GT-48 strain cultured on artificial medium and then cloned was also highly lethal to suckling rats but produced no cataracts in the survivors (*18*) receiving cultures diluted from 10^{-2} to 10^{-5}.

We confirmed the association between spiroplasmas and the classical pathogenicity in animals by recovering organisms from infected tissues and demonstrating that the recovered organisms were serologically related to, or identical with, those employed in challenge. Twenty-nine rats exhibiting cataracts were autopsied 3 to 4 weeks after intracerebral challenge with cultured SMCA (30th passage in media). Brain and eye tissue pools were cultured separately in SP-4 medium. Ten rats that had received uninoculated culture medium intracerebrally were examined in the same way. Spiroplasmas were recovered from the brain and eye pool of all infected rats, but never from control rat tissues. At least ten isolates, recovered from the eye and brain, were examined in the deformation test and shown to be serologically indistinguishable from SMCA. Thus, our pathogenicity experiments establish that both cultured and cloned SMCA and GT-48 spiroplasmas are able to induce disease manifestations typical of those initially described (*10*), and that recoverable organisms indistinguishable from the challenge organisms persist in the tissues of affected rodents.

The natural reservoir of SMCA spiroplasmas is unknown. However, the recovery of two isolates from rabbit ticks (*10*), the reported recovery of a third, possibly similar, organism from the same tick species (*19*), and the demonstrated persistence of the agent in animal tissues suggest that SMCA might be maintained in a tick-vertebrate cycle in nature. Certainly, an affinity for arthropods is at present a common link for all known spiroplasmas.

The ability of SMCA to multiply over a higher temperature range (30° to 37°C) distinguishes it from all known plant and insect spiroplasmas, which have an optimal temperature range of 25° to 32°C and fail to grow at temperatures above 34°C. Our observation that SMCA could be isolated only on a specially formulated medium explains previous failures to cultivate the agent. The inability of a variety of standard artificial media and cell cultures to support growth of SMCA provides a strong indication that other unrecognized mycoplasmas, including perhaps human or animal pathogens, await discovery. Thus, these organisms represent a new class of microbial agent, incapable of being cultured on the usual laboratory medium and undetected by current serological procedures, yet possessing the ability to provoke disease in vertebrates and to persist for prolonged periods in host tissue. We believe a continued search for the agent in human and arthropod hosts, and for development of information on their possible role in human diseases of unknown etiology would seem worthwhile.

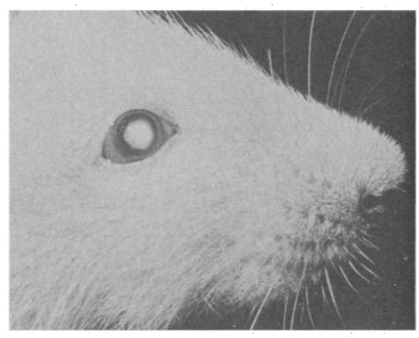


Fig. 2. Rat, 25 days old, with severe bilateral cataracts following intracerebral inoculation as newborn (24 hours) with cloned SMCA passaged 26 times on artificial medium.

JOSEPH G. TULLY
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014
ROBERT F. WHITCOMB
Plant Protection Institute, Agriculture Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705
H FRED CLARK
Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104
DAVID L. WILLIAMSON
Department of Anatomical Sciences, State University of New York, Stony Brook 11794

References and Notes

1. R. E. Davis and J. F. Worley, *Phytopathology* **63**, 403 (1972).
2. P. Saglio, D. Laflèche, C. Bonissol, J. M. Bové, *Physiol. Veg.* **9**, 569 (1971); A. E. A. Fudl-Allah, E. C. Calavan, E. C. K. Igwegbe, *Phytopathology* **61**, 1321 (abstr.) (1971); *ibid.* **62**, 729 (1972).
3. R. M. Cole, J. G. Tully, T. J. Popkin, J. M. Bové, *J. Bacteriol.* **115**, 367 (1973).
4. P. Saglio, M. L'Hospital, D. Laflèche, G. Dupont, J. M. Bové, J. G. Tully, E. A. Freundt, *Int. J. Syst. Bacteriol.* **23**, 191 (1973).
5. P. G. Markham, R. Townsend, M. Bar-Joseph, M. J. Daniels, A. Plaskitt, B. M. Meddins, *Ann. Appl. Biol.* **78**, 49 (1974).
6. T. A. Chen and C. H. Liao, *Science* **188**, 1015 (1975); D. L. Williamson and R. F. Whitcomb, *ibid.*, p. 1018.
7. C. Malogolowkin, *Genetics* **43**, 274 (1958); ——— and D. F. Poulson, *Science* **126**, 32 (1957); D. F. Poulson and B. Sakaguchi, *ibid.* **133**, 1489 (1961).
8. D. L. Williamson and R. F. Whitcomb, *Colloq. Inst. Natl. Sante Rech. Med. (Paris)* **33**, 283 (1974).
9. J. G. Tully, R. F. Whitcomb, D. L. Williamson, H. F. Clark, *Nature (London)* **259**, 117 (1976).
10. H. F. Clark, *J. Infect. Dis.* **114**, 476 (1964); *Progr. Med. Virol.* **18**, 307 (1974).
11. J. Hotchin, *Cur. Top. Microbiol. Immunol.* **40**, 33 (1967).
12. R. F. Zeigel and H. F. Clark, *Infect. Immun.* **9**, 435 (1974); J. W. Bastardo, D. Ou, R. H. Bussell, *ibid.*, p. 444.
13. L. Hayflick, *Texas Rep. Biol. Med.* **23** (suppl. 1), 285 (1965); M. F. Barile, *Colloq. Inst. Natl. Sante Rech. Med. (Paris)* **33**, 135 (1974); P. Whittlestone, *ibid.*, p. 143.
14. A. L. Jones, R. F. Whitcomb, D. L. Williamson, M. E. Coan, *Phytopathology*, in press.
15. Subcommittee on the Taxonomy of Mycoplasmatales, *Int. J. Syst. Bacteriol.* **22**, 184 (1972).
16. J. G. Tully, R. F. Whitcomb, J. M. Bové, P. Saglio, *Science* **182**, 827 (1973).
17. D. Taylor-Robinson, N. L. Somerson, H. C. Turner, R. M. Chanock, *J. Bacteriol.* **85**, 1261 (1963).
18. Suckling rats (Sprague-Dawley) were challenged intracerebrally on day 2 after birth with 0.03 ml of an inoculum of the GT-48 strain (triply cloned line at the 6th to the 22nd passage level) diluted in broth from 10^{-2} to 10^{-5}. Mortality, recorded at 4 to 5 weeks, for challenge dilutions 10^{-2} through 10^{-5} was 100, 83, 76, and 65 percent, respectively (N = 20, 59, 58, and 60, respectively).
19. E. G. Pickens, R. K. Gerloff, W. Burgdorfer, *J. Bacteriol.* **95**, 291 (1968); E. P. Brinton and W. Burgdorfer, *Int. J. Syst. Bacteriol.* **26**, 554 (1976).
20. We thank N. Ramsburg, D. Rose, C. Blood, and M. Coan for excellent technical assistance. We are grateful to L. Cline for the complement fixation tests.

10 September 1976; revised 26 October 1976

Vision in Insects: Pathways Possibly Underlying Neural Adaptation and Lateral Inhibition

Abstract. *Like horizontal cells in vertebrate retinas, horizontal amacrine cells beneath the insect eye intervene between receptors and interneurons at the first level of synapses. Synaptic arrangements between amacrines and interneurons that give rise to regular networks of axon collaterals may explain recent electrophysiological observations of lateral inhibition beneath the insect retina. Neural adaptation mechanisms acting on single retinotopic channels or assemblies of channels can also be referred to reciprocal relationships between receptors and first-order interneurons as well as to centrifugal cells from levels of so-called photopic receptor endings.*

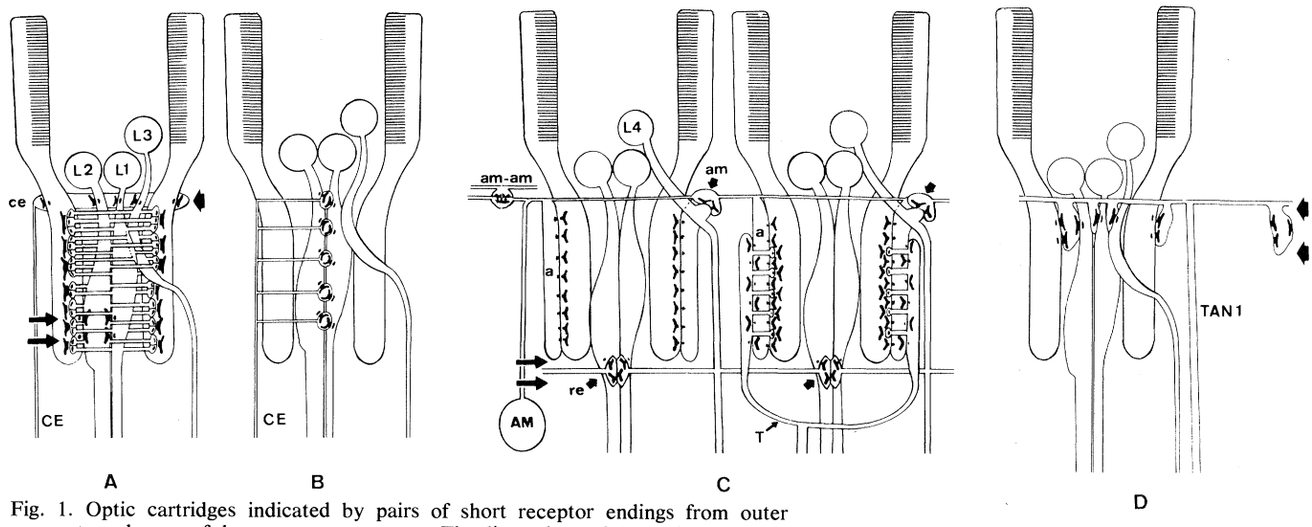
In arthropods, lateral inhibition and neural adaptation mechanisms have been observed at the level of visual receptors or their endings. In *Limulus*, contrast enhancement has been proposed to be mediated by pathways between ommatidia (*1, 2*), and, in insects, lateral inhibitory processes have been referred to axon collaterals of peripheral neurons in the lamina's external plexiform layer (EPL) (*3, 4*). Also, recordings from EPL-derived interneurons revealed light adaptation phenomena which are similar to those in their possible vertebrate ana-

logs, the bipolar cells (*5*). However, unlike investigators of the vertebrate retinas, insect visual physiologists have not had recourse to information about the whole spectrum of cell types and synaptic connections in the arthropod EPL.

We have used combined Golgi impregnations and electron microscopy of the EPL of the dipteran *Musca domestica*. Our previous studies resolved synaptic relationships between some centripetal cells and receptors and clarified controversies about the presence of anaxonal neurons at this peripheral level (*6–8*). However, only recently do we have knowledge about the dispositions and connectivities of neurons that provide information about the most feasible pathways for adaptation and lateral inhibition.

The cell components of the EPL (*6*) may be simply summarized; the description from the neural superposition eye of *Musca* can then be analogized to EPL's of diurnal apposition and superposition eyes of other insect orders (*9*). In flies, each retinotopic column ("optic cartridge") consists of a set of six short receptor endings (R1 to R6) derived from an asymmetric rhomboidal pattern distributed among six ommatidia (*10, 11*). The projections to a single cartridge share the same optical alignment, and the cyclic geometrical relationships of R1 through R6 endings is invariant across the planar neuropil (*10*). Golgi–electron microscope studies show EPL interneurons to have characteristic geometrical relationships to receptor placings (*6, 12*). Thus, morphological sampling of one cartridge is representative of any other.

There are 12 classes of neurons in the lamina, which are summarized in Fig. 1. Each is represented in every cartridge (*12*). The classes are (i) receptor endings, (ii) five forms of monopolar cells (L1 to L5) that originate from perikarya above the EPL, (iii) one form of T cell derived from above the second synaptic region, the medulla (*8*), (iv) four forms of centrifugal neurons derived from the medulla (*9*), and (v) one form of anaxonal neuron, intrinsic to the EPL (*8*). The L cells and T cells are centripetal elements that project to the outer strata of the medulla, a level that is topographically equivalent to the vertebrate inner plexiform layer. Two types of centrifugal cells (ce and CE) have narrow-field endings in the EPL, restricted to single cartridges. Two other centrifugals (TAN 1 and 2) have extensive telodendritic fields (*13*) that spread through assemblies of as many as 40 cartridges. The lamina contains about 500 amacrine cells distributed throughout 2800 cartridges. Amacrines are complex: each cell consists of horizontal processes that pass over the outer surface of the EPL and give rise to boutons and rosettelike specializations as well as bulbous alpha components that appose



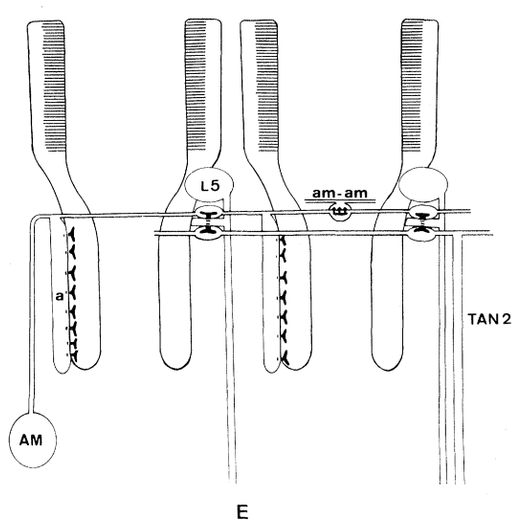


Fig. 1. Optic cartridges indicated by pairs of short receptor endings from outer segments and some of the component neurons. The dimensions of the optic cartridges are: diameter, 8 to 12 μm; length, 35 to 50 μm. (A) A cartridge with its triplet of spiny monopolar cells L1, L2, and L3 indicating the comb arrangement of their dendrites. In the outer third of the cartridge L1, L2, and L3 dendrites form triads opposite T-shaped presynaptic ribbons. Elsewhere L1 and L2 dendrites are arranged as dyads. The single arrow at the distal end of a cartridge indicates presynaptic relationships between one type of small field centrifugal *(CE)* onto L1, L2 and L3 and onto receptors above spiny monopolar dendrites *(ce)*. Paired arrows at the proximal end of a cartridge indicate the level of reciprocal synapses between L2 and receptors and onto L1. (B) L1, L2, and L3 monopolars (shown without their dendrites) and the postsynaptic relationships of L1 and L2, but not L3, to the second form of small centrifugal *(CE)*. (C) A simplification of complex interrelationships. The cardinal element is the amacrine cell *(AM)* which is postsynaptic to receptors via its alpha components *(a)* and presynaptic to other amacrines at *am-am* and presynaptic to L4 dendrites at *am*. The L4 collaterals are presynaptic to L1 and L2 proximally at the level indicated by double arrows. They are also presynaptic to each other *(re)*. The third form of centripetal cell, which derives input from receptors, is the T1 neuron, here shown in one cartridge only *(T)*. Its basketlike dendrites are postsynaptic to receptors via pinniform projections, postsynaptic to amacrine alpha processes *(a)* and reciprocally presynaptic to amacrines at *a*. (D) The simple relationships of one wide-field centrifugal. Here the type-1 tangential centrifugal endings *(TAN 1)* are presynaptic to L1, L2, and L3 and receptors of as many as 40 cartridges in its telodendritic domain, which is also at a level above the majority of SLM dendrites (double arrows). (E) The second form of wide-field centrifugal *(TAN 2)* presynaptic to *L5* midget monopolars. These cells are also postsynaptic to amacrines. The telodendria of TAN 2 neurons also extend through oval, vertically oriented domains of as many as 40 cartridges.

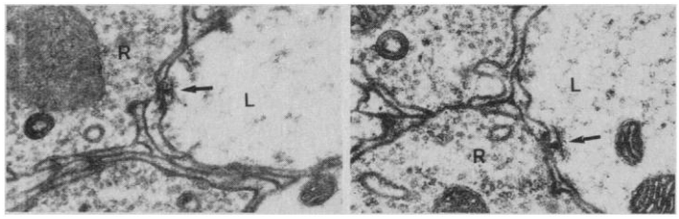


Fig. 2. A pair of electron micrographs showing presynaptic ribbons in the L2 neuron (*L*) opposite the receptor (*R*) membrane (× 25,560).

the whole length of receptor terminals. Each cartridge contains at least six alpha components derived from at least three amacrine cells (*8*).

We have mapped the synaptic relationships of lamina neurons from conventionally fixed material (*14*) after initially identifying (*6*) characteristically positioned profiles belonging to particular cell types by Golgi electron microscopy. These are also summarized in Fig. 1.

Synaptic relationships fall into two categories, intra- and extracartridge. Intracartridge relationships are either simple, as exemplified by the relationships of L3, or exceedingly complex, as are all intercartridge pathways. Only three of the five monopolar cells (spiny monopolars, SLM's) are postsynaptic to receptors. A pair (L1, L2), axial to the ring of six receptor endings, gives rise to 12 rows of 18 or more dendrites arranged as combs through the EPL. Each dendrite has between four and six swellings, which abut the receptor membranes of the six terminals, opposite T-shaped presynaptic ribbons. Dendrites of L1 and L2 are arranged as dyads (Fig. 1A) opposite single ribbons. A third type of centripetal cell, L3, has between six and eight dendrites, which also branch to all six receptor endings. There are three times more presynaptic sites on L1 and L2 than on L3. Where L1, L2, and L3 dendrites coincide they form triad arrangements opposite single T-shaped ribbons in receptors (Fig. 1A). This arrangement probably facilitates the summation of the receptor signal and improves the signal-to-noise ratio (S/N) at low light intensities (*11*). Because of the relative paucity of dendrites of L3, we suggest that it is a noisy cell with a low S/N. Synaptic relationships of L3 are also simpler than any other EPL neuron. With L1, L2, and receptors, L3 shares only one form of small-field centrifugal terminal (ce in Fig. 1A) above the level of its dendrites, and parts of the type-1 wide-field centrifugal terminals (TAN 1 in Fig. 1D).

The remainder of the EPL connections are complex. Cells L1 and L2 receive inputs from three centrifugals, including a narrow-field element that is exclusively presynaptic to L1 and L2 at the level of their dendrites (CE in Fig. 1B). In addition, the L2 neuron is reciprocally presynaptic to receptors near the base of a cartridge (Fig. 2) and presynaptic to its mimetic partner, L1 (double arrows, Fig. 1A). This relationship may have important consequences for neural adaptation, working on a single retinotopic channel (*5*), since narrow-field centrifugals usually have dendritic spreads in the medulla that pass to several columns and reside at the level of so-called photopic receptor endings, the long visual fibers (*6, 9*). Intracellular recordings of L3 cells, using Procion yellow, is feasible in Diptera (*3*). If some adaptation phenomena are shared by L1 and L2 but not by L3 it may indicate that reciprocal monopolar-receptor synapses contribute, at least in part, to intrachannel adaptation (*15, 16*). However, experiments must be designed with respect to both the intensity ranges of short receptor endings [across at least 4 log units (*5*)] and their spectral sensitivities, both of which are known to differ from those of long visual fibers (*17*). We suggest that, at high light intensities, small field centrifugals and one type of large field centrifugal may relay information from long visual "photopic" receptor axons to, respectively, the receptor elements and SLM's of single cartridges, and to assemblies of cartridges.

Our Golgi–electron microscope studies of lateral connections reveal three major substrates, in which EPL amacrine cells have a cardinal position. These are (i) inputs to L1 and L2, via L4 monopolar cell collaterals, from receptor assemblies via amacrines (Fig. 1C); (ii) inputs to T1 centripetal cells, via amacrines, from receptor assemblies (Fig. 1C); and (iii) inputs to the midget monopolar cells (L5), via amacrines and the type-2 centrifugal (TAN 2), from assemblies of short receptors and, possibly, long photopic receptors, respectively (Fig. 1E).

The L4 collaterals form a regular network beneath the inner face of the lamina (*7*): each L4 cell is presynaptic to L1 and L2 of its own and two neighboring cartridges as well as pre- and postsynaptic to other L4's within these same cartridges. Amacrines of the EPL are postsynaptic to receptors, via their alpha processes, and presynaptic to L4 dendrites; since at least three wide-field amacrines converge to each cartridge (*8*), input to L4 cells and hence to L1 and L2 may be derived from a very large field. Also, like horizontal cells of vertebrates, EPL amacrines are presynaptic to one another.

Lateral inhibition of L1 and L2 neurons has been detected in dragonflies (*16*) and in flies (*18*) by investigators using off-angle illumination or slit stimuli (*4*) whereby the receptive field of SLM cells is narrower than receptors and the plateau of their response diminishes or is abolished depending on the angular divergence of the light source. The second lateral pathway, to T1, involves only amacrines. Structurally, T1 cells have six climbing fibers that ascend the six receptor endings of a cartridge and are postsynaptic to them. The T1 fibers are also postsynaptic to amacrines, and some reciprocal synapses have been seen between T1 and alpha processes (Fig. 1D) (*8*). Recordings from T1, with Procion yellow identification, illustrated that the receptive field of this centripetal cell was even narrower than those of L1 and L2 neurons (*3*).

From the structure of the EPL, we suggest that its amacrine cells play the central role in lateral inhibition; they may also serve as receptor pools for neural adaptation phenomena by working on constellations of optic cartridges. Recent electrophysiological studies of the dipteran *Calliphora stygia* by Mimura (*4*) support this hypothesis. He has described a neuron whose complex receptive field is composed of small fields that apparently correspond to many single cartridges. The entire receptive field of the neuron is star-shaped with spokes radiating along four axes: vertical, horizontal, and two oblique. Amacrine cells are the only laminar neurons whose processes are mapped in this way, whereas L4 collaterals are arranged along rectilinear coordinates, 45° to the vertical and horizontal axes. Thus we suggest that large fields of the kind described were obtained from the amacrine input to an L4 neuron and that, together, amacrines and L4 perform adaptative and inhibitory functions analogous to those of the horizontal cells of the vertebrate retina.

Mention should be made of the last class of lateral pathways, namely, onto the minute dendrites of L5, via amacrines and the wide-field telodendria of type-2 centrifugals. These relationships (Fig. 1E) imply that L5 neurons may receive wide-field information from both short receptors, via amacrines, and long visual receptors, via a type of interplexiform neuron (TAN 2). Electron microscopy of Golgi-impregnated terminals

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*).

N. J. STRAUSFELD
European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany
J. A. CAMPOS-ORTEGA
Institut für Biologie III, University of Freiburg, Freiburg, Federal Republic of Germany

References and Notes

1. H. K. Hartline and F. Ratliff, *J. Gen. Physiol. (London)* **235**, 133 (1956).
2. W. H. Miller, cited by F. Ratliff, H. K. Hartline, D. Lange, in *The Functional Organization of the Compound Eye*, C. G. Bernhard, Ed. (Pergamon, London, 1965), p. 399; R. Whitehead and R. L. Purple, *Vision Res.* **10**, 129 (1970); M. Gur, R. L. Purple, R. Whitehead, *J. Gen. Physiol.* **59**, 285 (1972).
3. M. Järvilheto and F. Zettler, *Z. Zellforsch.* **136**, 291 (1973).
4. K. Mimura, *J. Comp. Physiol.* **105**, 65 (1976).
5. S. B. Laughlin, *Z. Naturforsch.* **30**, 306 (1975); *J. Comp. Physiol.* **84**, 335 (1973).
6. N. J. Strausfeld and J. A. Campos-Ortega, in *Information Processing in the Visual System of Arthropods*, R. Wehner, Ed. (Springer-Verlag, Berlin, 1972), p. 24: J. A. Campos-Ortega and N. J. Strausfeld, *Z. Zellforsch.* **124**, 561 (1972).
7. N. J. Strausfeld and J. A. Campos-Ortega. *Brain Res.* **59**, 97 (1973).
8. J. A. Campos-Ortega and N. J. Strausfeld, *ibid.*, p. 119.
9. N. J. Strausfeld, *Atlas of an Insect Brain* (Springer-Verlag, Berlin, 1976), pp. 134–138; *Philos. Trans. R. Soc. London Ser. B* **258**, 144 and 156 (1970).
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)].
11. K. Kirschfeld, *ibid.*, p. 248. *Fortschr. Zool.* **21**, 229 (1973).
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.
16. S. B. Laughlin, *J. Comp. Physiol.* **92**, 377 (1974).
17. H. Eckert, *Kybernetik* **9**, 145 (1971).
18. F. Zettler and M. Järvilheto, *Z. Vergl. Physiol.* **76**, 233 (1972).
19. This material has been partly summarized by N. J. Strausfeld, in *Neural Principles in Vision*, F. Zettler and R. Weiler, Eds. (Springer-Verlag, Berlin, 1976), p. 245.
20. We thank Dr. K. Hausen, Sir J. C. Kendrew F.R.S., and Dr. S. B. Laughlin for critical reading of the manuscript. We acknowledge the support of the Max-Planck-Institut für Biologische Kybernetik, Tübingen, where much of this research was performed.

26 July 1976; revised 9 November 1976

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 2.5×10^5 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 2.5×10^5 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. †$P < .001$.