

has shown that some of the shrunken eggs can survive without adverse effect.

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TEH PING LIN

Department of Anatomy, School of Medicine,
University of California Medical Center, San Francisco.

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¹ Lin, T. P., *Science*, **151**, 333 (1966).

² Reverberi, G., *Publ. Staz. Zool. de Napoli*, **11**, 168 (1931).

³ Daleq, A. M., *Introduction to General Embryology*, 65 (Oxford University Press, 1965).

⁴ Lin, T. P., Sherman, J. K., and Willett, E. L., *J. Exp. Zool.*, **134**, 275 (1957).

Melanin in an Insect, *Lucilia cuprina* (Wied.)

MELANINS, although somewhat ill defined, are dark pigments which occur in animals and plants and they are usually bound to protein. Thomson¹ in a review has pointed out that there are no satisfactory histochemical tests for the identification of melanin. Alkali solubility and reversible reduction merely indicate acidic and quinonoid properties—properties common to many different types of pigments. Mammalian and cephalopod melanins in particular have been extensively studied and some have been isolated and examined chemically. Melanins are formed by the action of a phenolase on a phenolic substrate, but their structure is not known. Those derived from tyrosine, however, are considered to be built up from indolyl units. Nicolaus *et al.*² classified melanins as "indole" or "catechol" types, depending on the degradation products formed on alkali fusion and permanganate oxidation. All melanins so far examined from animal sources are of the indole type, as are the melanins prepared by the oxidation of tyrosine and dopa.

In insects, except for one investigation³, identification of melanin has relied on inadequate histochemical tests. Sometimes a search has been made for an accompanying phenolase system, but this is usually of little value because of the phenolase activated process of sclerotization which occurs in insects. Nevertheless, sclerotization and blackening (said to be melanin) have been shown to be independent processes in a few insects (for a review, see Hackman⁴).

I have a laboratory strain of the blowfly *Lucilia cuprina* (Wied.) in culture which is homozygous for three recessive mutants carrying yellow eyes, rusty body and a black puparium (the puparium of the normal wild strain is brown). Electron microscopy shows a layer of fine black granules at the inner surface of the epicuticle of the puparium. When empty puparia, from which pupal cuticles, other residues and lipids have been removed, are hydrolysed (6 normal hydrochloric acid) thin insoluble membranes remain which are "ghosts" of the original puparia. In the normal wild strain these membranes are colourless, but in the mutant they are an intense black and thicker.

When radioactive tyrosine⁵ was injected into fully grown last instar larvae of the mutant, 79 per cent of the radioactivity was recovered from the puparia and 49 per cent of this was in the black membrane remaining after hydrolysis. With the normal wild strain these percentages were 59 and 16, respectively.

The black pigment was extracted (10 per cent aqueous sodium hydroxide) from the insoluble membranes remaining after acidic hydrolysis of puparia of the mutant and recovered by acidifying the extract and yielded 4.6 per cent on weight of puparia taken. The pigment was again subjected to acidic hydrolysis (6 normal hydrochloric acid) for 24 h. In alkaline solution the pigment showed only general absorption in the ultraviolet and visible regions of the spectrum. The pigment was subjected to alkali fusion² and 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid were identified among the degradation products. Permanganate oxidation⁶ of the pigment gave pyrrole-2,3,5-tricarboxylic acid as the principal product together with smaller amounts of pyrrole-2,3-dicarboxylic acid and pyrrole-2,3,4,5-tetracarboxylic acid. These degradation products are the same as those obtained by Nicolaus and his collaborators^{2,3} from invertebrate melanins (from squid, cuttlefish and octopus inks and from *Drosophila melanogaster* tumours and *Tropinota glabra* elytra) and from melanin prepared by oxidation of dopa.

The black pigment in the puparia of the *L. cuprina* mutant has the physical properties of a melanin and gives degradation products characteristic of melanins of animal origin. This pigment is therefore classified as an indole melanin. Experiments with radioactive tyrosine show it to be formed from tyrosine, which is confirmed by the nature of the degradation products.

R. H. HACKMAN

CSIRO Division of Entomology,
Canberra City.

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¹ Thomson, R. H., in *Comparative Biochemistry* (edit. by Florin, M., and Mason, H. S.), **3**, chap. 13 (Academic Press, New York, 1962).

² Nicolaus, R. A., Piatelli, M., and Fattorusso, E., *Tetrahedron*, **20**, 1163 (1964).

³ Fattorusso, E., Piatelli, M., and Nicolaus, R. A., *Rend. Accad. Sci. Fis. Mat. (Soc. Naz. Sci., Lett. Arti Napoli)*, **32**, 200 (1965).

⁴ Hackman, R. H., in *The Physiology of Insecta* (edit. by Rockstein, M.), **3**, chap. 8 (Academic Press, New York, 1964).

⁵ Hackman, R. H., and Saxena, K. N., *Austral. J. Biol. Sci.*, **17**, 803 (1964).

⁶ Nicolaus, R. A., *Rass. Med. Sper.*, **7**, suppl. 2, 1 (1960).

Retraction of the Claim that Host Pregnancy affects Pupal Production by the Tsetse Fly

DURING the first two seasons of an investigation into the effect of host pregnancy on female *Glossina austeni* we claimed that the performance of the flies fed on pregnant goats was significantly better than that of those fed on non-pregnant goats¹⁻³. We must now retract this claim. The combined results for season 2 are given in Table 1, followed by the figures for the first experiment in season 3 in which there was a complete reversal.

Table 1. SURVIVAL AND PUPAL PRODUCTION BY FEMALE *G. austeni* WHEN FED ON PREGNANT AND NON-PREGNANT GOATS

| No. of experiment | Age of flies (weeks) | No. of survivors | | No. of pupae deposited | |
|--|----------------------|-----------------------------|---------------------------|-----------------------------|---------------------------|
| | | Pregnant goats | Control (male and female) | Pregnant goats | Control (male and female) |
| Season 2 (all experiments combined) | 0 | 1,048 | 1,044 | 0 | 0 |
| | 9 | 639 | 406 | 2,865 | 1,852 |
| Season 3 Expt. 1 | 0 | 600 | 600 | 0 | 0 |
| | 9 | 163 | 367 | 1,032 | 1,766 |
| Expt. 2A | 0 | Female goats (non-pregnant) | Male goats | Female goats (non-pregnant) | Male goats |
| | 9 | 300 | 300 | 0 | 0 |
| Expt. 2B (same goats as in 2A) | 0 | 211 | 210 | 1,067 | 1,053 |
| | 9 | (Pregnant) | 600 | (Pregnant) | 0 |
| | 0 | 600 | 441 | 2,038 | 2,104 |
| | 20 | 129 | 145 | 4,078 | 4,048 |