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Migratory patterns of pigment granules in *Puntius* melanophores after treating them with colchicine

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Abstract
Intracellular transport comprises the movement of organelles along microtubules or actin filaments by means of opposite polarity of microtubule motor (MT) or actin dependent motor of myosin family. When the pigment granules migrate from the cell center, they result in increased colouration of the animal means dispersion of melanophors and alternatively they may aggregate in the cell centre and the animal appears less coloured. To investigate a role of colchicine, we examined the isolated scale equilibrated in physiological saline, than immersed in colchicine at varying concentration (10^{-6} to 10^{-4}M) and next treated with epinephrine. Colchicine (10^{-4}M) effectively blocked the epinephrine induced melanosome aggregation in melanophores on scale preparation, implicated a role for microtubules in transport of melanosomes in the cell. The drug affect the microtubules intracellularily and colchicine (10^{-4}M) effectively blocked the epinephrine induced melanosome aggregation in melanophores, implicating a role for microtubules in transport of melanosomes in the cell.

Keywords: Dispersion, aggregation, colchicine, microtubule, fish

Introduction
In order to adapt to the environment or to signal to other individual's animal group have developed abilities to rapid change colour using specialized pigment cell called chromatophores. Tyrosin derived melanin is deposited in pigment granules known as melanosomes and the black and brown Chromatophores containing melanosomes are called melanophores. Melanophores can aggregated their melanosomes to the cell centre or disperse them throughout the cytoplasm making them ideal as a system for studying intracellular transport or for cytoplasmic organization. The regulation of melanosomes movement involves multiple hormones and neuronal signals. Studies across various fish species have indicated that color changes in teleosts are typically governed by both endocrine and nervous systems, as supported by research by Fuji (1969, 1993a, b, 2000) [8, 9, 11], Abbott (1973) [1], Bagnara and Hadley (1973) [2], and Fujii and Oshima (1986, 1994) [12, 13]. There is some evidence that the endocrine system controls the mortality of melanophore in some fish, while the nervous system dominate in others.
There is considerable evidence that cytoplasmic microtubules are involved in three important processes: Spatial organization of the cytoplasm (Lane and Allan, 1998) [18], intracellular transport (Vale, 2003; Caviston and Holzbour, 2006) [30, 5] and cell locomotion (Wittman and Waterman-Storer, 2001; Burakov *et al*., 2021) [33, 4]. An array of microtubules is usually polarized with minus ends clustered around a center for micro tubular organization, such as a centrosome, and plus ends extending out the periphery of the cell.
In lower forms of life, multicellular Chromatophores are known to exist but in higher forms, including the teleost fishes, they are generally unicellular (Fingerman, 1963) [6]. Though earlier it was thought that melanophores are amoeboid-like cell, which extend pseudopodia during pigment dispersion but Mathews (1931) realized that the concept was incorrect and proposed that melanophores had a rigid cell shape and that only the pigment granules within the cell moved during the two phases *i.e.*, the dispersion or aggregation (Fig.1). When the pigment granules migrate out from the cell centre, they result in increased colouration of the animal alternatively they may aggregate in the cell centre and the animal appears less coloured.

These dispersed and aggregated stages of the same melanophores from isolated scale preparations of the fish under study have also been shown here as Fig-1.

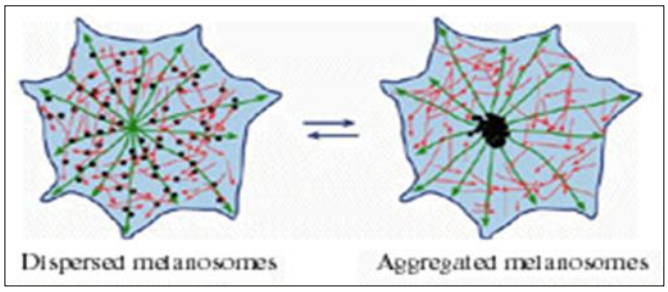

Fig 1: A diagram of a motile light-absorbing or light-reflecting Chromatophores with chromosome aggregated (right) and dispersed (left) throughout the cell.

The ultrastructure of Chromatophores have revealed that a considerable change in cell shape occurs during pigment migration (Fujii, 1971) [7]. When observed under scanning electron microscope, the melanophores of *Oryzias latipes* appeared rather flat. The surface was somewhat rugged but without prominent blebs or microvilli. Obika, 1975 [21], found that aggregated melanophores had very thin radiating dendrites about the same length as those at dispersed states. Besides the chromosomes *i.e.*, the pigment granules, other organelle reported in Chromatophores include in general the nuclei, centrioles, mitochondria, vesicular smooth-surfaced endoplasmic reticulum, micropinocytotic vesicles and ribosomes. The other important cell organelles that have been reported to exist and which are assigned to have a centrol role (the mechanochemical basis) in bidirectional movement of chromosomes within Chromatophores are the microtubules (Bikle *et al.*, 1966; Green, 1968) [3, 14] and actin filaments (Rodionov *et al.*, 2003) [26], which serve as "Rails" for the movement of cargo, the organelles (*i.e.*, the chromosomes in the Chromatophores). It is generally believed that microtubule motors (kinesins and dyneins) support long distance movement of organelles whereas actin filament-dependent motors, myosins are responsive for local transport (Kelleher and Titus, 1998, Hasegawa, *et al.*, 2019) [17, 15]. Pigment dispersion is activated by a cAMP levels while pigment aggregation occurs when cAMP levels are reduced. Microtubules and microfilaments to be essential for rapid transport of pigment granules in fish Chromatophores. Reduced cAMP levels lead to agglomeration, whereas elevated cAMP levels promote pigment dispersion. A cell surface receptor that epinephrine binds to then interacts with a G protein. GTP hydrolysis and binding are capabilities of G proteins. The inhibitory G protein that binds to adrenaline is called a G protein. Adenylate cyclase is inhibited when it is activated. APT is converted into cAMP by active adenylate cyclase. When cAMP levels fall, PKA is blocked, cAMP-dependent protein kinase (PKA) is activated, and pigment granules consolidate. Through the activation of phospholipase C, which in turn causes a shift in intracellular Ca2+, activation of alpha-1 receptors may result in the breakdown of phosphatedyl-inositol in membranes. According to Fujii and Fujii (1965) [35], the goby Chosmichthys gulosus's sympathetic nerve terminal releases catecholamines only when Ca2+ is present. In order to control pigment aggregation and dispersion in melanophores, Ca2+ and cAMP work against each other. Ca2+ enters the cell from the extracellular environment when epinephrine attaches to a receptor on the cell surface. The focus of current research is on clarifying how colchicine affects pigment granule movement in Puntius melanophores.

2. Materials and Methods

The fresh water teleost, *Puntius species*, irrespective sex were used as the experimental material. The collection of fish were done from Tighra reservoir located 23 km from Gwalior (M.P) and they were transported in fresh water aquaria (90 X45X45cm) in our facilities for at least a week for acclimatization. All the experiments were carried out at room temperature between 24 °C and 28 °C. The scale slips were gently plucked by fine forceps from the dorsal trunk surface of the animal. The isolated scale were immediately immersed in a physiological saline solution, Physiological salines had the following composition in mm (NaCl; 128.3, KCl; 2.8, Glucose; 5.6, $CaCl_2$; 1.8, 0.5M Hepes-NaOH with pH value 7.4). Hogben and Slome (1931) [16] evaluated the effects of drugs on the response of specific groups of amphibian melanophores, where 1, represents maximum aggregation, 5, represents maximum dispersion, and 2, 3, and 4 represent intermediate stages of aggregation dispersion (Fig-2).

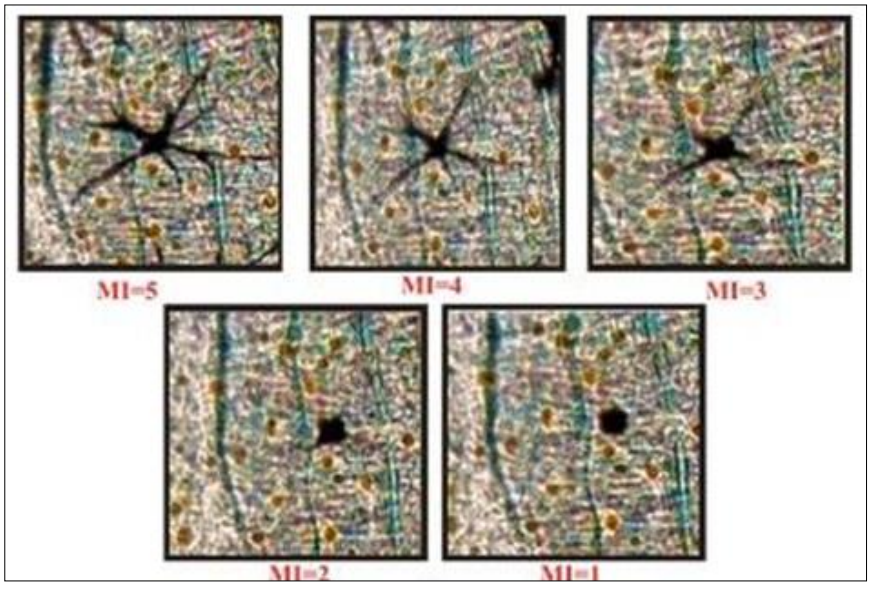

Fig 2: Melanophore indices (5-1) as were used for measurement of melanophore responses in the study.

3. Results

3.1 Effect of Epinephrine

It is adrenomimetic or sympathomimetic agent. Epinephrine is nonselective and interacts with α_1, α_2 and β_1, β_2 adrenoceptors. Its effect on some body systems depend on the concentration of epinephrine as well as the type of receptor. At low concentrations, β effects are predominant. Because of its properties to produce responses in effector cells by directly interacting with α-adrenoceptors, it is also referred to as directly acting adrenomimetic drug. In the present study, epinephrine at a concentration of 10^{-6}M induces rapid and quite potent aggregation of pigment in PS equilibrated melanophores. The effect starts within a minute and the full aggregation (M.I. =1) was achieved within 5 min (Fig-3).

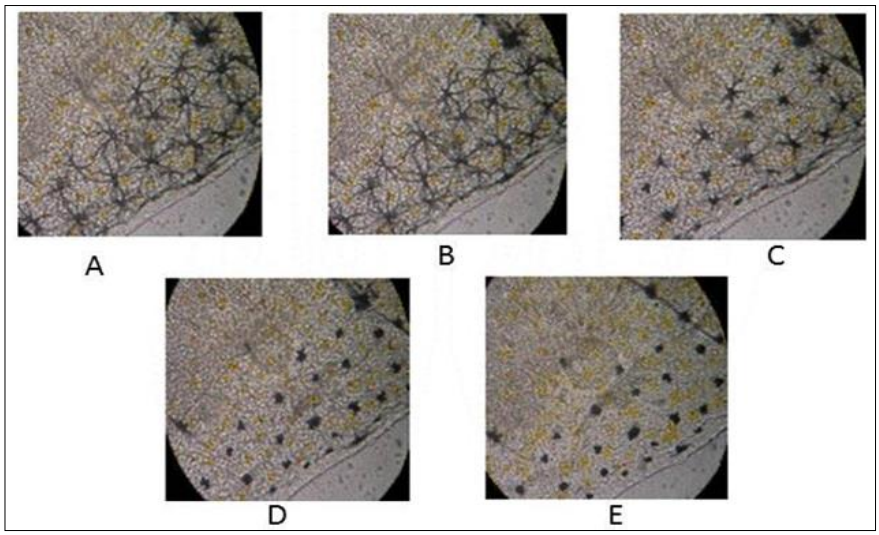

Fig 3: Sequence of photomicrographs showing responses of innervated melanophores in an isolated scale of *Puntius* to epinephrine

A: Melanosomes are fully aggregated when the scale is perfused with Physiological saline for 15 minutes; B: 1 minute after epinephrine (10–6M); C: 2 minutes after epinephrine; D: 3 minutes after epinephrine; and E: 5 minutes after epinephrine administration.

3.2 Effect of colchicine

Originally produced from plants of the genus Colchicum (Autumn crocus, Colchicum autumnale), popularly known as "Meadow saffron", colchicine is a poisonous natural product and secondary metabolite. The alkaloid colchicine was first discovered in 1820 by PS Pelletier and J Caventon. Colchicine inhibits microtubule polymerization by binding to tubulin, one of the main constituent of microtubules. Availability of tubulin is essential to mitosis, and therefore colchicine effectively functions as a "mitotic poison" or spindle poison. . In neurons axoplasmsic transport is disrupted by colchicine and so it affects the melanosome translocations within melanophores

The isolated scale preparations were equilibrated in physiological saline for 15-20 min to attain their dispersed state and then immersed in colchicine solution at varying concentration (10^{-6}-10^{-4}M) for 30 min. The melanophores maintained their dispersed state. These melanophores were next treated with epinephrine at a concentration of 10^{-6}M (10 min). In all the cases epinephrine caused aggregation of the pigment but this response was a decreased response as compared with the control. Colchicines 10^{-4}M significantly inhibited the response to epinephrine in a concentration dependent manner over the effective concentration range that was studied (10^{-6} to 10^{-4}M). This indicates that the drug affects the microtubules intracellularily (Fig-4, 5).

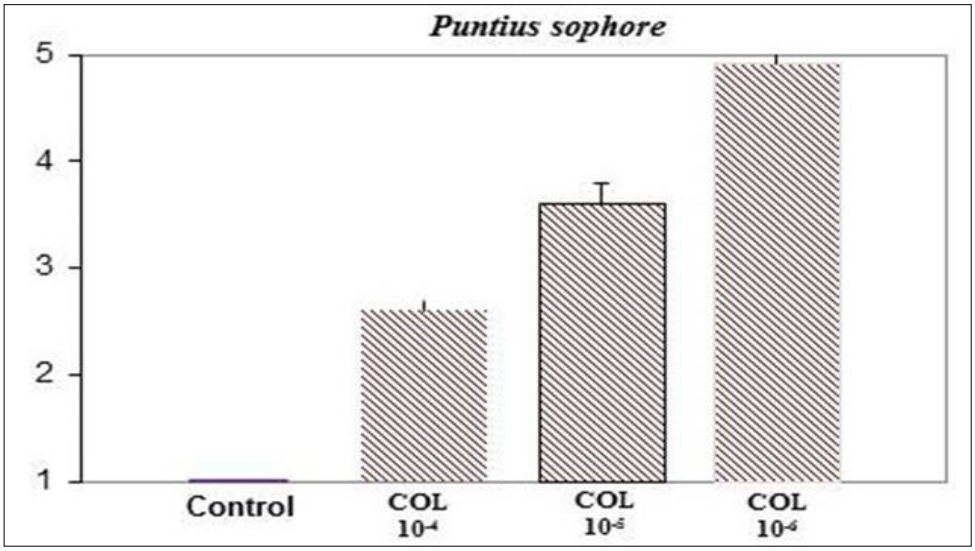

Fig 4: The response of melanophores to epinephrine (10^{-6}M) for 10 min after pretreatment with varying concentration of colchicine for 30 min. Control represent preincubation in PS for 30 min.

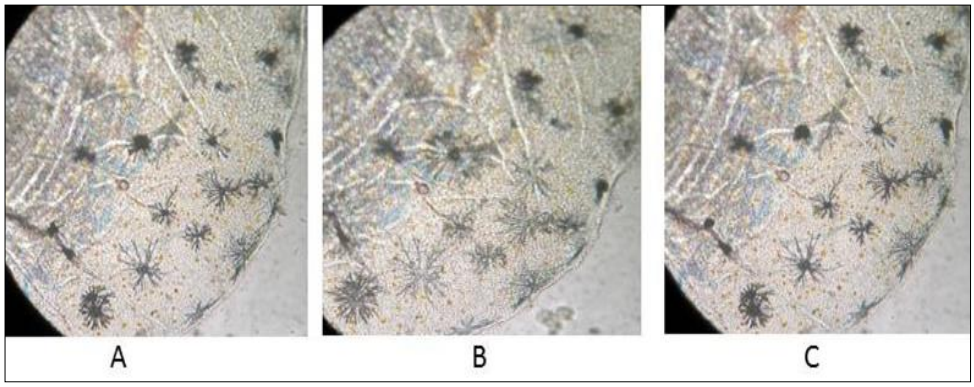

Fig 5: Successive treatments of the innervated scales melanophores. Sequecne of procedures was as follows: In PS after 15 min. (A), Col ($5X10^{-4}$M) for 5min (B), Epinephrine (10^{-6}M) for 5 minutes

4. Discussion

The observations pertaining to inhibitory effect of colchicine on pigment aggregation responses in *Puntius* melanophores due to a highly potent drug epinephrine, are in accordance with the results presented by earlier workers (Wikswo and Novales, 1969, Murphy and Tylney, 1974, Patil and Jain, 1996, Yadav and Jain, 2017) [32, 20, 25, 34]. However, the effect of drugs such as colchicine, is not always related to the disruption of cytoplasmic microtubules (Obika, 1986) [23], as in medaka melanophores, colchicine at 5mM produces a retardation in pigment aggregation although a substantial number of microtubules survive even after a prolonged exposure to the drug. That colchicine may act at a site unrelated to microtubules and still can influence the pigmentary response is suggested by the action of lumicolchicine, which has little binding activity to tubulin but has been found to be capable of preventing pigmentary response in the same manner as colchicine does (Obika *et al*., 1978) [24]. Drugs like vinblastin, nocodazole, colcemid and taxol having different mechanism of action on the microtubules may be tested for their effects on melanosome movements to elucidate the mechanochemical basis of pigment translocation in fish melanophores. In addition to this as actin filaments have also been implicated to have a definite role, it would be interesting to include the studies pertaining with drugs that are known to disrupt the cytoplasmic filaments. It is important to note that, in contrast to melanophores from *Fundulus heteroclitus, Gymnocorymbus ternetzii*, *Oryzias latipes*, and *Pterophyllum scalare*, which are inhibitors of temperate and tropical zones, Obika and Mayer-Rochow (1986) [23] worked with the Antarctic teleost *Pagothenia borchgrevinki* and were able to report a cold-resistant microtubule system on which melanosome movements depend. In contrast, melanophores exposed to low temperatures (0 to 50C) for 30 to 60 minutes completely disassemble microtubules, and the cells that result are then devoid of microtubules and cannot respond to aggregative stimuli by rapidly aggregating pigments (Murphy and Tilney, 1974; Obika *et al*., 1978 [24]; Schliwa and Enteneuer, 1978) [20]. Bright-field microscopy recording the aggregation of melanosomes carried by dynein in zebrafish melanophores at a high recording rate of 800 frames per second.

It is valuable observation recorded that microfilament and microtubules both are involved in melanosome movement in many higher teleosts. Similar to microtubules, filamentous actin possesses an inherent polarity. Short microfilaments, measuring 2–3 μm in length, are distributed in a certain way within melanophores with a higher density near the periphery than at the center, and they do not exhibit a preferred orientation. According to Rodinov *et al.* (1998) [36], actin filaments facilitate movement to local sites, whereas microtubules serve as pathways for long-distance transport. Microtubules provide tracks for initial fast motion toward the periphery, but a 2-D "random walk" on actin filaments achieves the final uniform distribution of pigment granules (Obika and Mayer-Rochow (1986) [23].

By attaching itself to tubulin, one of the primary components of microtubules, colchicine prevents the polymerization of microtubules. Because tubulin availability is necessary for mitosis, colchicine serves as a potent mitotic and spindle toxin. For 60 minutes at 4 °C, the isolated scale preparation was equilibrated in colchicine. In the scales, the melanophores had reached complete dispersion. Subsequently, the melanophores were exposed to epinephrine (10-6 M), which caused an odd accumulation of the pigment. The pigment aggregated at the center and the periphery, creating a hollow gap between them.

5. Conclusion

Colchicine (10^{-4}M) effectively blocked the epinephrine induced melanosomes aggregation in melanophores on scale preparation implicating a role for microtubule motors in transport of melanosomes in the cell.

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