS FROM DIFFERENT LAYERS OF EYE LENS OF JEWFISH *SCIAENA MACROPTERUS* (BLEEKER)

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ABSTRACT

Soluble proteins such as albumins and globulins were extracted from the aqueous humour and different layers of lens of the jewfish *S. macropterus* (Bleeker). Proteins from the nucleus gave consistent patterns and were most resistant to denaturation. Being most stable, the soluble proteins of the nuclear region of the eye lens appears to provide a suitable molecular system for studying genetically based differences in fishes.

INTRODUCTION

The proteins of different lens layers have been studied earlier in some fish species (Gutierrez, 1969; Smith, 1971) and also in other vertebrates and invertebrates (Dohrn, 1970; Cobb and Koenig, 1968). The lens nuclear protein was found to be resistant to heat in the yellowfin tuna *Thunnus albacares* (Smith, 1965). Similarly, resistance of nuclear protein to some physical conditions was demonstrated in the channel rockfish *Sebastolobus alascanus* (Smith, 1971).

Proteins of the eye lens were studied by electrophoresis to identify the different stocks and populations of the same species. A preliminary work was conducted to select a proper tissue which will resist denaturation when collected during the field work. Subsequently, the investigation was undertaken using aqueous humour and different layers of lens. *Sciaena macropterus*, a common large finned jewfish was selected for the present work, as a study of this type has not been previously conducted on this species.

Fresh samples of *S. macropterus* were collected from the local fish market and were divided into two groups for two different types of experiments.

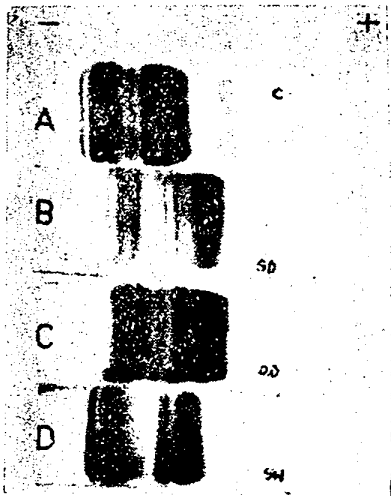
In the first experiment, lenses were removed and electrophoretic patterns were produced using cortices and nuclei of lenses. One lens was processed fresh and the others were given the following treatment in order to study the denaturation:

- i) Kept in decomposing fish head in non-circulating sea water at room temperature for five days.
- ii) Kept outdoor in the shade for five days.
- iii) Kept outdoor in the sun for five days.

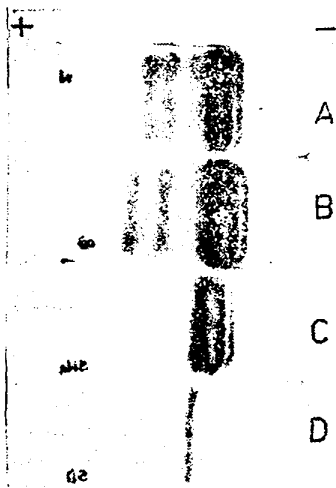
In the second experiment, electrophoretic patterns of proteins of aqueous humour, cortex and four different layers of lens nucleus from fresh frozen jewfish were obtained. In one lens the outermost layer of nucleus was not cleaned of the adhering tissue.

In both the experiments, the soluble proteins were extracted using 0.0189% sodium chloride solution. The extract was cleared by centrifugation at 3000 rpm for five minutes and the clear supernatant solution was used for electrophoresis. The electrophoresis was carried out on cellulose acetate strips (Shandon). The main points of the method are — Cellulose acetate strip size 16 × 125 mm, barbital buffer of pH 8.6, ionic strength 0.05, and a current of 200 volts was applied for one hour.

After electrophoresis, the cellulose acetate strips were soaked consecutively for five minutes in each of the three solutions. Naphthalene Black 12B (0.4% in 5% glacial acetic acid), 5% glacial acetic acid and absolute methanol. After methanol rinse they were immersed for few seconds in a solution of 80 parts of absolute methanol and 20 parts of 5% glacial acetic acid and dried on glass plate. Scanning of the protein was done by a densitometer (Toshniwal) using minimum slit.



CORTEX



NUCLEUS

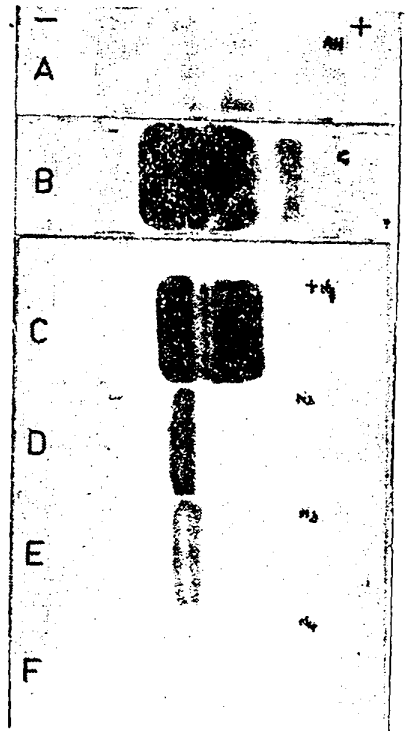


Plate 1 (b):- Electrophoretic patterns of proteins from the aqueous humor (A); Cortex (B); outermost layer of nucleus with some adherent cortex (C); and progressively deeper layers of nucleus (D-F) of a single lens of *S. macropterus*.

Plate 1 (a):- Electrophoretic patterns of proteins from the cortex and nucleus of *S. macropterus* lenses: Fresh (A), stored outdoors in the sun for five days; (B), stored outdoors in the shade for five days; (C), left in decomposing fish head in non-circulating sea water at room temperature for five days (D).

Electrophoretic separation and scanning of proteins from aqueous humour and from the different layers of lens are shown in plate 1 (a & b), Fig. 1 (a & b). In experiment 1, electrophoretic separation of protein from the nuclei of lens exposed to different physical condition produced simple and almost identical patterns (plate 1, Fig. 1). In contrast, the cortical protein patterns were rather complex and variable. For example, a broad and deep staining protein band which is near the point of application and slightly cathodic was clearly seen in the pattern A, while it is completely absent from the patterns B and C. In the pattern D, two additional protein

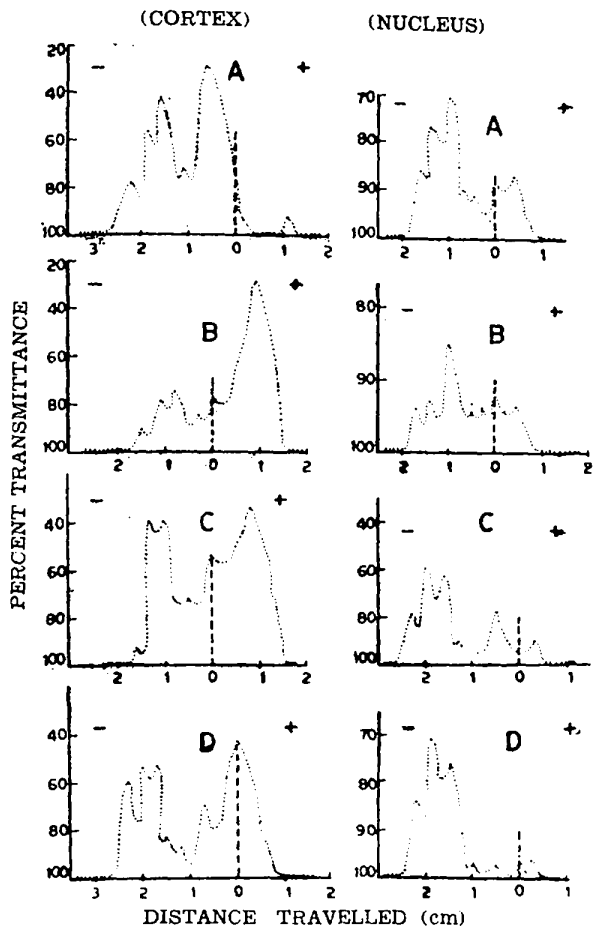


Fig. 1 (a):- Densitometric scanning of proteins as shown in plate 1 (a). Legend as in Plate 1 (a) Dashed line indicates the zone of sample application.

bands were present and anodic protein band is completely absent.

In experiment II, electrophoretic separation of proteins from the aqueous humour, cortex, whole lens and nucleus produced distinctly different patterns. Aqueous humour gave three bands which were lightly stained. The whole lens and cortex gave a complex protein composition, whereas there were six fractions in all the layers of the nucleus. However, the patterns produced by more superficial layers were more intensely stained.

In both the experiments, the electrophoretic patterns demonstrated a predominantly cathodal migration of lens proteins. Yet in both the tissues i.e. cortex and

nucleus, one anodic band was observed. Smith (1966), while studying the cortical lens protein pattern in the Pacific albacore, *Thunnus alalunga*, observed a clear anodal migrating protein band in the cortical tissue could possibly be a serum albumin. Since this molecule has a highly electronegative charge, it readily traverses the lens capsule (Orzalesi and Pirodda, 1955; Smith, 1971) and might normally be in the eye humour as are some other serum proteins (Utter and Hodgins, 1969).

The proteins in the lens cortex were found to be less stable than in the nucleus thus confirming the earlier finding of Smith (1971). The cortex is a metabolically active region of the lens (Baslow *et al.*,

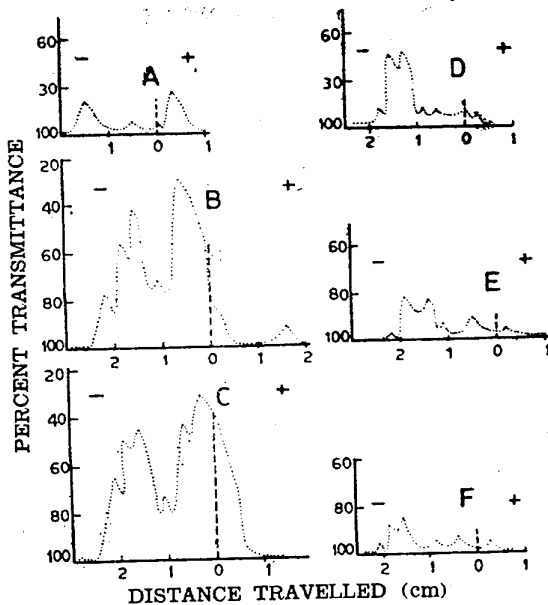


Fig. 1 (b):- Densitometric scanning of proteins as shown in plate 1 (b). Legend as in Plate 1 (b). Dashed line indicates the same as in Fig. 1 (a).

1966; Papaconstantinou, 1967) which synthesizes proteins, some of which may quantitatively and qualitatively be different from those of nucleus (Papaconstantinou, 1967). Smith (1966) reported that the cortex in contrast with the nucleus may contain proteins of the aqueous and vitreous humours and the proteins which are readily altered by the environment. Thus the complexity observed in cortex and whole lenses may be due to these proteins. The rapid denaturation of the lens proteins observed by Sibley and Brush (1967) is probably due to the alterations of "sensitive" cortical proteins. In contrast to this, the lens nuclear protein is metabolically inert and contains primarily such proteins which are structural and sclerosed; thus these proteins resist breakdown (Smith, 1971).

From the above findings it can be concluded that as compared to cortical proteins, the nuclear proteins are more resistant to denaturation. The proteins of the different layers of the nucleus give consistent pattern, although the deep layer showed a decrease in the staining intensity.

I am indebted to Dr. S. Z. Qasim, Director, National Institute of Oceanography, Goa, for his encouragement and facilities. I am also grateful to Dr. A. C. Smith, University of Hawaii, Hawaii, U. S. A. for his valuable help. I also wish to express my sincere thanks to Dr. S. N. Dwivedi, Director, Central Institute of Fisheries Education, Bombay and Dr. V. S. Bhatt, for critically reading the manuscript and offering valuable suggestions.

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