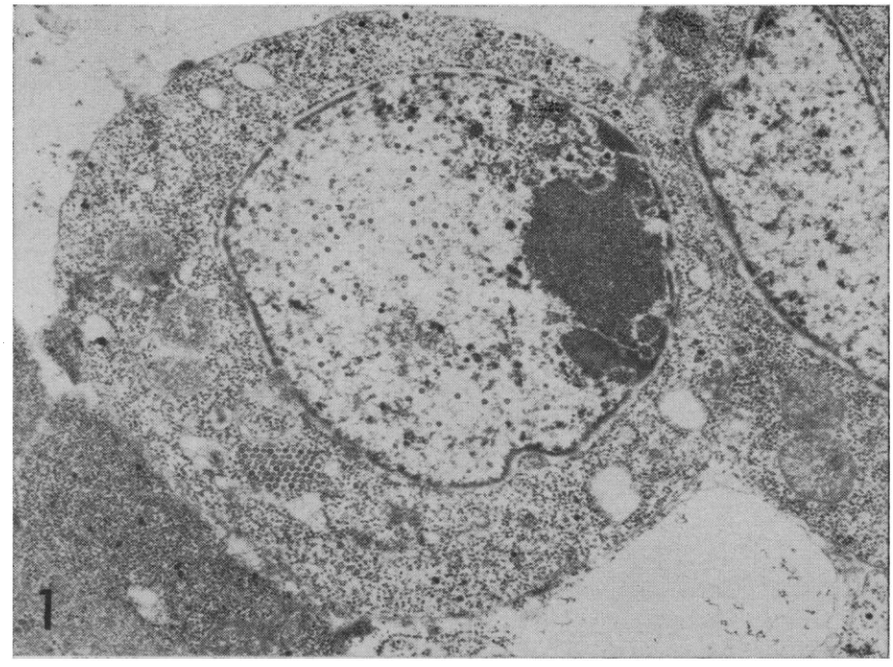
Virus-Like Particles in Normal and Tumorous Tissues of Drosophila

Abstract. *A new virus-like particle has been found in* Drosophila. *Thus far it has been detected only in electron micrographs of certain cells capable of division, such as those in larval imaginal disks, cell lines derived from imaginal disks, cells from a genetically controlled brain tumor, and adult gut cells. It appears to be slightly elliptical in shape, about 37 millimicrons by 45 millimicrons, and occurs in both the nucleus and cytoplasm. From the evidence it is suggested that the particle is a new virus.*

With the exception of the so-called sigma virus which causes sensitivity to carbon dioxide (*1*), viruses in *Drosophila* are virtually unknown. Our report describes a virus-like particle uncovered during electron microscopical studies of cell cultures derived from imaginal disks and from a genetically controlled brain tumor in *Drosophila melanogaster*. The imaginal disks, as well as the mutant strain bearing the brain tumor, were derived from a stock of *Drosophila melanogaster* cultured for many years in the laboratory.

For electron microscopic examination, fragments of different tissues were isolated and fixed in cold 6.25 percent glutaraldehyde buffered at *p*H 7.3 with 0.1*M* sodium cacodylate (*2*). The tissues were washed in several changes of buffered sucrose solution (*p*H 7.3) at 0° to 4°C and then treated with 1 percent osmium tetroxide (*p*H 7.3) containing sucrose (*3*). After fixation, the tissue was dehydrated in graded concentrations of ethanol and embedded in Maraglas (*4*). Sections were cut with glass knives on a Porter-Blum MT-2 or Cambridge ultramicrotome and were mounted on grids coated with a film of Formvar and reinforced with carbon. The sections were stained for 15 to 35 minutes in uranyl acetate (*5*) and then for 15 to 30 minutes in lead citrate (*6*). They were examined in an RCA-EMU 3H electron microscope.

The first virus-like particles were detected in cultures of *Drosophila* eye-antennal imaginal disks that had been propagated in vivo for nearly 1 year by the method of Hadorn (*7*). In this method, fragments of imaginal disks of late third-stage larvae are cultured for 2 weeks in the abdomens of young fertilized adult females, where they grow to considerable size but do not differentiate. To propagate a cell line derived from a disk or a disk fragment, a part



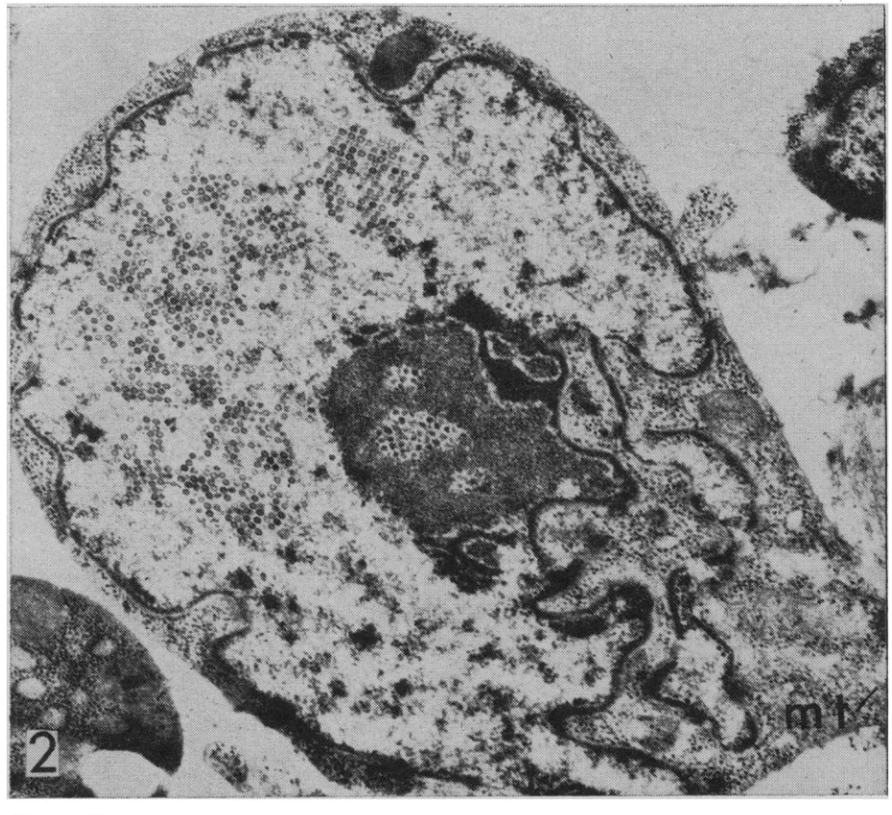
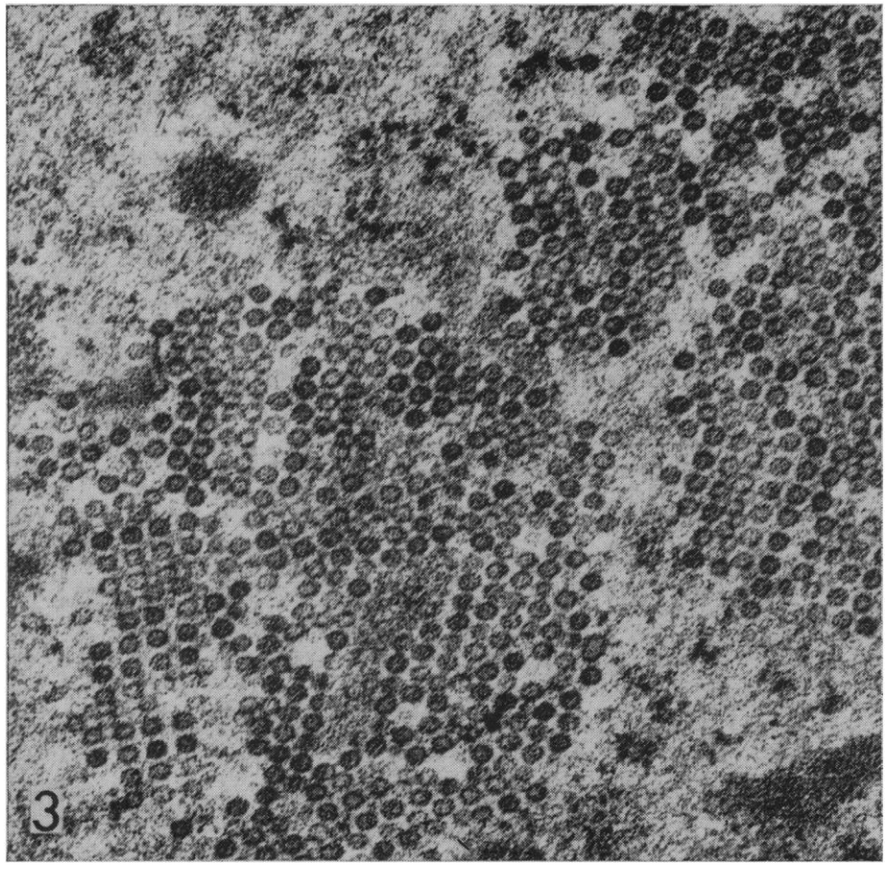
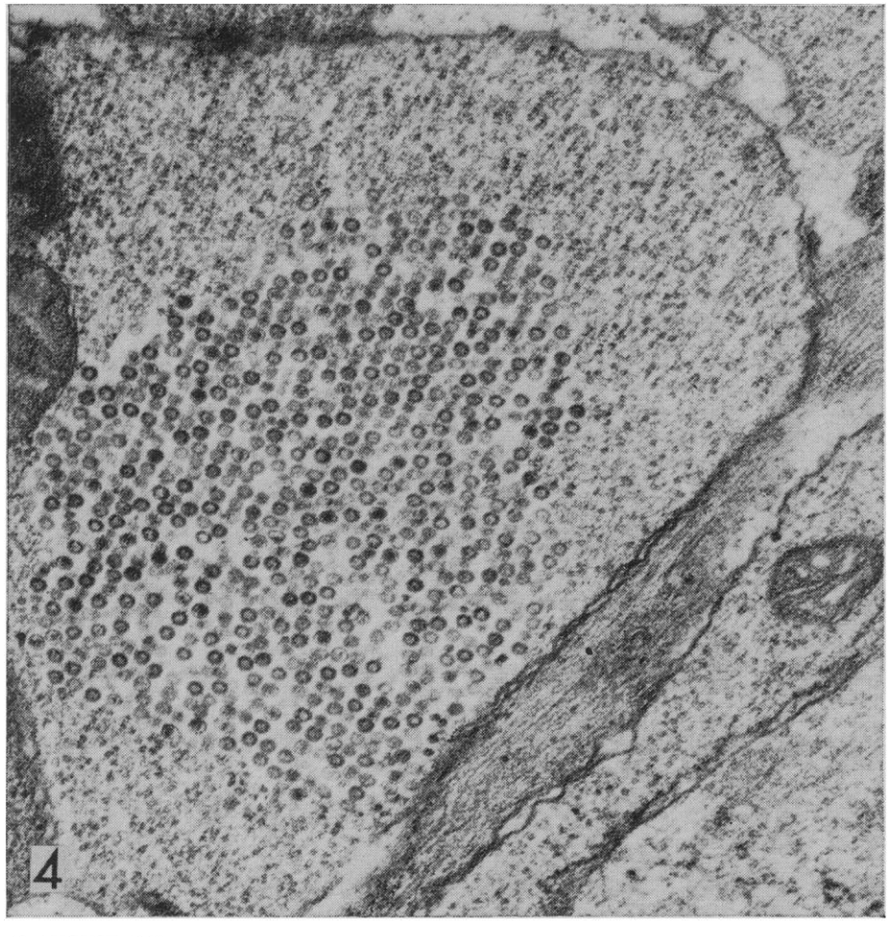


Fig. 1. Electron micrograph of a cell from the periphery of a line "C" implant in the 30th transfer generation. The nucleus contains scattered virus-like particles of different electron opacities. The cytoplasm contains a small cluster of regularly arranged virus-like particles. Conditions for staining and embedding are as described in text (× 22,000). Fig. 2. Electron micrograph of a cell from the periphery of a line "C" implant in the 30th transfer generation. The nucleus contains large clusters of virus-like particles in which some of the particles are irregularly distributed whereas others are arranged in well-oriented patterns. Microtubules (mt) of much smaller diameter are evident (× 22,000). Fig. 3. Electron micrograph showing part of the nucleus of an adult gut cell containing a large number of virus-like particles arranged in a fairly regular pattern. The individual particles seem to contain subunits (× 64,000). Fig. 4. Electron micrograph of *Drosophila* brain tumor cells showing large numbers of virus-like particles of both high and low electron opacity in the cytoplasm. The tumor tissue had been cultured for seven generations in the abdomens of adult female flies (× 48,000).





of the regenerated fragment—the stem piece—is reinjected into a female adult host to continue the line. To determine the developmental capacities of the line, another part—the test piece—is implanted into an 80-hour-old larva which is allowed to metamorphose and develop into an adult. When the adult emerges and is dissected one finds inside it the implanted test piece, now metamorphosed into a fragment of the integument of an adult fly. Thus, the metamorphosed test pieces disclose for each culture generation the capacities for differentiation of cells of the regenerated tissue at that time.

The virus-like particle was detected first in two sublines, both derived from half of an eye-antennal imaginal disk, which for convenience we have called line "B" and line "C" (Figs. 1 and 2) (*8*). Line "B" arose in the eighth transfer generation. It was characterized by extremely rapid growth and also by the fact that it differentiated not only autotypically into eye-antennal structures but also allotypically into foreign structures, such as anal plates. This change, which Hadorn calls "transdetermination" (*7*), was stable and has been inherited for more than 40 transfer generations.

Line "C" also arose in the eighth transfer generation from the same disk fragment. It exhibited extremely rapid growth (60-fold increase in 3½ days), but it had lost the capacity to differentiate. It has also been cultured for more than 40 transfer generations. Because of its rapid growth we routinely culture it in the abdomens of *Drosophila virilis*. This species is considerably larger than *D. melanogaster*, and its size permits us to prolong the transfer generation time of the implant from 1 week to 1 month, without reducing its growth.

The virus-like particles were subsequently detected in a spontaneous, malignant tumor of genetic origin and in cell lines derived from it. The mutant strain bearing this tumor arose spontaneously in our laboratory cultures. It is characterized by a tremendous proliferation and loss of organized growth of all cell types in the larval brain and all imaginal disks including the genital disk, which is located at the posterior end of the larva far away from the other disks (*9*). Tissue fragments derived from the tumorous brain of this mutant grew at a tremendous rate when cultured in adult abdomens, destroyed most of the adult organs, and usually killed the host within a week. The virus-like particles were evident in small num-

Table 1. Distribution of virus-like particles in *Drosophila melanogaster*. Key: 0, no virus; +, free particles; ++, small clusters of particles; +++, large clusters of particles.

Tissue	Generation (No.)	Nucleus	Cytoplasm	Approximate infected cells (%)
Normal larval brain				
	0	0	0	0
Normal imaginal disks				
	0	+	0	1
Normal adult				
Gut cells	0	+++	+	1–10
Blood cells	0	+++	?	10–30
Cell lines derived from normal imaginal disks				
"B"-line tissue	24	+++	+++	10–40
"B"-line tissue	34	+	0	10–40
"C"-line tissue	29	++	0	25–60
"C"-line tissue	30	+++	+++	25–60
Cell lines derived from mutant				
Larval brain tumor	0	+	0	5–20
Larval brain tumor	1	+	0	5–20
Larval brain tumor	2	0	0	0
Larval brain tumor	7	+++	++	5–20

bers in electron micrographs of the original brain tumor and in the second transfer generation. In the third transfer generation no particles were detected, but they were again detected in large numbers in the seventh transfer generation (Table 1). In this tissue, and in the "B" and "C" tissues mentioned above, the number of particles and the number of cells infected seemed to increase the longer the tissue was cultured in vivo. We do not know whether the presence of large numbers of these particles is causally connected with any of the changes that occur in the "B" and "C" tissues after prolonged culture (such as the rapid rate of proliferation and the loss of ability to differentiate) or whether the rapidly dividing tumor cells simply provide a good environment for the production of virus-like particles.

In host flies virus-like particles have thus far been detected only in gut cells and blood cells, but they have not yet been observed in hypodermis, muscles, fat body, tracheae, or nervous tissue. Virus-like particles have also been detected in small numbers in imaginal disks of normal larvae.

The virus-like particles are slightly elliptical in shape, about 37.7 mμ ± 1.62 by 45.5 mμ ± 1.06 (standard deviation). Within a given cell the long axes of the ellipses may be oriented in various directions. These particles do not resemble any insect virus that we know of. They are found singly or in clusters, mostly in the nucleus, but occasionally in the cytoplasm. The particles may differ from one another in their electron opacity. In general, younger cells contain fewer electron-opaque, virus-like particles than older cells do (Figs. 1 and 2). They cannot be cross sections of tubular structures because longitudinal sections of such structures have not been found in any of the hundreds of sections we have examined and because they have a much larger diameter than the usual sorts of microtubules have (Fig. 2). So far as we are aware these particles resemble no known cell organelles.

Table 1 summarizes the distribution of the virus-like particles in the different tissues that were examined. The particles appeared in five kinds of associations:

1) Scattered, free virus-like particles in the nucleus. These occur mostly in cells at the periphery of the implant, and usually only a few of the particles are electron opaque (Fig. 1).

2) Small irregular clusters of virus-like particles in the nucleus without specific orientation. These appear in cells at both the center and periphery of the implant. Here also most of the particles have a low electron opacity.

3) Large clusters of virus-like particles in the nucleus in which some of the particles are irregularly distributed and others are arranged in well-oriented patterns. These also occur in older cells, and most of the particles are not electron opaque (Figs. 2 and 3).

4) Large clusters of electron-opaque virus-like particles arranged in regular patterns. These are found only in the nuclei of degenerating cells.

5) Virus-like particles in the cytoplasm. These are much less abundant and occur either as free particles or clusters (Figs. 1 and 4).

Can one account for these variations in the pattern of virus-like particles? Autoradiographic studies (*8*) with the light microscope have shown that 8 days after a tissue fragment is implanted into an adult host, DNA synthesis ceases except in cells in the periphery of the implant. Thus peripheral cells are younger and also capable of DNA synthesis. This difference in biosynthetic ability is correlated with the different patterns of the virus-like particles. In general, younger cells located in the periphery of the implanted tissues contained scattered, free virus-like particles or small clusters of virus-like particles (Fig. 1). On the other hand, older cells located in the interior of the implanted tissues contained large clusters of particles. Older cells in general contained many more of the particles than younger cells did. Large geometric clusters of particles seemed to be restricted to the early stages of degenerating cells in the implants and to the nuclei of the gut cells of the host. Electron-opaque virus-like particles were found in high concentration in degenerating nuclei and in "lytic bodies" found in the tumor tissues.

What are these particles? If they are new organelles found in both the nucleus and cytoplasm of living cells, it would be both surprising and interesting. It seems far more likely that they are a new virus in *Drosophila*. They appear to infect only cells that divide, hence, their occurrence in gut cells and blood cells of the host, in tumors, and in rapidly growing lines of cells derived from imaginal disks, and their apparent absence in nondividing cells like adult epidermis, tracheae, and muscles. Their presence in other dividing cells of the adult, such as those of the gonads, may also be expected.

If their viral nature is confirmed by studies of infectivity, then their occurrence in *Drosophila*, a genetic organism of choice, assumes some special interest. Indeed, it introduces the prospect of using genetics as a tool to uncover the control of viral development in a multicellular organism.

Note added in proof: After this paper was submitted we had the privilege of examining some unpublished electron micrographs of Dr. R. P. Kernaghan of State University of New York at Stony Brook. He has independently discovered virus-like particles in the nucleus of certain cells of *Drosophila*.

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9. This lethal mutant has been mapped by E. B.

Lewis, and its properties are being described elsewhere.

10. Supported in part by grants from NSF and NIH. The original stock of *Drosophila melanogaster* for these experiments derived from a mass culture maintained for many years by Prof. A. G. Steinberg of Western Reserve University. We thank Mrs. Marilyn Ricklin for assistance; Drs. F. Sehnal and P. Lawrence for their helpful comments on the typescript; and Drs. K. Aizawa, J. Briggs, H. M. Mazzone, G. Stairs, and H. Swift for their comments on the electron micrographs.

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Molecular and Thermal Origins of Fast Photoelectric Effects in the Squid Retina

Abstract. *When a short, intense flash of light is absorbed by the outer segments of squid photoreceptors fixed in glutaraldehyde, a voltage appears briefly across the retina. The waveform depends on the relative amounts of rhodopsin and its various stable photoproducts present at the beginning of the flash. Each light-absorbing species present contributes a characteristic voltage component which is summed in the gross waveform. The heating effect of the absorbed light produces a small, long-lasting thermoelectric voltage as well. When this thermal effect is corrected for, interconversion of equal numbers of rhodopsin and acid metarhodopsin molecules by a flash results in a fast voltage waveform whose time integral is zero. Thus the charge flowing in one direction in the retina when rhodopsin is converted to acid metarhodopsin by one photon is apparently exactly reversed when acid metarhodopsin is reconverted to rhodopsin by another.*

Vertebrate retinas (*1*) as well as a variety of other light-absorbing tissues (*2*) show small transient voltages when illuminated with intense short flashes of light. In the vertebrate retina, these fast photoelectric effects depend specifically upon light being absorbed by rhodopsin (*3*), but they are little affected by the physiological state of the retina or by the ionic composition of the solution in which it is bathed (*4*). Moreover, the sizes and shapes of the voltage waveforms are influenced by the kinds and amounts of rhodopsin and its transient photoproducts present at the beginning of a test flash (*5*). Hence it is likely that the origin of fast photovoltages (FPV's) is somehow different from that of the usual electrodiffusive currents and voltages produced by live cells (*6*). Indeed, it has been suggested that FPV's arise from displacement of charges within the photoreceptors' outer segments or even in the rhodopsin molecules themselves (*7*). If so, the structural organization of the parts of the receptors bearing the photopigment and the nature and stability of rhodopsin's photoproducts would be expected to influence the size and shape of the FPV. Thus, it seemed useful to know what fast photoelectric effects would be produced by the squid retina, whose rhodopsin differs photochemically from the vertebrate photopigment and occurs in microvilli within rhabdomeres rather than in the flat lamellae of vertebrate rods (*8*). We find that the squid retina, live or fixed in glutaraldehyde, shows FPV's as large as those of vertebrate retinas and with waveforms which, like those of vertebrates (*5*), bear an apparently simple relation to the states of the photopigment chromophores at the beginning of a test flash. Light also produces an additional thermoelectric voltage which complicates the interpretation of the FPV in the squid retina and perhaps in other tissues as well. We now report a method for distinguishing the photochemical and thermal effects and for studying each separately.

Retinas from live squid adapted to the dark were isolated in darkness as described (*9*, *10*). Disks 4 to 8 mm wide were punched from the retinas and mounted between the two halves of a thin chamber. Cold electrolyte solution was pumped to the upper and lower chamber halves through silver tubes internally coated with AgCl to which electrical connections were made for voltage and current measurements. Electrical and optical shielding was used to reduce flash artifacts to 5 μv or less. A xenon flashtube (Edgerton, Germeshausen, and Grier, type FX33) delivered 100-joule flashes of about 100 μsec duration to the chamber through a set of heat filters, interference filters, and a 25-mm focal length *f*1.4 photographic objective. Only the rhodopsin-bearing inner surface of the retina was illuminated. Flash exposures were measured with a calibrated silicon photovoltaic detector. The rise time of the amplifier system coupled to the direct current was set at 5 μsec.

A live retina gives a large, slow photocurrent (*9*, *10*) when its inner surface is illuminated, but after 30 minutes at 0°C in darkness without 0_2, this physiological response disappears, leaving only fast photoelectric waveforms of the types shown in Fig. 1. Like the FPV of vertebrate eyes, the responses are not suppressed by substitution of either 1*M* KCl or .01*M* KCl for normal seawater. Fixation for 30 minutes in 5 percent glutaraldehyde in buffered seawater at 0°C (which does not destroy squid rhodopsin) also leaves the waveforms intact, though somewhat more rapid than those of the unfixed retina. Freezing to −80°C and thawing the retina, however, abolishes the FPV without destroying the normal complement of rhodopsin. In all of the experiments to be described, fixed retinas were used since these were available throughout the year. In early work, seawater was used as the bathing solution, while in later experiments a solution with a high electrical resistance containing 1.2 mmole of glycerol, 10 mmole of KCl, and 5 mmole of tris(hydroxymethyl)aminomethane (tris) buffer per liter was used to get the largest possible voltages. Such a solution increased the size of the FPV without appreciably altering its shape.

Illumination of the outer surface of the retina produces neither a slow photocurrent nor an FPV, since the light is totally absorbed by an intraretinal layer of black screening pigment before reaching the outer segments. Light absorbed by the black pigment itself yields no net transretinal voltage. In the dark-adapted retina, a test flash that activates less than 1 percent of the rhodopsin molecules present gives a simple outer-segment-positive wave whose form is independent of wave length (λ) and whose action spectrum is consistent with the absorption spectrum of squid rhodopsin. The voltage rises linearly without detectable latency, reaches a single maximum, and decays exponentially (*11*) to zero. If we allow for the finite duration of the flash, the voltage can be represented mathematically as the content of the second compartment of a three-compartment sequential kinetic system (*12*). The size of the response, called hereafter the "rhodopsin response," or "R wave," is proportional to the number of rhodopsin molecules absorbing one or more quanta from the flash, but the rate constants are independent of light intensity