titers, or both, were similar to those in appropriate control cultures (*10*) tested simultaneously. No change in sensitivity to viruses was noted for the various passages tested.

When DBS-FRhL-2 cultures were inoculated with adenovirus types 3, 4, or 7, parainfluenza types 1 or 2, influenza A2, herpes simplex, measles, cytomegalovirus, or rabies, vital titers were less than those observed in control cultures.

A second cell line, DBS-FCL-1 (*7*) was derived from lung tissue of a male fetus of *Cercopithecus aethiops* on 18 November 1969. Cultures were prepared and maintained under conditions identical to those for DBS-FRhL-2. During passages 1 through 5, epithelial cells predominated. After passage 6, however, the population was comprised largely of fibroblasts. Cells grew rapidly for 35 passages, with a population doubling time of 33 hours, and were transferred every 3 to 4 days with division at a 1:3 ratio. Around the time of passage 36 a decrease in growth rate was observed, and the cells were subcultured at 3- to 10-day intervals with division at a reduced 1:2 ratio. After passage 53 no further multiplication of the cells was noted. At an early passage, a seed stock of this cell line was prepared and stored in liquid nitrogen in a manner similar to that for DBS-FRhL-2. As with DBS-FRhL-2, a low frequency of polyploidy (1.3 to 6.0 percent) was observed between passages 1 and 34. Tests for the presence of adventitious agents and for tumorigenicity, as described for DBS-FRhL-2, have been negative.

The spectrum of susceptibility to viruses of DBS-FCL-1 is similar to that of DBS-FRhL-2. Sensitivity was demonstrated to poliovirus types 1, 2, and 3, adenovirus type 3, parainfluenza type 3, herpes simplex, mumps, rhinovirus HGP, rubella, Coxsackie A9, and vaccinia.

In order for these cell lines to be economically feasible as substrates for vaccine production, they must provide (i) adequate cell population, (ii) rapid growth rate, and (iii) yields of virus sufficient for vaccine production. An adequate population is assured by the ability of both cell lines to progress through at least 50 doublings before senescence. Thus, growth potential of these lines is equal to or greater than that reported for human diploid cells (*11*) and rhesus monkey diploid cells (*12*). The doubling time of both cell lines (approximately 30 hours) compares favorably with other values found for primate lung cells (*2, 13*). The stability of these cells during extended periods of storage is another factor assuring their adequacy for production of virus. No changes have been recognized in either cell line after 6 to 12 months of storage in liquid nitrogen.

The results of this program for development of new cell lines suggest that it is possible to develop populations of cells from subhuman primates which meet basic requirements for use in the production of human biologics. However, further evaluation by other independent investigators will be necessary to increase the level of confidence in the safety of these cells.

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5. Contract NIH-69-100 with Lederle Laboratories; P. J. Vasington, project director; R. E. Wallace, principal investigator.
6. Samples of DBS diploid cell lines are available to all investigators by contacting J. C. Petricciani. A more complete report on development and characterization of these cell lines is in preparation.
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8 June 1971, revised 12 July 1971

Limulus Lateral Eye: Properties of Receptor Units in the Unexcised Eye

Abstract. *Single receptor units in the compound eye of the horseshoe crab were illuminated, and their impulse discharges were recorded without removing the eye from the animal. The receptors were spontaneously active in darkness and responded without saturation over a light intensity range of 10^{10} to 1. When the eye was excised, the receptors did not discharge in darkness and had an intensity range of 10^{5} to 1, as is usually found. Experiments show that these and other differences result from cutting off the blood supply to the eye when it is excised. In addition, the range and shape of the intensity characteristic suggest that more than one receptor mechanism encodes light intensity in this eye.*

The lateral eye of the horseshoe crab, *Limulus polyphemus*, has been studied for many years (*1*). In most experiments the eye was removed from the animal and placed in a recording chamber (*2*). Although the preparation is well suited for experimentation, the sensitivity to light of the receptor units (ommatidia) often declines steadily after the eye is excised. To try to overcome this problem, we recorded from the optic nerve fibers without removing the eye from the animal.

Here we describe a method for recording from the intact *Limulus* eye. We show that responses from single ommatidia can be recorded for periods up to 30 hours and that the sensitivity of the eye remains stable for at least one week. We describe some response characteristics of ommatidia in the intact preparation and show how these characteristics differ from those of the excised eye.

Our method was to clamp an adult *Limulus* to a rigid platform that was placed in an aquarium filled with recirculated artificial seawater (*3*). The dorsal surface of the animal, including the lateral eyes, remained above the

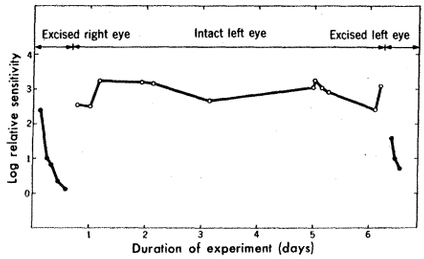

Fig. 1. Stability of visual sensitivity for units in excised and intact lateral eyes of the same animal. Log relative sensitivity (see text) is plotted on the ordinate, and time (in days) after the right eye was excised is plotted on the abscissa. The left eye was excised at 6.3 days. The open and closed circles represent measurements made on excised and intact eyes, respectively. Each data point corresponds to a different unit.

water level. Freshly aerated seawater washed the gill structure through slots in the platform. A small section (1.9 cm in diameter) of shell anterior to one of the lateral eyes was removed to expose the optic nerve. The nerve was cut and drawn into a small recording chamber that was filled with seawater (*4*) and attached to the shell. The optic nerve was then dissected with fine glass needles until a single active unit was isolated. Using glass suction electrodes,

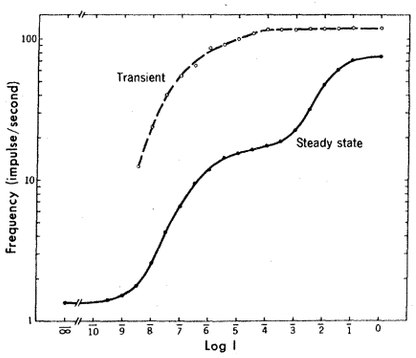

Fig. 2. Intensity (I) characteristics for the initial transient and steady-state responses from a dark-adapted receptor in an unexcised eye. The unit's response is plotted on the ordinate as a function of the log of the relative light intensity plotted on the abscissa. The steady-state response was the mean firing rate during the last 5 seconds of a 10-second flash. The initial transient response was measured by the highest frequency (reciprocal of shortest interspike interval) of the discharge during the early part of the record. The unit did not respond with a well-defined transient to intensities less than log $I = -8.5$. In darkness (log $I = -\infty$) the unit's spontaneous activity was 1.4 impulse/sec (spontaneous rates of 0.2 to 2 impulse/sec have been recorded from receptors in other eyes). At log $I = 0$ approximately 5×10^{12} quanta/sec are incident on the unit at the cornea from 400 to 650 nm.

filled with seawater (*4*), we could record spikes from the optic nerve fibers for periods of up to 30 hours. Single ommatidia were optically isolated with fiber-optic light pipes (*5*). At no time during the preparation of the animal or in the subsequent experiment were anesthetics used.

To test the stability of the "intact" preparation, we did the following experiment. First we excised the right eye of an animal and determined the sensitivity to light of several ommatidia. Sensitivity is the reciprocal of the relative light intensity required to elicit 55 percent frequency of responding to brief (20 msec) flashes of light (*6*). The results in Fig. 1 (closed circles) show that the sensitivity of ommatidia in the excised right eye decreased continuously during the first 14 hours after the eye was excised at which time it was discarded. Over the next 5 days we recorded, as described above, from the optic nerve fibers of the left eye of the same animal without removing the eye. During that period of time (Fig. 1, open circles) the sensitivity of the ommatidia from which we recorded was nearly constant. We then excised the left eye and found that its visual units declined in their sensitivity to light at about the same rate as did units in the excised right eye. We conclude from these results that our method of recording from the optic nerve fibers of the intact eye results in a stable preparation.

The stability of the intact preparation enabled us to determine reliably certain physiological characteristics of single ommatidia. The intensity characteristics for the transient and steady-state responses from a single dark-adapted ommatidium are shown in Fig. 2. The transient response was measured by the highest frequency of the impulse discharge at the onset of illumination. The steady-state response was the mean firing rate during the last 5 seconds of a 10-second flash. All the data in Fig. 2 were recorded from a single ommatidium that was optically isolated with a single glass fiber. Each of the data points was obtained when the unit was fully dark adapted.

Figure 2 reveals four interesting properties of an ommatidium in an intact eye: (i) a 10^{10}-fold range of light intensity for the steady-state response, (ii) a distinct plateau in the steady-state intensity characteristic at moderate levels, (iii) a monotonically increasing transient response over a relatively narrow range (10^4) of light intensities, and (iv) spontaneous activity in complete darkness.

In comparison, ommatidia in excised eyes respond over a relatively narrow range (10^5) of light intensities and are not usually active in the dark (Fig. 3a) (*7*). These differences probably result from cutting off the eye's blood supply in the excised preparation. A direct comparison of the two curves in Fig. 3a may not be justified, however, because the curves were not obtained from the same receptor. To clarify this point, we did the following experiment. An intensity characteristic was determined for a fully dark-adapted unit in an intact eye. Without disturbing the optical isolation of the unit or its state of adaptation, we cut the animal's heart in half (*8*). Intensity characteristics were then determined at various

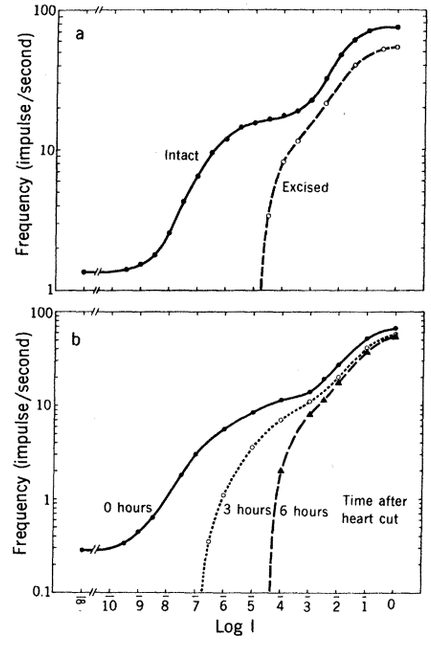

Fig. 3. (a) Intensity characteristics of receptor units in intact and excised lateral eye preparations. The "intact" characteristic (also shown in Fig. 2) was determined first. This eye was then excised and a second characteristic (labeled "excised") was obtained for a different unit. The unit in the excised eye did not respond to intensities less than or equal to log $I = -5$. (b) Effects of cutting the heart on the intensity characteristic of a single receptor unit. The "0-hour" curve is the intensity characteristic before the heart was cut. Each point on this curve was obtained when the receptor was fully dark-adapted. Intensity characteristics of the same unit were determined 3 and 6 hours after the animal's heart was cut in half (*8*). The unit did not respond to log $I = -7$ after 3 hours, and after 6 hours the unit did not respond to log $I = -4.5$. The data in (a) and (b) represent steady-state responses. All the curves were fit by eye.

times after the heart was cut (Fig. 3b).

The first noticeable effect on the eye of cutting the heart was a steady decline of the spontaneous discharge from the recorded unit. Two hours after the heart was cut the spontaneous discharge stopped. At 3 hours the range of the intensity characteristic had constricted by about 10^3, and after 6 hours the characteristic stabilized with a range of less than 10^5 to 1. The 6-hour characteristic is similar in shape and in range to the excised eye characteristic shown in Fig. 3a. It also agrees well with the results reported by Hartline and McDonald (*7*) for units in excised eyes. We conclude, therefore, that the differences in the properites of units in intact and excised eyes shown in Fig. 3a were caused primarily by cutting off the eye's blood supply.

We should point out that the optic nerve is closely associated with a blood vessel that is cut in our experimental procedure. This vessel is a branch of the hepatic artery (*9*), which joins the optic nerve a few millimeters behind the eye. Blood in this vessel flows toward the brain, so that cutting the vessel does not interfere with the blood supply to the eye.

The range of stimulus energies (10^{10}) encoded by a single ommatidium in the intact eye appears too large to be served by a single receptor mechanism. To our knowledge no other sensory receptor has been found with a similar range. For example, the range for some vertebrate photoreceptors is 10^4 (*10*); for auditory receptors in the noctuid moth it is 10^5 (*11*), and for the Pacinian corpuscle about 10^2 (*12*). It appears, then, that more than one receptor mechanism encodes light intensity in the *Limulus* eye.

The shape of the steady-state intensity characteristic in Fig. 2, especially the plateau region, further suggests that two mechanisms are involved. Measurements on light and dark adaptation and on the variability of the interspike intervals (*13*) also support the existence of two mechanisms. Dowling (*14*) pointed out that regenerative responses recorded intracellularly from the eyes of small *Limuli* may represent a second, more sensitive mechanism for encoding light intensity. Electroretinogram studies by Wulff (*15*) also suggest two mechanisms.

The range of the human visual system measured psychophysically (*16*) is about equal to that of ommatidia in the intact *Limulus* eye measured physiologically. Intensity coding in the human as well as in many other vertebrates is served by two receptors—rods and cones.

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Potassium Ion Release and Enzyme Secretion: Adrenergic Regulation by α- and β-Receptors

Abstract. *Epinephrine caused amylase secretion and K^+ release in rat parotid slices. Propranolol, which blocks β-receptors, inhibited amylase secretion; phentolamine, which blocks α-receptors, inhibited K^+ release. Since enzyme secretion was associated with fusion of secretory granules to the cell membrane and K^+ release was associated with vacuole formation, it could be shown that both α- and β-receptors are present in the same exocrine cell. The findings appear to exclude cyclic 3',5'-adenosine monophosphate as an intermediate in the α-receptor response.*

Potassium release is a component of excitation and activation phenomena in a number of tissues (*1–3*) including the salivary glands (*4*). Recently we observed in rat parotid slices that epinephrine causes K^+ release and vacuole formation concurrently with enzyme secretion. While monobutyryl cyclic 3',5'-adenosine monophosphate efficiently induced enzyme secretion, it failed to cause K^+ release (*5*). It is possible that the exocrine parotid cell has two epinephrine receptors, one controlling enzyme secretion via adenyl cyclase; the other regulating K^+ release via a different pathway. Findings substantiating this hypothesis are reported below.

Phentolamine, an α-adrenergic blocking agent, completely inhibited the epinephrine-induced K^+ release in rat parotid slices (Fig. 1A). Enzyme secretion caused by epinephrine was not affected by the α-adrenergic blocking agent (Fig. 1B). Electron micrographs revealed the typical picture of enzyme secretion: enlargement of the acinar lumens due to fusion of the secretory granules with the cell membrane. However, vacuole formation was completely depressed by phentolamine (compare Fig. 2, A and B). The β-adrenergic blocking agent propranolol had no effect on K^+ release, but it completely abolished epinephrine-induced enzyme secretion (Fig. 1, A and B). Addition of propranolol was characterized morphologically by extreme vacuolation of the acinar cells, constricted lumens, and the absence of fusion of secretory granules with the cell membrane (compare Fig. 2, A and C). Norepinephrine caused K^+ release and enzyme secretion as