of L5 neurons shows them to be interposed between L1-L2 terminal pairs in the medulla and some second-order neurons whose position is analogous to that of vertebrate ganglion cells. Possibly this relationship provides yet another adaptation channel whose input is derived from two separate receptor systems, scotopic and photopic.

Our studies show that although the insect external plexiform layer is complex neuropil in terms of its cell types, it is highly ordered in terms of synaptic loci and neuronal geometry. Also, electrophysiology has revealed many characteristics of the insect EPL that suggest analogies with the vertebrate counterpart. Whether or not the present functional proposals, derived from structural investigations, are confirmed will depend on future electrophysiological studies of the lamina neurons and their identification by dye or cobalt injection (*19*).

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10. The cyclic order of receptors and other elements is, though, mirror symmetrical between the upper and lower eye halves [V. Braitenberg, *Exp. Brain Res.* **3**, 271 (1967)].
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12. Golgi impregnations (Golgi Rapid, Golgi Cox, and Golgi Colonnier) [M. Colonnier, *J. Anat.* **18**, 327 (1964)] resolve some of the population of stainable neurons in any one lamina. All the cell types summarized here, but no others, have been seen on several hundred occasions in as many laminae. Golgi-impregnated neurons have also been matched to gestalts resolved by reduced silver procedures [see (*7*, *8*) and N. J. Strausfeld, *Z. Zellforsch.* **121**, 377 (1971)]. Combined Golgi impregnation and electron microscopy resolves the positions and the space occupied by a cell's EPL components in an optic cartridge. Together, the components of all cell types would leave no space available within a cartridge for an element that was hitherto refractive to silver impregnation.
13. The term "anaxonal" means axonless; for insect visual centers it applies to neurons that lack an identifiable axon that links separate and distinctive receptive and donative fields. In the EPL, cells without axons are termed "amacrines," in a plagiarism of Cajal's nomenclature [S. R. Cajal, *Cellule* **9**, 17 (1893)] for axonless cells in the inner plexiform layer of the vertebrate retina. The term "telondendron" is applied as for vertebrate neurons and refers to a wide-field terminal arborization whose processes are predominantly presynaptic.
14. Optic lobes were fixed in a mixture of paraformaldehyde and gluteraldehyde [M. J. Karnovsky *J. Cell Biol.* **7**, 137A (1965)] and postfixed in phosphate-buffered 1 percent osmium tetroxide. Silver-gray ultrathin sections were mounted on one-hole holders and double stained in uranyl acetate and lead citrate [J. H. Venable and R. Coggeshall, *J. Cell Biol.* **25**, 407 (1965)].
15. Recordings from dragonfly SLM's (*5*, *16*) may be favorably compared with those from dipterous L1 and L2 neurons under the assumption that the principles of connectivities of spiny LM neurons are the same in both insect orders. The studies of dragonfly laminar synaptology (C. Kibel, I. Meinertzhagen, J. E. Dowling. *Proc. R. Soc. London Ser. B*, in press) show that dyadic and triadic relationships of SLM's are essentially the same as in Diptera and that only one of the two SLM's is presynaptic to receptors, as is L2 in dipterous laminae.
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## Surface Molecules of Hematopoietic Stem Cells: Requirement for Sialic Acid in Spleen Colony Formation

Abstract. *Enzymatic treatment was used to test the function of some surface peptides and carbohydrates in hematopoietic spleen colony formation. Proteases and most glycosidases had no effect on spleen colony formation, whereas treatment with* Vibrio cholerae *neuraminidase reduced colonies by one-half. Intact sialic acid (residues) appear to play an important role in colony formation.*

Suspensions of bone marrow cells injected into lethally irradiated mice cause discrete colonies of proliferating hematopoietic tissue on the recipient's spleen within 7 days after injection (*1*). These colonies are clones (*2*), each arising from a single cell (colony-forming unit, or CFU-S), the progeny of which can ultimately restore hemopoietic function (*3*). Since at least some CFU-S accumulate specifically in the spleen and bone marrow, the processes of site recognition and selective adhesion should precede implantation and colony formation.

Cell-surface carbohydrates and glycoproteins have a role in cell-to-cell recog-

Table 1. Effect of selective removal of surface components on the number of spleen colonies. Each enzyme group was incubated with enzyme for 1 hour at 37°C. Lethally irradiated recipients were transfused with $2.5 \times 10^5$ nucleated marrow cells. Each treatment group had an appropriate control, handled identically, except that buffer was substituted for enzyme. After 7 days, mice were killed, their spleens were removed and fixed, and colonies were counted. The *P* values are from Student's *t*-test; each treatment group was compared to its corresponding control.

| Treatment group | Colonies per $2.5 \times 10^5$ cells | Spleens (No.) |
|---|---|---|
| Control | 18.1 ± 1.8 | 8 |
| Elastase (2 unit/ml) | 15.4 ± 1.8* | 7 |
| Pronase (0.5 unit/ml) | 17.0 ± 1.7* | 6 |
| CONTROL | 23.0 ± 2.6 | 7 |
| Papain (1.2 unit/ml) | 20.4 ± 2.5* | 7 |
| Control | 27.6 ± 3.7 | 14 |
| Trypsin (0.005 percent) | 26.8 ± 1.5* | 8 |
| Trypsin (0.05 percent) | 31.2 ± 2.6* | 8 |
| Control | 27.6 ± 3.7 | 14 |
| Trypsin (0.25 percent) | 24.0 ± 3.7* | 13 |
| Control | 25.5 ± 1.3 | 8 |
| α-D-glucosidase (2 unit/ml) | 23.2 ± 1.6* | 7 |
| β-D-glucosidase (2 unit/ml) | 24.3 ± 2.3* | 6 |
| Control | 13.5 ± 3.3 | 16 |
| α-L-fucosidase (20 unit/ml) | 14.4 ± 3.4* | 14 |
| Control | 25.3 ± 5.5 | 14 |
| β-galactosidase (20 unit/ml) | 22.0 ± 5.5* | 16 |
| Neuraminidase (20 unit/ml) | 14.3 ± 1.2† | 16 |
| Heat-inactivated neuraminidase (20 unit/ml) | 24.7 ± 4.6* | 14 |

*Not significant. †$P < .001$.

nition processes (*4*) and cellular adhesion (*5*). Because implantation of CFU-S involves a site-recognition process, we set out to characterize the involvement of some surface peptides and carbohydrates in the CFU-S recognition-implantation process.

Bone marrow suspensions from 6-week-old CF1 mice were prepared in glucose-free Hanks solution (GFH) and passed through a 200-mesh stainless steel screen. Nucleate cells were enumerated by a Coulter counter. Cells at $3.5 \times 10^7$ per milliliter were incubated with or without enzyme in GFH for 1 hour at 37°C. All enzymes were assayed for activity (*6*). Trypsin-incubated cells were treated with excess trypsin inhibitor prior to washing. Cells were washed once by a 30-fold dilution in GFH, and resuspended in GFH for injection. Recipient mice received 850 rads (*7*) 24 hours before transfusion of $2.5 \times 10^5$ nucleated marrow cells via tail-vein injection. After 7 days, mice were killed, and their spleens were fixed in a mixture of alcohol, formalin, and acetic acid. Colonies were counted under a dissecting microscope at a magnification of ×15.

Table 1 shows the effect of selective removal of cell-surface components on spleen colonies. None of the proteases tested—elastase, Pronase, papain, or trypsin—had any effect on CFU-S. Similarly, α-D- and β-D-glucosidase, β-galactosidase, and α-L-fucosidase had no effect on CFU-S. However, *Vibrio cholerae* neuraminidase (VCN) reduced CFU-S by about one-half (56 percent). Trypan blue viability of the inoculum was unaffected. VCN exhibited no measurable nonspecific enzymatic activities. Heating VCN for 10 minutes in a boiling water bath abolished both enzymatic activity and the reduction of CFU-S, indicating that the action was enzymatic rather than a nonspecific phenomenon. In addition, enzyme concentrations far in excess of that required to achieve maximal reduction in colonies (Table 2) had no additional effect, further supporting the idea that VCN exerts its effect by removal of sialic acid rather than by cytotoxicity.

Surface sialic acid residues, therefore, appear to play a role in the fate of approximately half of the CFU-S in the inoculum. The mechanism of this VCN-induced reduction of spleen colonies is not clear. Several possible explanations, however, could be put forward—that is, killing of some CFU-S, a rapid clearing of VCN-susceptible CFU-S from circulation, or the blocking of some CFU-S from implantation.

Table 2. The effect of *Vibrio cholerae* neuraminidase (VCN) concentration on the number of spleen colonies. Bone marrow cells ($3.5 \times 10^7$) in 1 ml were incubated with appropriate amounts of VCN for 1 hour at 37°C. After being washed once in GFH, $2.5 \times 10^5$ nucleated marrow cells were transfused into lethally irradiated mice. After 7 days mice were killed, their spleens were removed and fixed, and colonies were counted. The results are expressed as the means ± standard error of at least seven spleens.

| VCN (units) | Colonies per $2.5 \times 10^5$ cells |
|---|---|
| 0 | 19.4 ± 3.4 |
| 0.01 | 20.0 ± 1.2 |
| 0.1 | 17.4 ± 2.3 |
| 0.5 | 15.2 ± 3.2 |
| 1.0 | 9.7 ± 2.3 |
| 25.0 | 9.9 ± 2.0 |
| 50.0 | 10.2 ± 3.0 |

Selective killing of some CFU-S could occur if cryptic antigenic sites were exposed by VCN treatment, as occurs with human lymphocytes (*8*). A naturally occurring antibody to these exposed antigens has also been reported (*9*). Similarly, VCN-induced exposure of cryptic sites on a heterogeneous subpopulation of the CFU-S could be a mechanism for selective killing of those CFU-S.

Removal of sialic acid from plasma proteins (*10*) and erythrocytes (*11*) results in their clearance from circulation, and VCN-treated rat lymphocytes exhibit altered circulation patterns (*12*). Therefore, the possibility that VCN-treated CFU-S are removed from circulation or bound at another site must be considered.

Some receptors are exposed on the surface of cells for only a portion of the cell cycle (*13*). If the presence of sialic acid–containing receptor is also a function of cell cycle, VCN could effectively block the implantation of only those CFU-S with exposed implantation receptors. While the actual mechanism of the VCN-induced reduction in spleen colonies is unclear, an important role for surface sialic acid in spleen colony formation is demonstrated.

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## Membrane Potential of Mitochondria Measured with Microelectrodes

Abstract. *The membrane potentials of giant mitochondria from cuprizone-fed mice were found to be independent of metabolic state. Experiments are described in which the presence of the microelectrodes in the inner mitochondrial space, and the metabolic viability of the impaled mitochondria, are validated.*

In some previous studies (*1–4*), membrane potentials were measured in isolated mitochondria (3 to 4 μm in diameter) of *Drosophila*, by means of microelectrodes driven by a piezoelectric drive. The mitochondria were coupled, and the potentials, which ranged from 10 to 20 mv, inside positive, were found not to depend on metabolism (*2*). The membrane resistance ranged from 1 to 4 ohm-