JOURNAL OF INVERTEBRATE PATHOLOGY 24, 37–40 (1974)

Topology of the Caudal Fat Body of the Tumor^{w} Mutant of *Drosophila melanogaster*[1]

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Received December 14, 1973

Hereditary melanotic tumors in the *tumor^{w}* strain of *Drosophila melanogaster* are known to involve encapsulation of the caudal fat body by the larval hemocytes. The encapsulated masses are subsequently melanized. The present study shows that the chain of events preceding encapsulation includes disintegration of the basement membrane of the caudal fat body and the appearance of particulate materials between and around the dissociating fat cells.

INTRODUCTION

Larvae of the *tumor^{w}* (*tu^{w}*) mutant strain of *Drosophila melanogaster* develop melanotic masses shortly before they pupate, and the amorphous capsules are retained throughout life (Wilson et al., 1955). It was previously reported that these abnormal masses consist of caudal fat body cells that have been encapsulated by hemocytes (Rizki, 1957). In forming the cellular sheath, spherical hemocytes (plasmatocytes and podocytes) assume a flattened shape (lamellocytes) and layers of lamellocytes adhere to the fat body cells to yield compact masses that become melanized. This same morphological transformation of the larval hemocytes in *D. melanogaster* normally occurs at pupation in wild-type *Ore-R* larvae, but can be triggered in *Drosophila* larvae infected with parasites (Walker, 1959) and bacteria (Rizki, 1969) as well as under the influence of the *tu^{w}* gene. No evidence of bacterial or viral infection was detected in an examination of *tu^{w}* cells with the electron microscope (Rizki and Soliman, 1965) and melanotic masses could not be induced in nontumorous larvae by parabiotic ligation to *tu^{w}* larvae (Rizki, 1958). Nevertheless, the *tu^{w}* caudal fat cells show signs of dissociating from one another prior to hemocyte aggregation about the region, indicating that some abnormality of this tissue must be an underlying factor for the encapsulation site.

The *tu^{w}* mutant offers an excellent opportunity to study the interaction of hemocytes and fat body cells in melanotic tumor formation with particular reference to the phenomenon of autoimmunity diseases of insects. The process of melanotic capsule formation is limited to the cells of the caudal fat body, and with the proper feeding conditions penetrance of the inherited syndrome approaches 95–100% in a selected strain (*tu^{w}rc*). Fat bodies of *tu^{w}rc* mutant larvae were therefore examined with the scanning electron microscope and compared with fat bodies of the *Ore-R* normal strain to determine whether changes in the topology of the caudal region of the mutant precede encapsulation of this tissue by the hemocytes.

[1] Supported in part by USPHS Research Grant CA12600 from the National Cancer Institute and NIH Biomedical Institutional Grant 011295.

Material and Methods

Third-instar *Ore-R* and *tu*^{w}*rc* larvae were used for this study. Larval age was recorded from the time of egg hatching and at various intervals throughout the third instar, groups of larvae of known age were fixed in buffered formaldehyde followed by osmic acid. The specimens were dehydrated in ethyl alcohol, transferred to amyl acetate, and dried by the critical point method of Anderson (1953) using liquid CO_2. They were then attached to 12-mm diameter coverglasses mounted on aluminum stubs, coated with gold, and examined with a JEOL (Model JSM-U3) scanning electron microscope.

Results and Discussion

No differences were noted in the topology of *tu*^{w} and *Ore-R* fat bodies in the early third instar. The fat body is covered by basement membrane and its texture is distinct from other body tissues since it shows craterlike depressions over its entire surface (Fig. 1). This pocked appearance may be a result of slight shrinkage due to loss of lipid contents during the fixation and dehydration procedures. At approximately 72 hr the caudal fat region of *tu*^{w} differs markedly from anterior *tu*^{w} fat body as well as *Ore-R* fat bodies. An example of this change is illustrated in Fig. 2, where it is apparent that the basement membrane of the fat body is disintegrating and the cells are covered with shredded and torn pieces of the membrane. The boundaries of individual fat cells thus become visible, and this feature can be noted in this photograph as well as in the *tu*^{w} caudal fat body shown in Fig. 3. In addition to membranous debris, globular particles are found between and on the surfaces of the fat cells. The particles resemble the cytoplasmic inclusions found in fat body cells, and occasionally a globule is positioned directly in a pore at the cell surface giving the appearance that it is exiting from the cell. Hemocytes aggregate in this region and during the interval from approximately 72 to 86 hr of larval age, plasmatocytes and podocytes can be observed on the surfaces of the fat body cells as they flatten and form layer upon layer of lamellocytes. The surface of a completed capsule following melanization is pictured in Fig. 4, and the outlines of lamellocytes are evident on the surface of this capsule from a larva at age 93 hr. The smoothness achieved by wrapping the dissociating fat cells with lamellocytes is apparent by comparing photographs 3 and 4.

In a recent review, Salt (1970) has tabulated the variety of foreign objects that induce cellular defense reactions in the insect hemocoel. He notes that it is unlikely such diverse objects as nonbiological materials, insect parasites, microbes, and interspecific and intergeneric tissue implants contain a common factor that would stimulate the hemocyte encapsulation process; he suggests therefore that absence of a surface feature might underlie the distinction between "self" and "not-self" in insects. Since insect tissues are coated with basement membrane, the presence of the latter would provide a recognition site for "self." The present study supports this suggestion as well as confirms the earlier observations on the dissociation of the cells of the *tu*^{w} caudal fat body (Rizki, 1957). However, examination of the microtopography of the *tu*^{w} caudal fat body shows the accumulation of particulate material on and around the dissociating cells, and the possibility that this material may be responsible for exciting hemocyte encapsulation must therefore be considered. Surface features of the encapsulation process are revealed exceptionally well by scanning electron microscopy, and the use of this technique for a reexamination of surface phenomena associated with hemocyte reactions to foreign objects may be valuable in determining the nature of the specific factors that induce cellular defense reactions in insects.

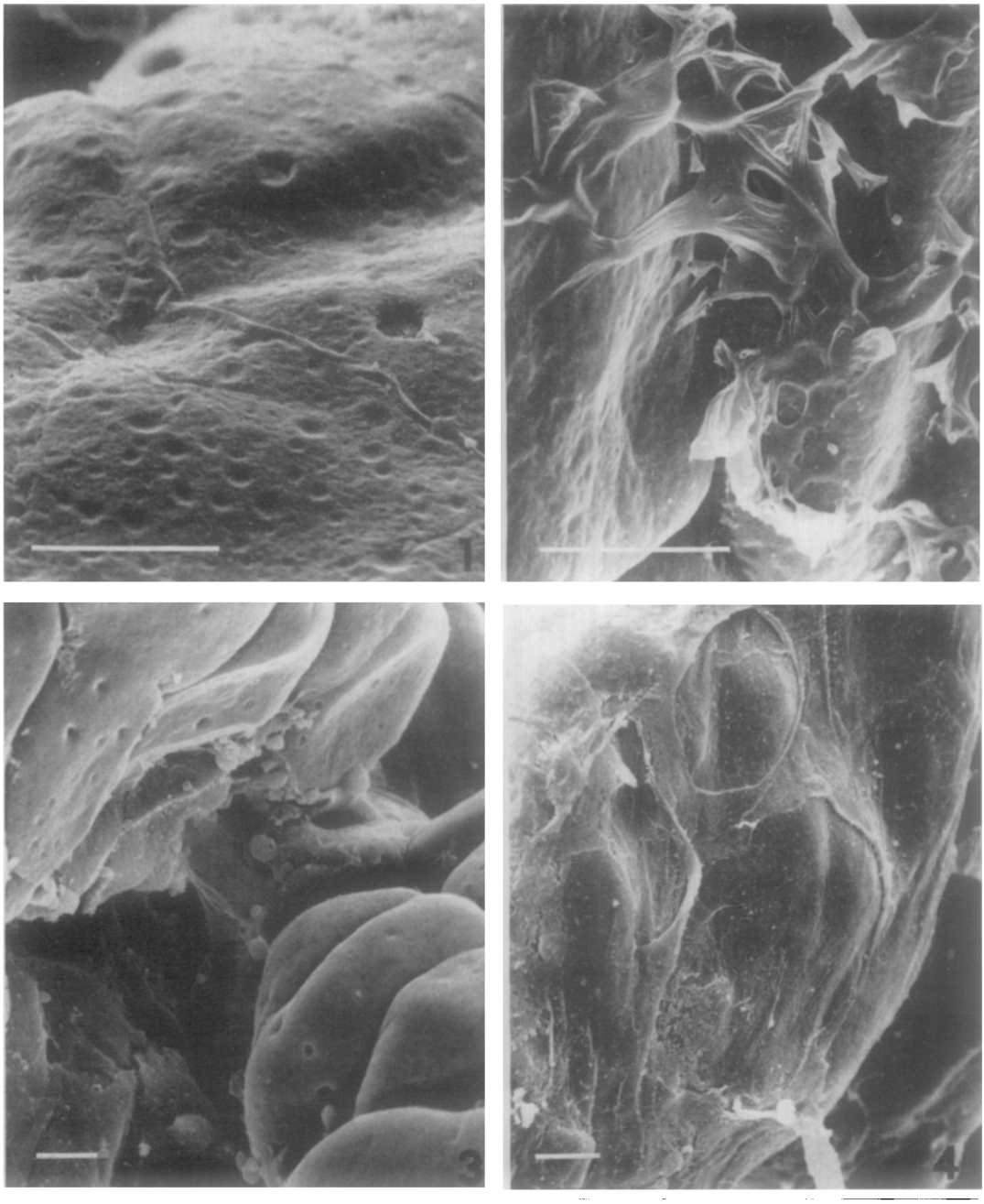

FIG. 1. The normal topographic features of caudal fat body (*Ore-R* larva, 72 hr of age). Note the continuity of basement membrane over the surface and the distribution of crater-like depressions of varying sizes over this tissue. ×2700.

FIG. 2. The caudal fat body of a 72-hr old tu^w mutant larva. The disintegration of the basement membrane covering the fat body cells is evident and the individual cells are now being dissociated. ×2700.

FIG. 3. Tu^w caudal fat body which has lost its basement membrane. Groups of fat cells are clearly dissociated in this specimen and the pockmarks on the surface of the fat cells which were earlier covered by basement membrane (such as in Fig. 1) are now visible as well as small spherules between the crevices of the dissociating cells. ×900.

FIG. 4. Topography of an encapsulated caudal fat body of tu^w larva (93 hr old). The outlines of lamellocytes wrapped about the surface of the mass can be discerned. ×900.

(The bar in each photograph represents 10 μm.)

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