STUDIES ON THE CONTROL OF TAPETAL PIGMENT GRANULE MIGRATION IN THE SPINY DOGFISH, *SQUALUS ACANTHIAS*.

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Intracellular organelle transport is a fundamental process underlying a number of major cellular functions, including axoplasmic streaming, chromosome separation during cell division and pigment granule movement in both vertebrate and invertebrate chromatophores. In the visual system, pigment granule translocation is of particular importance in a number of vertebrate phyla (notably the fishes), where it plays a role in the response of the neural retina to light and dark adaptation. This has been most thoroughly studied in the retinas of teleost fish. The retinal epithelial cells of these animals possess long, filamentous processes which interdigitate intimately with the photoreceptor cells of the neural retina itself. During dark adaptation, melanin pigment granules move towards the basal region of the epithelial cells, away from the retina. During light adaptation, the pigment granules move in the opposite direction until, by virtue of the anatomical arrangement of the processes, they reach a point where screening of photoreceptors from scattered light is possible (Burnside and Basinger, Invest.Ophthalmol.Vis.Sci. 24:16-23, 1983). Screening pigment granule movement is also found in the elasmobranchs. In these animals the retinal epithelium is usually non-pigmented. However, underlying the retinal epithelium in many species is a specialised layer, the tapetum. This consists of two basic types of cells, melanophores and guanophores, which contain pigment granules and reflective guanine platelets respectively. In the light, the pigment granules migrate distally towards the retina obscuring the tapetum and preventing light reflection back through the retina. In the dark, pigment granule migration is reversed, the tapetum is exposed and light reaching it is reflected back (Nicol, J.mar.biol.Ass.U.K. 45:405-427, 1965). The process of control of this phenomenon has so far not been elucidated and, indeed, the mechanisms by which organelles are translocated inside cells in general are not well understood. We have begun studies on the tapetum in the Spiny Dogfish, *Squalus acanthias* in order to investigate both these aspects of the problem.

Our approach has been to use short-term culture of isolated pieces of dogfish eyecup. Briefly, light or dark adapted dogfish were sacrificed by decapitation and enucleated. The eyes were opened, drained of vitreous, and pieces approximately 25 - 35 mm^2 were cut from the back of the eye using razor blades. These pieces were transferred to a bicarbonate buffered culture medium identical to that previously described for use with frog retinas (Heath and Basinger, Vision Research 23:1371-1377, 1983) with the addition of NaCl to give a final molarity of 280 mM, and urea to 350 mM. Culture was carried out under the appropriate lighting conditions with the constant passage of 95% O_2/ 5% CO_2 over the tissue. At the end of an experiment, the tissue was fixed, plastic embedded and sectioned for light microscopy. It was found that qualitiative inspection of the tissue prior to fixation was a reliable indicator of the state of screening pigment migration. Tissue that was dark and non-reflective was found on sectioning to have dispersed pigment, tissue that was shiny and highly reflective was found to have aggregated pigment (see also Nicol, ibid). However, the results described here are based on examination of tissue sections. Our strategy was to add chemical effectors of various types that have been shown to affect intracellular translocation in other systems. Of particular interest are the cyclic nucleotides. In teleost fish, cyclic adenosine monophosphate (cAMP) induces dark adaptive pigment granule movement in a culture system similar to ours (Burnside and Basinger, ibid). Accordingly, we concentrated on the effects of this drug.

Our major findings were as follows. In isolated pieces of tissue transferred from light-adapted animals to darkness, complete exposure of the tapetum occurs in 2 hours. Conversely, isolated tissue transferred from dark-adapted animals and cultured in the light shows complete occlusion over the same time course. We found that addition of dbcAMP to the culture medium at concentrations of greater than 0.5 mM will (1) inhibit dark-adaptive pigment aggregation in light-adapted tissue, (2) promote light-adaptive pigment dispersion in DA tissue. These effects occurred in the absence of phosphodiesterase (PDE) inhibitors. However, methyl xanthine PDE inhibitors such as isobutylmethylxanthine (IBMX), theophylline and caffeine produced similar effects alone, in the case of IBMX at concentrations of 10 - 20 μM. Subthreshold doses of dbcAMP in combination were also effective. Inhibition of pigment aggregation due to caffeine was reversible. In control tissues, we found that non-derivatized cyclic AMP, dbcGMP and adenosine were without effect.

This study has linked for the first time the control of pigment granule migration in the shark tapetum lucidum with a cyclic nucleotide system, that is cyclic adenosine monophosphate and its catabolic enzyme, phosphodiesterase.

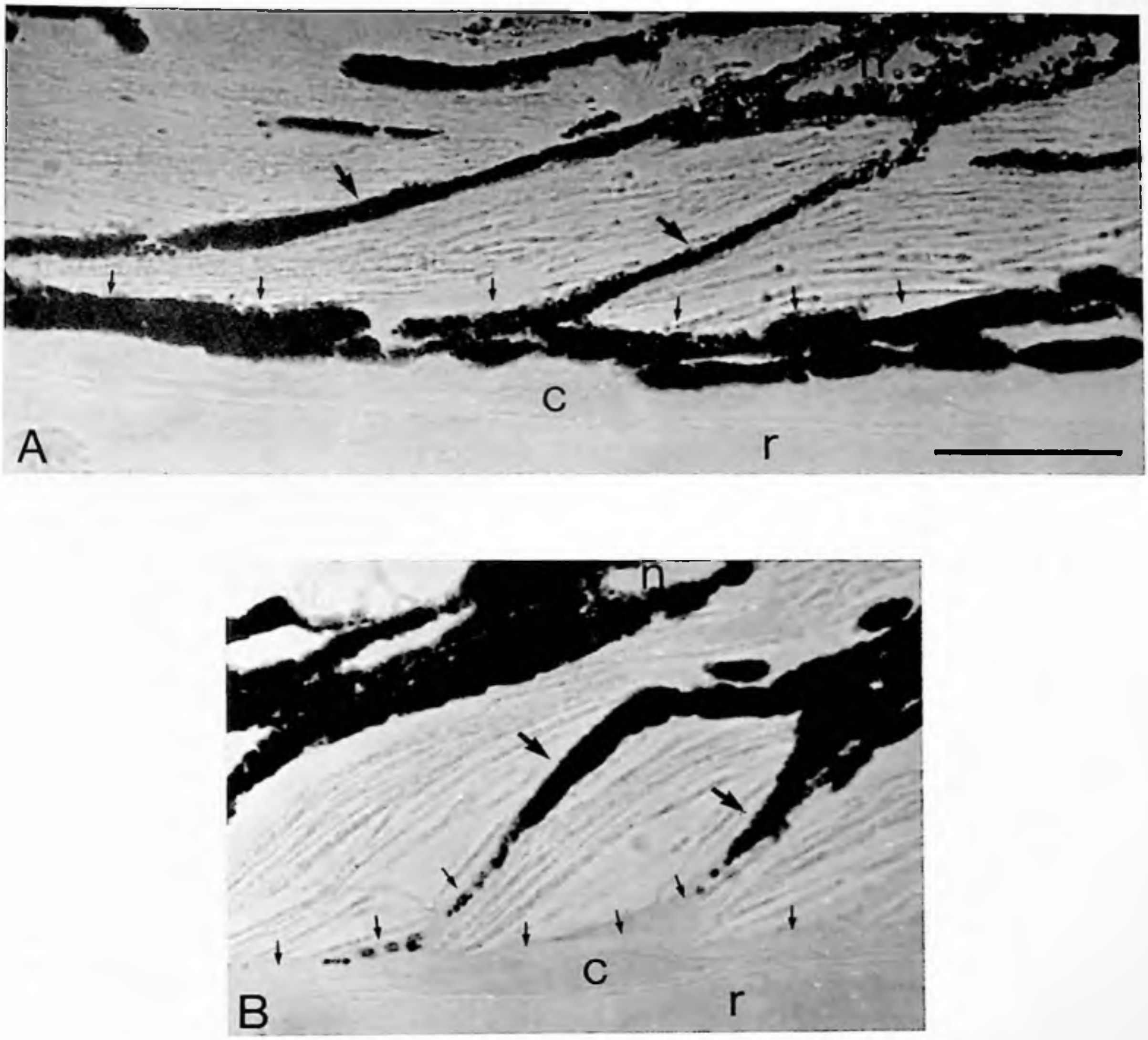

Figure 1. Effect of dbcAMP on dark-adapted <u>Squalus</u> choroidal tapetum, <u>in vitro</u>. (A) With 5 mM dbcAMP, pigment granules migrate into distal melanophore processes (small arrows) which overlay the choroid. (B) In tissue incubated without drug, melanin granules remain in the proximal processes (large arrows). Little pigment is seen above the choroid. Key: (c) choroid, (n) tapetal cell nucleus, (r) retinal epithelium. Size and orientation of the tapetal cells varies with their location in the eye. Unstained, 2 μM plastic sections. Scale bar 20 μM.

Interestingly, the effect of dbcAMP observed in this study was exactly opposite to that previously observed in studies of pigment granule migration in teleost retinas. In the latter studies, dbcAMP induces pigment aggregation and has been described as a form of "chemical darkness". In the case of the shark tapetum, however, it appears to act as a form of "chemical light". Further investigation will no doubt help to explain these differences. We are particularly concerned to determine whether there exists in Squalus tapetal tissue an anabolic (adenylate cyclase) system controlling endogenous cyclic AMP levels. For example, will pigment granule migration be affected by agents known to modulate adenylate cyclases in other tissues (e.g. adrenaline, isoproterenol, prostaglandins)?

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