The Influence of Farnesenic Acid Ethyl Ester on the Differentation of *Kalotermes flavicollis* Fabr. (Isoptera) Soldiers

LÜSCHER'S[1] experiments demonstrated the role of the corpora allata in the differentiation of *Kalotermes flavicollis*; moreover[2,3] it was found that the juvenile hormone and several analogous substances, when administered in various ways, induce differentiation of soldiers and intercastes both in *K. flavicollis* and in *Reticulitermes lucifugus*. But while the fact itself is now a certainty, observations are lacking on the relationship between dose and effect, as well as on the side effects of treatment.

We treated *K. flavicollis* pseudergates with different doses of a crude preparation of farnesenic acid ethyl ester (FAEE)[4]. The preparation was dissolved in acetone, spread on No. 3. Whatman filter paper and left to evaporate at room temperature. 9 different amounts of the crude product were used (Table I), per 1000 parts of filter paper, weighed at room conditions. Paper treated in the same way with pure acetone was used for the control pseudergates. The insects were placed in closed plastic boxes whose bottoms were covered with the treated paper. The substance thus could act through contact, through the gas phase (perhaps by way of the tracheal system?) and by being eaten along with the paper (the insects were given no other food). The paper was changed every 10 days. The treatment continued until all the pseudergates had moulted; the insects were removed in each case after the ecdysis. Pseudergates of 2 different wild colonies gathered in Sardinia were used. The groups were composed of about 20 pseudergates, all orphans, with no soldiers.

The results are reported in Table I. In experimental groups mortality was high. The pseudergates refused the paper treated with the largest doses of FAEE; in these cases the mortality was largely due to cannibalism. Larval moults, moults into supplementary reproductives, soldiers, royalsoldiers and pseudergate-soldiers were observed[5].

Even the smallest doses of FAEE lowered the number of larval moults, but higher doses were needed to reduce the number of moults into supplementary reproductives. The larval moults and moults into supplementary reproductives were replaced by moults into intercastes and soldiers: at the highest doses of the substance, these were almost the only types of moults that took place. Higher doses of FAEE are necessary for the differentiation of soldiers than for the formation of intercastes: a few true white soldiers were obtained only with doses from 16 to 128, and these moulted again 10 to 14 days later.

The intercastes did not have constant external morphological characteristics. The shape and size of their head and mandibles varyied widely: in some cases these features were more or less the same as those of the pseudergates and the reproductives, in others they resembled those of the soldiers. The variability seems continuous, the biometric study has just begun. The heterogeneity of these features was observed in insects belonging to all the treated groups and thus cannot depend solely on the dose of substance used in the treatment, but must also be related to the physiological conditions of the treated pseudergates. None of the royal-soldiers intercastes had pigmented compound eyes; the females, however, all lacked styles. The intercastes did not moult any more (some were observed for as long as 90 days), their intestine filled up with ingested material, and the distal and median parts of their mandibles darkened.

The period of time from the beginning of the treatment until the larval moults and moults into supplementary reproductives is significantly longer for the treated pseudergates than for the control insects (Table II), whereas for the moults into soldiers and into intercastes it was much shorter than in other experiments done using untreated orphan pseudergates[6]. In our studies on the differentiation of castes[6,7], we have always found

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Table I. Moults of orphan pseudergates, no soldiers, treated with farnesenic acid ethyl ester

Dose[a]	No. of pseudergates treated	No. deaths	Moults						
			No. total	S.R. No.	S.R. %	Larval No.	Larval %	Soldiers[b] No.	Soldiers[b] %
controls	40	2	38	23	60.53	15	39.47	0	0.00
1	40	3	37	24	64.86	9	24.32	4	10.81
2	40	5	35	25	71.43	3	8.57	7	20.00
4	40	4	36	22	61.11	7	19.44	7	19.44
8	40	4	36	14	38.89	2	5.56	20	55.56
12	40	12	28	1	3.57	1	3.57	26	92.86
16	40	3	37	1	2.70	4	10.81	32	86.49
32	40	13	27	1	3.70	1	3.70	25	92.59
64	41	19	22	1	5.54	2	9.09	19	86.36
128	42	26	16	1	6.25	0	0.00	15	93.75

[a] Parts per 1000 parts of paper by weight. [b] Including intercastes. The percentages are calculated on the total number of moults observed; S.R. = supplementary reproductives.

Table II. Average length of time elapsed between the formation of the experimental groups and the moults: cumulative data

Type of moult		No.	Days	Log	P
Larval	Control	15	22.17	1.346 ± 0.079	< 0.05
	Treated	29	39.90	1.517 ± 0.040	
Supplementary reproductives	Control	23	15.53	1.191 ± 0.054	< 0.01
	Treated	90	22.74	1.357 ± 0.027	
Soldiers and intercastes	Control[a]	58[a]	43.09[a]	1.634 ± 0.035	< 0.01
	Treated	155	16.03	1.205 ± 0.016	

[a] Data collected by the author in other researches (only soldiers).

that on the average the moults into supplementary reproductives take place first, followed by larval moults and, simultaneously, moults into soldiers. In the groups treated with FAEE, moults into soldiers and into intercastes are the first to take place, followed by moults into supplementary reproductives and then, last of all, by larval moults. Evidently the substance does not act only on pseudergates that are in the well-defined period of competence for differentiation into soldiers.

The frequency of moults into soldiers and intercastes increases as the dose of FAEE administered to the pseudergates is increased; on the other hand, larval moults and moults into supplementary reproductives diminish; only at very high doses are white soldier obtained. There was an increase in the average time between the beginning of the treatment and the larval ecdyses and ecdyses into supplementary reproductives, whereas the times of ecdysis into intercastes and soldiers became shorter, compared with the times observed in other experiments[8].

Riassunto. Per studiare l'influenza del farnesato di etile (FAEE) sulla differenziazione delle caste, si sono trattate pseudergati di *Kalotermes flavicollis* con dosi differenti di sostanza. Si è ottenuta la differenziazione sia di soldati, sia di intercaste: tra soldato e pseudergate e tra soldato e reale di sostituzione; la frequenza di questi tipi di mute aumenta con la dose di FAEE usata, mentre diminuisce la frequenza delle mute larvali e a reale di sostituzione. Il tempo medio intercorso tra l'inizio del trattamento e le mute larvali o a reale di sostituzione è stato più lungo che per i controlli, è stato invece più breve per le mute a soldato e a intercasta di soldato.

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Antitumor Effect of a New Retinoic Acid Analog

Retinoic acid[1] has been shown to have a prophylactic and a therapeutic effect on chemically induced benign and malignant epithelial tumors in mice (BOLLAG[2–4]). Skin papillomas as well as skin carcinomas of mice induced by Dimethylbenzanthracene and croton oil could be made to regress either partially or completely by systemic administration of retinoic acid. In clinical studies the treatment of actinic keratoses and basal cell carcinomas with local application of retinoic acid led to partial or complete regression of these lesions (BOLLAG and OTT[5]). Further positive therapeutic results were obtained when retinoic acid was administered orally to patients with premalignant conditions of the skin or mucous membranes, e.g. leukoplakias of the mouth, tongue and larynx (RYSSEL et al.[6]). Papillomas of the urinary bladder, too, have been influenced favourably by the oral administration of retinoic acid (EVARD and BOLLAG[7]). Although from a scientific point of view interesting results have been achieved, this treatment cannot be recommended for practical purposes because of side effects. Retinoic acid induces, in animals as well as in man, a series of toxic effects, well known under the name of the hypervitaminosis A syndrome. The main symptoms in man are headache and alterations of the skin and mucous membranes. In small rodents bone fractures are a prominent feature. These side effects limit higher and therapeutically more efficacious dosages. Our aim was therefore to find derivatives with a better therapeutic ratio, possessing a more favourable relation between the tumor-active dose and the hypervitaminosis A causing dose. Among a large series of retinoic acid analogs, synthesized by RUEGG and RYSER in the Roche Laboratories[8], the aromatic analog all-trans-9-(4-methoxy-2, 3, 6-trimethylphenyl)-3, 7-dimethyl-2, 4, 6, 8-nonatetraenoic acid, as well as its esters and amides, proved to be particularly active preparations. The following investigations have been carried out with the ethyl ester I (Figure) of the above-mentioned free acid.

[1] Retinoic acid = all-trans-β-retinoic acid = vitamin A acid = Retinsäure.

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[8] Belgian Patent Application No. 142589 of March 29, 1974.