TYROSINE METABOLISM IN BLOWFLY LARVAE AT PUPATION*

By R. H. Hackman† and K. N. Saxena†‡

In insects tyrosine has been regarded as participating in metabolic reactions which lead, not only to its incorporation in various proteins, but also to sclerotization of the cuticle (i.e. quinone cross-linking of cuticular protein). Three different metabolic pathways have been suggested, all of which lack direct evidence to support them. The suggested pathways are (i) oxidative deamination leading to the formation of *o*-quinones, (ii) non-specific hydroxylation and elimination of the side-chain leading to the formation of *p*-quinones, and (iii) conversion to *N*-acetyldopamine which is the phenolic precursor of the sclerotizing quinone. For a review of these theories see Pryor (1962) and Hackman (1964).

Karlson (1960) has reported that when radioactive tyrosine was injected into *Calliphora erythrocephala* larvae 1 day before pupation, about 80% of the recovered activity appeared in the puparium and the side-chain of the tyrosine was not eliminated. No radioactive tyrosine was found in hydrolysates of the puparium. Similarly dopa, but not hydroquinone, was incorporated into the puparium. In later papers (Karlson, Sekeris, and Sekeri 1962; Karlson and Ammon 1963) it was shown that dopamine, *N*-acetyldopamine, the acetyl group of *N*-acetyldopamine, and, to a much lesser extent, tyramine and *N*-acetyltyramine were also incorporated into the puparium. Nothing further is known about the manner in which tyrosine is metabolized when injected just before pupation although Karlson and Sekeris (1962) have studied the metabolism of tyrosine in immature larvae. They consider that the immature larva is able to form all the above-named compounds from tyrosine. Information on the metabolism of tyrosine in larvae of the blowfly *Lucilia cuprina* (Wied.) at pupation is now reported.

Fully grown last instar larvae of *L. cuprina* were starved for 24 hr, washed in water, blotted dry, and chilled until they were immobile by being placed on a block of ice which had been covered with a paper tissue. Each larva was injected with 5 μl of an 0·3 mM solution of L-[^{14}C]tyrosine hydrochloride§ in water which had been dyed with 0·1% neutral red. Activity of the solution was 0·005 μc/μl. The hypodermic needle was withdrawn through a small drop of melted paraffin wax placed on the puncture and the wax, when solid, sealed the wound. The larvae were allowed to return to room temperature and were kept for some hours on filter paper to enable the ready detection of any liquid that might be lost from the wound. Concentrated aqueous extracts of the filter papers on which they were kept were not radioactive so confirming that no liquid had been lost. The larvae pupated in 1–2 days at 25°C.

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† Division of Entomology, CSIRO, Canberra.

‡ Present address: Department of Zoology, Delhi University, Delhi, India.

§ Uniformly labelled.

Two days after pupation the pupae were removed from the puparia. Pupae and puparia were hydrolysed separately in constant-boiling HCl (1 ml acid per 1 mg material) at 100°C for 16 hr. Insoluble material was removed from the puparial hydrolysate and washed, both hydrolysates were evaporated to dryness *in vacuo*, and the residues dried *in vacuo* over solid KOH. Each residue was dissolved in a small volume of water (100 μl for each 5 mg puparia or pupae) and the amounts of radioactivity present in the two solutions and in the insoluble material (from the puparial hydrolysate) measured. Scintillation counting could not be used because of the quenching effects of the extracts. Radioactivity in the solutions was most conveniently measured by 4 π counting of known volumes as spots on filter paper. In all 79·3% of the activity injected into the larvae was recovered; 25·6% of this activity was in the pupal hydrolysate, 62·2% as soluble material in the puparial hydrolysate, and 12·2% in the insoluble material remaining after hydrolysis of the puparia. This insoluble material was an intact thin membrane.

The hydrolysates were subjected to paper chromatography on Whatman No. 1 filter paper (solvent n-butanol–acetic acid–water, 4:1:5 v/v). Separate chromatograms were sprayed with ninhydrin, Pauly reagent, aqueous ferric chloride, and Folin–Ciocalteau reagent. Tyrosine was the only radioactive substance present in the pupal hydrolysate. Trace amounts of [^{14}C]tyrosine and [^{14}C]phenylalanine were detected in the puparial hydrolysates but almost all the radioactivity remained at the base line. A non-radioactive substance (R_F 0·11) which gave a red colour with Pauly reagent was present in the puparial hydrolysate.

The radioactivity present in the soluble fraction of the puparial hydrolysate was separated into two parts by paper chromatography with 50% aqueous ethanol as solvent. 41% of the activity remained at the origin (fraction A); it did not give any colour with ninhydrin or ferric chloride but gave a light buff colour with Pauly reagent and a blue colour with Folin–Ciocalteau reagent but only after it had been made alkaline. 53% of the activity moved as a compact yellow spot (R_F 0·80) which was in that area occupied by amino acids. The yellow material was separated into two fractions by paper chromatography (overnight) with aqueous n-propanol as solvent. The chromatograms were run twice with 85% propanol and once with 60% propanol, air-drying the papers between each run. 77% of the radioactivity remained at the origin as a yellow spot (fraction B) completely separated from amino acids, and this material gave the same colour reactions as did fraction A. 23% of the activity moved as a pale yellow spot (fraction C): it reacted with Pauly reagent but was in the area occupied by amino acids. Fraction A could be made to travel along the paper when 10% aqueous sodium carbonate or n-butanol–ammonia, sp.gr. 0·88 (4:1 v/v) were used as solvents but no separation into additional spots occurred. Fraction A represents 25·4% of the activity originally recovered from the intact puparia (puparia +pupae), fraction B 25·3%, and fraction C 7·6%.

From the above it can be concluded that when tyrosine was injected into a blowfly larva just before pupation a small fraction of it was incorporated as such in the pupa but most of it appeared in the puparium, i.e. in the sclerotized cuticle. Over 80% of the tyrosine incorporated into the puparium was present as phenolic material, probably derived from the compounds used to cross-link the protein to form

the hard, dark puparium. In view of the colour reactions this material was not recovered in the form of simple *o*-dihydric phenols. The remainder was incorporated into a thin membrane which was insoluble in constant-boiling HCl at 100°C. This membrane is perhaps part of the "outer epicuticle" or "paraffin layer" reported by Dennell (1946) and Dennell and Malek (1955) in the puparia of blowflies—the protein component of the outer epicuticle would have been removed during the hydrolysis. The manner in which the tyrosine has been incorporated into this membrane is unknown but in view of the stability of the membrane it may be in the form of cross-linkages.

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