WHITE PIGMENTARY EFFECTORS (LEUCOPHORES) IN KILLIFISHES

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In interpreting their observations on birefringent materials in the scales of *Fundulus heteroclitus* examined with polarized light, Shanes and Nigrelli (1941) seem to deny the morphological individuality of what Odiorne (1933) called guanophores. After first identifying the birefringent masses they observed as the guanophores of Odiorne, they advocate the conclusion that the doubly refractive material is an integral part of the scale melanophores and xanthophores. The question thus raised as to the status of the guanophores, about which relatively little is known (Parker, 1940), may lend heightened interest to observations of mine on certain similar integumentary structures found in other, related species. In any event, these observations add to available pertinent information concerning species found very useful for study of the complexities of the hormonal and nervous control of their often independently responding different kinds of pigmentary effectors.

While a guest in the U. S. Fish and Wildlife Laboratory at Beaufort, N. C.—where I was hospitably and helpfully accorded all facilities by the Director, Dr. H. F. Prytherch—I had occasion to study the chromatophores of the dorsal aspect of local Cyprinodontidae, especially the striped killifish, *Fundulus majalis* Wahlb. Very numerous, chromatophorelike, whitish structures at once attracted my attention.

The component material that made these bodies conspicuous in *F. majalis* was not iridescent and appeared to share actively in chromatic adjustments; also, they occurred in addition to iridescent bodies as well as melanophores and xanthophores. They resembled published photographic representations of the "guaninophores" (Ginsburg, 1929) and silvery halolike "iridosomes" (Sumner and Wells, 1933) of *Lebistes*, which is classed in the same order as *Fundulus*, and were evidently the same kind of chromatophore as these and as the ones Odiorne described in *F. heteroclitus*.

Odiorne adhered to long-established views in classing the non-iridescent structures as guanophores. His conclusion that the minute, not obviously crystalline particles characterizing their contents was probably guanine rested on comparative grounds laid down in papers too numerous to relate (but traceable through the bibliographic citations given by Odiorne, Sumner and Wells, Ginsburg, and Foster). For my part, I did not determine by chemical means whether the whitish material in the structures that are the subject of the present paper was guanine. But there seems

to be no reason to doubt that it is the same as one of the forms in which MacMunn, for example, by his extensive chemical and cytochemical tests, identified guanine in divers teleosts (Cunningham and MacMunn, 1893). Modern analytic methods have not led to revision of the older determinations of guanine in different integumentary granules, etc., of whitish, silvery or iridescent appearance (Peschen, 1939; cf. also Millot, 1923).

Although the non-iridescent chromatophores in question would accordingly be guanophores, it seems to me preferable to call such bodies *leucophores*, i.e., white or colorless chromatophores. This designation, first used, it seems, by Keller (1895) in reference to similar structures in the chameleon, usefully indicates their distinctive chromatic character and does so without further (and perhaps rash) implications. It is more logically alternative to *iridocyte*, *iridophore* or *iridosome* than is *guanophore*, since the iridescent material of the former is just as probably guanine (in crystalline form) and justifies classing them as another type of guanophore, as Odiorne himself did. In construction and meaning, *leucophore* corresponds advantageously to *xanthophore*, *erythrophore*, etc., terms that conveniently distinguish chromatophores according to their color without reference to the chemistry of their pigments, which is not necessarily the same for chromatophores of one color.

Unlike *F. heteroclitus*, in which the "guanophores" occur only by rare and isolated exception in the outer dermal layer with its rich studding of melanophores, *F. majalis* is abundantly equipped with these white chromatophores, or leucophores, in the outer dermal layer over the dorsal and dorsolateral aspects of the whole body excepting the fins (into which few stray). Both species have also iridophores, commonly combined as iridosomes (which, like Odiorne, I distinguish from the "iridosomes" that Sumner and Wells record for *Lebistes*). The leucophores of *F. majalis* are characteristically, at least in the outer layer, closely associated with melanophores and melaniridosomes (described for *F. heteroclitus* by Foster, 1937); their central masses lie next internal to and usually hidden by the associated pigmentary bodies. Most of these outer chromatophores and chromatophore complexes are aggregated in diagonal bands crisscrossing the back and sides much the same as in *Lebistes* (cf. photographs of Sumner and Wells); they are grouped in less geometric pattern over the head. Intervening gaps of skin are rather transparent as deep as the inner dermal chromatophore layer beneath the scales, which is characterized by many more massive iridosomes or clusters of iridocytes and often larger and less delicately branched melanophores than those of the outer layer. (Superficial to all the other chromatophores is a sparse sprinkling of mostly smaller, simple melanophores. The relatively small but numerous xanthophores occurring both within the inner layer and just under the main outer dermal layer of other chromatophores, are fairly accessible to view

in the gaps of the latter.) Exceptionally, a leucophore of the outer dermal layer may occur apart from any melanophore or melaniridosome. The existence and mode of occurrence of these teleostean white chromatophores recalls certain Crustacea described as having polychromatic chromatophores including a white component and other species having white pigment in separate, or monochromatic, chromatophores (for bibliography, see Parker's review, 1940).

To determine whether the leucophores, combined or separate, do in fact play an active rôle in color changes shown macroscopically by the striped killifish, I tested them for response to background, i.e., to bottoms of different shades and hues. These were provided by 8-inch and 10-inch glass culture bowls painted externally with enamel (black, white, yellow or light blue). I enclosed each fish for observation in a shortened test tube opened to admit inflow of water at the head end and half-stoppered to permit outflow at the opposite end (Butcher, 1939). With the tube held in place, immersed in sea water in a painted bowl, by a metal strap pinched on the end away from the fish's eyes, the dorsal chromatophores could be examined through a binocular dissecting microscope, while the fish stayed in a situation inducing continued chromatic response to the given color. The bowls stood inside a window exposing the fish to a broad expanse of diffuse daylight. A Spencer "universal" microscope lamp provided good illumination of the microscopic field from above, without interfering detectably with the pigmentary adjustments called forth by the colored bowls.

The critical tests involved two male and four female *F. majalis* about 7 cm. long. In each, a group of favorably exposed chromatophores was selected for ready recognition and repeated inspection as the fish stayed now in one, now in an oppositely colored bowl. Such exchanges between black and white environments established the fact that the leucophores concentrated their "pigment" in response to the black and dispersed it in response to the white. After a stay of two hours or longer over the white bottom, the visible contents of every leucophore was spread finely through a delicate lacework of processes extending from the leucophore's center; they partly overlay the now concentrated pigment of the melanophores, obscuring them and the other chromatophores. When the fish was transferred to a black dish, the black pigment spread out in a few seconds partway into the melanophore processes, thereby covering up much (but not yet the farthest processes) of any associated leucophores. The gray-whiteness of the slenderest and most peripheral lacy processes of each leucophore disappeared almost simultaneously. The means of this disappearance could not be the screening effect of the melanin, which was not yet so widely dispersed. As the melanophores continued showing rapid pigment spread, a markedly slower centripetal accumulation of the white

material was discernible in the leucophores (usually increasingly obscured by the melanophores, but with substantial opaquely white stumps often to be seen between the roots of the melanophore processes). After longer sojourn over black, the melanophores in the maximum pigment dispersal never duplicated the gossamerlike appearance characterizing the leucophores in their maximum dispersal. The extreme dispersed form of a melanophore was not identical with that of its associated leucophore. Evidently the processes of the two were complexly interlaced, not confluent. Upon reverse transfers of the fully black-adapted fish to a white or other pale bowl, a great reduction of melanin dispersal occurred in a few seconds, whereas it took longer for the white processes to appear; and the melanin concentration seemed practically complete well before the dispersing response of the white chromatophores approximated its maximum. The leucophores took more than half an hour (at about 20°C.) to bring their dispersing change close to completion; this was twice as long as the melanophores required for equivalent pigment concentration. Whether, indeed, the leucophore changes consisted of such dispersing and concentrating migrations of particles outward and inward in the processes as are familiar in melanophores could not be seen with the magnifications used (higher than 3.4× objectives and 12.5× oculars proved impractical, especially because of the fish's breathing movements). In any case, the conclusion seems clear: the leucophores of this species are active effectors; they assist in the color changes whereby the fish becomes less conspicuous over different grounds and they do so by responding, inversely as compared with the quicker reacting melanophores, to the shade of the bottom.

Examination of the leucophores after similar sojourns over, and transfers between, yellow and blue revealed no sure differences. Comparative examination of six fish macroscopically well adapted to blue and a larger lot of others well adapted to yellow added support to the conclusion that leucophore changes are probably correlated only with the shade and not with the hue of the environment. There appeared at most a mere suggestion of more filmy, extreme, leucophore dispersal and complete screening of the black and iridescent chromatophores in the blue than in the yellow fish; such an apparent difference might be illusory, resulting from the condition of the other, deeper-lying pigments (the dorsal xanthophores showed decided pigment dispersal in the yellow, versus concentration in the blue, fish).

Leucophores are very plentiful, in addition to iridophores, also in the sheepshead minnow, *Cyprinodon variegatus* Lacépède. They occur apart from melanophores less rarely than in *F. majalis*. This made it easier to determine that they quickly initiated dispersal in response to white and concentration in response to black. One of these fish, 3 cm. long, was tested through repeated stays in the black, white, yellow and blue bowls;

it showed no significant difference from *F. majalis* in the behavior of the leucophores. It was particularly in *Cyprinodon*, however, that a close relationship between iridophores and non-iridescent leucophores was indicated by a similarity of the reactive leucophores, in size and glitter of some of their contained bodies, to the same fish's iridophores, which were unreactive (constant in form).

Intraperitoneal injections into eight *F. majalis* of ergotamine tartrate (0.01–0.03 ml. of Sandoz's "gynergen" per gram of fish) demonstrated that the response of the leucophores can take place without the opposite changes occurring in the melanophores.* While the latter remained in the concentrated state regardless of what vessel the fish was kept in, the leucophores still effected concentration in the black bowls and dispersal in the white. The demonstration was especially convincing in the case of two fish that had been hypophysectomized fifteen days before the ergotization. After a two-hour stay over black or white, following transfer from the opposite color, repeatedly in these fish the leucophores responded as already described, while the melanophores remained practically punctate. Assuredly, then, whatever the longer-term biochemical relation may be between the black and white chromatophores, and however intimately they are associated, the white chromatophores are not undissociable parts of other chromatophores, but chromatically individual functional entities, significant especially for the maximum pallor achieved in this species. Their resemblance to the independently reactive white pigmentary effectors of crustaceans accordingly embraces their function.

It follows from these results of ergotization that the mechanisms mediating the presumably visually initiated response of the leucophores to pale or dark ground colors must differ from those subserving the similarly adaptive changes of the melanophores and xanthophores. That the mechanisms exclude direct innervation of the leucophores suggests itself but is unproved by present data, since the ergot dose that blocks melanophore changes does not paralyze such nervous functions in the fish as equilibration, breathing, etc. On the other hand, it follows from the same experiments performed on pituitaryless fish (five of the eight that were ergotized) that the leucophores do not depend on variation in the supply of a pituitary hormone for their changes in response to the shade of the bottom.

Summary.—White chromatophores like the non-iridescent guanophores of *Fundulus heteroclitus*, but preferably called leucophores, are abundant in the outer derma of *F. majalis* and *Cyprinodon variegatus*.

They participate actively, but slower than the melanophores, in chromatic responses to "backgrounds," effecting concentration of their whitish contents in fish kept in a black bowl and presenting a very different, dispersed appearance, which augments pallor, in those kept over a white, light blue or yellow bottom.

Ergotization of *F. majalis* kept the melanophores in the concentrated state without stopping the leucophore changes. This confirmed that the leucophores are functionally individual, rather than integral parts of the melanophores with which most are combined.

They do not (in *F. majalis*) depend on the pituitary for mediation of their responses.

* A fuller report of the effects of ergotamine on the several chromatophores in this species is in preparation for publication elsewhere.

Butcher, E. O., *Biol. Bull.*, **77**, 258–267 (1939).
Cunningham, J. T., and MacMunn, C. A., *Phil. Trans. Roy. Soc. (London)*, **B184**, 765–812 (1893).
Foster, K. W., *Jour. Exp. Zoöl.*, **77**, 169–213 (1937).
Ginsburg, J., *Zool. Jahrb., Abt. Anat.*, **51**, 227–260 (1929).
Keller, R., *Arch. ges. Physiol.*, **61**, 123–168 (1895).
Millot, J., *Bull. Biol. France Belg.*, **57**, 261–363 (1923).
Odiorne, J. M., *Proc. Nat. Acad. Sci.*, **19**, 750–754 (1933).
Parker, G. H., *Proc. Amer. Acad. Arts Sci.*, **73**, 165–195 (1940).
Peschen, K. E., *Zool. Jahrb., Abt. Physiol.*, **59**, 429–462 (1939).
Shanes, A. M., and Nigrelli, R. F., *Zoologica (N. Y. Zool. Soc.)*, **26**, 237–243 (1941).
Sumner, F. B., and Wells, N. A., *Jour. Exp. Zoöl.*, **64**, 377–403 (1933).

DISTORTION OF STRATIGRAPHIC THICKNESSES DUE TO FOLDING

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Introduction.—Discussions of causes or mechanism of folding include as one vital component the thicknesses of the folded strata. Geosynclines, troughs or furrows are regions in which sedimentation has led to greater stratigraphic thicknesses and out of which folded zones, like the Appalachians, emerge. Thousands of papers have been written with the assumption that thicknesses as measured today are indicative of and permit conclusions of depths of troughs, basins of sedimentation, location of geosynclines, correlation of increase or decrease of thicknesses of formations and many others. Swells and deeps within geosynclines have been discussed and Schuchert's paleogeographic maps are well known to every geologist. The concept of the geosyncline has become one of the pillars on which tectonic speculation rests. The author, however, feels obliged to cast some serious doubts on the underlying assumptions which are contained in the determination of stratigraphic thicknesses.

The general assumption may be phrased simply as follows: "Folding