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*COLOR CHANGES IN FUNDULUS WITH SPECIAL REFERENCE
TO THE COLOR CHANGES OF THE IRIDOSOMES*

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As suggested by Connelly (1925), the iridocytes (Pouchet, 1876; guano-phores, Ballowitz, 1912) play an inactive rôle in the color changes incited in the fish *Fundulus heteroclitus* L. by the colors of the backgrounds over which they are placed. For example, the yellow chromatophores (xanthophores) when partly "expanded"¹ cover the blue iridocytes which comprise the stratum argenteum (Cunningham and MacMunn, 1893; l'argenteure of Pouchet, 1876; reflecting layer of Connelly, 1925). The blue color which emanates from the guanin crystals of these iridocytes viewed together with the translucent yellow of the xanthophores gives the green color which is characteristic of fish that have been kept upon a green background. Likewise, in fish kept over blue backgrounds, the xanthophores are maximally "contracted"¹ (Fries, 1931), revealing the blue of the stratum argenteum. The black cells (melanophores) are partly "expanded," shading the stratum argenteum beneath, and darkening the shade, but not greatly obscuring the blue color.

Certain iridosomes (Cunningham and MacMunn, 1893; Ballowitz, 1912), i.e., isolated groups of iridocytes, in *Fundulus* are associated with large melanophores, located in the deeper portions of the dermis but external to the stratum argenteum. These iridosomes form "chromatophore combinations," called "melaniridosomes" in other species of fish by Ballowitz (1912), and by Becher (1929). These iridosomes, together

with the reflecting patches (Connelly, 1925) composed of iridocytes found in male *Fundulus*, play a passive though not as important a rôle in the adaptive color changes of the fish as do the iridocytes of the *argenteum*. These iridosomes and iridocytes, composed of guanin crystals, are always blue or green in color, regardless of the color of the background upon which the fish has sojourned, except under certain conditions to be described below.

No account of changes in color of iridosomes, independent of the effects of chromatophores mentioned above, has been found in the literature. It is purposed here to report briefly certain interesting color changes of the iridosomes of *Fundulus*. These activities have developed in connection with a problem suggested by Prof. G. H. Parker.

When stimulated by direct sunlight, or the light from a Silverman Illuminator lamp, the iridosomes and the reflecting patches change color very rapidly. They appear blue or green in color when first exposed to the light, and within approximately five seconds change to an orange or wine red color. After this change, within forty seconds to two minutes of the time they were stimulated, either in the dark or in light, the iridosomes regain their original color. After responding to a stimulus the iridosomes remain refractory to further stimulation for approximately twenty minutes. If the intensity of the Silverman Illuminator light is reduced, so that the color of the iridosomes is just visible with the microscope, the change in color progresses more slowly. When the light is first thrown on, the general character of the color of the iridosome is green or blue; while certain regions in it, one or two plate-like guanin crystals, are light green or yellowish in color. The color of the iridosomes in general changes through yellowish green, to yellow, to orange or wine red. At the end of such a color change the portion of the iridosome which was originally blue or green becomes orange, while the few crystals which were originally yellow or light green become wine red in color. Rarely is an iridosome found showing only one color. Generally they show a mixture with one color predominating, as in the case described. The recovery phase which follows the initial color change progresses in the reverse order, from the red toward the blue end of the spectrum. These color changes on the part of the iridosomes occur in greater or less clearness, depending upon the state of "expansion" or "contraction" of the melanophores and xanthophores. They show most clearly in fish which have been upon a blue background and which are blue in color. The melanophores are then partly "expanded" and the xanthophores are maximally "contracted" (Fries, 1931). The colors of the iridosomes are darker in shade because of the melanophore just behind them. The iridosome colors are not conditioned in their appearance by the overlying layer of xanthophores; for when expanded, the xanthophores are a very light translucent yellow, and cause the iridosomes

below them, which are therefore viewed more or less through the xanthophores, to appear more green or yellow than they really are. This appearance is probably due on the one hand to blue light from the iridosome, which, passing between processes of the expanded xanthophores, mingles with the yellow from the xanthophores to give a green color. On the other hand, those xanthophores which cover an iridosome in the dispersed condition of their pigment probably do not absorb all the blue light from the iridosome, but transmit some of it. This transmitted light, mixed with yellow light from the xanthophores, presents a green, or even a yellowish green, the exact color depending upon the amount of the transmitted blue light.

The fish studied in Cambridge showed less reactivity on the part of their iridosomes than those studied at the United States Bureau of Fisheries Laboratory at Woods Hole. This may be due to the less favorable condition of the material used in Cambridge, or to the fact that the fish studied at Woods Hole were observed through the summer months, during and after the breeding season. It has been noted by others (Parker, 1930), that the color changes of fish called forth during the breeding season by the color of the background are much more pronounced than during the rest of the year. The humoral state of the body is undoubtedly very different during the breeding season, and this may place the iridosomes in a particularly reactive condition.

The iridocytes of the *stratum argenteum* have not been observed to change color, and only those of the melaniridosomes and the reflecting patches, which are more superficially located in the skin, show this phenomenon.

Under the high magnifications possible with the Ultropak microscope, the individual crystals may be more or less clearly seen in certain iridosomes of the living fish. During the color change of the iridosomes no movement of the crystals was observed. The crystals appear to be long thin plates lying more or less flat on a plane parallel with the surface of the skin. Occasionally a crystal is seen turned up on edge, and faint striations are noted. This seems to indicate either that the crystals are laminated or that they adhere with their broad surfaces together. According to Pfund (1917) and Bancroft (1919) mother-of-pearl in mollusk shells owes its colors to two causes: first, to the diffraction of light by the grating-like structure of the terminal edges of alternating laminae of aragonite (CaCO_3) and conchiolin, which overlap one another at the surface like slates on a roof; and secondly, to the interference of light reflected from parallel laminae of equal thickness. The colors that emanate from the guanin crystals are probably not caused by diffraction, since not much evidence of a grating is observed in the crystals. The interference of light reflected from parallel laminae of the crystals, if such laminae exist, or

perhaps from the thin plate-like crystals themselves, seems the best explanation of these colors. If the crystals are laminated, they must nevertheless be composed of only one substance, for the more recent investigators (Taylor, 1925) are of the opinion that the guanin in such crystals is pure and not combined with other substances.

The iridosomes will respond locally to mechanical or electrical stimulation. They do not respond to heat, except that at higher temperatures than room temperature they respond more generally and completely upon stimulation by light. Heat may then be regarded as having an accelerating effect upon the color change.

The environmental medium about the iridosomes has a profound effect upon their color and their reactivity when stimulated. If two or three scales are removed from a fish, thus breaking the epidermis and subjecting the iridosomes to the osmotic and ionic effects of the environmental medium, the following effects are noted. If such a fish is placed in tap water, in five minutes the iridosomes of the scale-free region are yellow in color rather than blue or green. If the same fish is placed in sea water, in five minutes the same iridosomes become dark blue or green. This reaction may be reversed several times with the same iridosomes. In such fish, in which only a relatively small area of the dermis is exposed to the environmental medium, the responses of the iridosomes to light stimulation are but slightly inhibited in tap water; but greatly inhibited in sea water. Isolated pieces of scale-free skin placed in tap and later in sea water show similar reactions; but the color changes of scale-free skin in response to light are inhibited. It is evident from these results that the environment may condition the color of the iridosomes, and also that it may inhibit their response to light in proportion to the degree of exposure.

The question arises as to whether these reactions of the iridosomes are under the control of the nervous system. If light is allowed to stimulate a very small region of the skin under observation, the iridosomes respond in this region by changing color. When a neighboring region of the skin of the same fish is illuminated in the same fashion, the iridosomes are found not to have changed color in response to the illumination of the first region, but respond only to the second stimulation. This indicates that there is no conduction to the neighboring iridosomes of the excitation which initiated the response of the first group of iridosomes, but does not remove the possibility of a local reflex arc in which the receptor is located in or near the effector. To test for this possibility, the body wall was cut in such ways, on several fish, that the sum of the cuts would have isolated a piece of body wall from the fish, provided all the cuts had been made in one fish. In the center of this piece of body wall the iridosomes responded to the stimulation of light regardless of which side of this point the body wall was cut. This experiment proves that the iridosomes in their re-

sponses to stimulation are independent of the nervous system. The local and extremely rapid nature of the response would also seem to remove the possibility of the intervention of any humoral controlling factor in these reactions.

It may be concluded that the iridosomes of *Fundulus* respond directly to certain definite stimuli. They show fairly definite reaction, recovery and refractory phases in their response and are independent of the nervous system in their reactions. Their color changes progress from the colors at the blue end of the spectrum through the intervening colors, in the order of increasing wave-lengths, to colors at the red end of the spectrum. In the recovery phase, following almost immediately upon the reaction phase, the colors go down the spectrum in the order of decreasing wave-lengths, finally reaching a color near the blue end. This indicates a progressive change in the reaction of the iridosomes to stimulation, and also in their recovery a reverse process. This in turn suggests that possibly the laminae of the crystals, if they exist, or the crystals themselves, may in some way be thickened progressively during the reaction phase and thinned progressively during the recovery phase. The iridosomes appear to be active structures in *Fundulus*, though they are not active in producing the color responses of the animal to the color of the background upon which it is placed. In this latter connection the iridosomes play an important but only a passive rôle. The adaptive color changes in *Fundulus* skin are due entirely to the activity of the melanophores and xanthophores in covering or uncovering the guanin deposits by their processes of "expansion" or "contraction."

¹ The terms "expansion" and "contraction" as applied to the activities of chromatophores are used for reasons of convenience and precedence and should not be construed as implying amoeboid activity on the part of pigment cells.

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THE CAUCHY-GOURSAT THEOREM FOR RECTIFIABLE JORDAN CURVES

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In a recent paper¹ Kamke stated that the property expressed by Cauchy's integral theorem had never been proved for the case of a function analytic interior to an arbitrary rectifiable Jordan curve, continuous in the corresponding closed region. A proof was then supplied by Denjoy.² The following proof is much more immediate than that of Denjoy, although not so elementary.

THEOREM I. *If C is a rectifiable Jordan curve and if the function $f(z)$ is analytic interior to C , continuous in the corresponding closed region, then we have*

$$\int_C f(z) dz = 0.$$

The integral of an arbitrary polynomial $p(z)$ over C is zero, for that integral can be expressed as the limit as n becomes infinite of the integral of $p(z)$ over a suitably chosen closed polygon π_n whose vertices lie on C ; the latter integral is clearly zero. The function $f(z)$ of Theorem I, being analytic interior to C and continuous on and within C , can be represented in the closed interior of C as the limit of a uniformly convergent sequence of polynomials.³ This sequence can be integrated over C term by term, so Theorem I is established.

Theorem I extends easily to the case of a limited region D bounded by a finite number of non-intersecting rectifiable Jordan curves, if $f(z)$ is analytic interior to D , continuous in the corresponding closed region. In such a closed region the function $f(z)$ can be expressed as the limit of a uniformly convergent sequence of rational functions of z whose poles lie exterior to the closed region.⁴ The integral of such a rational function over the complete boundary of D is zero; hence the corresponding integral of $f(z)$ is also zero.

In particular, Cauchy's integral formula is valid under the hypothesis of Theorem I, or under the more general hypothesis just mentioned.