stance may play a regulatory role in initiating or inhibiting spermatogenesis. In the rat, germ cells of the seminiferous tubules are not all at the same stage of spermatogenesis. The heterogeneous staining we observed might reflect the various stages of the cycle of the seminiferous epithelium encountered in random histological sections through the seminiferous epithelium.

It is unlikely that the LHRH-like substance is synthesized in the nuclei of the spermatogonia. The mode of action of this substance could be similar to that of androgens, which are also produced in the interstitial cells. In this paradigm, the LHRH-like substance might be synthesized in the Leydig cells and transported by diffusion, or perhaps in conjunction with a carrier molecule, to the seminiferous tubules. Upon gaining access to the germ cells it may be translocated to the nucleus. The LHRH-like substance could then affect the mitotic rate of the cell in a fashion similar to that of steroids.

The concept of a peptide gaining access to the nuclear compartment is new. Marchisio *et al.* (*11*) have recently demonstrated by immunofluorescence and autoradiographic methods that nerve growth factor can be localized within the nuclei of pheochromocytoma cells. These authors suggest that nerve growth factor may serve to form or modulate nucleation sites for pools of tubulin and actin. The testicular LHRH-like compound might serve a similar function in initiating spermatogenesis within the testes.

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Fluorescence of Photoreceptor Cells Observed in vivo

Abstract. *Most rhabdomeres in the eye of the fly* (Musca domestica) *are fluorescent. One kind of fluorescent emission emanates from a photoproduct of the visual pigment, other kinds may be ascribed to photostable pigments. These phenomena provide not only a means of spectrally mapping the retina but also a new spectroscopic tool for analyzing the primary visual processes in vivo.*

Fluorescence is not the most salient property to be expected from a visual pigment. Rather than waste excitation energy in such a "trivial" process, molecules of visual pigment would be expected to have a high efficiency for photoisomerization, as they have (*1*). When rhodopsin fluorescence was reported (*2*), it was found to have a low quantum efficiency of less than 1 percent. These considerations may explain why fluorescence methods, despite their selectivity, have not been used extensively for studying the primary steps in the visual process. But now more than a century has passed since Helmholtz first reported fluorescence of the vertebrate retina and subsequent studies have ascribed the various fluorescence colors, in part, to intermediates of visual pigment bleaching (*3*).

Using a technique of ommatidial fundus fluoroscopy applied to an intact animal, we show that retinula cells of flies may exhibit various fluorescence colors closely related to the properties both of visual pigments and of the recently discovered accessory photostable pigments contained in the rhabdomeres.

In the compound eye of diurnal insects, the receptor cells are separated from the outside world by transparent components (crystalline cone and cornea) whose total thickness rarely exceeds 0.1 mm. Taking advantage of this situation, which is encountered in no vertebrate eye, we recently devised several techniques for studying photoreceptor cell processes in live animals (*4*, *5*). One of these techniques consists of covering the "waffled" corneal surface with a medium such as nail polish, immersion oil, or even water, whose refractive index approximately matches that of chitin. Optically neutralized in this way, each corneal lenslet becomes a porthole behind which the seven receptor endings of a retinula can be viewed with a microscope (*4*).

We have combined this technique with epifluorescence microscopy (Fig. 1B), using excitations at various wavelengths within the spectral range relevant to fly vision. The basic observations we made under steady-state conditions are illustrated in Fig. 2 and summarized in Table 1. (For the numbering of receptor cells in a fly retinula, see Fig. 1F.)

When excited by blue light (400 to 500 nm), all rhabdomeres R1 to R6 of *Musca domestica* (white-eye) emit red light (Fig. 2A), the emission maximum of which ($\lambda > 620$ nm) was estimated by substituting a pupil spectroscope (Zeiss) for the microscope eyepiece. In contrast, the distal tip of the central rhabdomere R7 may exhibit three different colors: green, black (no fluorescence), or red, depending on the ommatidium.

Under ultraviolet (UV) excitation (300 to 400 nm) all R1 to R6 exhibit a pinkish color (Fig. 2B). By contrast, the three types of R7 and R8 exhibit the following colors: black, black, and pink, respectively (Table 1) (*6*).

To ensure that the emission arose from the rhabdomeres, we cut the eye with a vibrating razor blade and examined the eye stump and eye slice (Fig. 1, C to E). In all cases the characteristic red color of R1 to R6 as well as the green color of some R7's could still be observed under blue excitation. Hence, we conclude that the rhabdomere itself is an extended fluorescent light source, which, through its light-piping property, channels part of the emitted light up to the microscope.

We then examined the retinas of flies deprived of vitamin A obtained by rearing their larvae on a β-carotene and vitamin A–free Sang's synthetic medium (*7*). The fluorescence of all rhabdomeres appeared to be reduced to very low levels, suggesting that the colors observed emanate from the visual pigment, β-carotene, or vitamin A photostable derivatives, or a combination thereof.

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Incorporating recent knowledge about the pigment content of the fly retina (*8, 9*), we will try to identify the fluorescing molecules. Rhabdomeres R1 to R6 contain a photosensitive pigment (P) rhodopsin P_{490}, which is in dynamic equilibrium with its photoproduct metarhodopsin M_{580} (*8*). They also contain an ultraviolet sensitizing pigment X, which increases the absolute sensitivity of cells 1 to 6 by making them panchromatic (*9*).

The red emission from R1 to R6 shows four properties that may suggest from which of substances P, M, or X it emanates. (i) It is not seen at first (in contrast to the green emission from R7) and requires that the retina initially be exposed to blue light; (ii) it decreases in intensity under orange excitation and is recovered by subsequent blue excitation (Fig. 1, G and H); (iii) it is approximately 20 times more intense during an orange flash (577 nm) than during a blue flash (436 nm) of equal duration (40 msec) and equal quantal content; and (iv) it is still present after 1 hour of dark adaptation following blue adaptation.

All four properties suggest that the red-fluorescing substance is M. The concentration of M increases during exposure to blue and decreases during exposure to orange (*8*). Moreover, M absorbs nearly 20 times as much at 577 nm than at 436 nm. [A factor of 17 is predicted from the most recent absorption spectrum determined by Schwemer (*10*).] Finally, M is quasi-stable in the dark (*8*). In view of their absorption spectra (*8–10*), neither P nor X could emit more intensively under orange than under blue excitation.

Table 1. Fluorescence colors exhibited in vivo by the various rhabdomere types under blue and ultraviolet (UV) excitation (Fig. 2, A and B).

Rhabdomere type	Excitation	
	Blue	UV
1 to 6	Red	Pink
7 and 8	Green	Black
	Black	Black
	Red	Pink

Stark *et al.* (*11*), however, could not detect increased fluorescence during a monitored conversion of P to M in *Drosophila*. By combining in vivo transmission and epifluorescence microscopy on the deep pseudopupil (*5*) of *Musca*, we found that the time course of fluorescence increase under exposure to blue was much slower than the time course of metarhodopsin formation. Although our continuous excitation by blue led to a new equilibrium between P and M within less than 0.1 second, it took at least 100 seconds for the red emission to reach a steady state (Fig. 1H). This conspicuous difference in time scale is evidence that the red emission is not strictly related to M as we know it. We therefore assume that it emanates from a special fluorescing form of metarhodopsin, M′. Different kinds of M have already been proposed in a recent model of pigment states (*12*), and fluorescence may provide a means of discriminating among them.

The pink color of R1 to R6 under UV excitation (Fig. 2B) could result from two types of emission. The red fluorescence of M′ would now be superimposed on a whitish, UV-excited fluorescence of X. Pigment X sensitizes not only P but also M (*13*) and possibly M′, whose red emission could then be induced indirectly by energy transfer from X to M′. [Compare with the "sensitized fluorescence" of chlorophyll a when the accessory carotenoids or phycobilins are excited (*14*).]

Let us now consider the central receptor cells R7 and R8 (Fig. 1F). Approximately 70 percent of the R7's in the fly retina (the so-called R7 yellow, or R7y) contain, in addition to the visual pigment, a blue-absorbing photostable pigment (*15*), which acts as a screen and modifies the spectral sensitivity of receptor cells R7 and R8 (*15–17*). We have examined eye slices (cut at such a depth that R8 was certainly absent) with both

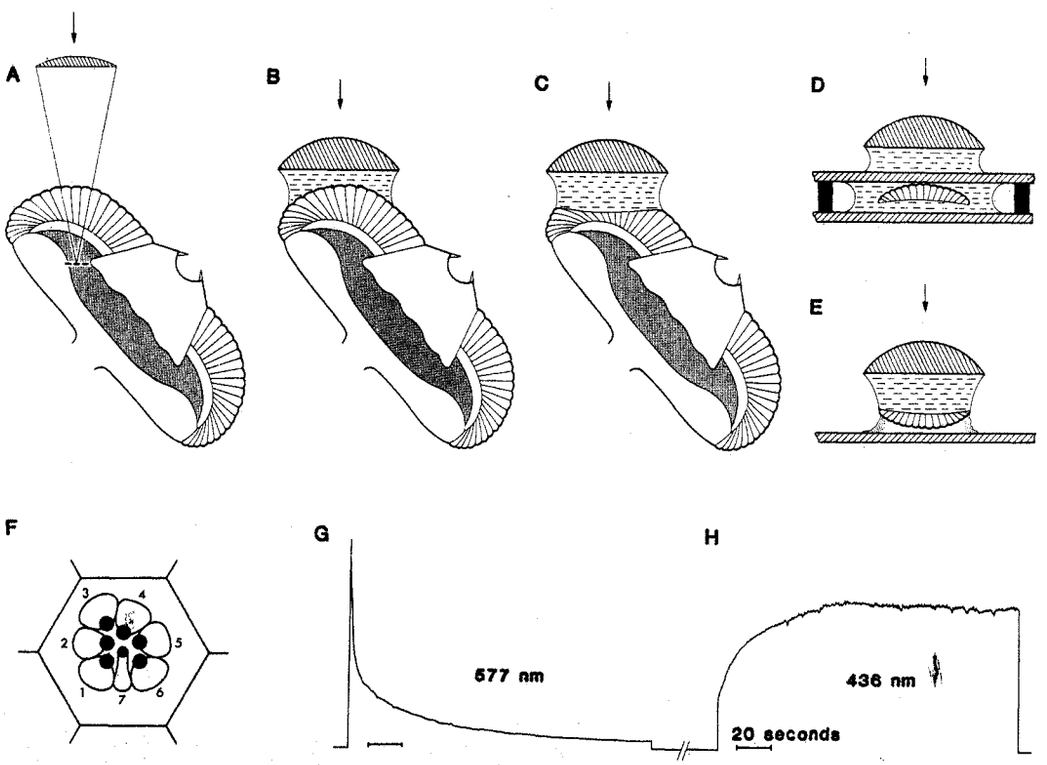

Fig. 1. (A to E) Observation of the retina with epifluorescence microscopy. The lens above the eye represents the microscope objective. (A) Method of the deep pseudopupil (virtual image of the retina in the depth of the eye) (*5*). (B) Method of optical neutralization of the cornea (*4*). (C to E) Successive observations of eye stump and eye slice with water immersion, after cutting the retina. (F) Arrangement and numbering of the seven photoreceptor cells in a fly ommatidium. The rhabdomeres are hatched to indicate the direction of the microvilli. Cell 8 is not seen in this distal section. Its rhabdomere R8 is in the proximal prolongation of R7. (G and H) In vivo recording of the change in red light emission (measured at $\lambda > 610$ nm) of rhabdomeres R1 to R6 during orange (577 nm) and blue (436 nm) excitation, by the deep pseudopupil method (objective lens, ×6; numerical aperture, 0.18). Orange and blue lights do not have the same quantal content here, and photomultiplier sensitivity was increased between (G) and (H). The fast rise of the signal at the onset of the blue excitation (H) is due to the relatively strong greenish (but broadband) fluorescence of the cornea. The two records are broken by 80 seconds of darkness.

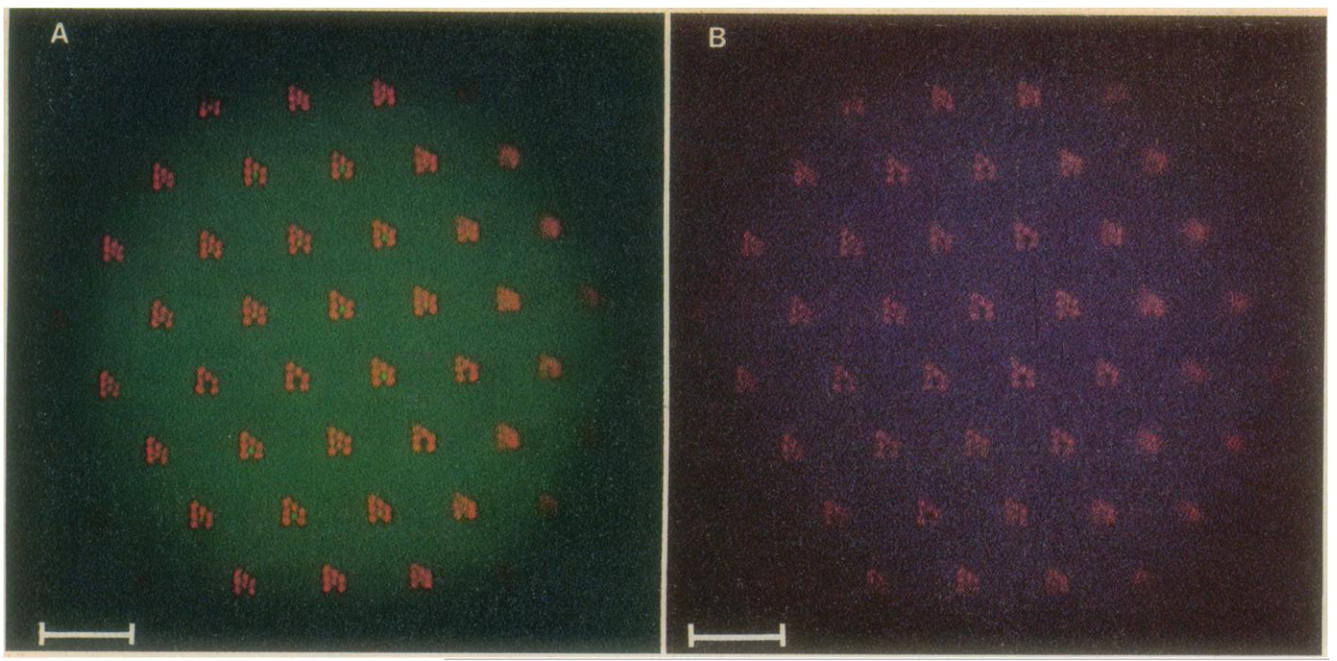

Fig. 2. In vivo observation of the light emitted by single rhabdomeres of the fly retina under blue (A) and ultraviolet (B) excitation. Only the rhabdomeres emit light; the cell bodies do not (Fig. 1F). Technique of optical neutralization of the cornea (Fig. 1B) used with water immersion (objective, ×25; numerical aperture, 0.65). An aperture diaphragm placed in the microscope viewing tube passed selectively the (directionally radiated) light of the rhabdomeres while filtering out the strong (isotropic) autofluorescence of the cornea. The homogeneity of the R1 to R6 population, which all emit red (A) or pink (B) light, contrasts with the diversity of R7 and R8, which appear either green, black, or red, under blue excitation (A). The retina is usually shared by 70 percent green-fluorescing R7 and R8 and 30 percent nonfluorescing ones. The red-fluorescing R7 and R8 are encountered in the dorsal part of the male eye exclusively (*20*). These red R7 and R8 [which are numerous in (A)] are the only ones that fluoresce under ultraviolet excitation (B). A white-eyed fly has been used here to permit a longer exposure. Similar phenomena are seen in the wild-type fly, but their observation is complicated by the pigment migration (*5*) which drastically attenuates the excitation light within a few seconds. Kodak Ektachrome ASA 160 film exposed 15 seconds (A) and 8 seconds (B), developed as ASA 400. Scale bars, 30 μm.

fluorescence and transmission microscopy (Fig. 1D) and found that green-fluorescing R7 absorbed strongly in the blue, whereas nonfluorescing R7 did not absorb blue light conspicuously. We therefore conclude that the green fluorescence emanates from R7y rhabdomeres. Whether it stems from their peculiar M (*18*) or from their blue-absorbing photostable pigment [which we have tentatively identified as β-carotene (*15*)] is uncertain. The relative stability of the green emission would support the latter hypothesis. Although β-carotene fluorescence is hardly detectable in vitro (*19*), the microvillar membrane may provide a suitable milieu allowing fluorescence emission.

The third class of R7 and R8 exhibits the same fluorescence colors as R1 to R6 (red under blue excitation, pink under UV excitation) (Fig. 2), as if they had the same pigment system. A combined study incorporating fluoroscopy, microspectrophotometry, electron microscopy, and intracellular recordings has shown that the red-fluorescing R7's are virtually indistinguishable from their six neighbors in the ommatidium (*20*).

Rhabdomeres of other Diptera (*Drosophila, Calliphora, Sarcophaga,* and *Eristalis*) exhibit similar fluorescence phenomena, including the greenish emission by some of their R7's. We have also observed a reddish emission under blue excitation from the fused rhabdom of many insects (bee, wasp, locust, butterfly, and mantis); the phenomenon may reveal a general property of insect or invertebrate visual pigments.

Intracellular recordings in *Musca* and *Calliphora* have shown that the spectral sensitivity of a cell is correlated with the autofluorescence of its rhabdomere (*17*). Both green-fluorescing and nonfluorescing R7's seem to be UV receptors, the green-fluorescing ones having, in addition, a tail of sensitivity over the blue part of the spectrum. Each fluorescence color appears as a natural color tag, which can henceforth be used reliably to map out the various spectral types of the retina in vivo. Such retinal mappings have already disclosed a unique example of sex-specific retinal organization (*20*): only male *Musca domestica* are equipped with the red-fluorescing type of R7.

The combined observations demonstrate that the characteristic red fluorescence exhibited by the great majority of fly photoreceptor cells is somehow related to their metarhodopsin. Fly rhodopsin does not fluoresce detectably over the "visible" spectral region. Though contrasting with the observation of fluorescence from vertebrate rod outer segments (*2*), this result may reflect the fact that retinal itself does not fluoresce in the 11-*cis* form whereas it does in the *trans* form (*21*).

As demonstrated by the antagonistic changes in the intensity of red emission under orange and blue excitation (Fig. 1, G and H), analysis of fluorescence emission in the live animal provides a new spectroscopic tool for dissecting the primary processes of visual transduction. Even though fluorescence emission may represent but a spillover of excitation energy, it may shed a new light on the conformational changes of various molecules involved in the generation of the bioelectrical signal.

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Autoantibodies from Vasectomized Guinea Pigs Inhibit Fertilization in vitro

Abstract. *Immunoglobulin G and Fab antibodies were isolated from the serum of vasectomized guinea pigs, and the effects of the antibodies on fertilization in vitro were investigated. These antibodies had profound inhibitory effects on (i) sperm-to-sperm adhesion, (ii) the acrosome reaction, (iii) sperm-zona binding, and (iv) sperm-ovum fusion. This finding may explain certain cases of infertility after vasovasostomy in men.*

Because vasectomy is a widely accepted option of fertility control, it is imperative to assess whether vasectomy results in long-term detrimental effects and whether production of antibodies to sperm attendant to vasectomy (*1*) may prevent reversal of fertility control through vasovasostomy.

Davis (*2*) reported a high incidence of infertility in vasovasostomized men. The hypothesis that some cases of infertility have an immunological basis is plausible because vasectomy results in a high incidence of circulating autoantibody directed against sperm (*1*), and infertility after vasovasostomy is correlated with sperm agglutinins in serum (*3*) and semen (*4*). Infertility in vasovasostomized rhesus monkeys is also correlated with a high titer of circulating antibody to sperm (*5*). This general appraisal led us to investigate whether autoantibody to sperm produced after vasectomy has biological effects on fertilization. We used the guinea pig as an experimental model for the following reasons. First, guinea pigs are widely used to study sperm autoimmunity (*6*), including its genetic basis (*7*). Second, conventionally prepared autoantibody to guinea pig sperm inhibits the sperm acrosome reaction (*8*) and fertilization in vitro (*9*). Finally, owing to the extraordinarily large size of the acrosome, we can readily distinguish acrosome-intact from acrosome-reacted spermatozoa by using phase contrast microscopy (*10*); this enables us to determine whether the observed effects of antibodies are due to interference with the acrosome reaction itself or with events preceding or following the acrosome reaction.

Bivalent immunoglobulin G (V-IgG) and univalent Fab fragments (V-Fab) were prepared (*11*) from the serum of strain 13 guinea pigs that had been bilaterally vasectomized 9 to 13 months earlier and that showed high levels of antibodies to surface antigens of guinea pig spermatozoa and spermatids (*7*). Animals with sham vasectomies served as the source of control reagents (SV-IgG and SV-Fab, respectively). All reagents were dialyzed against and stored in potassium-containing minimal capacitation medium (K-MCM) (*12*) at 10 mg/ml. We designed experiments to assess the effects of V-IgG and Fab on (i) sperm capacitation and (ii) sperm-ovum interactions in vitro.

Mammalian spermatozoa must reside for a time in the female reproductive tract before they become competent to fertilize ova (*13, 14*). Austin (*13*) termed this process capacitation. It is possible to capacitate spermatozoa from the guinea pig cauda epididymis by incubating them in defined media, such as K-MCM (*8*). Initially, the majority of spermatozoa are in "rouleaux," in which eight to ten cells adhere in orderly stacked arrays (*10, 15*); the rest are single cells ($\leq$5 percent) or doublets (10 to 20 percent), in which two cells are attached like those in a rouleau. Within 5 minutes in K-MCM, sperm rouleaux spontaneously agglutinate in a head-to-head fashion; within 1 hour, more than 95 percent of the entire motile sperm population joins into large clusters of spermatozoa (*8*).

In V-IgG (5 mg/ml), agglutination of rouleaux was largely inhibited, over 80 percent of the spermatozoa continuing to swim as individual rouleaux, singlets, or doublets; SV-IgG had no inhibitory effect (Fig. 1A). It is not clear why V-IgG did not augment agglutination of rouleaux, but similar observations have been made with a conventionally prepared autoantibody to guinea pig sperm (*8*). Additionally, an antibody directed against discoidin, a cell adhesion molecule in the slime mold, does not agglutinate target cells in adhesion assays (*16*).

Treatment of spermatozoa with V-Fab (5 mg/ml) immediately dispersed all sperm rouleaux to single cells (approximately 25 to 30 percent) or to doublets (70 to 75 percent) (Fig. 1B). The V-Fab effect was half-maximal at 1 mg/ml and absent at 0.1 mg/ml (data not shown). Control SV-Fab did not perturb normal rouleau agglutination. The V-IgG and V-Fab results indicate that serum from vasectomized guinea pigs reacts with surface determinants involved in homotypic sperm adhesion.

Under ordinary conditions in vitro, spermatozoa that have undergone acrosome reactions detach from stacked or agglutinated configurations and swim individually with "activated" motility (*10, 17*). Spermatozoa suspended in K-MCM containing V-IgG (5 mg/ml) did not un-

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