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# *Drosophila* Larval Fat Body Surfaces

## Changes in Transplant Compatibility during Development

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**Summary.** The hemocytes of *tu-Sz*$^{ts}$ melanotic tumor larvae of *Drosophila melanogaster* encapsulate heterospecific and surface-modified homospecific tissue implants, but do not encapsulate unmodified homospecific implants (R. Rizki and Rizki 1980). In the present study we used *tu-Sz*$^{ts}$ hosts to assay changes in larval fat body surfaces during development. Donor fat bodies from various ages of larvae were accepted (remained unencapsulated) in *tu-Sz*$^{ts}$ hosts whereas fat bodies from donors with everted spiracles and all subsequent stages of development that were tested were rejected (encapsulated). Since the demarcation between acceptance and rejection by the *tu-Sz*$^{ts}$ blood cells did not coincide with the gross morphological changes that appear in the fat body during metamorphosis (dissolution of the basement membrane and dispersal of the freed fat body cells at pupation), we compared acceptable and nonacceptable fat body surfaces by three other methods. Fat body surface ultrastructure was examined, fat bodies were treated with fluorescein isothiocyanate-conjugated lectins, and fat body surfaces were reacted with a monoclonal antibody specific for basement membrane. These approaches did not uncover fat body surface changes associated with eversion of the anterior spiracles, suggesting that recognition of tissue surface heterogeneities by the insect hemocytes exceeds the resolving power of the other three methods. However, the monoclonal antibody fails to bind to the basement membrane of *D. virilis* larvae, whose fat body is always rejected in *tu-Sz*$^{ts}$ hosts. This supports our suggestion that the molecular architecture of the basement membrane may be important in eliciting the encapsulation response.

**Key words:** Fat body – Basement membrane – Ageing – Transplantation – Monoclonal antibody

### Introduction

The larval fat body of *Drosophila melanogaster* is a monolayer of large cells surrounded by a thin covering of basement membrane, or basal lamina. Its cells with polytene chromosomes do not undergo cell division but grow in size during the larval stages. During this time they remain in a continuous sheet extending from the first thoracic segment to the posteriormost segment of the larva. Regions of this tissue can be recognized by the morphological arrangement of cell groups and their positional relationships with other organs such as the salivary glands and gonads. There are also physiological differences between the cells from different regions of the tissue (Rizki 1978). In the pupal stage the cells of this organized structure separate from one another and disperse as individual cells in the hemocoel. They survive metamorphosis but disappear in the young adult (Wigglesworth 1949; Butterworth et al. 1965).

The fat body of the larva can be dissociated into individual cells by digesting the basement membrane with collagenase. Most of the dissociated fat body cells are free of basement membrane as confirmed by examination in the transmission electron microscope (TEM) (Rizki and Rizki 1980). When such experimentally isolated fat body cells are implanted in *tu-Sz*$^{ts}$ larvae, lamellocytes, which are flattened hemocyte variants, layer around the implants forming small melanotic capsules, i.e., the dissociated fat body cells are treated as "foreign" and rejected. Large pieces of fat body whose surfaces have been mechanically damaged are likewise rejected when implanted in *tu-Sz*$^{ts}$ hosts, but undamaged larval fat body implants remain unencapsulated and healthy. Heterospecific fat body implants, except those from sibling species, are encapsulated by *tu-Sz*$^{ts}$ lamellocytes even though no physical damage has been inflicted to their surfaces during experimentation. It seems that the lamellocytes of *tu-Sz*$^{ts}$ larvae are capable of discriminating against fat body that is not covered by intact homospecific basement membrane. Thus, the *tu-Sz*$^{ts}$ larval host might be a useful bioassay for normal basement membrane, and possibly for tissue surface changes during development such as those of the larval fat body at pupation. In the present study we demonstrate that this is so by implanting fat bodies from larvae and pupae into *tu-Sz*$^{ts}$ hosts. In addition, we examined the surfaces of fat bodies from specimens of the ages used as donors by three other methods: (1) the presence or absence of basement membrane on fat body cell surfaces was established by TEM; (2) fat body surfaces were reacted with a monoclonal antibody specific for basement membrane, Drosox 305; (3) lectin binding sites of fat body surfaces were examined. We also investigated the binding of Drosox 305 to fat body of *D. virilis* and of *D. simulans*, a sibling species of *D. melanogaster*, since implants of larval fat body from *D. virilis* are encapsulated in *tu-Sz*$^{ts}$ larval hosts but fat bodies from a number of *melanogaster*-sibling species are not (Rizki and Rizki 1980).

## Materials and Methods

*Drosophila Strains.* The phenogenetics of the temperature-sensitive melanotic tumor mutant, *tu-Sz*$^{ts}$ (1, 34.3±), was described previously (Rizki and Rizki 1980). When *tu-Sz*$^{ts}$ larvae are grown at 26–27° C, they develop melanotic tumors in their caudal fat bodies whereas 18–19° C is non-permissive for melanotic tumor formation. At the lower temperature, however, *tu-Sz*$^{ts}$ larvae still develop lamellocytes, the flattened blood cell variants that form the capsule walls of melanotic tumors. These larvae are ideal hosts for testing the encapsulation response since they have competent lamellocytes yet their own tissues do not develop melanotic tumors which might deplete the lamellocyte population available for encapsulation of foreign implants.

The *tu-Sz*$^{ts}$ larvae were grown at 18° C on cream of wheat medium seeded with live yeast. An *Ore-R* wild-type strain grown under the same conditions served as the source of donor fat bodies. Hatching larvae were collected within a two hour interval and ages for the larval stages were recorded ±1 h. Morphological features rather than age were used to select donors from these same dishes once the mature larvae left the food surface to undergo pupariation on the 8th day.

For lectin binding studies a $v^{51c}$ mutant strain and the *Ore-R* strain were used. The former was selected since its larval fat body lacks kynurenine whose intense autofluorescence might overshadow the fluorescent lectins (Rizki 1961). We later noted that this was not the case; the greenish fluorescence due to fluorescein isothiocyanate (FITC) was distinct from intracellular autofluorescence although the latter increased background for photography.

*Implantation Studies.* Donor fat bodies were dissected and rinsed in *Drosophila* Ringer solution, and implanted in etherized host larvae as described previously (Rizki and Rizki 1980). As in the earlier study, region 6 (Rizki 1978) fat bodies were used since these elongate dorsal strips of tissue can be easily dissected and implanted without injury to their surfaces. Region 6 implants were taken from the following donors: 4, 7, and 8 day old larvae, prepuparial larvae with everted spiracles and soft cuticle (8 days old), white puparia (8.5 days old), and tan puparia (9 days old). Clumps of fat body cells from the dorsal thorax and abdomen were taken from donor pupae (after head eversion on day 10) and pharate adults (15 days old).

The *tu-Sz*$^{ts}$ larvae received implants when they were 6 days old, or 2 days prior to pupariation under the conditions used in this study (18° C). Implants remained in the host larval environment 48 h to assure adequate time for assaying the hemocyte response (Rizki and Rizki 1980). Donor tissues were removed from host larvae in Ringer solution and examined with phase optics.

*Transmission Electron Microscopy.* Fat body tissues from specimens at each age used as donors were fixed in phosphate-buffered 2% formaldehyde-1% glutaraldehyde, post-fixed in osmium tetroxide, and embedded in epon. Thin sections from at least two blocks of each batch of embedded material were stained with uranyl acetate and lead citrate. The sections were examined in a Zeiss EM 109.

TEM study of lectin reacted fat bodies was limited to the mid third instar. For these experiments, ferritin-conjugated wheat germ agglutinin (WGA) and WGA-coated gold colloidal particles (20 nm size) obtained from E–Y Laboratories were used to treat freshly dissected, Ringer-rinsed fat bodies from *Ore-R* larvae for 3 min. The treated fat bodies were rinsed in Ringer solution and transferred to formaldehyde-glutaraldehyde fixative. They were then divided into two groups, one of which was postfixed in osmium and the other transferred directly to phosphate buffer. Subsequent preparation of the tissues was the same as above except that the sections from these epon blocks were stained in vanadatomolybdate solution (Callahan and Horner 1964).

*Monoclonal Antibody Binding Assay.* The production and characterization of antibody Drosox 305 has been described previously (Spragg et al. 1982). It labels basement membranes but is not detectable on cell surfaces in the third instar larva.

Indirect immunofluorescence assays were carried out as follows. All incubations were at room temperature. Fat bodies were dissected and fixed in *Drosophila* Ringer solution containing 2% paraformaldehyde and were washed once in Ringer solution in the wells of a Terasaki microtiter plate. They were then incubated for 20 min in monoclonal antibody (undiluted culture supernatant from the hybridoma cell-line). As a negative control, some fat bodies were also incubated in culture supernatant from the myeloma P3/X63-Ag8 (Köhler and Milstein 1976) which secretes an irrelevant antibody. After washing with two changes of Ringer solution, the tissues were transferred to FITC-conjugated rabbit anti-mouse IgG (FITC-RAM; Miles), diluted 1/20 in Ringer solution, for a further 20 min. They were then washed with 3 changes of Ringer solution and examined under epifluorescent illumination using a Leitz microscope. Photographs were taken using Kodak Tri-X film, developed in Acuspeed (Paterson) at an effective ASA of 1250.

To test whether Drosox 305 antibody recognizes the same carbohydrate moiety as wheat germ agglutinin, the antibody was pre-incubated with 0.05 M N,N′-diacetylchitobiose (Sigma) for 5 min before incubating with the tissues as above.

*Lectin Binding Studies.* The following fluorescein isothiocyanate (FITC)-labeled lectins obtained from Vector Laboratories were diluted to a concentration of 100 µg/ml in phosphate-buffered saline (Seecof 1971) at pH 6.8: wheat germ agglutinin (WGA), peanut agglutinin (PNA), soybean agglutinin (SBA), *Dolichos biflorus* agglutinin (DBA), *Ulex europaeus* agglutinin I (UEA I). Concanavalin A (Con A) at the same concentration and pH was used in 20 mM $CH_3COONa$, 0.4 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$. High lectin concentrations were used because it was previously demonstrated that *Drosophila* cell surfaces are immobilized by this treatment (Rizki et al. 1975, 1977). Fat bodies were dissected in *Drosophila* Ringer solution on microscope slides, rinsed in fresh Ringer solution, and treated with lectin solutions for 1–3 min. The slides were then flooded with 4 changes of Ringer solution to remove unbound lectin and a coverglass was placed gently on the tissue which was examined immediately with a Zeiss fluorescence microscope equipped with a darkfield ultracondenser. To test for specificity of FITC-WGA binding, the lectin was pre-incubated for 5 min with 0.05 M N,N′-diacetylchi-

tobiose before treating the cells; 0.05 M D(+) mannose was used to test for the specificity of FITC-Con A binding.

Fat bodies treated with FITC-PNA, UEA I, SBA, and DBA did not differ from controls in buffer; those treated with FITC-Con A had smooth, greenish fluorescent surfaces; fat bodies exposed to FITC-WGA showed intense fluorescence distributed in minute aggregates giving a speckled appearance to the tissue surface. Fat body fluorescence was not observed when the lectins were pre-incubated with their specific haptenic sugars. Since the fluorescence following FITC-WGA treatment was stronger than that with FITC-Con A and displayed a distinct pattern, we selected the former to study fat body surfaces during development.

## Results

For the first implantation experiment, region 6 fat bodies of 4 and 7 day old *Ore-R* larvae were implanted in *tu-Sz*$^{ts}$ hosts at 18° C. None of these fat bodies were encapsulated, confirming the earlier study that fat bodies from larvae remain healthy in *tu-Sz*$^{ts}$ hosts (Rizki and Rizki 1980). Clumps of larval fat body cells from pharate adults were then implanted in *tu-Sz*$^{ts}$ larval hosts. All of these implants were encapsulated and melanized, suggesting that fat body cells from specimens after pupation are no longer acceptable in *tu-Sz*$^{ts}$ larvae.

To determine the age at which larval fat body is no longer acceptable in a *tu-Sz*$^{ts}$ larval host, we implanted fat body cells or intact region 6 fat bodies from successively younger *Ore-R* donors. These data are summarized in Table 1. Fat bodies from larvae that had crawled up the sides of the dishes and everted their spiracles were encapsulated in *tu-Sz*$^{ts}$ hosts whereas fat bodies from mature larvae of the same age group that were still on the food and whose anterior spiracles had not yet everted were not encapsulated. For the spiracle-everted donors, we selected specimens still showing mobility when submerged in Ringer solution and capable of contracting and telescoping the body wall when touched with forceps. Thus, the major apparent difference between these two donor groups was indicated by the condition of the anterior spiracles.

To determine whether fat body surface morphology of susceptible and nonsusceptible stages differs we examined epon sections of fat body tissues from each of the stages used as donors. The fat body of the larva is covered by basement membrane which forms a continuous sheath around the tissue. This structure is not a tightly-formed membrane but consists of a meshlike array of fine fibrils, many of which project at various angles from the surfaces of the cut section. The fibrils form open spaces which presumably allow passage of materials in and out of the underlying fat body cells. Some of the fibrils appear thicker than others, particularly at points where they meet to form the network. The arrangements of the fibrils can be seen best in tangential or grazing sections which also reveal projections from the fat body cells and fibrillar attachments between these protuberances and the overlying basement membrane. In 6–7 day old larvae the basement membrane is 40–60 nm wide.

We did not detect any differences between the basement membranes of larvae, larvae with everted spiracles, white puparia, and tan puparia (Figs. 1 A–C). Basement membranes of tan puparia seemed thinner, but such comparison is difficult since the angle of the cut through the basement membrane affects this measurement, and the inner and outer surfaces of the membrane are irregular with fibrous projections. For these reasons we did not undertake quantitative comparison of basement membrane thicknesses. After pupation when the fat body cells separated from each other and were distributed as individual cells in the pupal hemocoel, the majority of the cell surfaces lacked basement membrane (Fig. 1 D). Some fine fibrillar material was occasionally seen on the free cells and, in some instances, these were obviously small remnants of basement membrane adhering to the cell surfaces.

The presence of Drosox 305 antigen at various stages of development of *Ore-R* larvae and pupae was examined. The antibody binding assay showed that Drosox 305 antigen is present on fat body surfaces throughout the larval period, including the spiracle everted stage, and up to the tanned puparium stage (Fig. 2 A). However, the surfaces of dissociated fat body cells from the pupal stages showed no detectable antibody binding (Fig. 2 B). These results combined with the TEM observations (Table 1) confirm that Drosox 305 is specific for structural elements of basement membrane as reported by Spragg et al. (1982). We also used this antibody to examine the distribution of Drosox 305 antigen among other drosophilid basement membranes, including *D. virilis* and *D. simulans*, a sibling species of *D. melanogaster*. *D. simulans* basement membrane possesses the antigen but *D. virilis* showed no detectable binding with the antibody.

Figure 2 C shows the distribution of FITC-WGA binding to the fat body of a mid third instar larva. We did not detect any differences in this pattern of FITC-WGA binding to region 6 fat body surfaces as long as the tissue remained intact, that is, in larvae and puparia (Table 1). This method of examination would not uncover subtle changes in the spatial distribution of the fluorescent lectin binding sites nor quantitative differences in lectin binding. Clumps of fat body cells that were still adhering to each other in pupae at the head eversion stage also showed this fluorescence pattern. Free fat body cells in pupae had fewer fluorescent specks distributed over the cell surfaces (Fig. 2 D).

To determine whether WGA binding was limited to the basement membrane of the intact fat body, tissues of mid third instar larvae were treated with ferritin-conjugated WGA or gold particles coated with WGA, and sectioned for TEM. Ferritin particles were found in the basement membrane as well as on the underlying cell surfaces (Fig. 1 E). Gold particles which are larger in size were limited to the basement membrane (Fig. 1 F). We conclude that within the time interval used in the studies with FITC-WGA, the lectin was bound to both the basement membrane and the fat body cell surfaces. The WGA binding sites in the basement membrane are lost when this covering disintegrates in the pupal stage and the binding sites localized on the fat body cell surfaces beneath the basement membrane disperse over the surfaces of the freed cells. Details of this redistribution of lectin binding sites will be reported elsewhere.

In order to test whether the monoclonal antibody recognizes the same carbohydrate moiety as WGA, the antibody was pre-incubated with 0.05 M chitobiose. No inhibition of binding was detected after this treatment. The binding of another antibody, Drosox 301 which labels cell surfaces

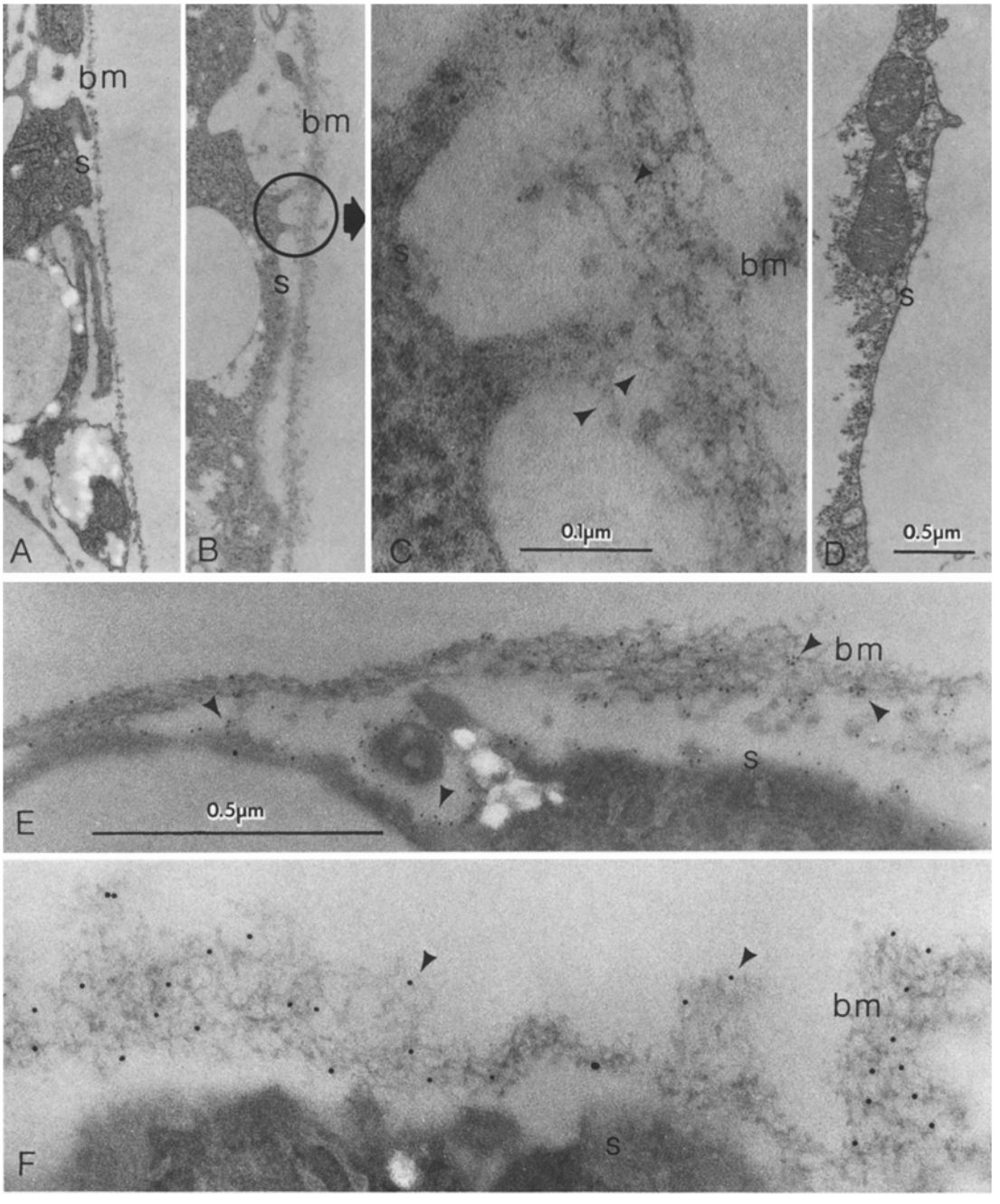


**Fig. 1A–F.** Micrographs of sections through *Ore-R* fat body surfaces; *bm*, basement membrane; *s*, surface of fat body cell. Magnifications of (**A, B,** and **D**) are the same with the scale given in (**D**); the scale is the same for (**E** and **F**). **A** Basement membrane over the surface of a fat body cell of a 7 day larva. ×29,000. **B** A slightly tangential cut through the surface of basement membrane of a larva whose spiracles had been everted. This region was selected to illustrate the connections (circled) between the fat body cell surface and the basement membrane. ×29,000. **C** Higher magnification of the circled region to show the fibrous nature (*arrowheads*) of the basement membrane and its attachment to the cell surface. At s, the plasma membrane elements are apparent. ×242,000. **D** Surface of a fat body cell from a pupa after head eversion. Basement membrane is absent; the cells which have now rounded do not show the highly convoluted surfaces characteristic of earlier stages, such as A. ×29,000. **E** Section through larval fat body (mid third instar) treated with ferritin-conjugated WGA. Ferritin particles (a few indicated by *arrowheads*) are located in the basement membrane and along the fat body cell surfaces. This specimen was not postfixed in osmium so the intracellular contents are not well preserved. However, fixation of the basement membrane is adequate and compares with material postfixed in osmium. This micrograph illustrates the differences in basement membrane thicknesses relative to the angle of cut through the tissue. ×160,600. **F** Larval fat body treated with WGA-coated gold colloidal particles, 20 nm size. The gold particles, some of which are indicated by *arrowheads,* are restricted to the basement membrane. ×106,600

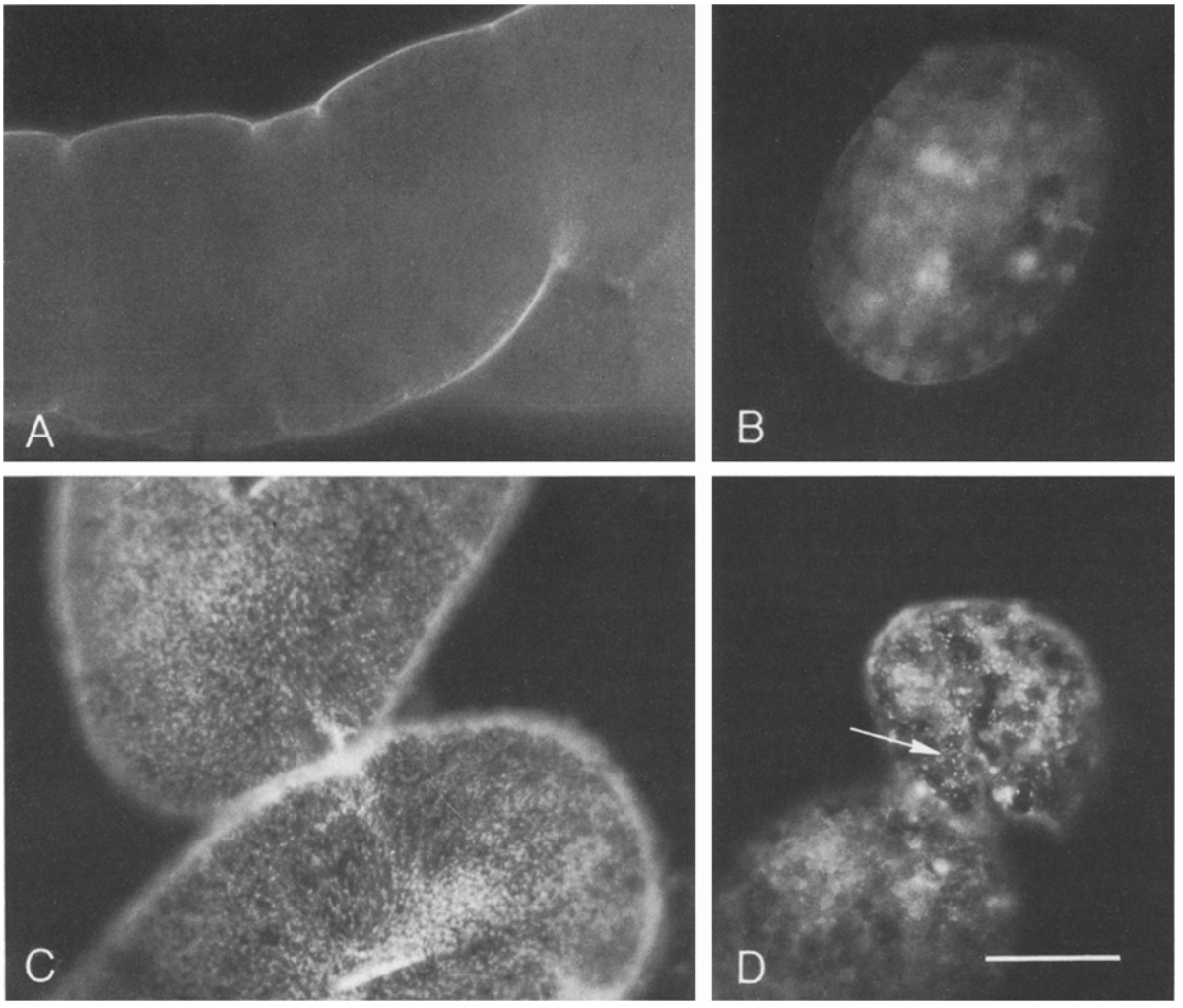


**Fig. 2A, B.** An immunofluorescence assay on *Ore-R* fat bodies using Drosox 305 as the first antibody. Binding is revealed by epifluorescence illumination. **A** Region 6 fat body from a tan puparium. The antibody binds to the basement membrane. **B** A single fat body cell from a late pupa, after dissolution of the basement membrane, showing absence of antibody binding. Autofluorescence can be seen in the protein granules within the cell. (**C** and **D**) *Ore-R* fat bodies treated with FITC-WGA (darkfield illumination). **C** Illustration of the larval pattern (L P in Table 1) of WGA binding to tissue surface covered by basement membrane. **D** Illustration of the cell pattern (C P in Table 1) of WGA binding on fat body cells from a late pupa. The fluorescence is localized in small specks (*arrow*). Large autofluorescent granules and nonfluorescent lipid droplets are visible within the cells. Scale = 50 μm for (**C** and **D**)

**Table 1.** Status of *Ore-R* fat body surfaces detected by *tu-Sz*$^{ts}$ lamellocyte response to implants, TEM, Antibody Drosox 305, and FITC-WGA binding

| Donor | Number of implants | | Presence of basement membrane | | FITC-WGA Binding[a] |
|---|---|---|---|---|---|
| | Accepted | Rejected | TEM | Drosox 305 | |
| Larva/age 4 days | 3 | 0 | yes | yes | L P |
| Larva/age 7 days | 16 | 0 | yes | yes | L P |
| Larva/age 8 days | 15 | 0 | yes | yes | L P |
| Spiracles everted | 0 | 16 | yes | yes | L P |
| White puparium | 0 | 29 | yes | yes | L P |
| Tan puparium | 0 | 16 | yes | yes | L P |
| Pupa/head everted | 0 | 14 | no | no | C P |
| Pharate adult | 0 | 7 | no | no | C P |

[a] L P = larval pattern; C P = cell pattern (see Fig. 2C and D)

and not basement membrane (Spragg et al. 1982), was blocked by chitobiose at this concentration so this antibody was used as a positive control for inhibition.

## Discussion

The larval fat body of holometabolous insects is not only a storage site for proteins, lipids and carbohydrates, but is highly active in protein synthesis as well (Thomson 1975). In *D. melanogaster* third instar larvae, the fat body secretes large quantities of larval serum proteins into the hemolymph (Roberts et al. 1977). Near the middle of this instar the cells of the fat body begin to sequester proteins from the hemolymph in the form of proteinaceous granules (Butterworth et al. 1965; Thomasson and Mitchell 1972). Presumably these materials are stored for use in the pupal stage, a time during which reorganization of the larval fat body itself occurs. Shortly after the head is everted in the pupa, the bulk of the fat body cells are no longer in the

sheet in which they had been held since their development in the embryo (Poulson 1950). This dispersion of the fat body cells in the pupa is associated with the breakdown of the basement membrane which previously surrounded them.

Larval fat body implants are not a target for encapsulation in *tu-Sz*$^{ts}$ larval hosts at non-permissive temperature whereas fat bodies from donors committed to pupariation are. This demarcation in susceptibility to encapsulation does not coincide with the overt reorganization of the fat body cells in the pupa, nor did our examinations of surface morphology, antibody labelling, or lectin binding sites uncover changes in the fat body surfaces at this specific time. We limited our study to the condition of the basement membrane and the cell surfaces immediately beneath it, as our earlier work suggested tissue surface changes are crucial for initiation of encapsulation in melanotic tumor mutants (Rizki and Rizki 1979, 1980). It is obvious that the surface covering of a dissociated fat body cell in the pupa differs from that of a fat body cell in the larva, for basement membrane no longer surrounds the cells in the pupa. We may presume that the disintegration of the basement membrane and dispersal of the fat body cells are the culmination of biochemical events initiated earlier. Perhaps it is these early modifications in the molecular architecture of the basement membrane to which the *tu-Sz*$^{ts}$ lamellocytes are responding. If so, then the changing hormonal milieu associated with pupariation (Garen et al. 1977; Hodgetts et al. 1977) may be a major factor in modifying the fat body surface.

There is little or no information on important aspects of insect basement membranes including their formation, chemistry, and functions. Indeed, there is controversy whether these tissue sheaths are a product of the hemocytes or of the tissues with which they are associated (Whitten 1962) although evidence supporting the latter is more convincing (Ashhurst 1968). Hydroxyproline, an indicator for collagen, has been identified in insect neural lamellae (Harper et al. 1967), and collagen components of the locust have been characterized, including fibrous collagen molecules showing the same banding pattern as mammalian type I collagen and a type IV basement membrane collagen (Ashhurst and Bailey 1980). The basement membrane of *Drosophila* fat body is digestible by collagenase, suggesting the presence of collagen (Rizki and Rizki 1980). This surface covering consists of nonoriented fibrillar materials lacking a defined periodicity at the magnifications available for the present study. Preparations of vertebrate basement membrane collagens contain glycosylated hydroxylysyl residues involving a number of sugars (Adams 1978). The presence of α-D-galactopyranosyl groups in a variety of vertebrate basement membranes was demonstrated by histochemical localization of a fluorescein-conjugated lectin from *Bandeiraea simplicifolia* seeds (Peters and Goldstein 1979). Of the six FITC-conjugated lectins utilized in the present study, only WGA and Con A resulted in detectable fluorescence of fat body basement membrane. Three of the lectins giving negative results for fat bodies (DBA, SBA, PNA) did show fluorescence with other tissues, suggesting that the probes were at least adequate for some *Drosophila* tissues, but no fluorescence was obtained with UEA I (specific for L-fucosyl-). Both DBA and SBA bind N-acetylgalactosaminyl groups while PNA is specific for α-*D*-galactosyl groups. WGA is an N-acetyl-*D*-glucosamine binding lectin and Con A binds *D*-mannose (*D*-glucose). Since the binding of these two lectins to *Drosophila* fat body was inhibited by their haptenic sugars, it is likely that *Drosophila* basement membrane contains these sugar groups.

The molecular nature of the antigen recognized by Drosox 305 is unknown but it seems to occur only in the basement membrane and is not detectable on the underlying cell surface, despite the fact that IgG antibodies can penetrate the basement membrane (Spragg et al. 1982). The differences in surface distribution, and the fact that Drosox 305 binding is not inhibited by chitobiose, strongly suggest that the antibody and WGA recognize different components in the basement membrane.

Lectin binding to fat body surfaces was not detectably altered at the spiracle eversion stage nor did we perceive changes in ultrastructure or antibody binding to fat body basement membrane at this time. The bioassay provided by the *tu-Sz*$^{ts}$ lamellocytes clearly indicates that some biochemical changes at the tissue surface must take place at this point in development. In a previous paper (Rizki and Rizki 1980) we suggested that the distinction made between *D. melanogaster* fat body and *D. virilis* fat body when implanted into *tu-Sz*$^{ts}$ hosts must reside in the molecular architecture of the basement membrane. The observation that Drosox 305 recognizes a basement membrane antigenic site in *D. melanogaster* but not in *D. virilis* supports this suggestion. Moreover, sibling species such as *D. simulans* have fat body which is not rejected by *tu-Sz*$^{ts}$ but is recognized by Drosox 305. However it cannot be the presence of this antigen which prohibits rejection of fat body in *D. melanogaster* for as Table 1 shows this rejection process takes place even when the antigen is still present and the fat body is still surrounded by basement membrane.

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