Sclerotization in the Blowfly Imago

Abstract. *N-acetyldopamine has been identified as a dihydroxyphenylalanine metabolite in the blowfly imago during eclosion. The activity of the dihydroxyphenylalanine decarboxylase, the main enzyme responsible for its formation, which is minimal during the pupal stage, increases 1 day before eclosion. The similarity of dihydroxyphenylalanine metabolism in* Calliphora *during eclosion and during puparium formation justifies the assumption that N-acetyldopamine can be regarded as the sclerotizing agent of the imaginal cuticle. It is concluded that the formation of this substance is under the control of ecdysone.*

Sclerotization of the insect cuticle during pupation has been ascribed to the interaction of phenolic substances with the cuticle proteins (*1*). In a study of the sclerotization process in *Calliphora*, co-workers and I identified a tyrosine metabolite, *N*-acetyldopamine, as the sclerotizing agent (*2*). The biosynthesis of this substance was elucidated and was shown to proceed by way of dihydroxyphenylalanine (DOPA) and dopamine (*3*). The biosynthetic pathway to *N*-acetyldopamine is under the control of the molting hormone, ecdysone, through induction of the key enzyme, DOPA decarboxylase (*4*). Most of the *N*-acetyldopamine formed is incorporated in the cuticle while the rest of the substance is converted to the 4-β-glucoside. This glucoside could be a detoxication product but could also serve as a potential source of *N*-acetyldopamine for the tanning process which takes place after eclosion of the imago.

The sclerotization process of the imago has not received the attention given to puparium formation. However, recent work of Fraenkel and Hsiao (*5*) and of Cottrell (*6*) showed that tanning of the blowfly imago is under the control of an unknown factor, probably a protein hormone. The significance of ecdysone in this process is doubted; Fraenkel postulated that this tanning process is different from the one taking place during puparium formation.

We believe that tanning in the imago and tanning of the puparium have the same biochemical basis and that ecdysone participates in both processes. The following evidence supports this view:

1) Metabolism of radioactive labeled tyrosine and DOPA during eclosion follow the same pathway as in the prepupa. I have detected dopamine and *N*-acetyldopamine as metabolites in the newly emerged flies (see Fig. 1).

2) During development of the blowfly, *Calliphora erythrocephala*, the activity of the DOPA decarboxylase is at a maximum twice, first shortly before pupation and then during eclosion (Table 1).

3) In mature larvae, *N*-acetyldopamine-glucoside is accumulated. The concentration of this compound, determined by labeling it with 2-^{14}C-dopamine, remained constant during pupal life until 1 day before eclosion and then decreased. No glucoside was detectable in 1-day-old flies; presumably, the glucoside has been used as the source of the tanning agent in the sclerotization process.

Thus the biochemistry of the tanning process of the fly and of puparium formation is principally identical. *N*-acetyldopamine can be regarded as the sclerotizing agent not only of the puparium but also of the imago. The biosynthesis of *N*-acetyldopamine in the prepupa is under the control of ecdysone through induction of the DOPA decarboxylase; the possibility exists that the same process in the last-day pupa is controlled by the same hormone.

Table 1. DOPA decarboxylase activity during the development of *Calliphora erythrocephala*. The activity is expressed as percent transformation of DOPA to dopamine (see *3*).

Source	Activity
7-day larvae	3
White pupa	35
1-day pupa	24
3-day pupa	<1
7-day pupa	<1
10-day pupa	4
Shortly before eclosion	14
1 hour after eclosion	24
1-day-old imagoes	9

This interpretation of the role of ecdysone in tanning of the imago should not invalidate the observations of Fraenkel and Hsiao (*5*) and Cotrell (*6*). These authors showed that a blood-borne factor which is not ecdysone initiates the hardening of the cuticle in newly emerged flies. Ecdsone is believed to be responsible only for the production of the tanning agent, that is, *N*-acetyldopamine, through induction of the key enzymes. The factor of Fraenkel and Cotrell is concerned with the actual tanning process; it could be either a hormone controlling the interaction of the sclerotizing agent, *N*-

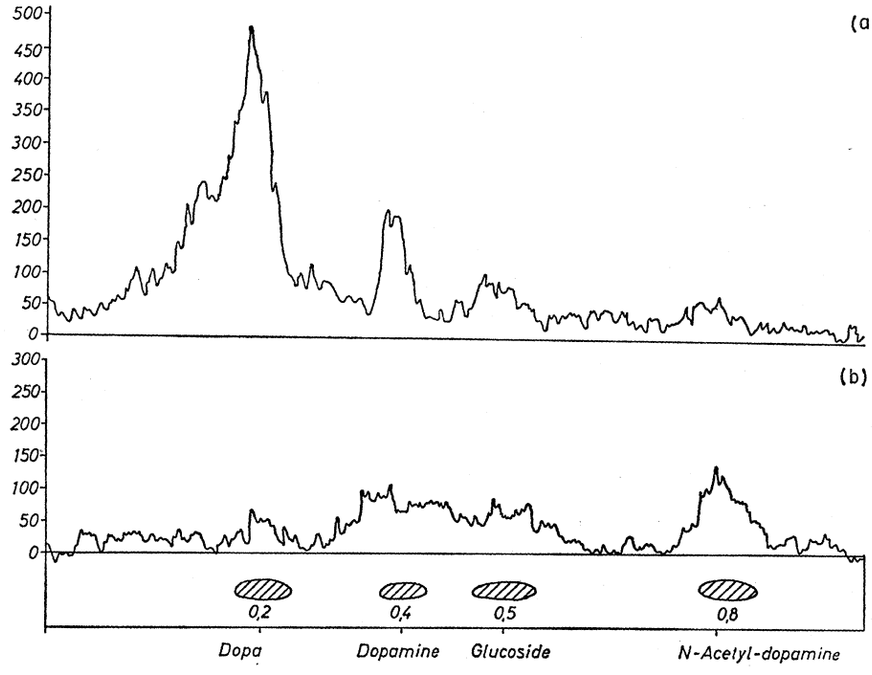

Fig. 1. Paper chromatogram of DOPA metabolites of a methanol extract of (*a*) white prepupae 15 minutes after injection of DOPA (40,000 count/min), and (*b*) fresh eclosed imagoes 5 minutes after injection of DOPA (20,000 count/min). The shaded areas show the Pauly positive metabolites; the curve, the radioactivity measured. Ordinates: impulses per minute.

acetyldopamine, with the cuticular matrix, or an enzyme, possibly one of the compounds of the complex phenoloxidase system (*7*).

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Fertility Restoration and Its Inheritance in Cytoplasmic Male-Sterile Wheat

Abstract. *Male-sterile* Triticum aestivum *L. 'Bison,' possessing cytoplasm of* T. timopheevi *Zhuk., typically produces male-sterile progeny when pollinated with common wheat cultivars. A fertility restorer developed by transferring genes from* T. timopheevi *to* T. aestivum *produced fertile hybrids when used as the pollen parent in a cross with male-sterile Bison. Data on the* F_2 *and testcross plants indicate that two dominant genes, designated* Rf_1 *and* Rf_2*, condition fertility in wheats with* T. timopheevi *cytoplasm.*

Male sterility due to the interaction of nuclear genes with specific cytoplasm is known in many plant species (*1*). This kind of male sterility frequently makes possible mass production of crossed seed of economic species and thus permits commercial culture of vigorous hybrids.

Three cases of male sterility attributable to interactions of genes and cytoplasm have been reported in common hexaploid wheat, *Triticum aestivum* L. ($2n = 42$). The first two were produced by breeding procedures which resulted in substitution of the chromosomes of common wheat in the cytoplasm of related species of goatgrass, *Aegilops caudata* L. (*2*) and *A. ovata* L. (*3*). Common wheats used thus far as pollinators of these two male-sterile types have produced only male-sterile hybrids. Recently, a more promising sterility system was developed (*4*) by backcrossing so that the chromosomes of the common wheat Bison were substituted in the cytoplasm of *T. timopheevi* Zhuk. ($2n = 28$). Male-sterile Bison was pollinated with a hexaploid having *T. timopheevi* ancestry and fertile plants were produced (*5*). The report is concerned with the inheritance of fertility-restoring genes transferred from *T. timopheevi* to hexaploid wheat.

A hexaploid fertility restorer with *T. timopheevi* cytoplasm was developed by crossing *T. timopheevi* × *T. aestivum* 'Marquis' and then backcrossing twice to Marquis as the pollinator. The resulting F_2 generation had the pedigree *T. timopheevi* × Marquis[3] (*6*). A selected plant from this population proved to be homozygous for genes conditioning fertility in *T. timopheevi* cytoplasm. All progeny resulting from self-pollination were fully fertile. Furthermore, when male-sterile Bison was pollinated with this selected plant, seven hybrid plants were produced in which the pollen and the number of seeds developing was normal showing restoration of fertility to be completely dominant. The fertile members of the F_1 generation were self-pollinated and also backcrossed with male-sterile Bison. Plants of the resulting F_2 and testcross generations were classified according to the microscopic appearance of the pollen as normal, partially fertile, or sterile. Distribution of segregating F_2 and testcross plants into these three fertility classes is recorded in Table 1. Chi-square tests indicate that a two-factor hypothesis is in accord with the observations.

Table 1. Frequency distribution in the F_2 generation and testcross populations segregating for male sterility compared with expectations from digenic inheritance.

Pollen class	Normal	Partly fertile	Sterile
F_2, male sterile × fertility restorer			
Ratio tested	9	6	1
Observed	30	28	3
Calculated	34	23	4
Chi-square probability .41			
Testcross, male sterile × fertile F_1			
Ratio tested	1	2	1
Observed	31	46	29
Calculated	26.5	53	26.5
Chi-square probability .40			

These results can be explained by assuming that the newly-developed fertility restorer is homozygous for two dominant genes, designated Rf_1 and Rf_2, which produce normal fertility in wheats with *T. timopheevi* cytoplasm. Male-sterile Bison exemplifies the recessive genotype rf_1rf_1 rf_2rf_2 in combination with *T. timopheevi* cytoplasm. Normal Bison, essential as a pollinator in propagation of the male-sterile form, has the same recessive genotype and owes its pollen fertility to possession of *T. aestivum* cytoplasm. The heterozygote, Rf_1 rf_1 Rf_2 rf_2, produced by crossing male-sterile Bison with the new fertility restorer, proved to be fully fertile. The partially fertile segregates encountered presumably possess either the dominant gene Rf_1 or Rf_2 but not both.

Experience obtained by breeding wheat with the kind of male sterility developed in Bison has shown that it can be readily transferred to many common wheat cultivars by backcrossing. In view of its mode of inheritance, no problem is anticipated in transferring fertility restoration to other wheats as desired. Thus it is clear that the genetic requisites for experimental production of hybrid wheat are now available.

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Histones from Developing Tissues of the Chicken: Heterogeneity

Abstract. *Electrophoretic analysis of the molecular heterogeneity of histones from developing and adult chicken tissues demonstrates that differentiation need not be correlated with changes in the relative proportion of individual histone molecules during development.*

One of the chief reasons for thinking that histones may be regulators of genetic activity is that histones display molecular heterogeneity, a possible provision for selective regulation of genetic activity widely thought to occur in tis-