with a mixture of 95 percent O_2 and 5 percent CO_2 no significant effect on the viability of the tissue or the size of the signal could be demonstrated.

Thus, merocyanine 540 seems to bind to the cardiac cell membrane without any deleterious effect on contraction and is capable of monitoring the change in the myocardial membrane potential fluorometrically when excited at 540 nm. Fluorometric measurement of the optical signal combined with the use of a rapid scanning system could provide an accurate measure of the exact propagation pathway of the electrical signal in normal and diseased heart.

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11 September 1975; revised 18 November 1975

Color Receptor Identities of Goldfish Cones

Abstract. *Goldfish retinas were exposed to spectral lights, then incubated with nitro-blue tetrazolium chloride. Diformazan deposits revealed that five morphologically distinct cone types were segregated into three color classes: red long double and long single cones, green short double and long single cones, and blue short single and miniature short single cones.*

Receptive fields of retinal neurons must eventually be described in terms of the types and distributions of photoreceptors that provide input through synaptic contact and indirect pathways. Recent advances have been made in elucidating the color-specific cone contacts of horizontal cells (*1*) and bipolar cells (*2*) in cyprinid fish. These studies depend on correlation of cone morphology with chromatic sensitivity by microspectroscopy of visual pigments in single receptor cells. Determining the organization of color receptors in certain species, such as mammals, is substantially more difficult because of the small size of the cone outer segments and the lack of morphologically distinguishing features. Our efforts to elucidate the anatomy of goldfish color receptors were undertaken with the additional goal of establishing procedures that could be applied to species where microspectroscopy of visual pigments in single receptors is difficult.

We have been investigating the color coding of goldfish cones by a state-dependent cytochemical method in flat mounts of whole retinas in which the mosaic arrangements of cones are preserved. Metabolic events triggered in cones by photon capture can be assayed by the use of a redox probe (*3*) such as nitro-blue tetrazolium chloride (NBT). When goldfish cones are irradiated with light and exposed to NBT, they reduce NBT to NBT-diformazan (NBT-DF) faster than do nonirradiated cones (*4*). The dark blue precipitate, NBT-DF, is localized in the cone ellipsoid.

Retinas were isolated from dark-adapted animals under infrared light and mounted receptor side up on a nylon mesh support moistened with oxygenated teleost saline (*5*). Retinas were stimulated with light (*6*) for 5 minutes, or left in darkness for 5 minutes as a control, and subsequently incubated for 5 minutes in a saline-succinate-NBT medium (*7*). The retinas were fixed in 10 percent formalin–isosmotic phosphate buffer (*p*H 7.4) and examined in whole mounts. All experiments were performed at room temperature (about 22°C).

Schematic illustrations of the major types of dark-adapted goldfish cones are shown in Fig. 1a. Double (D) cones are composed of long members (LD) and short members (SD) apposed at the ellipsoid and myoid levels. Three types of single cones are found (*8*): long single cones (LS), short single cones (SS), and miniature short single cones (MSS). Arrays of D and LS cones in the "sclerad" plane and arrays of SS and MSS cones in the "vitread" plane are visible in whole mounts.

Long double and most long single cones respond to red lights by forming NBT-DF in their ellipsoids. In D cones stimulated by dim 650-nm light (1.2×10^4 photon sec^{-1} μm^{-2}) LD cones form NBT-DF, SD cones do not (Fig. 2a). In more than 10,000 D cones examined in experiments with dim 650-nm light, LD cones always responded and SD cones never did. Short single and miniature short single cones were never found to respond to dim 650-nm light. If the flux density of 650-nm

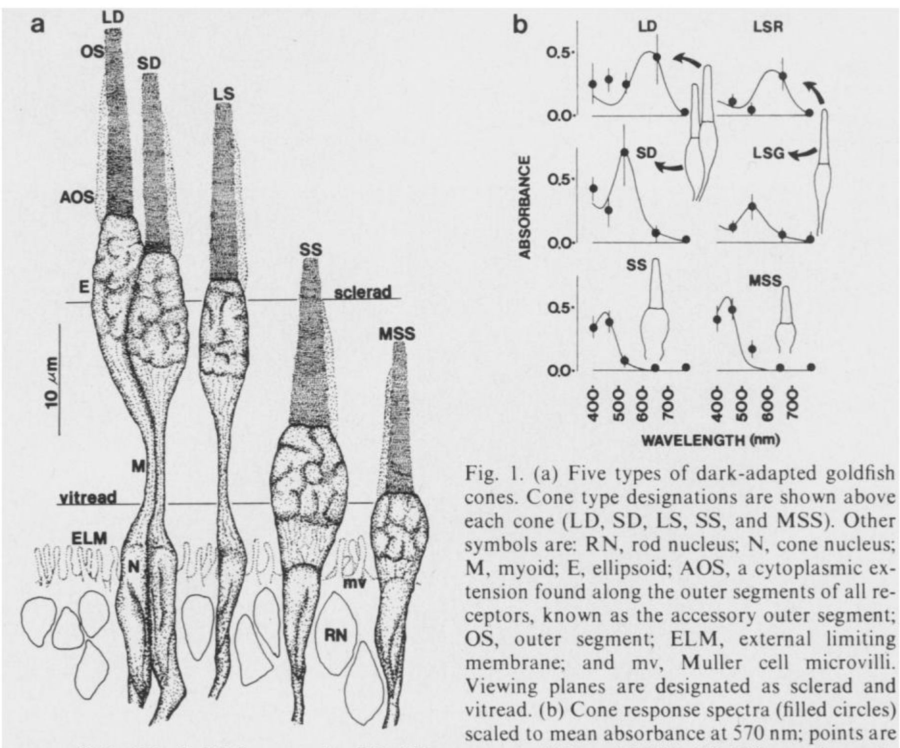

Fig. 1. (a) Five types of dark-adapted goldfish cones. Cone type designations are shown above each cone (LD, SD, LS, SS, and MSS). Other symbols are: RN, rod nucleus; N, cone nucleus; M, myoid; E, ellipsoid; AOS, a cytoplasmic extension found along the outer segments of all receptors, known as the accessory outer segment; OS, outer segment; ELM, external limiting membrane; and mv, Muller cell microvilli. Viewing planes are designated as sclerad and vitread. (b) Cone response spectra (filled circles) scaled to mean absorbance at 570 nm; points are mean ± S.D. (*11*). Solid lines are for VP 625_2 cyanopsin (LD and LSR), VP 530_2 porphyropsin (SD and LSG), and VP 455_2 porphyropsin (SS and MSS) scaled to the maximum response for each cone type. Symbols: LSR, red LS cones; LSG, green LS cones.

light is increased to 6.7×10^6 photon sec^{-1} μm^{-2} (Fig. 2c), both LD and SD and all LS cones respond strongly, but SS and MSS cones do not (*9*). Holding the flux density roughly constant and changing the wavelength to 750 nm (8.5×10^6 photon sec^{-1} μm^{-2}) caused only LD and most LS cones to respond (Fig. 2d).

Green light (535 nm, 3.0×10^3 photon sec^{-1} μm^{-2}) induced strong responses in SD and a few LS cones, and weak responses in LD and most LS cones. Figure 2b shows a D cone response after exposure to green light. At this flux density SS and MSS cones remained insensitive to green lights.

Short single and miniature short single cones were sensitive to blue lights. As all cones exhibited high blue sensitivity, low flux densities of 470-nm light (5.0×10^3 photon sec^{-1} μm^{-2}) were necessary for the selective demonstration of SS and MSS cone responses (Fig. 2e).

Response magnitudes were evaluated by microspectrophotometry of NBT-DF in the ellipsoids of cones. The spectral absorbance of NBT-DF in cone ellipsoids after light exposure correlates well with that published by Altman (*10*) and is maximal at 570 nm. Cone response spectra (Fig. 1b) were obtained from five experiments with equal flux density (3.0×10^4 photon sec^{-1} μm^{-2}) at 750, 650, 535, 470, and 400 nm. Response magnitudes were determined by measuring ellipsoid absorbances at 570 nm for members of each cone class in each experiment (*11*). Responses were expressed as mean absorbance ± 1 standard deviation (S.D.). The solid curves in Fig. 1b are the absorption spectra of VP 625_2 cyanopsin (*12*) and VP 530_2 and VP 455_2 porphyropsins (*13*) as reported by Harosi and MacNichol (*14*). These are scaled to the maximum absorbance at 570 nm for each cone class. Our response spectra are consistent with these pigment absorptance spectra (*15*).

Most LS cones (77 ± 6 percent, mean ± 1 S.D.) and all LD cones are red sensitive, the remainder of the LS cones (23 ± 6 percent) and all SD cones are green sensitive, and SS and MSS cones are blue sensitive. The red-sensitive LS cones are designated LSR cones, and green-sensitive LS cones designated LSG cones. These color assignments agree with those of Stell and Harosi, as cited by Stell and Lightfoot (*1*), and with the morphologies of blue single (SS) and green accessory (SD) cones in the rudd (*2*).

We have shown NBT to be a successful probe of spectral selectivity in the various classes of goldfish cones, but we also believe it to be of general applicability. Promising results have been obtained in our efforts to use NBT as a probe of color coding in primate cones. Thus the NBT method may be a potential probe of photoreceptor responses in many species of vertebrates and may even be useful in studying other sensory systems.

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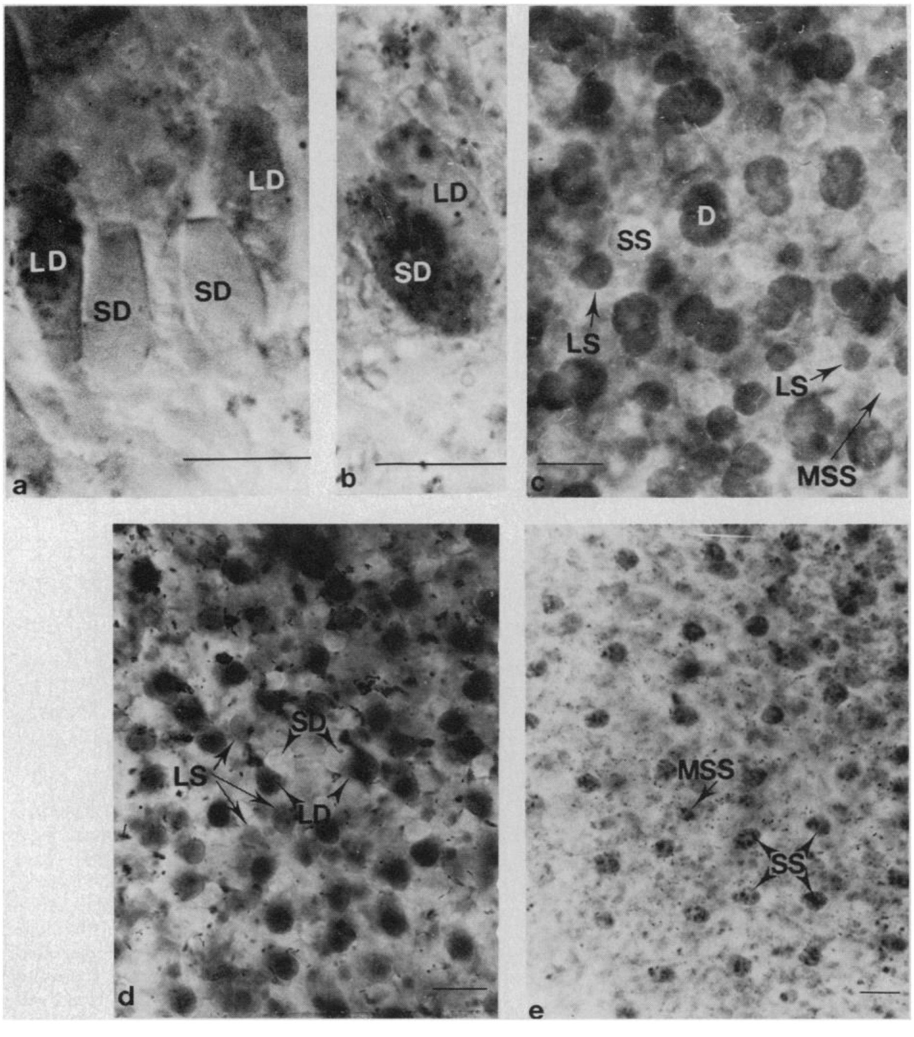

Fig. 2. Light micrographs of cones exposed to spectral lights and NBT. (a) Double cones stimulated with dim 650-nm light. (b) A double cone stimulated with dim 535-nm light. (c) Mosaic view of cones stimulated with bright 650-nm light. (d) Mosaic view of D and LS cones stimulated with bright 750-nm light. (e) Mosaic view of SS and MSS cones stimulated with dim 470-nm light. Scale markers, 10 μm.

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6. Wavelengths were selected by either a Bausch and Lomb monochromator (470 and 535 nm, 5-nm half-peak bandwidth) or interference filters (400, 470, 650, and 750 nm, 7- to 10-nm half-peak bandwidths).
7. Incubation medium stock solution: 35 percent (by volume) disodium-monosodium phosphate buffer (80 m*M*, *p*H 7.4), 35 percent teleost saline (*5*), 30 percent disodium succinate solution (75 m*M*). Before each experiment 5 mg of NBT (Sigma) was dissolved in 1 ml of stock solution. The final *p*H was 7.35.
8. A sixth class of cones, most appropriately referred to as miniature long single cones (MLS), has been seen by W. K. Stell and ourselves, albeit infrequently. No spectral analysis of the MLS cones has been made.
9. In formalin-fixed preparations of dark-adapted retinas, not all classes of cones can be viewed in a single plane of the retinal mosaic (for example, Fig. 2, d and e). As shown in Fig. 1a, LD, SD, and LS cones are sclerad to SS and MSS cones. That all classes of cones may be viewed in one plane in Fig. 2c is due to the fact that this retina was not fixed in formalin and the fresh tissue became compressed by the cover slip until the ellipsoids of all cone types were visible in a single focal plane.
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11. Each point on the response spectra shown in Fig. 1b represents measurements from a single retina with a minimum of 20 cones measured, except the point at 650 nm for the infrequently occurring LSG cones, which represents 12 cones. In some cases as many as 50 cones were sampled. The LD, SD, LSR, and LSG cones were measured side-on and the SS and MSS cones were measured axially. Measurements were made with a 3-μm-diameter aperture centered on the widest point of ellipsoids measured side-on or concentrically centered in ellipsoids viewed axially.

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16. This work was supported in part by NSF grants GB-27561-X and GB 41460, NIH grant EY-00381, and grant FD-00687 from the Food and Drug Administration.

23 June 1975; revised 20 October 1975

Acceleration and Clocks

Cannon and Jensen (*1*) have given a review of the development of timekeeping and the establishment of internationally recognized standards of time. They have emphasized the presence of relativistic effects at the different locations which would introduce rate differences even if there were no systematic or random errors from the rates of perfect atomic clocks. From the records of intercomparisons of the clocks at seven contributing laboratories for a 1-year period, Cannon and Jensen develop statistical evidence which they see as supporting a theory that adds to the time dilations derived from general relativity a new term attributed to the centripetal accelerations of the clocks. They refer to this evidence as a discovery.

In Cannon and Jensen's derivation from general relativity of the rate differences for the different geographical locations, their equation 44, they have introduced the elevations of the stations relative to the geoid as the relevant parameter. The geoid is a surface on which the combined effects of the velocity due to the earth's rotation relative to an inertial frame and the effect of the gravitational potential combine to a constant, an equipotential surface of gravitation and acceleration. Because the earth's surface is so shaped that it comes close to the geoid, the net corrections are very small except for the National Bureau of Standards station at Boulder, Colorado. The small predicted rate differences are tabulated in their table 4 in the column labeled elevation. Their alternative analysis adds a term attributed specifically to acceleration, which they claim to derive by analogy to the derivation of the gravitational redshift from the principle of equivalence. In the use of the geoid, however, they have already invoked the concept of an acceleration potential as a calculating device to account for the rotational velocities. The principle of equivalence was devised as a method allowing the calculation of the effects of gravity as if they were kinematic. It must surely be circular to analyze an effect which is indeed kinematic, such as acceleration itself, via the equivalence principle. The kinematic effect of acceleration is to allow the stations to move at different velocities and yet conserve their physical separations.

In essence, because the elevations relative to the geoid are small and general relativity predicts only small rate differences, the addition of a second term derived from the rotation, which is exactly the normal one but not compensated by a gravitational effect, results in a net correction which is just that which would come about were there no gravitational redshift. The evidence, interpreted in this way, seems hardly strong enough to be accepted.

There is, in fact, much evidence in support of the straightforward interpretation of the effect of the rotating frame, which is, in itself, just special relativity. The comparison by Hafele and Keating (*2*) of the time displacements of clocks carried around the world in aircraft, eastward and westward, was fit to formulas that contained straightforward kinematic terms (including the airplanes' velocities) as well as the gravitational ones.

The measurement of the gravitational redshift by Pound and Snider (*3*) was compared to gh/c^2, where h was the vertical path length traversed, c was the speed of light, and g was the local acceleration in free fall and included the effect of the noninertial frame at about –0.3 percent of the effect of gravity. The experimental precision was just less than could resolve that contribution. However, several experiments that demonstrate the validity of the simple kinematic time dilation for systems possessing far larger accelerations than that of the earth's rotation are well known (*4*).

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5 May 1975

In their article Cannon and Jensen (*1*) compared the Coordinated Universal Time (UTC) time scales during 1972 of seven laboratories and concluded that the scale of the Royal Greenwich Observatory (RGO) was "running at an anomalous rate," even though they recognized that it was "the most stable clock of all." They went on to say that "the reason for RGO's anomalous behavior is unknown and merits investigation." It is unfortunate that the authors did not seek comments on their comparisons before publication since the explanation is obvious to us. Since the beginning of 1972 several of the UTC scales have been adjusted empirically (or "steered") in order that they should conform closely with UTC (BIH), which is determined in arrears by the Bureau International de l'Heure; UTC (BIH) has the same rate as International Atomic Time (TAI), but differs from it by an integral number of seconds. The rate of the RGO scale was, however, deliberately made equal to that of the stable, independent Greenwich atomic time scale, GA2. No further explanation of the differences in rate and stability of the UTC scales is required.

Since the beginning of 1974 the rate of UTC (RGO) has also been adjusted to bring it into conformity with UTC (BIH), but every effort has been made to maintain the independence, uniformity, and stability of the Greenwich atomic time scale; in particular, no attempt has been made to steer it to bring its rate close to that of TAI. The current evidence suggests that the second of GA2 is closer to the SI (International System) second than is the second of TAI.

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16 June 1975

We wish to clarify some essential points relative to the article by Cannon and Jensen (*1*). These authors have developed two contradictory equations (their equations 44 and 45) based on different relativistic assumptions and have attempted to "resolve the contradictions between Eqs. 44 and 45" using data tabulated by the Bureau International de l'Heure (BIH) on atomic clocks located at various places in North America and Europe. The principal effects of interest are the transverse Dop-