Myeloblasts collected from leukemic chickens[4], and washed twice with medium 199 containing 50% chicken serum, were stored at −20 °C until needed. The cells were disrupted by 2 methods, one by breakage for 30 sec in a wig-L-Bug instrument, as described before[5] except the homogenizing medium used was 0.01 *M* phosphate buffered saline (PBS), pH 7.4, and the other by homogenization with a glass pestle. For the latter, 1 ml of frozen and thawed myeloblasts diluted to 10 ml with PBS were broken with 10 pestle strokes.

Cell membrane plus nucleus fractions were obtained from both types of preparations. Disruption with the wig-L-Bug yielded a layer of membranes of characteristic morphology, but only traces of the structures were seen in the phase contrast microscope after pestle breakage. The membrane-nucleus pellets were treated with sodium dodecyl sulphate (SDS) in 0.05% concentration. Mitochondria were sedimented at $10{,}000 \times g$ for 10 min from the supernate of membrane-nucleus pellet. The resulting supernate was then centrifuged for 2 h at $100{,}000 \times g$ to yield the microsome fraction, and the soluble fraction was the supernate after sedimentation of the microsomes. Nuclei without membranes were prepared from myeloblasts either by the wig-L-Bug or glass-pestle, isolated in a discontinuous sucrose gradient[5], washed with PBS and treated with SDS, as were the nucleus-membrane fractions.

Microtechnique of Sever, as already described[6], was used for measuring complement fixation (CF) values. The CF titre is expressed as the reciprocal of the highest antigen dilution giving 100% fixarion. Serum pool obtained from hamsters bearing transplanted S-R tumors[7] used in this study had a CF titre of 64 or more and did not react with normal chick tissue. The serum was inactivated at 56 °C for 30 min.

The results revealed a marked difference in antigen distribution between the fractions obtained by pestle homogenization and those derived by wig-L-Bug breakage.(Table) In the latter, most of the antigen was associated with the fraction containing membranes, in contrast to the pestle preparations in which the antigen was primarily in the soluble fraction and in much greater concentration than in the soluble fraction obtained with the wig-L-Bug. Variable and relatively small amounts of antigen were associated with the mitochondrial and microsome fractions. The preparations of nuclei showed no trace of antigen. The data indicate that the antigen is primarily concentrated in the cell membrane which can be isolated in quantity from wig-L-Bug preparations but of which only traces remained after pestle homogenization. In this latter case, the antigen was extracted from the destroyed membranes and appeared in the soluble fraction.

Percent of the total whole cell homogenate CF activity in different cell fractions obtained from wig-L-Bug and pestle homogenization

| Cell fractions | Wig-L-Bug | Pestle homogenization |
|---|---|---|
| Membranes | 62.5 | 3.7 |
| Mitochondria | 12.5 | 7.4 |
| Microsomes | 6.3 | 14.8 |
| Soluble fraction | 18.7 | 74.1 |

Electron microscopic[2] studies have not shown many virus particles in the myeloblast cell cytoplasm, although there was virus elaboration by budding at the cell surface. Fluorescent[3] antibody studies with GS hamster serum have demonstrated the GS antigen inside the viral particle at the cell surface in the cells actively elaborating the virus. Biochemical studies done with the virus infected chick fibroblasts also involve trypsin released cell surface structures as the site of virus synthesis[8]. In this study, when intact myeloblast cell membranes were prepared and tested for GS antigen against hamster serum, most of the whole cell homogenate activity was found in the membranes whereas the cell soluble fraction in the same preparation showed a correspondingly decreased titre. Since in all homogenization procedures cell membranes are destroyed, it seems very plausible that some membrane antigen is released into the soluble fraction during homogenization.

The finding that the viral antigen is associated with cell membrane may be of importance for the understanding of the site of the synthesis of this virus in the myeloblast host cell. The present evidence, coupled with the fluorescent antibody and electron microscope studies, strongly suggests that the BAI strain A virus is synthesized in association with the cell membrane of the infected cell.

*Zusammenfassung.* Zellfraktionen von Myeloblasten leukämischer Hühner wurden auf das Vorliegen des für die Hühnerleukoseviren gruppenspezifischen Antigens untersucht. Das Antigen fand sich vorwiegend in der Fraktion der Zellmembranen.

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## The Influence of Farnesenic Acid Ethyl Ester on the Differentiation 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,

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. | % | Larval No. | % | Soldiers[b] No. | % |
| 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 | 4.54 | 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.

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 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

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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 | $1.346 \pm 0.079$ | $<0.05$ |
| | Treated | 29 | 39.90 | $1.517 \pm 0.040$ | |
| Supplementary reproductives | Control | 23 | 15.53 | $1.191 \pm 0.054$ | $<0.01$ |
| | Treated | 90 | 22.74 | $1.357 \pm 0.027$ | |
| Soldiers and intercastes | Control[a] | 58[a] | 43.09[a] | $1.634 \pm 0.035$ | $<0.01$ |
| | Treated | 155 | 16.03 | $1.205 \pm 0.016$ | |

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

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 using untreated orphan pseudergates[6]. In our studies on the differentiation of castes[6,7], we have always found 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 soldiers 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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## Basement Membrane Abnormalities in Melanotic Tumor Formation of *Drosophila*

Two general mechanisms are utilized by insects to combat an invasion of the hemocoel by foreign objects. In the case of a small infective particle, individual hemocytes destroy the intruder by a phagocytic reaction[1]. When the size of the infective agent or the intensity of infection is such that a large segment of the hemocyte population is mobilized, the invaders are surrounded by layers of blood cells forming a capsule which then becomes melanized. Striking examples of the effectiveness of the latter mechanism are the aggregation of hemocytes about the cynipid *Pseudeucoila* in parasitized larvae[2], and encapsulation of *Streptococci* in infected larvae of *Drosophila melanogaster*[3]. This cellular defense mechanism corresponds precisely to that occurring in 'melanotic tumor' strains in this same species of *Drosophila*. The initiating site for the hemocyte reaction in melanotic tumor formation, however, is the larva's own tissues, and in the *tumor*$^{w}$ (*tu*$^{w}$) mutant the caudal fat masses are specifically singled out for this process of encapsulation and melanization[4]. Ultrastructural examination of the caudal fat masses and the hemocytes of *tu*$^{w}$ larvae did not reveal any foreign infectious agent[5], nor could transmission of an infective agent be demonstrated by parabiotic ligation of *tu*$^{w}$ larvae to larvae of nontumorous strains[6]. In other tumorous strains of *D. melanogaster* PEROTTI and BAIRATI[7] were not able to locate infective bacteria or viruses specific to the melanotic masses.

The analogy between hemocyte response to a foreign agent and encapsulation of the *tu*$^{w}$ caudal fat masses in the absence of an infective agent prompted our suggestion that this region of the fat body in the mutant was defective or 'abnormal' for this stage of development and consequently a reaction to 'foreignness' was aroused to contain the aberrant tissue[8]. Several observations support this consideration. The caudal fat body in *tu*$^{w}$ loses its firm texture in the 3rd larval instar and the individual fat cells separate from one another easily when *tu*$^{w}$ larvae are dissected. This problem is not encountered in handling nontumorous larvae; in the latter strains dissociation of individual cells of the fat body is a normal developmental process that occurs during tissue reorganization accompanying pupal metamorphosis. Furthermore, the morphic transformation of the spherical plasmatocytes (hemocytes) to the flattened lamellocyte variants which normally is associated with pupal development in *D. melanogaster*[9], occurs during the 2nd molt of *tu*$^{w}$ larvae[10]. If the stimulus for hemocyte transformation is emitted from metamorphosing tissues in the normal sequence of events, premature appearance of lamellocytes in *tu*$^{w}$ larvae might result from the condition of the caudal fat body which precociously assumes 'pupal' fat body status. Both the processes of cell transformation and the binding of the hemocytes to the caudal fat cells involve changes in cellular surface properties, and the topological aspects of these phenomena are now open to direct observation with the scanning electron microscope. The present communication describes a comparative examination of the caudal fat bodies of the *tu*$^{w}$ strain and a nontumorous strain; an additional control included comparison of the anterior fat bodies of these two strains.

Larvae from a wild type strain (*Ore-R*) and a tumor strain (*tu*$^{w}$*rc*) were collected within 1 h after eclosion from eggs and maintained on cream of wheat-molasses

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