COMPARATIVE ELECTROPHORETIC STUDIES ON THE TISSUE PROTEINS OF SOME CAT FISHES

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ABSTRACT

A comparative study on the soluble eye lens proteins, muscle myogens and multiple haemoglobins of four cat fishes has been made using starch gel electrophoresis.

The whole eye lens protein patterns in air-breathing fish species remain consistent, while those of non-air-breathing species showed minor variations towards the cathode. However, white and red muscle as well as multiple haemoglobin electropherograms were devoid of individual differences in all the species.

In the acid formate gels, globins of *Heteropneustes fossilis* did not resolve into constituent polypeptide chains, but three were obtained in urea starch medium. Two of them had electrophoretic mobilities equal to the chains of *Clarias batrachus*, which were resolved into the same number of chains in both the media. Haemoglobins of air-breathing species were resolved into the same number of multiple forms over a pH range of 6.8 to 8.5; but those of non air-breathing fishes moved in aggregated form at pH lower than 7.0. It is suggested that this property may be advantageous to air-breathing species which have to withstand drought conditions.

INTRODUCTION

During the past few years, classical morphometric data have been, to a large extent, substantiated by chemical evidences derived from tissue protein electropherograms. Although, chemical work is largely confined to fishes from the temperate region (Nyman, 1965; Barrett *et al.*, 1966; Barrett and Williums, 1967; Tsuyuki *et al.*, 1965, 1968; Tsuyuki and Roberts, 1966; Koehn, 1969; and Yoshiyasu, 1973), the evidence suggests that polymorphism, as compared to marine fishes, is more pronounced in fresh water species (Tsuyuki and Roberts, 1965; Uthe *et al.*, 1966). Unfortunately, information on fishes of tropical waters, which might possess

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more stable proteins is quite scanty. It is also probable that air-breathing fishes, having endurance to fight drought conditions, might be least sensitive to physiological variations. In fact, only one air-breathing species has been found to have polymorphic haemoglobins (Hasnain *et al.*, 1973a), while its eye lens protein and muscle protein, patterns have remained fairly consistent. *Ophicephalus marulius*, another air-breathing species, exhibited constancy not only in the patterns of lens protein and muscle protein, but also in the multiple forms of its haemoglobins (Hasnain *et al.*, 1973b).

In the present study, we have attempted to compare tissue proteins of several cat fishes.

MATERIALS AND METHODS

Fish samples were collected from the localities given in Table I, during the usual commercial fishing operations. Eye lenses and desired quantities of muscle were removed from the live specimens, after draining out the blood by severing the caudal peduncle. From distant localities, air-breathing fishes were transported alive. Tissue from the two non air-breathing species (see Table I), were kept under ice.

Preparation of the tissue extracts and electrophoresis procedures were the same as reported elsewhere (Hasnain *et al.*, 1973a-b). Acid formate and urea starch gels were prepared using the methods of Muller (1960) and Poulik (1962) respectively. Slabs of 12 × 7 cm

TABLE I

Details of Samples used for Electrophoretic Studies

Name of the species	Number of individuals	Size in cm	Locality
Wallagonia attu	62	10-45	Aligarh
	13	15-65	Moradabad
	5	42-59	Pilibhit
	16	28-39	Keetham Lake, Agra
Rita rita	25	20-26	Aligarh
	16	17-27	Moradabad
	33	16-25	Pilibhit
Heteropneustes fossilis	102	7-19	Aligarh
	31	7-20	Moradabad
	8	15-20	Pilibhit
Clarias batrachus	98	11-27	Aligarh
	23	19-26	Moradabad
	13	22-25	Pilibhit

* Samples included both males and females with the varying conditions of gonads.

were run at a constant current of 50 mA for 8 hours.

RESULTS AND DISCUSSIONS

In the patterns of eye lens proteins of *W. attu* (Fig. 1a) from the Aligarh area, a total of four zones appeared towards both the electrodes. Pattern-*b*, a variant, lacked the minor anionic zone present just near the origin in pattern-*a*. Electropherograms of the specimen from other localities were identical to pattern-*a*.

R. rita eye lens proteins showed three distinct patterns (Fig. 1). Pattern-*c* was common to the fishes from all regions, but *d* and *e* were only found in the specimens from Pilibhit region.

Eye lens protein patterns of air-breathing species showed constancy towards both electrodes. A total of 5 zones towards the cathode and 3 towards the anode appeared in *H. fossilis*. The respective number of zones in *C. batrachus* was three on each side. Since

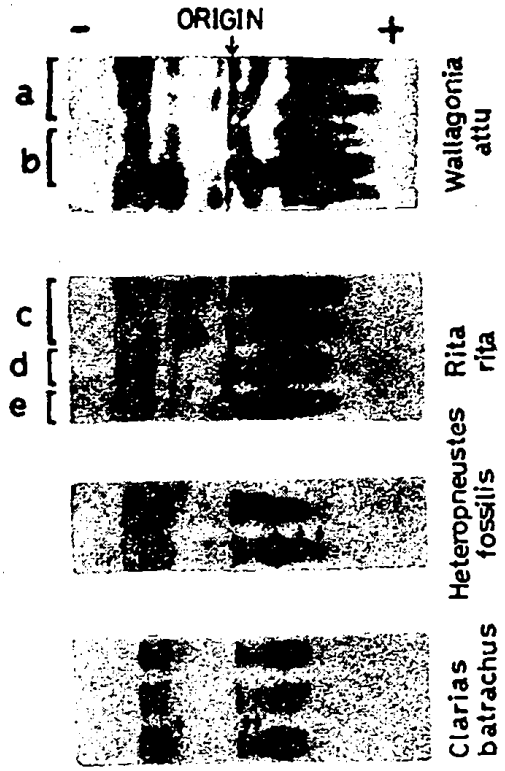

Fig. 1 Starch gel electropherograms of uoluble whole eye lens proteins.

a, b, c, d and e stand for the variants of respective species.

Arrows indicate the positions of minor zones, which could not be clearly produced in the photographs.

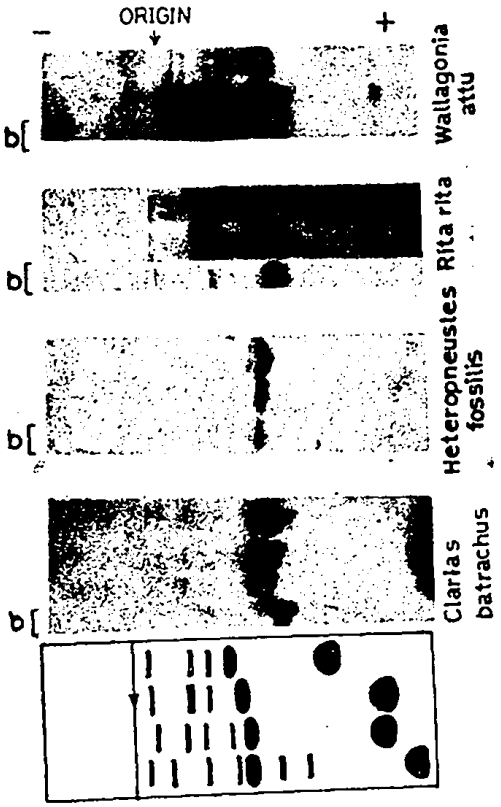

Fig.2 Starch gel electropherograms of the muscle myogens.

Strips b indicate the patterns of lateral line red muscle extracts.

Below : A graphic presentation of white muscle protein electropherograms in the same sequence.

photography of very minor zones poses difficulties they have been marked by arrows.

White muscle patterns of all the four species were devoid of individual variations (Fig. 2). Though their respective electrophoretic mobilities were not the same, three minor zones followed by one major and an adjacent minor, constantly appeared in the muscle myogen patterns of *W. attu* and *R. rita* from each locality. A single zone of fastest mobility was shared by all species. Likewise, this zone was absent from the lateral line red muscle patterns of all species.

The number of minor zones in *H. fossilis* and *C. batrachus* was four. Both the species lacked the minor zone present adjacent to the major, in the other two species. As compared to *H. fossilis*, two additional zones appeared in the electropherograms of *C. batrachus*, between the major and the fastest moving zone. To further clarify the affinity shown by sarcoplasmic protein patterns, a graphical presentation has also been added.

At optimum pH (8.5), *W. attu* haemoglobins were resolved into 4 subforms (Fig. 3), whereas that of *R. rita* into two minor and two majors. Separations in both the cases were towards the anode, and the over all mobilities of multiple forms of the latter species were higher than those of the former.

At a pH below 7.0 (*e. g.* 6.8), haemoglobins of these two species moved in an aggregated form towards the cathode. On the contrary, multiplicity was maintained by the haemoglobin of air-breathing species, in spite of the shift in

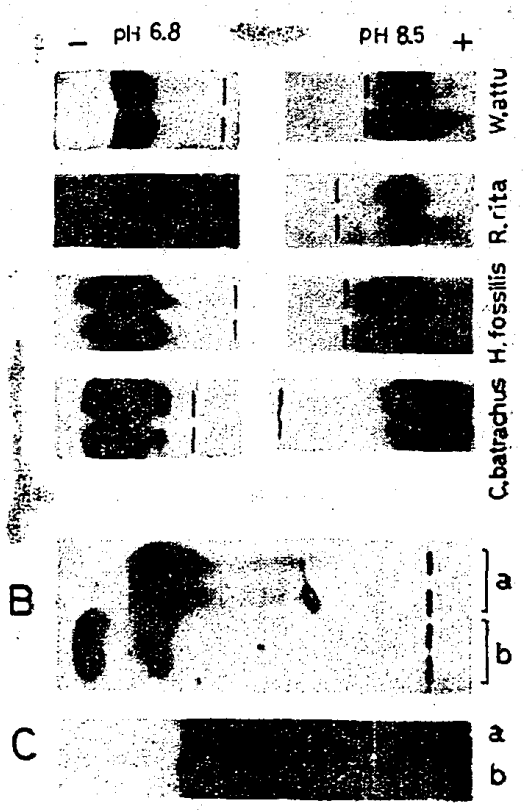

Fig. 3 Starch gel electropherograms of the multiple hemoglobins.

Black line marks the origin.

Below : Separations of polypeptide chains of the globins of *H. fossilis* (a) and *C. batrachus* (b) in the acid formate (A) and urea starch (B) gels.

the charge. At the optimum pH value, haemoglobins of air-breathing species were resolved into six subforms. The mobilities of *C. batrachus* multiple haemoglobins were more than twice the mobilities of *H. fossilis* subforms.

Haemoglobins of the air breathing species were further investigated by attempting the resolution of constituent polypeptide chains in acid media, in the presence of B-mercaptoethanol. In the acid formate gels prepared using

the method of Muller (1960), haemoglobins of *H. fossilis* moved in the form of a single aggregated zone, while those of *C. batrachus* got resolved into two polypeptide chains (Fig. 3 B). The mobility of the first chain of the later species was equal to the aggregated single zone of the former. Assuming that the acid formate gels of pH 1.5 may not be a suitable medium to distinguish between the chains of *H. fossilis*, their separation was attempted in urea starch gels, where a lower pH value of 4.5 could be used. In this system, the separation of haemoglobins of this species into three chains was achieved (Fig. 3C). Two of them had equal mobilities with those of *C. batrachus*, which gave two chains in this system also.

The eye lens protein of fishes in starch gels resolve into more zones than they do in cellulose acetate (Smith, 1960) or agar gels (Rabaey, 1964). Despite the minor differences observed in the non air-breathing cat fishes, the patterns seem diagnostic of each species. In *R. rita* at least, there exist the possibility that pattern–*a* may be heterozygote of homozygous *b* and *c*; a condition already reported in a scombroid fish (Barrett and Williums, 1967). Since the present data do not give enough base for the frequency analysis, further studies are required to determine the exact nature of these variants. The point made by Rabaey (1964) that the variations seem limited to minor protein zones, is supported by our work too.

The muscle myogen patterns show an overall similarity among all the four cat fishes. The affinity is, in particular, distinct in the case of air-breathing species. Since the patterns of red muscle extracts also do not seem to be influenced by individual variations, they can be of equal significance for the purpose of species identification.

As far as we know, no report has yet been published on haemoglobins of *W. attu* and *R. rita*. Haemoglobins of both air-breathing species, however, have been studied by Chandrasekhar (1959) in agar gels, where only one anionic zone appeared in both cases. His patterns showed haemoglobin zone of *C. batrachus* to have a greater mobility than that of *H. fossilis*- a finding concomitant with ours; though six multiple forms appeared in starch gels.

Cyclostomes and fishes are mainly characterised by a large number of multiple forms of haemoglobins, which are considered to allosterify respiratory process in a variety of ways (Li *et al.*, 1972). In the case of air–breathing fishes, the maintenance of multiple forms over a wide range of pH may be of selective advantage, under conditions of water scarcity. Already, there is some evidence that plasma contents are affected during asphyxia. This may be associated with a change of pH in the muscle and blood (Siddiqui and Siddiqui, 1973).

Sasakawa *et al.* (1963) have demonstrated that several haemoglobins exist as a single polypeptide chain at a pH value below 3. Probably for this reason the resolution of *H. fossilis* globin chains could not be achieved in the gels of pH 1.5. Since the main polypeptide chains of the two air-breathing species share the electrophoretic mobilities in the urea starch gels, a similar chemical affinity might exist at the other levels of molecular organization.

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