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.

A. SPRINGHETTI

Istituto di Zoologia, Università di Ferrara, Via Previata 24, I-44100 Ferrara (Italy), 1 November 1973.

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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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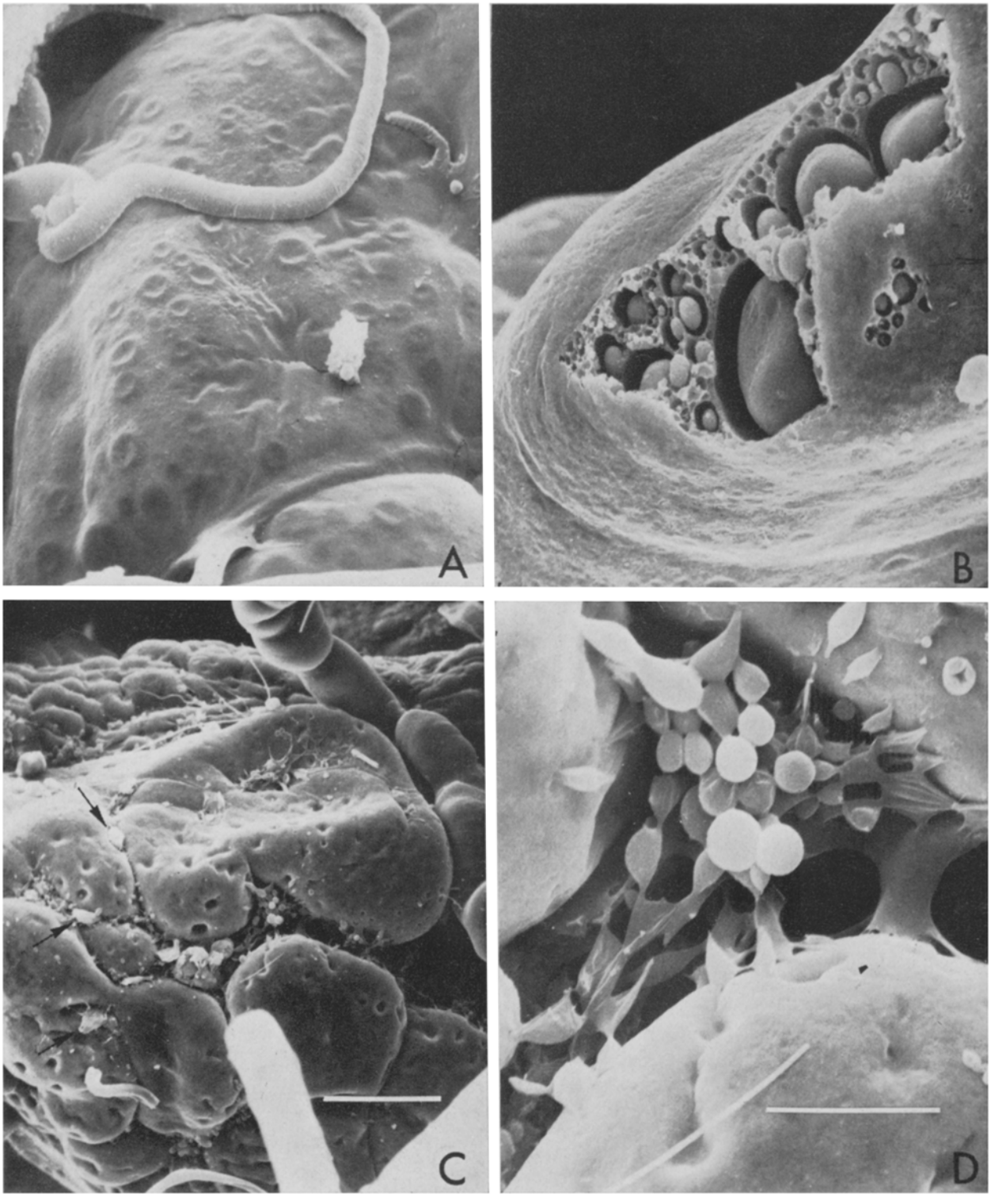

Fig. 1. A) The posterior fat body of a 60 h *tu*^{w} larva illustrating the surface with crater-like marks; a tracheal branch is overlying the surface. Note particularly the continuity of basement membrane over the surface of the adipose cells. SEM ×3000. B) The chipped surface of anterior fat body of a 72 h *tu*^{w} larva shows the various spherical elements and their distribution within a cell. SEM ×3000. C) The caudal fat body of an 86 h *tu*^{w} larva shows the first visible signs of tumor formation including loss of basement membrane and dissociation of the individual cells. The surface of the adipose cells has deep folds and gullies, and several hemocytes can be seen in this region (arrows). SEM ×500. The scale is represented by a white bar, 40 μm. D) A detailed view of the specimen in Figure C) demonstrates the membranous elements stretched between 3 cell surfaces. The spherical elements are similar to those seen within the cell in Figure B) and these globules may escape from the cells as surface membranes disintegrate. SEM ×3000. Scale, 10 μm.

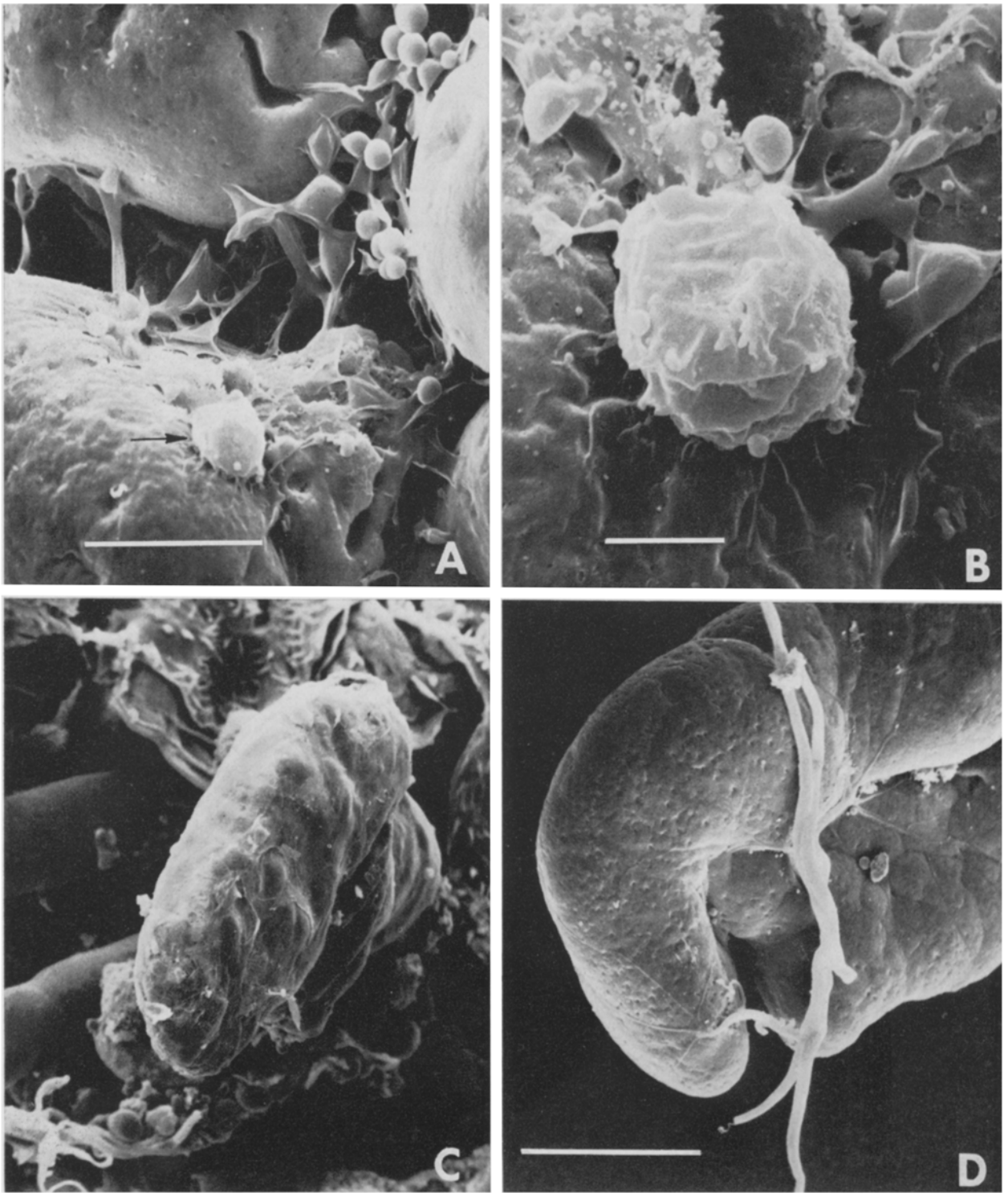

Fig. 2. A) Caudal fat body of an 86 h *tu*^{w} larva with a hemocyte (arrow) in the tumor forming site. Fat cell surface in the lower left of the frame has been modified by hemocyte activity and can be compared with the surface texture of the 2 other fat cells in the frame. SEM ×3000. Scale, 10 μm. B) Enlargement of the hemocyte shown in Figure A. The specimen has been tilted to show that the cytoplasm of the hemocyte is extending over the surface of the underlying fat cell. This cell is rapidly transforming to a lamellocyte. SEM ×10,000. Scale, 2 μm. C) A melanotic tumor of the caudal fat body of a 93 h *tu*^{w} larva. The margins of encapsulating lamellocytes of the upper layer of the tumor mass are apparent. SEM ×300. D) A caudal fat body from an *Ore-R* larva at 93 h from the same region illustrated in Figure C shows normal fat body texture, typical craters, and normal tracheation. SEM ×300. Scale, 100 μm.

medium at 25 °C. At the ages of 60, 72, 82, 86, 93, 96, and 104 h, groups of *Ore-R* and *tu^{w}rc* larvae/pupae were fixed in buffered formadehyde followed by osmic acid[11]. The specimens were dehydrated in a graded series of ethanol and transferred to amyl acetate. Liquid CO_2 was used to replace the amyl acetate in the critical point drying method essentially as described by ANDERSON[12], and the specimens were attached to cover glasses mounted on metal stubs. The specimens were then coated with gold and examined with a JEOL (Model JSM-U3) scanning electron microscope.

The topology of the caudal and anterior adipose tissues of the tumor and normal strains do not differ during the early 3rd instar. Adipose tissue is covered with basement membrane and its topography is marked by crater-like depressions (Figure 1, A). Fat body cells at this stage of development contain large lipid droplets[13] and loss of lipid contents during the fixation and dehydration procedures might account for the circular depressions overlying locations of lipid droplets. Mechanical brushing and peeling during mounting of the specimens illustrates the relationship between cytoplasmic content and the cell surface (Figure 1, B). The topography of the fat body changes slightly as development proceeds and at 82 h the depressions over the surface of the fat body cells begin to take on the appearance of folds and gullies. During this period (72–82 h) the surface of the *tu^w* caudal fat masses differs distinctly from *Ore-R* caudal fat masses. The basement membrane overlying the *tu^w* fat cells is lost and individual fat cells or groups of cells begin to separate from one another (Figure 1, C). Small droplets of material not seen in younger *tu^w* larvae or normal larvae appear between and around the dissociating cells. These droplets resemble inclusions found in the fat body cells and they may represent seepage from cells which have lost their surrounding basement membrane (Figure 1, D). Blood cells invade the affected area (Figures 1, C; 2, A and B). As reported previously[4, 10], spherical hemocytes (plasmatocytes and podocytes) undergo a morphological transformation to form extremely flattened cells that have been designated lamellocytes. A hemocyte in the process of cellular transformation is pictured in Figure 2A and enlarged in Figure 2B where the foldings of the cell's surface are illustrated as well as the manner in which the margin is extended in extremely flattened sheets (upper left corner of the photograph B). The process of cell transformation continues until the entire cell becomes a flat sheet spread over the surface of the underlying substrate. This layering of lamellocytes binds the fat cells into a relatively smooth, compact mass. These masses become melanized at approximately 93–96 h of age and the melanotic masses are retained throughout the life of the individual. Figure 2C is a *tu^w* melanotic mass which has been positioned for comparison with this same region of a normal caudal fat mass from an *Ore-R* larva (Figure 2, D). Basement membrane surrounding *Ore-R* fat body remains intact during larval development and anterior fat body of *tu^w* larvae is indistinguishable from *Ore-R* anterior fat body at 93 h of age. Only after pupation do changes in the basement membrane of *Ore-R* fat body cells become noticeable when dissociation of the adipose cells from each other sets in.

The origin of the basement membrane in insects has not been established, although a number of observations suggest epithelial cells as its source. WIGGLESWORTH[14] has recently reviewed the reported variety of sources of basement membrane material and presented evidence that the hemocytes contribute to basement membrane formation in *Rhodnius*. In *Drosophila* the nature of the glycoproteins in the basement membrane is unknown, and it is not clear whether the acellular membrane is a product of the cells underlying a given tissue or a product elaborated by the hemocytes. This distribution is relevant to an analysis of 'melanotic tumor' formation in *Drosophila*; if the hemocytes contribute to basement membrane formation, their neutrality to surfaces covered by products of their own metabolic activity might provide the factor for recognition of 'self' as opposed to foreign entities, and this mechanism would apply to the organism's own tissues denuded of their membranous cover as in *tu^w*. On the other hand, the appearance of specific intercellular fat body contents in the hemocoel following loss of the overlying basement membrane may also serve as a stimulus for hemocyte aggregation about this area to contain the affected cells within an enclosed capsule. The latter stimulus, however, must be considered a consequence of changes at the cell surface supporting our conclusion that the etiology of 'melanotic tumor' formation in *Drosophila* includes a hereditary factor affecting the integrity of basement membrane.

Zusammenfassung. In *Tumor^w*-Mutanten von *Drosophila melanogaster* kapseln Hämocyten den hinteren Teil des Fettkörpers ein und bilden melanotische Tumoren. Mit Hilfe des Rasterelektronenmikroskops wurde nachgewiesen, dass mit dem Beginn der Tumorbildung ein Zerfall der Basalmembran, und eine Auflösung des hinteren Fettkörpers in Einzelzellen gekoppelt sind.

R. M. RIZKI and T. M. RIZKI[15]

Department of Zoology, The University of Michigan Ann Arbor (Michigan 48104, USA),
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Development of an Established Cell Line Derived from *Dasypus novemcinctus* (Armadillo), a Laboratory Animal Susceptible to Infection by *Mycobacterium leprae*

The search for a classical bacteriological medium applicable to the rapid and reproducible laboratory growth of *Mycobacterium leprae* has not been successful[1]. The problems encountered tend to support the possibility that the human leprosy bacillus is an obligate intracellular parasite. If such is the case, the need for a viable, susceptible eukaryotic cell system is quite evident. Tissue culture systems long exploited in the study of