spectrophotometry of perfused rat heart provides valuable information on changes in pO_2 during contraction (*16*). The NMR method is potentially superior, however, because it is applicable to intact organs in vivo by use of topological surface probes. The method may be especially useful in monitoring the effects of perfusates designed to maintain aerobic conditions in the heart. In addition, the effects of oxidant drugs in inducing metmyoglobinemia may be followed, as the spectrum of metmyoglobin also shows characteristic resonances in the paramagnetic region.

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$$\Delta = \frac{\gamma g \beta}{r^3} \text{ (cgs units)}$$

where g is the spectroscopic splitting factor and β is the Bohr magneton.

$$S_c = g\beta S(S+1)\frac{B_0}{3kT} = \frac{\mu_{eff}^2 B_0}{3g\beta kT}$$

[since $\mu_{eff} = g\beta[S(S+1)^{1/2}]$ where S is the spin quantum number.

$$\frac{1}{T_2} = \left(\frac{\Delta^2 S_c^2}{5}\right) 4\tau_r + \frac{7}{15}\Delta^2 S(S+1)T_{le} \; (\omega_I^2\tau_r^2 >> 1)$$

where T_2 is the spin-spin relaxation time and ω_I is the proton Larmor frequency.
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Blood Cell Surface Changes in *Drosophila* Mutants with Melanotic Tumors

Abstract. *When wheat germ agglutinin conjugated to fluorescein isothiocyanate is bound to hemocytes from larvae of* Drosophila melanogaster, *two populations of hemocytes are distinguished. One shows a fluorescent speckled surface (spk$^+$) and the other lacks this characteristic (spk$^-$). In mutant larvae with melanotic tumors and in larval hosts with heterospecific implants, most of the lamellocytes (a hemocyte variant involved in capsule formation and tissue rejection) are spk$^+$, whereas the lamellocytes in nontumorous larvae are spk$^-$. This suggests that spk$^+$ lamellocytes are necessary for encapsulation of aberrant tissues in the mutant larvae and are responsible for rejection of foreign tissue implants.*

Subpopulations of vertebrate lymphocytes have been recognized and separated by use of plant lectins specific for carbohydrate moieties on the cell surfaces (*1*). Immunocompetent cells are distinguishable from immature cells by these methods. Although insects lack an immune system capable of antibody production, their blood cells do recognize foreign substances (*2*). Insect hemocytes readily phagocytize bacteria and small particulate materials that enter the hemocoel. Larger foreign objects are surrounded by layers of flattened hemocytes, known as lamellocytes, that adhere to each other, forming compact melanized capsules. In *Drosophila melanogaster*, encapsulation is also used to enclose aberrant tissues in mutant strains that develop melanotic tumors (*3*) and for rejection of heterospecific tissue implants (*4*).

Capsule formation requires binding between lamellocytes that previously existed as separate cells in the hemocoel. Therefore, the surfaces of the lamellocytes adhering to each other during encapsulation of a foreign object must be altered so that they have adhesive sites or adhesive materials. If this is so, then lamellocytes stimulated to form capsules in the mutants with melanotic tumors should be distinguishable from lamellocytes not actively engaged in such a response. To test this possibility we used a temperature-sensitive mutant strain that develops melanotic tumors at permissive temperature. Wheat germ agglutinin (WGA) conjugated to fluorescein isothiocyanate was used to show binding to lamellocytes. The percentage of lamellocytes showing WGA binding as a speckled surface (spk$^+$) was increased at the tumor-permissive temperature; these

Table 1. Blood cell response to wheat germ agglutinin. Data from nontumorous and tumorous larvae were obtained from two samples of hemocytes each taken from a pool of hemolymph from three specimens (except *Ore-R* lamellocyte counts, which were taken from four to six samples). Since data from the samples in each group were consistent ($P > .5$; χ^2_{1df}), they were pooled. Samples from four nontumorous larvae with *Drosophila virilis* implants were grouped into two consistent pools. Larvae were grown at temperatures used previously to study melanotic tumor formation or to study the blood cells of each strain (*3*, *5*). N.D., none detected.

Strain, temperature, and age	Cell type	Number of cells	Percentage of spk$^+$ forms
	Nontumorous larvae		
Ore-R			
26°C, 72 hours	Lamellocyte	22	N.D.
26°C, 88 hours	Lamellocyte	60	N.D.
26°C, 72 hours	Plasmatocyte	833	N.D.
tu-Sz^{ts}			
18°C, 6 days	Lamellocyte	470	3
18°C, 7 days	Lamellocyte	234	5
18°C, 6 days	Plasmatocyte	600	< 0.01
	Tumorous larvae		
tu-Sz^{ts}			
26°C, 50 hours	Lamellocyte	247	81
26°C, 72 hours	Lamellocyte	179	79
26°C, 72 hours	Plasmatocyte	984	12
tu-W			
24°C, 72 hours	Lamellocyte	210	46
24°C, 90 hours	Lamellocyte	276	62
tu bw			
24°C, 72 hours	Lamellocyte	218	72
	Nontumorous larvae with D. virilis *implants*		
tu-Sz^{ts}			
18°C*	Lamellocyte	196	71
18°C*	Lamellocyte	392	53

*Host age was 6 days, and hemocytes were sampled 26 hours later.

appear to be the capsule-competent cells since the lamellocytes of two other nonallelic mutants with melanotic tumors also showed this characteristic binding of WGA. Furthermore, the percentage of competent lamellocytes was increased when heterospecific tissue implants were used to arouse the encapsulation response of host blood cells at nonpermissive temperature.

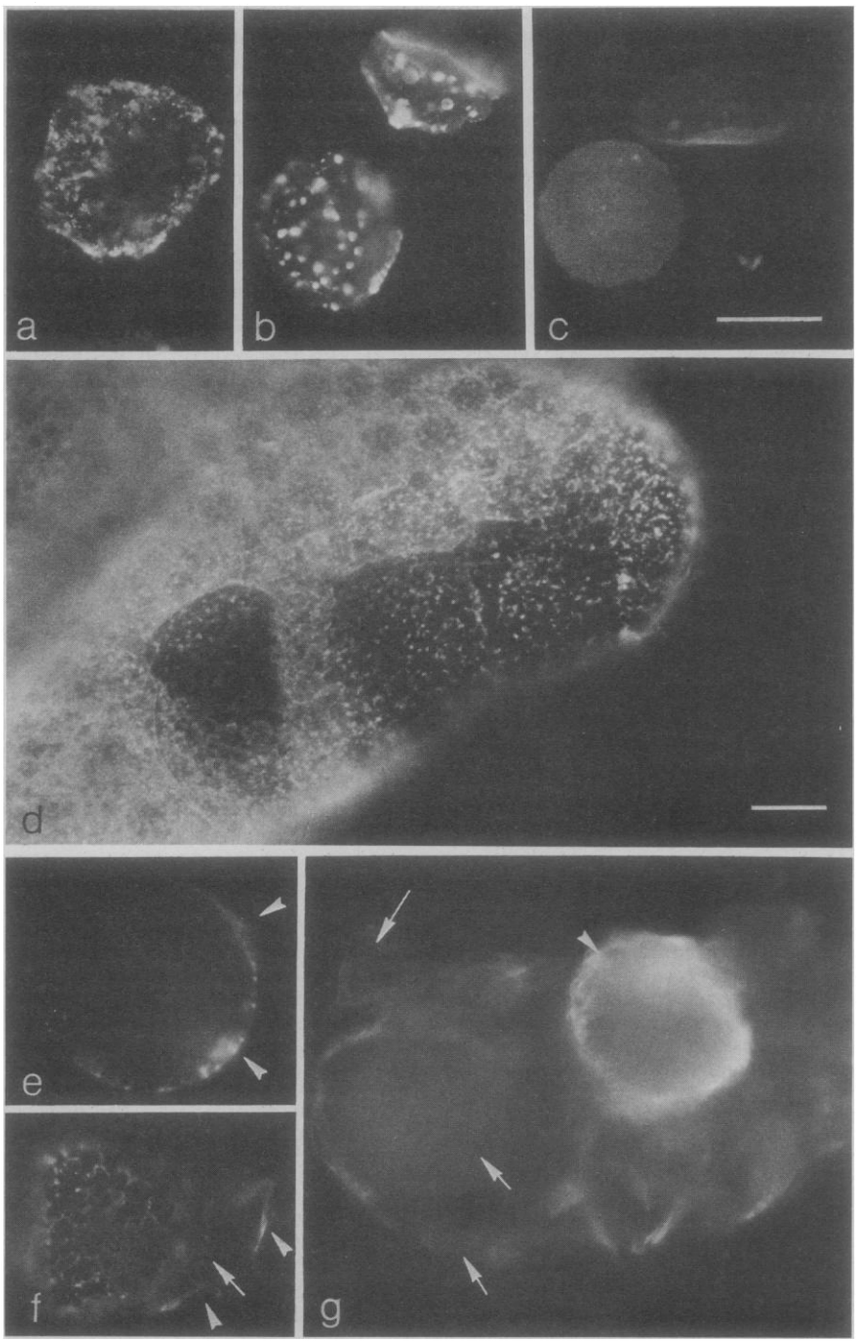

Fig. 1. Cells and tissues of *tu-Sz*^{ts} larvae treated with WGA. Scale bars represent 25 μm. (a and b) Lamellocytes from larvae grown at 26°C show spk$^+$ fluorescence. One side of the flattened cell in the upper right of (b) is folded upward. (c) Two spk$^-$ lamellocytes from a larva raised at 18°C. Contrast for this photographic print was modified to accentuate the cells since their fluorescence is considerably weaker than the fluorescence of spk$^+$ cells; with conditions comparable to those for the cells in (a) and (b), these cells would not be seen. The lower border of the right cell is folded upward. (d) The caudal tip of the larval fat body of a *tu-Sz*^{ts} larva at 26°C showing three aberrant cells with decreased WGA binding. At this early stage, hemocytes were not yet on the aberrant tissue surfaces (*3*). (e) A single fat body cell in an early stage of encapsulation; two spk$^+$ lamellocytes (arrowheads) are attached to the adipose cell surface, which did not bind WGA. (f) A fat body cell coated with WGA material; spk$^+$ (arrowheads) and spk$^-$ (arrow) lamellocytes are adhering to this cell. (g) An encapsulated mass of caudal fat body showing spk$^-$ (arrows) lamellocytes at the outermost layer of a fully compacted region of the capsule, and a mixture of spk$^+$ and spk$^-$ lamellocytes on a single fat body cell in an earlier stage of encapsulation (arrowhead). Wheat germ agglutinin treated with *N,N'*-diacetylchitobiose did not bind to the cell and tissue surfaces.

Unlike *Ore-R* wild-type larvae of *D. melanogaster*, third-instar larvae of mutant strains with melanotic tumors contain many lamellocytes. Two strains, *tu-W* and *tu-Sz*^{ts}, which show encapsulation of the caudal fat body, have been used most extensively to study encapsulation (*3*). The *tu-W* gene is autosomal (2-66.2); the *tu-Sz*^{ts} mutant is sex-linked (1-34.3) and develops melanotic tumors at 26°C but not at 18°C. Selected lines of these mutants are ideal for studying capsule formation since they have high penetrance and expressivity. Furthermore, the large nondividing fat body cells that become encapsulated contrast markedly with the hemocytes, thus making recognition of cell types easy for study. Larvae with another mutation, *tu bw* (synonym *mt*^{A}), develop melanotic tumors in their lymph glands. In this mutant the melanized capsules are often found floating in the hemocoel (*5*).

Hemolymph samples from each of the above-mentioned strains were prepared in *Drosophila* Ringer solution on microscope slides and diluted approximately 1:1 with WGA (100 μg/ml) in phosphate-buffered saline for *Drosophila*. This high concentration of lectin was selected because it immobilizes *Drosophila* cell surfaces (*6*). After 2 to 3 minutes the treated cells were rinsed in a continuous stream of Ringer solution by simultaneous addition and removal of liquid through fine pipettes held at opposite ends of the microscope slide. The cells were examined immediately with a fluorescence microscope. Both the lamellocytes and plasmatocytes were divisible into two types: those that showed intense fluorescence in small areas or specks over their surfaces (spk$^+$) and those that did not show such fluorescence (spk$^-$) (Fig. 1, a to c). The spk$^-$ lamellocytes are not negative for WGA binding but show a weak, evenly dispersed fluorescence.

The percentage of spk$^+$ lamellocytes was determined for each of the strains that developed melanotic tumors; a larger number of *Ore-R* larvae were sampled to locate a sufficient number of lamellocytes. Tumorous larvae had significantly more spk$^+$ lamellocytes than *Ore-R* larvae had (Table 1). Furthermore, the lamellocytes of *tu-Sz*^{ts} larvae at the nonpermissive temperature were mostly spk$^-$, suggesting that the high incidence of spk$^+$ lamellocytes in *tu-Sz*^{ts} larvae at 26°C is related to capsule formation. Lamellocytes of *tu-W* larvae and of *tu-Sz*^{ts} larvae at 26°C were sampled shortly before or during early stages of encapsulation of fat body cells, as well as during

later stages of encapsulation. The spk^+ lamellocyte frequency was high at both early and late stages.

That the absence of melanotic tumors in *tu-Sz^{ts}* larvae at 18°C is due to normalization of the presumptive site of melanotic tumor formation and not to the inability of the lamellocytes to encapsulate at this temperature had been shown earlier by implanting tissues with modified surfaces and heterospecific tissues into *tu-Sz^{ts}* larvae maintained at 18°C (*4*). Such implants are encapsulated by lamellocytes and are usually melanized 24 to 48 hours later. To determine whether the presence of heterospecific tissue in the hemocoel elicits spk^+ lamellocytes, we implanted *D. virilis* imaginal disks into *tu-Sz^{ts}* larvae at 18°C and subsequently examined samples of the hosts' hemocytes. All of the hosts had a preponderance of spk^+ lamellocytes, whereas control larvae at 18°C had about 5 percent spk^+ lamelloctyes (Table 1).

Lamellocytes differentiate from plasmatocytes, which are spherical cells (*7*). Therefore, hemolymph samples with spk^+ lamellocytes should contain spk^+ plasmatocytes. This was indeed the case for the tumorous larvae. In *tu-Sz^{ts}* larvae, the percentage of spk^+ plasmatocytes at 26°C was significantly higher than the percentage of spk^+ plasmatocytes at 18°C. However, the percentage of spk^+ plasmatocytes at 26°C was much lower than the percentage of spk^+ lamellocytes at this temperature (Table 1). No spk^+ plasmatocytes were found in *Ore-R* samples that lacked spk^+ lamellocytes.

The increased frequency of spk^+ hemocytes in larvae in which tissues are being encapsulated suggests that the spk^+ sites on the hemocyte surfaces may play a role in encapsulation. One obvious function of these sites might be cell-to-cell binding. Therefore, we sought to determine whether spk^+ hemocytes are the sole participants in capsule formation and whether the tissue surfaces to which lamellocytes bind in capsule formation show a differential reaction to WGA.

We reported earlier (*3*) that the basement membrane of caudal fat bodies of *tu-Sz^{ts}* changes before the lamellocytes begin to accumulate at these tissue surfaces. The tissue surface changes are accompanied by a loss of WGA binding (Fig. 1d). Furthermore, in early stages of encapsulation, we find spk^+ lamellocytes adhering to affected adipose cell surfaces (Fig. 1e), and spk^+ and spk^- lamellocytes adhering to affected adipose cells that are coated with WGA material (Fig. 1f). Distinguishing between spk^+ and spk^- cells was difficult when lamellocytes were layered, but fully formed capsules showed mostly spk^- lamellocytes in their outermost layers (Fig. 1g).

To summarize the above observations and provide a mechanistic basis for capsule formation, we propose the following sequence of events:

1) The presence of an aberrant (nonself) surface in the hemocoel evokes a stress signal, a diffusible factor, that stimulates the differentiation of spk^+ hemocytes. This suggestion relies on the observation that spk^+ hemocytes circulate in the hemocoel and are not always found in direct contact with the aberrant surfaces. Whether a clone of spk^+ plasmatocytes gives rise to spk^+ lamellocytes is not known but is likely because both cell groups were found and we know from previous work (*5*) that plasmatocytes differentiate into lamellocytes.

2) The aberrant surfaces are coated with WGA material (vesicles), which we presume is released from the spk^+ plasmatocytes as they acquire the lamellocyte shape. This material, which has been described (3, 8), is seen only on nonself surfaces. We suggest that the binding of this material to the aberrant surfaces creates the recognition site for lamellocyte aggregation.

3) The spk^+ lamellocytes may bind directly to aberrant tissue surfaces, but spk^- lamellocytes bind to these surfaces via the coating material or spk^+ lamellocytes.

4) Lamellocyte-to-lamellocyte adhesion involves spk^+-to-spk^- surfaces as well as spk^--to-spk^- surfaces via the extruded materials that are the products of the competent cells (3).

5) Lamellocyte layering continues until the competent hemocyte population and its products in the hemolymph are exhausted and the stimulus for the stress signal has been obliterated by the formation of impenetrable capsular walls.

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Plant Chimeras Used to Establish de novo Origin of Shoots

Abstract. *When African violet leaf explants are cultured in vitro, buds and shoots develop directly from the upper leaf surfaces. Three developmentally different African violet chimeras were cultured, and in each case adventitious shoots that developed into plants had the parent chimera pattern. A multicellular origin of the adventitious buds accounts for these results.*

Since 1937 when Naylor and Johnson presented data that have been popularly interpreted to suggest that a single epidermal cell is the source of adventitious shoots from African violet leaf tissue, the notion has pervaded the literature that adventitious shoot formation is a result of the division of a single epidermal cell (*1*). To the contrary, in our opinion the data of Naylor and Johnson suggest that adjacent epidermal and parenchyma cells also participate in the formation of adventitious shoots. Using chimeral cultivars of African violets, we show here that all layers of leaf tissue are involved in adventitious bud formation. Thus, adventitious shoots are of multicellular origin.

The ontogeny of de novo buds from leaf explants in tissue culture has enormous implications for basic and applied research. As scientific interest focuses on using plant tissue cultures for novel genetic engineering of crop plants, the understanding of the cellular origins of the plants from culture becomes a matter of paramount importance.

A plant chimera is an individual composed of two or more genotypically different tissues. The components of a chimera may differ with respect to their chromosome or plastid constitution. Cramer (*2*) added the important qualification that the genotypically different tissues in the chimera must be represented by their respective cell lines in the