A Comparison of the Effects of Mitomycin C on the Bristle Organ Formation in Wild-Type and *sv^{de}* Strains of *Drosophila melanogaster*

During metamorphosis of *Drosophila melanogaster*, the bristle socket and the bristle shaft are secreted by two polytenic cells, the tormogen and the trichogen cell. These two cells arise by differential cell division from a single bristle mother-cell. Injection of mitomycin C or nitrogen mustard into late third instar wild-type larvae of *D. melanogaster* results in the formation of bristle organs without sockets[1-3]. Flies of the mutant strain *shaven-depilate* (*sv^{de}*: 4-2.0) are characterized by the differentiation of bristle sockets without shafts[4,5]. It was the purpose of the present investigation to analyse the effects of injection of mitomycin C into larvae of the mutant *sv^{de}*. Specifically, we wanted to know whether the bristle organs of *sv^{de}* mutants differ from wild-type flies in their susceptibility towards the action of the drug.

The mutant *sv^{de}* is recessive and the homozygote is lethal during the late pupal or early adult stage[4]. The balanced stock *sv^{de}/ey^{D}* (*eyeless-Dominant; ey^{D}*: 4–2.0), employed in our earlier study[5], was no longer used for the present experiments, since this stock does not allow the distinction between the homozygous *sv^{de}* and heterozygous *sv^{de}/ey^{D}* larvae. Consequently, a new stock was constructed utilizing the translocation *T (3;4) 89E*[4] and the mutant genes *red malpighian tubules* (*red:* 3–53.6) and *sv^{de}*. *Inter se* crosses of these flies yield homozygous *red; sv^{de}*, heterozygous *red; sv^{de}/T(3;4)* and homozygous *T(3;4)* progenies. This new stock showed the following properties: 1. Homozygous *sv^{de}* animals are also homozygous for the marker gene *red*. This allows the detection of the *sv^{de}* phenotype at the larval stage by the presence of red malpighian tubules. 2. Animals homozygous for the translocation *T(3;4)* are lethal at an early embryonic stage. Thus, the stock is balanced and only heterozygous animals are fertile.

Table I. Modification of the 4 scutellar macrochaetae in the homozygous mutant strain *shaven-depliate* (*sv^{de}*) of *Drosophila melanogaster*

Bristle organ	Complete (%)	Socket only (%)	Shaft only (%)	Missing (%)
Left anterior scutellar	51.2	48.8	0	0
Right anterior scutellar	63.4	36.6	0	0
Left posterior scutellar	53.7	43.9	0	2.4
Right posterior scutellar	58.6	39.0	0	2.4
Average	56.7	42.1	0	1.2

Number of examined flies (n) = 41.

Wild-type and *red; sv^{de}/T(3;4)89E* stocks of *D. melanogaster* were raised on standard food (corn, sugar, agar, yeast) at 25 °C. Mitomycin C[6] dissolved in insect Ringer solution (0.8 mg/ml) was injected into 96-h-old larvae. The dose was about 65 µg/g live weight resulting in a pupal mortality of 45%. 4–5 days after puparium formation, the dorsal integument of the thorax was liberated from the pupal case and mounted in Faure's solution on a glass slide. For a quantitative analysis of bristle organ modifications, this study was confined to an examination of the 4 scutellar macrochaetae, since they are always present in untreated control wild-type flies.

Homozygous *sv^{de}* mutants show the following effects on the formation of the scutellar macrochaetae (cf. Table I): 1. On the average, 42% of the bristle organs form sockets without shafts, whereas whole bristle organs are only very rarely absent (≈1%). 2. All 4 macrochaetal primordia are modified with equal frequencies (χ^2-Test: $p > 0.5$). If whole bristle organs are missing, the remaining bristle organs nevertheless differentiate at the same positions as in wild-type flies. This is also true when bristle organs fail to differentiate upon injection of mitomycin C (Figure).

Table II summarizes the effects of injection of mitomycin C into wild-type larvae of *D. melanogaster* on the formation of the 4 scutellar macrochaetae. The majority of the bristle organs formed socket-less shafts, in 30% the complete organ is missing, whereas normal bristle organs or sockets without shafts are differentiated at rather low frequencies of 1.2–3.5%. No difference in sensitivity of the 4 macrochaetae is noted towards the action of the drug (χ^2-Test: $p > 0.5$). Thus, like in *sv^{de}* mutants, all macrochaetal primordia of the scutellum are equally

[1] H. Tobler, Experientia *25*, 213 (1969).
[2] H. Tobler and V. Maier, Wilhelm Roux Arch. EntwMech. Org. *164*, 303 (1970).
[3] H. Tobler and H. Burckhardt, Experientia *27*, 189 (1971).
[4] D. L. Lindsley and E. H. Grell, Carnegie Inst. Wash. Publ. (1968), vol. 627.
[5] H. Tobler, V. Rothenbühler and R. Nöthiger, Experientia *29*, 370 (1973).
[6] Mitomycin C was purchased from Calbiochem.

The arrangement of the 4 macrochaetae on the scutellum of control (a) and mitomycin C injected wild-type (b) flies of *Drosophila melanogaster*. Note that the differentiated bristle organs in the mitomycin C treated flies occupy the same relative positions as in non-treated flies.

Table II. The effect of injection of mitomycin C into 96-h-old wild-type larvae of *Drosophila melanogaster* on the differentiation of the 4 scutellar macrochaetae (n = 43)

Bristle organ	Complete (%)	Socket only (%)	Shaft only (%)	Missing (%)
Left anterior scutellar	2.3	0	65.1	32.6
Right anterior scutellar	0	0	74.4	25.6
Left posterior scutellar	4.7	2.3	62.8	30.2
Right posterior scutellar	7.0	2.3	60.5	30.2
Average	3.5	1.2	65.6	29.7

Table III. Comparison of the frequency of observed and expected bristle organ aberrations in the homozygous mutant *shaven-depilate* of *Drosophila melanogaster* upon injection of mitomycin C into 96-h-old larvae

4 Scutellar bristle organs	Complete (%)	Socket only (%)	Shaft only (%)	Missing (%)
Observed (n = 41)	11.6	56.1	14.0	18.3
Expected	2.0	2.7	37.2	58.1

affected. Other experiments[7] have shown that the pattern of bristle organ modification is the same, whether mitomycin C is injected into wild-type larvae or into larvae of the phenotypically wild-type stock *red*; $sv^{de}/T(3;4)89E$.

To test whether the phenotypic effects of mitomycin C on wild-type and sv^{de} flies are cumulative, we compared the frequency of observed bristle organ modifications in the mutant sv^{de} upon injection of mitomycin C and the expected frequency of bristle organ aberrations. Such an expected frequency was calculated by multiplying the frequencies of the different bristle organ aberrations in non-treated sv^{de} mutants with those of mitomycin C treated wild-type flies. The results are depicted in Table III. It is evident that the expected and the observed frequencies for bristle organ modifications are not in accord (χ^2-Test: $p < 0.001$). On the one hand, too many complete bristle organs and sockets without shafts are formed, on the other hand there are not enough missing bristle organs and bristle shafts without sockets. The same disagreement between expectation and experimental result has also been noted upon injection of nitrogen mustard into wild-type and sv^{de} flies[7].

The data presented indicate that the bristle organ primordia of sv^{de} flies are much more resistant towards the detrimental effect imposed by mitomycin C than in wild-type flies. The reason for this relative resistance against the injuring effect of the drug is not known.

Zusammenfassung. Bei *Drosophila melanogaster* erlaubten genetische Methoden die Konstruktion eines balancierten Stammes, bei dem homozygote «*shaven-depilate*» (sv^{de}) Fliegen bereits im Larvalstadium als solche erkennbar sind. Die Primordien der Borstenorgane der Mutante sv^{de} sind gegenüber einer weiteren Schädigung der Borstendifferenzierung, die durch Injektion von Mitomycin C in verpuppungsreife Larven bewirkt wird, signifikant weniger empfindlich als Borstenprimordien des Wildtyps.

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[7] V. ROTHENBÜHLER, Diploma Thesis, University of Zürich (1975).

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Blockage of LSD Binding at its High Affinity Site on Synaptosomal Membranes by 1-Methyl-1,2,5,6-Tetrahydropyridine-N,N-Diethyl- carboxamide

SMYTHIES et al.[1] in an attempt to delineate the activity of hallucinogenic agents based on classical structure-activity relationships discovered a most interesting phenomenon related to the compound 1-methyl-1, 2, 5, 6-tetrahydropyridine-N, N-diethylcarboxamide (THPC). The structure of this compound as shown in Figure 1 indicates its relationship to LSD through the D-ring of the LSD molecule.

These investigators had postulated, on the basis of structure activity comparisons, that THPC in sufficiently high concentrations should, in fact, cross the blood brain barrier and act as a competitive antagonist of the hallucinatory activity of LSD. In order to test this hypothesis the modification of rodent behavior produced by LSD was quantified using Bovet-Gatti profiles on a Sidman avoidance schedule[2]. Having established these profiles on rats given LSD the ability of THPC to block this behavior was tested by premedicating the rat with a dose of THPC equivalent to 15 mg/kg of the drug. The drug was given i.p. approximately 45 min before 0.1 mg/kg of LSD was given to the animal. Results of these studies clearly indicated that THPC brought about an almost complete abolition of the behavioral disruption produced by LSD intoxication.

Since we have been working for some time in our laboratory characterizing the properties of the high affinity LSD binding site on synaptosomal membranes from rat brain, we were intrigued by the idea of obtaining molecular (rather than behavioral) evidence for the blockade of LSD binding by THPC.

[1] J. R. SMYTHIES, J. BEATON, F. BENINGTON, R. D. MORIN, Nature New Biol. *226*, 644 (1970).

[2] J. R. SMYTHIES, R. J. BRADLEY, V. S. JOHNSTON, F. BENINGTON, R. D. MORIN, and L. CLARK, Psychopharmacologia *10*, 379 (1970).