

black area of 8x8 sq mm within 30-45 sec. after the injection, lasting for 10-13 min. Under binocular microscope, the melanophores were found to be expanded.

A purified extract was made from the caudal fins according to the method of Chang, Hsieh, Lee, Li and Lim<sup>3</sup> and was tested on toad's rectus and heart, and leech, (*Whitmania acranulata*, Whitman). AC was identified by the ratio between the unknown and the AC-standard before and after eserine on the rectus (Chang and Gaddum<sup>4</sup>), the atropine test on the heart, and the acid-alkali test on the leech. The extract was found to contain 0.5-1.0 $\gamma$  AC (as chloride) per g wet tissue.

Injection of an extract equivalent to 0.009 $\gamma$  AC could produce blackness in the caudal fin comparable to that produced by an equal amount of the AC-standard.

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### Light-Pituitary Reflex and the Adrenergic-Cholinergic Sympathetic Nerve in a Teleost.

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Working on the snake fish (*Ophiocephalus argus*, Cantor), we found that its melanophores are under two types of control, one through the pituitary which is regulated by light and darkness, and the other through the sympathetic nerve which is partly adrenergic and partly cholinergic.

*The Pituitary Control.* After removal of the sympathetic chains at the level between the pectoral fin and cloaca, the fish developed, on a white background under the continuous illumination from a lamp of 100 W, a black color over the anterior denervated region. Subsequent enucleation of the eyes intensified this color into coal-black, whereas a previous hypophysectomy would prevent such an appearance. These results indicate clearly that the black color is due to the expansion of the melanophores produced by the pituitary principle whose continuous discharge is increased by darkness,<sup>1</sup> and

<sup>3</sup> Chang, H. C., Hsieh, W. M., Lee, L. Y., Li, T. H., and Lim, R. K. S., in press.

<sup>4</sup> Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

<sup>1</sup> Jores, A., *Klin. Wchschr.*, 1935, **14**, 1713; Stutinsky, F., *C. E. soc. Biol.*, 1938, **127**, 409.

whose effect is accentuated in the absence of the sympathetic influence. Similar results were obtained from frogs (*Rana nigromaculata*).

*The Sympathetic Control. Adrenergic fibers.* When the denervated area had become sufficiently sensitized, usually 3 days after the abdominal sympathectomy, stimulation of the myelencephalic sympathetic center would turn the black denervated area pale. From 10 days onward, the same phenomenon would occur spontaneously under continuous light illumination. This pallor is apparently due to diffusion into the denervated area of adrenaline-like substance liberated at the nerve-endings of the neighboring normal region in response to electrical or light stimulation. We may recall that Parker<sup>3</sup> has advanced similar explanation for the fading of the dark caudal band in *Fundulus*.

*Cholinergic fibers.* It was shown by Chin<sup>2</sup> that the paradise fish responds to very small dose of acetylcholine (AC) and that there is enough AC in the caudal fin to suggest for it a physiological rôle. Furthermore, we found that local mechanical stimulation such as cutting of the fin-rays could produce a dark band peripheral to the cut in the normal fish kept under light illumination, in keeping with the results of the earlier workers.<sup>3</sup> This phenomenon could also be shown in the hypophysectomized fish. On the other hand, we found that when nerve degeneration of the fins had well advanced after removal of the abdominal sympathetic chains similar cutting failed to produce the dark band, and the AC-content of the fin extract was markedly decreased, ranging from 1/6 to 1/2 of the normal, which was 0.077  $\gamma$ /g. These results were confirmed on the paradise fish.

It may thus be concluded that the melanophores of the snake fish (possibly the paradise fish also) are innervated anatomically by the sympathetic nerve, but some of its fibers, which cause their dispersion, are mediated by AC, while the others, which cause their concentration, are mediated by adrenaline.

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<sup>2</sup> Chin Yin-ch'ang, PROC. SOC. EXP. BIOL. AND MED., 1939, 40, 454.

<sup>3</sup> Wyman, L. C., *J. Exp. Zool.*, 1924, 39, 73; Mills, S. M., *J. Exp. Zool.*, 1932, 64, 231; Parker, G. H., *Humoral agents in nervous activity with special reference to chromatophores*, Cambridge Univ. Press, 1932; *Color changes in animals in relation to nervous activity*, Univ. Pennsylvania Press, 1936.