Lapita pottery-making people were in Tonga by 1140 B.C. (*12*) and in Samoa perhaps as early as 800 B.C. (*13*). If we accept A.D. 300 as about the time that our exotic sherds (in the form of pots) arrived in the Marquesas, then the people carrying them were probably too late in time to be proto-Polynesians. The early Lapita people of Tonga and Samoa were no doubt in this stage of development, but by A.D. 300 the cultures of Tonga and Samoa were Polynesian. The implication for Polynesian origins is a continuing infusion of Melanesians into Polynesia from Fiji (and perhaps elsewhere), and supports indirectly the suggestion that Fiji was the homeland of the proto-Polynesians (*14*). If our conclusions are valid we have, in any case, demonstrated the movement of pottery from Fiji to both eastern and western Polynesia at an early date.

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Lateral Diffusion of Visual Pigment in Photoreceptor Disk Membranes

Abstract. *Visual pigment molecules are found to move transversely, but not longitudinally, in both rod and cone outer segments of mud puppy and frog. This is consistent with the idea that they are immersed in a two-dimensional fluid disk membrane. The diffusion coefficient for the motion is about* 5×10^{-9} *square centimeters per second at 20°C, corresponding to a root-mean-square molecular displacement of 0.3 micrometer in 1 second.*

A variety of evidence supports the concept of a two-dimensional fluid mosaic structure for biological membranes (*1*). In particular, rhodopsin has been shown to exhibit fluid-like behavior in retinal rod disks (*2*). A quantitative measure of fluidity in several artificial and natural membranes has been made through the use of spin-labeled lipid probes. Values of the lateral diffusion coefficient estimated in this way range from 1×10^{-8} to 6×10^{-8} cm^2/sec (*3*). Microscopic observations of the spread of fluorescent labels attached to proteins in two types of biological membrane yield values for these proteins of 5×10^{-11} to 2×10^{-9} cm^2/sec (*4*). In this report, we describe evidence for lateral diffusion of visual pigment in rod disk membranes from experiments we did but could not interpret in 1964 (*5*), together with new work that provides a value for the diffusion coefficient (*6*).

Rhodopsin is a hydrophobic glycoprotein with a molecular weight of 36,000, which is known to be embedded in disk membranes of rod outer segments (ROS) (Fig. 1a). The diameters of the ROS range up to 8 μm in frog (*Rana pipiens*) and up to 13 μm in mud puppy (*Necturus maculosus*). Because of their great size and accessibility, the ROS can be readily isolated and visualized in the light microscope. Techniques have been developed for directly measuring absorption spectra of small regions of these organelles. Measuring and reference microbeams as small as 1 by 5 μm are projected onto a microscope stage and aligned with the ROS before being sent on to a photomultiplier and associated electronics for detection.

In our early work, with a split-beam microspectrophotometer of this general design, we found that we could determine visual pigment spectra in single outer segments if the measuring light was made sufficiently dim to limit loss of the photosensitive pigment by bleaching during the measurement (*5*). We could also determine the photosensitivity of the visual pigment by measuring the rate of loss of absorption in beams of higher intensity and constant wavelength. We were surprised to find that, although the bleaching rates of rods and cones 1 to 2 μm in diameter were nearly identical in beams 1 to 2 μm wide, the bleaching rate of larger amphibian rods was much slower in these beams. Moreover, the rate increased in direct proportion to

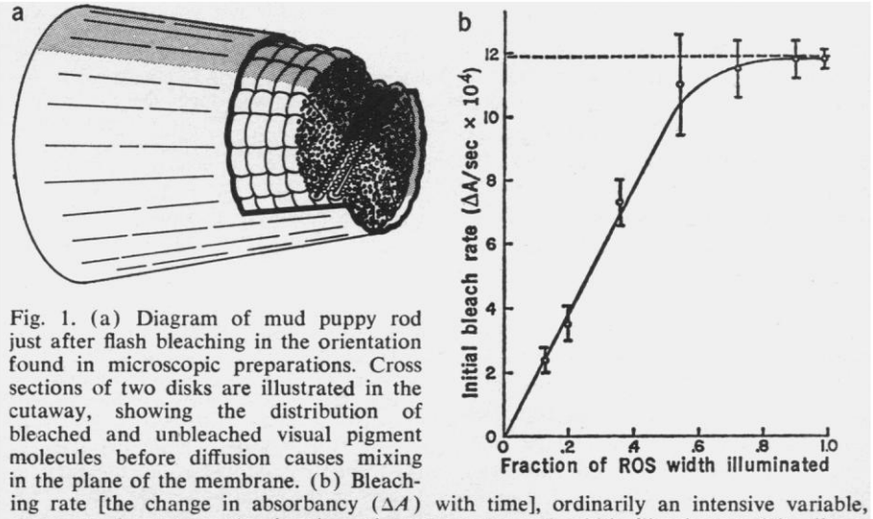

Fig. 1. (a) Diagram of mud puppy rod just after flash bleaching in the orientation found in microscopic preparations. Cross sections of two disks are illustrated in the cutaway, showing the distribution of bleached and unbleached visual pigment molecules before diffusion causes mixing in the plane of the membrane. (b) Bleaching rate [the change in absorbancy (ΔA) with time], ordinarily an intensive variable, seems to depend on the fraction of mud puppy rod width illuminated. The linear dependence is due to rapid diffusional redistribution of unbleached molecules. Molecules are bleached by the measuring beam. The dotted line is for glutaraldehyde-fixed cells.

beam width (Fig. 1b), beams as wide as the outer segments giving a bleaching rate comparable to that for the 1- to 2-μm rods and cones. We also found bleaching of one side of a rod to be accompanied by spontaneous loss of pigment density on the opposite side. We concluded that these unexpected results could only be explained by lateral diffusion of the bleaching photons' energy (resonance transfer) or of the absorbing molecules themselves (*7*). However, Hagins and Jennings (*8*) had argued that resonance energy transfer was unlikely on both theoretical and experimental grounds. We have tested this idea by repeating our experiments on glutaraldehyde-fixed ROS. These showed normal absorption spectra and pigment density, but the bleaching rate was independent of beam width. Elimination of the dependence of bleaching on beam width in the cross-linking fixative glutaraldehyde argues strongly that the observed effect in normal cells is due not to resonance transfer but rather to continual lateral diffusion of visual pigment molecules into the bleaching beam from adjacent dark regions.

Further experimnts were therefore designed to measure the lateral diffusion coefficient by using a more mathematically tractable beam configuration and instantaneous bleaching. A Honeywell Strobinar 300 linear flashtube, giving flashes 2 msec in duration, was masked down by a slit aperture at the tube envelope and mounted on a focusable assembly in the upper microscope. The aperture was minified to a slit measuring 13 by 60 μm at the microscope stage by an immersion objective (numerical aperture 40 $\times$ 0.5), and was located and sharply focused by photographing its image and the image of a stage micrometer projected backward through the microscope. Its precise position was noted on the micrometer and, in turn, on an ocular reticle. The microspectrophotometer measuring and reference apertures could be positioned with respect to these coordinates by using the centering mount of the microscope condenser.

The ROS were isolated on cover slips in Ringer or Ringer-gelatin solution by using infrared image converters, as previously described (*9*). These were visualized in the microspectrophotometer by deep red ($>$ 680 nm) illumination and aligned so that one long edge of the flash beam bisected the rod longitudinally (Fig. 2). The cell width was measured against the ocular reticle, and the measuring micobeam was placed precisely 1 μm in from the flashed edge or the dark edge (Fig. 2). An absorption spectrum was recorded in dim light. The monochromator was then set at a single wavelength, 420, 502, 530, 560, or 575 nm. After a 10-second recording of the absorbancy before bleaching, the flash was triggered and the absorbancy change was recorded as a function of time until an apparent steady state was reached (100 seconds or more). The spectrum after bleaching was recorded and the cell's coordinates were rechecked to determine whether any motion had occurred. In experiments with the measuring beam on the bleached side (Fig. 2a), the flash causes a jump down to one-third followed by a creep back to two-thirds of the original absorbancy along an approximately exponential time course. Measurements at 420 nm give deflections that appear to be mirror images of the longer-wavelength ones (*10*). When the measuring beam is on the dark side (Fig. 2d), a momentary flash artifact replaces the first phase, while the slow phase is of the same time course but opposite in sign to that on the bleached side. Control experiments in which the entire cell is bleached (Fig. 2b) show no slow phase. This implies that we do not measure the kinetics of appearance or disappearance of photoproducts in our experiment. Furthermore, glutaraldehyde-fixed cells show only the fast absorbancy jump on the bleached side but no recovery. On the dark side nothing but a flash artifact is seen.

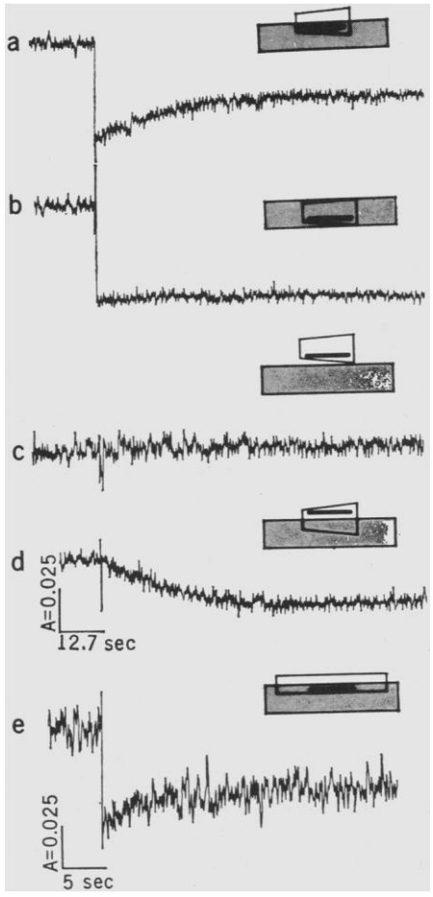

Fig. 2. Tracings of original experimental records. Insets show beam configurations on the ROS: the dark bar represents the dim measuring beam (560 nm) placed 1 μm in from the cell edge (the reference beam outside the cell is not shown); the shaded area is the locus of the yellow bleaching flash ($>$ 575 nm), which was 2 msec in duration. Records (a) to (d) are for mud puppy. (a) Fast loss of absorbancy when the lower half only was bleached, followed by slow half-recovery due to diffusion from the upper half of the ROS. (b) Photoproduct control when the flash covers the whole cell. Fixing the cell with glutaraldehyde converts (a) to this kinetic form. (c) Control experiment in which the flash did not strike the ROS; the measuring beam causes no absorbancy change. (d) Slow diffusional loss of unbleached pigment from the upper (unbleached) half after a flash. (e) Recording from frog rod under the conditions in (a). Note the faster time scale. All recordings were made with light plane-polarized perpendicular to the long axes of the ROS. Absorbancy changes in parallel polarized light was found to be negligible.

In other experiments, rods were oriented perpendicular to the flash beam axis so that a band 13 μm long was bleached completely across the rod (*11*). These rods show only the flash artifact in the dark area immediately adjacent to the bleaching edge and only a fast absorbancy loss in the bleached region. This confirms the expectation that diffusion of visual pigment cannot take place longitudinally between separate disk membranes.

We have calculated the diffusion coefficient for lateral translational motion for rhodopsin in frog rods and for porphyropsin in mud puppy rods from these measurements. We used a one-dimensional slab model for which a rapidly converging series solution to the diffusion equation can be found for the boundary conditions described above (*12*). This is

$$D = -\frac{L^2}{\pi^2 t_{\frac{1}{2}}} \ln\left(\frac{-0.393}{\cos\frac{\pi x}{L}}\right)$$

where D is the diffusion coefficient, L the cell width, x distance in centi-

meters from the unbleached edge to the measuring slit, and $t_{1/2}$ the time to half recovery evaluated from records such as Fig. 2a. Thirty records similar to Fig. 2a give $t_{1/2} = 15.4 \pm 3$ seconds for mud puppy and 4.0 ± 0.5 seconds for frog (*13*). The computed diffusion coefficients are $(4.7 \pm 0.9) \times 10^{-9}$ cm^2/sec for the former and $(5.5 \pm 0.6) \times 10^{-9}$ cm^2/sec for the latter.

Some of our 420-nm measurements in mud puppy suggest that bleached visual pigment molecules may diffuse only half as fast as unbleached molecules. However, we cannot be exact about this because the records must be corrected for a variable rate of loss from the cells of dehydroretinol, which also absorbs at 420 nm, after bleaching.

Finally, results such as those given in Fig. 1b show that mud puppy cone pigment also undergoes rapid lateral diffusion. Since electron micrographs show that cone lamellar membranes (unlike those of rods) are longitudinally continuous through hairpin turns on one edge and through the cell envelope membrane on the other, it was expected that fast longitudinal diffusion would also be found. Surprisingly, no diffusion occurred during 20 minutes. We conclude that motion of cone pigment protein is impeded at the highly curved membrane bends.

Using the Stokes-Einstein relation for diffusing spheres (*14*) together with our value for the diffusion coefficient and an assumed spherical visual pigment radius of 23 Å, we calculate an effective membrane viscosity of about 2 poise, in close agreement with that calculated from rotational relaxation measurements on the same assumptions (*15*). However, we do not conclude from this agreement that visual pigment is a sphere completely immersed in lipid, for the same agreement would occur if it were any partially or completely immersed object of revolution (*16*). The calculated membrane viscosity of 2 poise therefore constitutes a lower limit. Evaluation of the actual viscosity from our kind of experiment awaits more precise information on shape and degree of immersion of visual pigment.

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Stability of Catecholamines Immobilized on Glass Beads

M. S. Yong (*1*) gives the impression that biological activities for catecholamines covalently attached to glass beads (*2, 3*) are due to pharmacological concentrations of catecholamines leaching off the glass beads. The evidence is that [^{14}C]catecholamines are released from glass beads washed only with distilled water. This is not the washing procedure we outlined (*3*) and used (*2, 3*) prior to our biological assay of catecholamines immobilized on glass beads. Instead, the bead-bound catecholamines were extensively washed with several liters of 0.1*N* HCl to remove any noncovalently bound catecholamines. In addition, the beads were washed extensively with 0.8 percent NaCl immediately prior to use, to remove the acid and any free catecholamines that might have accumulated since the acid washing. During this procedure, the beads were washed with at least 12 liters of 0.1*N* HCl, and then with 18 liters of 0.8 percent NaCl or distilled water, prior to biological assay (*4, 5*).

The results of washing 1 g of [^{14}C]epinephrine–glass beads with acid (*4*) illustrate that when the [^{14}C]epinephrine-glass beads are washed slowly with 20 liters of 0.1*N* HCl over a 6-day period, 16 percent of the [^{14}C]epinephrine is removed in the first 10 liters of acid, and less than 1 percent is released in the next 10 liters of acid. The total amount of catecholamine liberated in the first 10 liters of acid would more than account for the free catecholamine that was reported by Yong (*1*).

The release of [^{14}C]epinephrine from [^{14}C]epinephrine–glass beads placed in an oxygenated Krebs solution was studied subsequent to washing in either 0.1*N* HCl or distilled water. Total radioactivity bound to the glass beads was determined by the hydrogen fluoride and dithionite techniques (*4, 5*). The release of [^{14}C]epinephrine from acid-washed glass beads was compared to the release from glass beads washed only with distilled water. Two grams of epinephrine–glass beads had bound a total of 0.10 mc of [^{14}C]epinephrine. One gram of these beads was washed with 5 liters of distilled H_2O, and 1 g with 12 liters of 0.1*N* HCl at a flow rate of 3 liters per day. Each gram was placed in 10 ml of oxygenated Krebs solution at 37°C and 50-μl samples were counted in 10-ml of Aquasol. The [^{14}C]epinephrine–glass beads that were washed only with distilled water released 4 percent of the labeled epinephrine in the first 24 hours, whereas the acid-washed beads released only 0.19 percent of the [^{14}C]epinephrine in the same period. There is a substantial difference in the observed rate of release, dependent upon the washing procedure used, indicating the necessity of acid washing.

Yong's method (*1*) for determining biological activity of the water-washed epinephrine–glass beads consisted of placing 25 mg of the glass [by our estimate, about 6500 glass beads of the size used in (*2, 3*)] into 2 ml of a Krebs solution for 5 minutes, in which time, he reports, approximately 0.5 per-