Histochemical Staining of Melanin Formed in a Mayfly in Response to Helminth Infection

G. O. Poinar, R. Leutenegger, and P. Götz (J. Ultrastruct. Res. 25, 293-306, 1968) tested the pigment formed about a nematode parasite of *Diabrotica* and found that this pigment satisfied several of the standard histochemical tests for melanin. The Masson-Fontana ammoniated silver nitrate test was positive, the pigment was bleached in 30% hydrogen peroxide, and

was soluble in concentrated sulfuric acid and sodium hydroxide, but not in xylene, acetone, benzene, chloroform, ether, or concentrated hydrochloric acid. However, results with Lillie's ferrous iron retention technique were considered inconclusive.

The ferrous iron test is highly selective for melanin if the presence of iron-containing pigments is ruled out by a negative test

| Stain used | Reference | Duration of infections | | |
|--|--|---|---------------------------------|---------------------------------|
| | | 24 Hr | 7 Days | 30 Days |
| Ferrous iron | Lillie, R. D. 1957. Arch. Pathol. 64, 100–103. | Pigment stained dark green, specific for melanin | Same | Same |
| Periodic acid- Schiff's reaction | McManus, J. F. A. 1948. Stain Technol. 23, 99–108. | Weakly PAS positive | PAS positive | PAS positive |
| Bleaching with 30% Hydrogen peroxide | Bancroft, J. D. 1967. "An Introduction to Histo- chemical Technique," p. 173. Appleton-Century-Crofts, New York | Pigment bleached in 36 hr | Pigment bleached in 48 hr | Pigment bleached in 48 hr |
| | Poinar, G. O., Leutenegger, R., and Götz, P. 1968. J. Ultrastruct. Res. 25, 293-306. | | | |
| Masson-Fontana's Silver nitrate | Pearse, A. G. E. 1972. "Histo- chemistry," 3rd ed., Vol. 2, p. 1379. Williams & Wilkins, Baltimore, Maryland. | Pigment stained black in 24 hr | Same | Same |
| Ziehl-Neelsen method for acid fastness | Barka, T., and Anderson, P. J. 1963. "Histochemistry," p. 154. Harper and Row, New York. | Negative, pigment nonacid fast | Negative | Negative |
| Toluidine blue | Bancroft, J. D. 1967. "An Introduction to Histo- chemical Technique." p. 83. | Pigment stained dark blue (basophilic) | Same | Same |
| Nile blue sulfate (Huek's test) | Pearse, A. G. E. 1972. "Histo- chemistry" Vol. 2, p. 1383. | Negative, pigment did not stain | Negative | Negative |
| Giemsa | Luna, L. G. (ed.) 1968. Armed Forces Institute of Pathology, Manual of Histologic Staining Methods, 3rd ed. p. 236. McGraw-Hill, New York | Pigment stained purple | Same | Same |

 TABLE 1

 HISTOCHEMICAL CHARACTERIZATION OF PIGMENT IN HOST CAPSULES



Fig. 1. Host reaction to Cercaria tremaglandis metacercaria; 48 hr infection of Hexagenia recurvata naiad, Lillie's ferrous iron method. Pigment layer (M) stains intensely. Loose hemocytes (H) and a more compact hemocytic capsule layer (HC) are located at the periphery. The parasite (P) shows nuclear degeneration and appears to have been killed by the host response. \times 960.

for iron (T. Barka and P. J. Anderson, "Histochemistry," p. 185. Harper & Row, New York, 1963), and other stains, though not specific, may contribute to the histochemical characterization (A. G. E. Pearse, "Histochemistry," 3rd ed., Vol. 2, p. 1090. Williams & Wilkins, Baltimore, Maryland, 1972).

In the present study a battery of histochemical tests (Table 1) has been completed with pigments located in cellular host capsules formed in naiads of the mayfly, *Hexagenia recurvata*, in response to a trematode larva, *Cercaria tremaglandis*. Solubility tests were based in part on direct immersion of host capsules in the test substance. Other tests were based on examination of paraffin-embedded, sectioned material.

The host pigment located within the capsule was laid down first in the area of the parasite cyst wall and was seen to have infiltrated the parasites in capsules examined after 7 days. Solubility of the pigment was identical to that noted by Poinar et al. (loc. cit.). Its histochemistry is presented in Table 1.

The pigment stained dark green with Lillie's ferrous iron method (Fig. 1). This, in conjunction with the positive Masson-Fontana silver nitrate test and the negative reactions obtained with Perl's test for iron and Heuk's test for lipofuchsins, strongly indicates that the pigment is a melanin. The negative Ziehl-Neelsen, weakly positive to positive PAS test, and the strongly basophilic nature of this substance when tested with toluidine blue and Giemsa's stain further indicate that a true melanin is present.

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