XXVIII. NEUROPHYSIOLOGY[*]

Academic and Research Staff

Dr. W. S. McCulloch
Prof. J. Y. Lettvin
Prof. J. E. Brown
Dr. T. O. Binford

Dr. S-H. Chung
Dr. E. Douglass
Dr. A. Natapoff
B. Howland

Lynette Levy
Diane Major
W. H. Pitts
J. R. Seitz

Graduate Students

R. J. Bobrow
R. E. Greenblatt
C. D. Jones

J. E. Lisman
M. Lurie
K. J. Muller

J. F. Nolte
S. A. Raymond
J. I. Simpson

RESEARCH OBJECTIVES AND SUMMARY OF RESEARCH

1. Basic Theory

Research on the functional organization of the reticular core of the central nervous system continues, in collaboration with Dr. William L. Kilmer of Michigan State University.

Our problem is to construct a theory for the reticular system which is compatible with known neuroanatomy and neurophysiology, and which will lead to testable hypotheses concerning its operation.[1,2]

Our first and second approaches to this problem[3] were outlined in Quarterly Progress Report No. 76 (page 313).

We can report that we are embarked on a kind of iterative net statistical decision theory[4] that is comprehensive, versatile, and penetrating enough to stand a reasonable chance of success.

The computer modeling is being done at the Instrumentation Laboratory, M. I. T., by members of Louis L. Sutro's group.

W. S. McCulloch

References

1. W. S. McCulloch and W. L. Kilmer, "Introduction to the Problem of the Reticular Formation," in Automata Theory (Academic Press, Inc., New York, 1966).

2. W. S. McCulloch, "What's in the Brain That Ink May Character?," Proceedings of the 1964 International Congress for Logic, Methodology and Philosophy of Science, Held in Jerusalem, August 26-September 2, 1964 (North-Holland Publishing Company, Amsterdam, 1965).

3. W. L. Kilmer, "On Dynamic Switching in One-Dimensional Iterative Logic Networks," Inform. Contr. 6, 399-415 (1963).

4. W. L. Kilmer, "Topics in the Theory of One-Dimensional Iterative Networks," Inform. Contr. 7, 180-199 (1964).

2. Project Plans

Besides the continuation of research in various sensory systems, the work of this group has now drifted into what might be a confrontation with both classical and current notions on the nervous handling of information. Two years ago, we announced this notion: We supposed every impulse in a train of impulses to convey information at the nervous terminals about the spectrum of pulse intervals preceding it in the train. We were driven to this notion by the records that we obtained from the dimming detectors of the frog in whose firing pattern there were at least two kinds of information conveyed by two separate bands of pulse intervals (i. e. , intraburst and interburst intervals). This work, which Dr. S. H. Chung has now brought nearly to completion, poses the question of what constitutes the information conveyed by a nerve fiber. Short of superstition, there seems to be no reason for deciding that one band or another, one kind of information rather than another, is most relevant. We developed the thesis, then, about the nature of the invasion of the impulse into the branching axonal tree on the supposition that the complex exponential change in threshold following the pulse in any single branch alters the threshold for the invasion of that branch by an impulse that comes to it; and the second supposition was that the complex exponential differs from one branch to another according to the diameter. Such an operation provides a display of excited terminals with each pulse such that the excited terminals are a subset of all terminals of that fiber, and the particular subset expresses something like a crude statistical view on the immediate past in the pulse train, not only of pulse intervals but of sequences of pulse intervals.

The search for evidence led to the elegant experiments of S. A. Raymond that appear as part of his doctoral thesis research. The results that he mentions in Section XXVIII-A provide supporting evidence for the model as proposed. This model leads, however, to considering that synapses do not work on the basis of a deterministic logic, and that a local memory is provided in every separate fiber. If this notion can be proved, as we now think it can, the whole strategy of considering the action of the nervous system will change profoundly. Our major projects for the coming year are, first, to pursue this idea as far as it can be shown to be applicable to real nervous systems; and second, to see how well it can work in confected nets.

J. Y. Lettvin

3. Status of Research

a. Our group, in collaboration with Dr. T. G. Smith, Jr. and Dr. W. Stell of the National Institutes of Health, has studied the mechanisms underlying the generation of the "receptor potential" in Limulus ventral eye photoreceptors. We have found that during the steady-state response to light, the current-voltage (I-V) relation for the membrane undergoes a current-source displacement away from the dark-adapted I-V curve (for the physiological range of light and voltage). Moreover, procedures that tend to diminish or abolish the activity of sodium-potassium sensitive adenosine triphosphatase (as determined in other systems) tend to diminish or abolish the receptor potential. We propose that since the generator of the steady-state receptor potential appears to be a current source (not a conductance increase) and the "pump-ATPase" seems to be required, an electrogenic sodium pump is part of the mechanism generating the receptor potential, and we hypothesize that light acts to change the electrogenicity of this pump.

b. Professor Brown and John F. Nolte have continued investigation of the median ocellus of Limulus. Three types of receptor cells have been found: (i) approximately 40% of the cells depolarize to a flash of visible light, and initially hyperpolarize to a flash of ultraviolet light. At the end of the stimulus, membrane potential rapidly returns to resting potential. An ultraviolet stimulus presented during a steady-state response to visible light produces an initial hyperpolarization followed by a further depolarization. (ii) Approximately 50% of the cells depolarize to UV and initially hyperpolarize to visible

The action spectra for these events (and those in (i)) peak at 360 nm and 525 nm, respectively, and are similar in shape to the absorption spectra for rhodopsins. Unlike the "visible-type" cells, these cells have a very long afterdepolarization to a bright UV stimulus which sometimes lasts for several minutes. During this afterdepolarization, the membrane is repolarized to resting level by presentation of visible light, and remains at the resting level when the light is removed. Also, a visible stimulus presented during a steady-state response to UV elicits a hyperpolarizing potential change across the membrane. (iii) The small number of remaining cells depolarizes to both visible and UV light; its action spectrum is broad and rather flat.

We have still found no electrotonic coupling between receptor cells in the ocellus. Nor have we found a reversal potential in the physiological range for either the initial-hyperpolarization or the repolarization phenomena. We propose that there are two photopigments acting on the mechanisms responsible for light-elicited potential changes in the same photoreceptor cell, and perhaps these responses are generated, at least in part, by an electrogenic pump mechanism.

J. E. Brown

A. INFLUENCES ON AXONAL CONDUCTION

The results of this investigation are part of the author's doctoral thesis, which will be presented to the Department of Biology, M. I. T., in May 1969. The chief findings are summarized in this report.

The conduction of impulses in the primary afferent fibers to the lumbar spinal cord is not 100% "safe." Some of the impulses are blocked in the region of the dorsal root entry zone; others may be blocked elsewhere.[1,2] This blocking has been studied in two sorts of preparation. In the first the recurrent collaterals, first described by Barron and Matthews,[3] served to indicate the sorts of anomalies in conduction that might be expected of branched axons in situ (Fig. XXVIII-1. A). The second preparation restricted investigation strictly to the well-known axial bifurcation of primary afferent fibers near the dorsal root entry zone (Fig. XXVIII-1. B).

All animals considered in this preliminary summary were under barbiturate anesthesia. Work on decerebrate preparations is in progress.

Recurrent collaterals were supposed by Barron and Matthews to be branches of primary afferent fibers that steered themselves outward from the dorsal column toward the periphery along the dorsal roots. Subsequent investigators have dealt harshly with this notion.[4-6] Recently, Scheibel[7] has reported the presence of such fibers in Golgi sections of cat spinal cord. Our results confirm the existence of short latency, bidirectional, "single-fiber" conduction paths between carefully selected pairs of rootlets. For these and other reasons, we have assumed that a continuous axonal connection exists in such cases.

Gaps in the reception of a continuous, supramaximally stimulated spike train occur in most of the fibers that have been studied. This is the intermittent conduction of Barron and Matthews. Such blocks may remove from one to ten or more spikes and
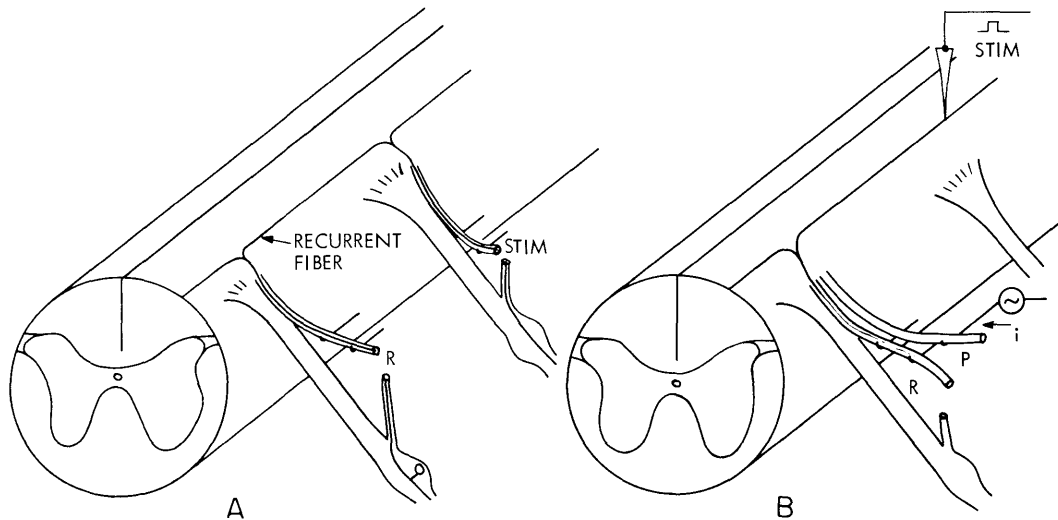
Fig. XXVIII-1. A. Showing a recurrent fiber running from one dorsal root to another. It has been severed from its cell body at the recording electrodes, R, and a rootlet in another root has been found that will stimulate the fiber at R only.

B. Showing a stimulating electrode in the dorsal columns at S, recording electrodes at R, and a polarizing electrode at P that is attached to a sine-wave current generator used either to inject or withdraw current very near those of rootlet R.

depend in surprising ways on the activity in adjacent afferent fibers.

Salient characteristics of this blocking are listed as follows:

1. The blocking is central; it occurs when the impulse is within the central nervous system and never in either of the rootlet extensions.

2. The degree of blocking depends to some extent on the frequency of stimulation. Sustained frequencies above 250/sec lead to sustained block, or to a large increase in the amount of time during which the fiber is blocked (Fig. XXVIII-2. A). Individual impulses can block at frequencies of 10/sec or lower. A period of "rest" permits fibers to conduct continuously for longer intervals (sometimes several minutes) before blocking begins.

3. In lively preparations blocking is more, rather than less, common.

4. Gross stimulation of adjacent rootlets causes a block to occur.

5. "Natural" stimulation (muscle stretch or touch) of the ipsilateral hindleg either reduces or enhances the tendency to block. The more dorsal roots left intact near the fibers, the stronger the effects of peripheral stimulation are likely to be. The control of blocking is quite dramatic; in several cases one portion of the leg would cause immediate blocking of an electrically stimulated spike train, whereas another portion of the leg would release that block and inhibit the formation of another.

6. Artificial polarization of the cord by applying current through a μ electrode is
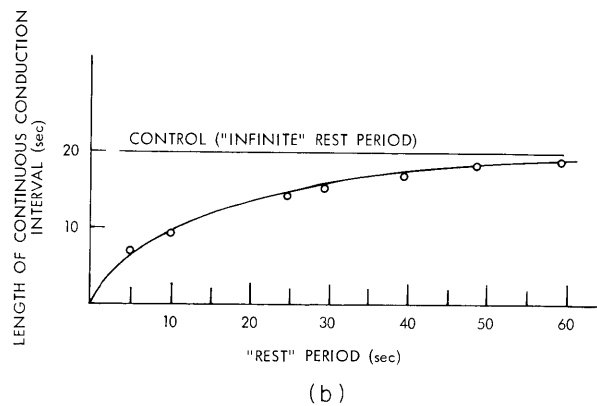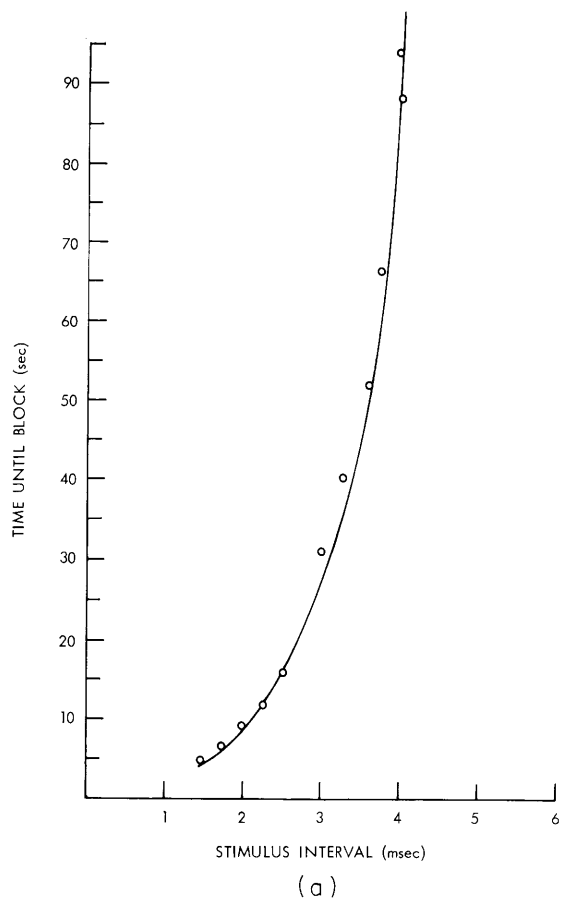
Fig. XXVIII-2. A. Relation between stimulus frequency and time to develop sustained
block. (Three-minute rest between trials.)
B. Relation between duration of rest period and the interval of continu-
ous conduction before sustained block is re-established. All points
were obtained at stimulus frequency of ~350/sec.

an effective way to control blocking. Currents of less than 1 μA are sufficient, and the areas of greatest sensitivity to current control are the dorsal root entry zones of the twin rootlets.

We believe that the block occurs at bifurcations in the primary afferents. The architecture of fibers connecting two dorsal roots is not known, but in primary afferent fibers, and in other systems, strong evidence that the branch points are regions of low conduction "safety" has accumulated.[8, 9, 1, 2] The characteristics mentioned above indicate the importance of "external" influences in controlling the passage of an impulse through the region of the block. The polarization sensitivity (see item 6) and the timing of the blocks following lumped stimulation of adjacent dorsal roots (see item 4) implicate the dorsal root potential[1] as the external sign of the membrane voltage shifts associated with the change in safety factor. The specificity apparent in controlling the block from the
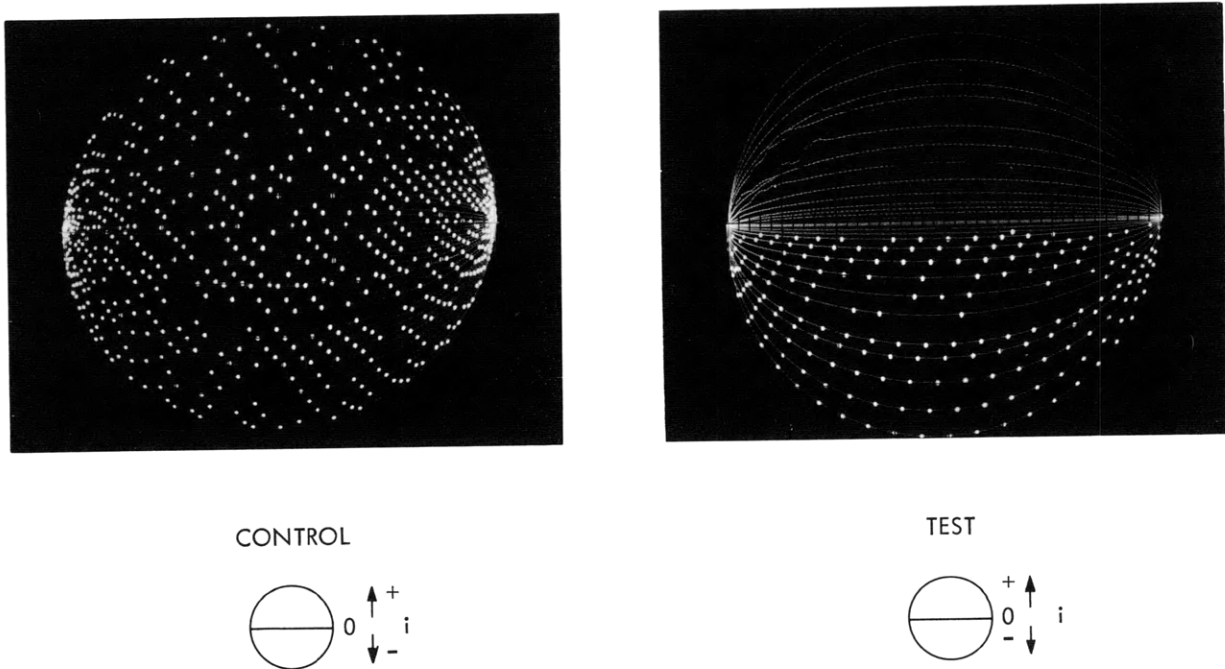

CONTROL

TEST

Fig. XXVIII-3. Showing the ability of various amplitudes of polarizing current applied at P in Fig. XXVIII-1 to modify the "success" of impulses initiated at S in Fig. XXVIII-2 in getting through the large branch near the dorsal root entry zone. The y axis is current magnitude with 0 current at the horizontal diameter. Maximum current is 8 μA, and the scale is linear. The x axis is varied by a sine wave 90° out of phase with that of the y axis, in order to spread out the data points. The control is just like the experimental situation, but the current generator is turned off so that no current flows down the polarizing root. As a result, it is clear that the test picture shows initiation of block (no dots) during + current, and release of block (continuous success) during – current. (Plus current is in the direction of positive charge flowing into the cord down the polarizing root.)

periphery differs from fiber to fiber, but some of the fibers had quite precise natural peripheral zones that controlled their blocking.

Can physiological events such as touching the skin or moving the legs influence axonal conduction in other situations? The answer seems to be in the affirmative. Results from antidromic stimulation in dorsal columns (Fig. XXVIII-3) show that even this very "safe" branch shows a reliable variation in the number of impulses passing through it when current is applied on adjacent rootlets. Although natural stimulation also influences the conduction between the dorsal columns and the dorsal roots, it has not yet been possible to rule out relative movement of the stimulating electrode as an artefactual cause of this effect. The polarization experiments, however, show beautiful modulation of the success factor at currents much lower than 1 μA. Potentials generated at such low currents are generally smaller than physiologically generated dorsal root potentials.

The closer the applied current is to the presumed branch point between ascending dorsal columns and dorsal root, the more effective it is. Whether or not + or - currents will cause or release the block depends on the state of the animal. In general, + currents block and - currents seem to alleviate blocks, although the opposite is the case in depolarized cords (anoxia, high K^+). We are checking this point to determine if the geometry of the terminals of the primary afferent is such that optimal penetration of an impulse from the parent axon into the terminals might depend on slight and local shifts in the transmembrane voltages — shifts that could be induced by activity in adjacent axons or cells, or, even more fascinating, shifts depending on prior activity of the fiber itself.

S. A. Raymond, J. Y. Lettvin

References

1. B. Howland, J. Y. Lettvin, W. S. McCulloch, W. H. Pitts, and P. D. Wall, "Reflex Inhibition by Dorsal Root Interaction," J. Neurophysiol. 18, 1-17 (1955).

2. D. P. C. Lloyd and A. K. MacIntyre, "On the Origins of Dorsal Root Potentials," J. Gen. Physiol. 32, 409-443 (1949).

3. D. H. Barron and D. H. C. Matthews, "Intermittent Conduction in the Spinal Cord," J. Physiol. 85, 73-103 (1935).

4. J. S. Habgood, "Antidromic Impulses in the Dorsal Roots," J. Physiol. 121, 264-274 (1953).

5. J. F. Toennies, "Reflex Discharges from the Spinal Cord Over the Dorsal Roots," J. Neurophysiol. 1, 378-390 (1938).

6. J. F. Toennies, "Conditioning of Afferent Impulses by Reflex Discharges over the Dorsal Roots," J. Neurophysiol. 3, 515-525 (1939).

7. A. B. Scheibel, Personal communication, 1968.

8. F. T. Dun, "The Delay and Blockage of Sensory Impulses in the Dorsal Root Ganglion," J. Physiol. 127, 252-264 (1955).

9. K. Krnjevic and R. Miledi, "Failure of Neuromuscular Propagation in Rats," J. Physiol. 140, 440-461 (1958).

B. DISCHARGE CHARACTERISTICS OF FROG'S "OFF" FIBERS

We have been studying the discharge characteristics of the "dimming detectors" in
frog's optic nerve. These fibers discharge very actively when the retina is dark-
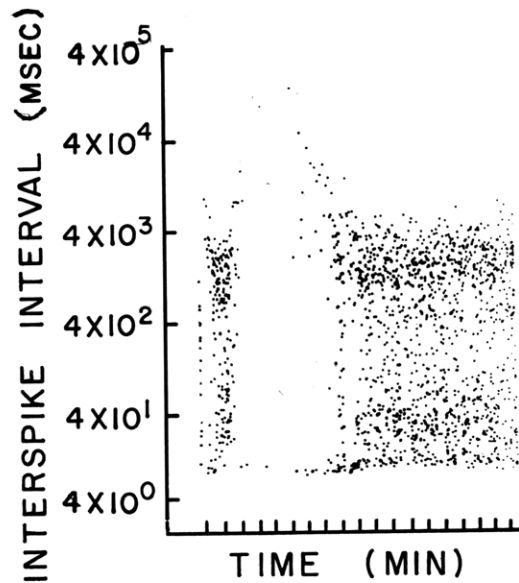adapted, and exhibit a prolonged, rhythmic response to a brief flash of light. Upon

Fig. XXVIII-4.   Prolonged, rhythmic discharge following a
brief flash of light.

cessation of illumination, there appears a period of active discharge, lasting a few
seconds to several minutes, depending on the stimulus parameter. Thereafter, the
fiber goes into a long period of inhibition before it returns to the resting level. These
processes are illustrated in Fig. XXVIII-4 where the logarithms of pulse intervals
are plotted against time.

The most salient feature of these cells is the prolonged inhibition that, under
certain conditions, lasts up to three hours. We believe that there is a direct relation-
ship between this prolonged inhibition and the regeneration of rhodopsin. Evidence for
this is based on the following observations.

1. The time of return to the resting discharge rate in the dark is a linear function
of the amount of light that had impinged on the retina (see Fig. XXVIII-5).

2. The recovery process from the inhibition is closely related to the sensitivity
changes in the dark. In Fig. XXVIII-6 the average discharge rate after the retina is
completely light-adapted is plotted against the time in the dark (filled circles). Super-
imposed on this curve are the threshold measurements obtained at fixed intervals
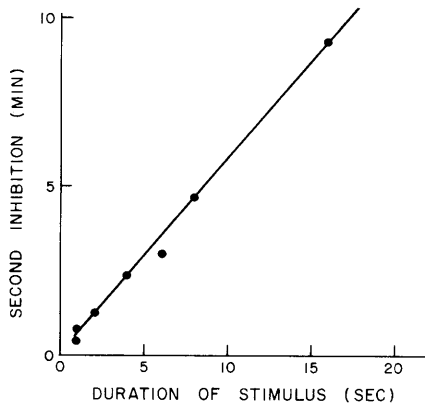
Fig. XXVIII-5.

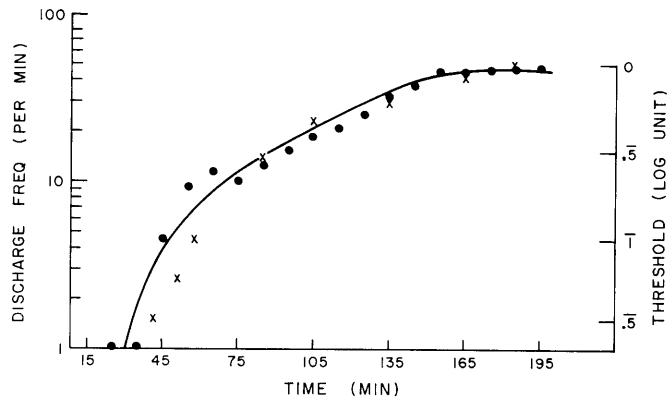Inhibition as a function of the stimulus duration.

Fig. XXVIII-6.

Recovery process of an "off" unit (filled circles), and the sensitivity changes in the dark (crosses).

during the adaptation (crosses). The threshold is defined as the minimum amount of light required to elicit "off" responses.

3. There is a linear relationship between rhodopsin concentration in the retina and the log threshold. This relationship has been convincingly demonstrated by Rushton in man, and by Dowling and Wall in rat.

4. Finally, the time course of rhodopsin regeneration in the frog's retina closely approximates that of the sensitivity change in the dark and of the recovery process of the "off" units.

Thus, there appears to be an intimate relationship between pigment concentration, the discharge frequency of "off" units, and the sensitivity of the retina. While the pigment is regenerating, the discharge frequency increases and the sensitivity improves. When the regeneration stops, the firing rate returns to normal and the improvement in sensitivity also ceases.

S. H. Chung, J. Y. Lettvin

C. ACTION OF TETRODOTOXIN ON FROG'S MUSCLE SPINDLE

The study described briefly in this report was conducted by Professor David Ottoson, of the Royal Veterinary College, in Stockholm, Sweden, and myself, late last year. A detailed account of our experiments will appear in Acta physiol. Scand.

Tetrodotoxin a concentrations of 0.8 to 1.0 $\times$ 10^{-7} g/ml completely blocked conducted activity of the afferent nerve of isolated frog muscle spindle without affecting the receptor potential. The blocking action of tetrodotoxin, however, was found to be influenced by changes in external calcium concentration. We could demonstrate this interaction by comparing the time course of the poison's blocking action at different concentrations of calcium in the extracellular medium. A fivefold increase in external calcium concentration produced a significant decrease in the blocking action of tetrodotoxin. Contrariwise, the effectiveness of the poison in blocking the conducted activity was enhanced when the bathing solution was decalcified. Representative findings are illustrated in Fig. XXVIII-7. In this experiment, the time course of the reduction in the amplitude of the spike after application of the toxin (2 $\times$ 10^{-8} g/ml) was determined (filled circles.) After recovery, the preparation was bathed with a calcium-free solution plus EDTA for a period of 7 min. With reapplication of the same concentrations of the toxin, the impulse decreased rapidly in amplitude in the first 5 min, and then declined more gradually (open triangles.) The response remaining after 10 min represents the underlying receptor potential. The blocking action of tetrodotoxin was appreciably
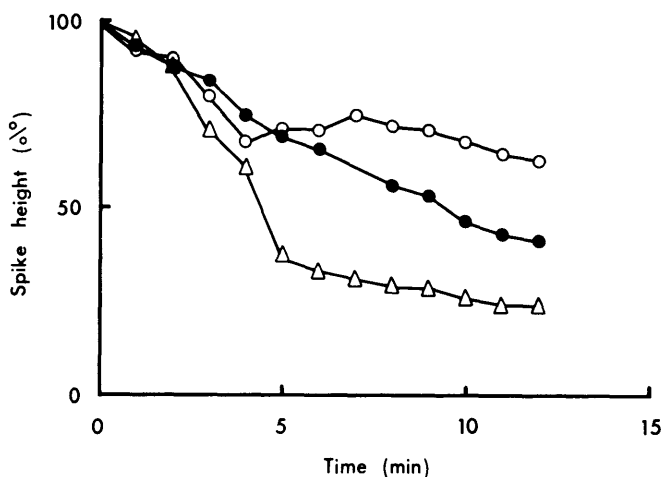

Fig. XXVIII-7. Effect of calcium on the blocking action of tetrodotoxin. Decrease in amplitude of spike evoked by given test stretch after application of tetrodotoxin at a concentration of 2 $\times$ 10^{-8} g/ml in normal Ringer (filled circles), in Ringer with five times normal calcium concentration (open circles), and in Ringer without calcium (triangles).

delayed when the calcium concentration in the bathing solution was increased fivefold (open circles).   Thus it appears that both calcium and tetrodotoxin act on the same site (or "carrier") that controls the early transient current.
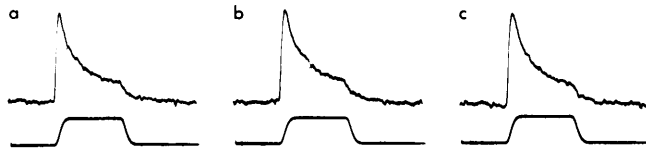

Fig. XXVIII-8.   Effect of tetrodotoxin on the receptor potential.   Records of receptor potential after treatment of spindle for 20 min with tetrodotoxin at a concentration of 2×10^{-8} g/ml (a), 1×10^{-7} g/ml (b), and 1×10^{-6} g/ml (c). Note that the size of the receptor potential remains unchanged.

Tetrodotoxin had no significant effect on the receptor potential, as illustrated in Fig. XXVIII-8.   Record a shows the responses obtained after blocking the conducted activity with tetrodotoxin at a concentration of 2×10^{-8} g/ml.   The concentration was then increased to 1×10^{-7} g/ml and 1×10^{-6} g/ml in records b and c, respectively. A close inspection of the records shows that the receptor potential was not affected by the toxin in concentrations that were from 5 to 50 times greater than those required to block the conducted activity.   That receptor potential remained unaffected by tetrodotoxin indicates that the sodium-carrying system of the receptor membrane is different from that of the membrane producing the propagated action potential.

S. H. Chung

D. TEGMENTAL AUDITORY FIBERS OF RANA PIPIENS

In collaboration with Dr. S. H. Chung, I have been investigating some of the aspects of the functional organization of the auditory system of Rana pipiens. Experiments are performed in a soundproof chamber; stimuli are provided by an audio-oscillator signal passed through an electronic switch to a loudspeaker, or by tape-recorded R. pipiens croaks. The frogs are curarized, the nervous system intact, and extracellular records are taken, with platinum-plated metal-filled glass microelectrodes having a tip diameter of 1-3 microns.

The investigation is still in its preliminary stages; we have characterized 25 single units. Spikes are generally triphasic, and latency measurements suggest that we are recording from fibers of cells that are of higher order than those of the medullary nuclei. The units appear to fall into two major functional groups: (a) those that respond poorly to croaks have a unimodal tuning curve with a maximum at approximately 400 Hz, and often detect seismic vibration also; and (b) those that respond well, or best, to croak stimuli. The latter generally respond to frequencies in the range 500-2000 Hz, and may be further classified into four types of units.

1. Cells of this type have little spontaneous activity. They have a unimodal tuning curve with maximum sensitivity at 600-700 Hz. They respond to the onset of a tone with a burst of spikes, generally giving a more active response to a louder tone. They adapt rapidly and do not respond to cessation of the tone.

2. These cells appear to be similar to type 1, but their maximum sensitivity lies at higher frequencies (1000-1500 Hz).

3. These cells also respond to the onset of a tone with a burst of spikes, adapt to prolonged tones, and do not respond to the cessation of the tone. They are characterized, however, by a bimodal tuning curve with maxima at 700 and 1500 Hz, and a minimum at 1400 Hz.

4. Units of this type have a high level of spontaneous activity, and are sensitive to frequencies at 500-2000 Hz. They respond to the onset of a tone in this range with an increased activity that adapts somewhat, but is maintained even in the presence of prolonged continuous stimulation. Cessation of the tone results in complete abolition of activity that may last for several hundred milliseconds.

The R. pipiens mating croak lasts for several seconds and is composed of 50-msec tone bursts with most of the energy at approximately 700 Hz and 1500 Hz. It appears, therefore, that the major portion of tegmental auditory fibers acts as a matched filter for the species' specific recognition of mating croaks.

<div align="right">R. E. Greenblatt</div>

E. APPARATUS FOR THE ACCURATE DETERMINATION OF
FLANGE FOCAL DISTANCE

The distance from the flange of a lens to its focal plane is called the flange focal distance; it is usually specified for the case of the conjugate focus at infinity. The accurate determination of this distance is important in many optical and photographic experiments. Conventional means for making this measurement utilize optical benches, autocollimators, or other expensive special-purpose apparatus. The method that we describe in this report has two advantages over previous methods: (a) it is designed to make direct use of the techniques and tools of dimensional metrology, and (b) it is free from the possibility of observational errors, resulting either from subjective factors or the state of ophthalmic refraction of the observer. Our method makes use of a bifurcated, randomly intermixed fiber optic light guide, and is an adaptation of a more general principle recently developed by the precision tool industry for extremely sensitive non-contacting measurement of linear displacements.

In Fig. XXVIII-9 we show the apparatus, which includes a light source, and a bifurcated light guide with one set of fibers going to the light source, and the other to a photomultiplier tube. The lens under test is mounted with its infinity conjugate pointing upward, in a precision steel flange. The flange, in turn, is mounted in a larger precision ring stand, supported by three stacks of gauge blocks, above a surface plate. The lens mounting flange is centered by removable pins. Flanges specific to the commonly used photographic lens mounts are machined, and have the same outer diameter. The construction is such that all parts can be finished by surface grinding to a flatness and parallelism of 0.0001 inch.

The fiber optic light guide, available on special order from the manufacturer, is prepared by randomly intermixing as thouroughly as possible two sets of glass fibers; these are epoxied into metal mounts, and the end surfaces are ground and polished flat. The double end of this assembly is held by set screws in a steel fixture, which is attached to a micrometer, thereby permitting vertical travel in measured increments of 0.0001 inch. In Fig. XXVIII-10 we show a cross section of the double end of our best fiber optic bundle using 3-mil fibers. The illumination of the separate sets of fibers was such that one set appears white, the other gray; broken fibers appear black.

The operation of this measuring instrument depends on this fact: When the end surface of the fiber optic guide is located coincident with the focal plane of the lens, the action of the lens and retroreflector is such as to reimage the illuminated fibers back onto themselves. Thus a minimum of light diffuses into the second, neighboring set of fibers connected to the photomultiplier. If, however, the end surface of the fiber optic guide is located a small distance either above or below the focal surface of the lens, the image recreated by the lens and retroreflector will be located the same distance below

RETRO-REFLECTIVE PRISM

LENS UNDER TEST

PRECISION FLANGE
(SPECIFIC TO LENS MOUNT)

DOWEL
PINS

BIFURCATED
FIBRE-OPTIC
LIGHT GUIDE,
GROUND AND
POLISHED
FLAT

TO PHOTOCELL
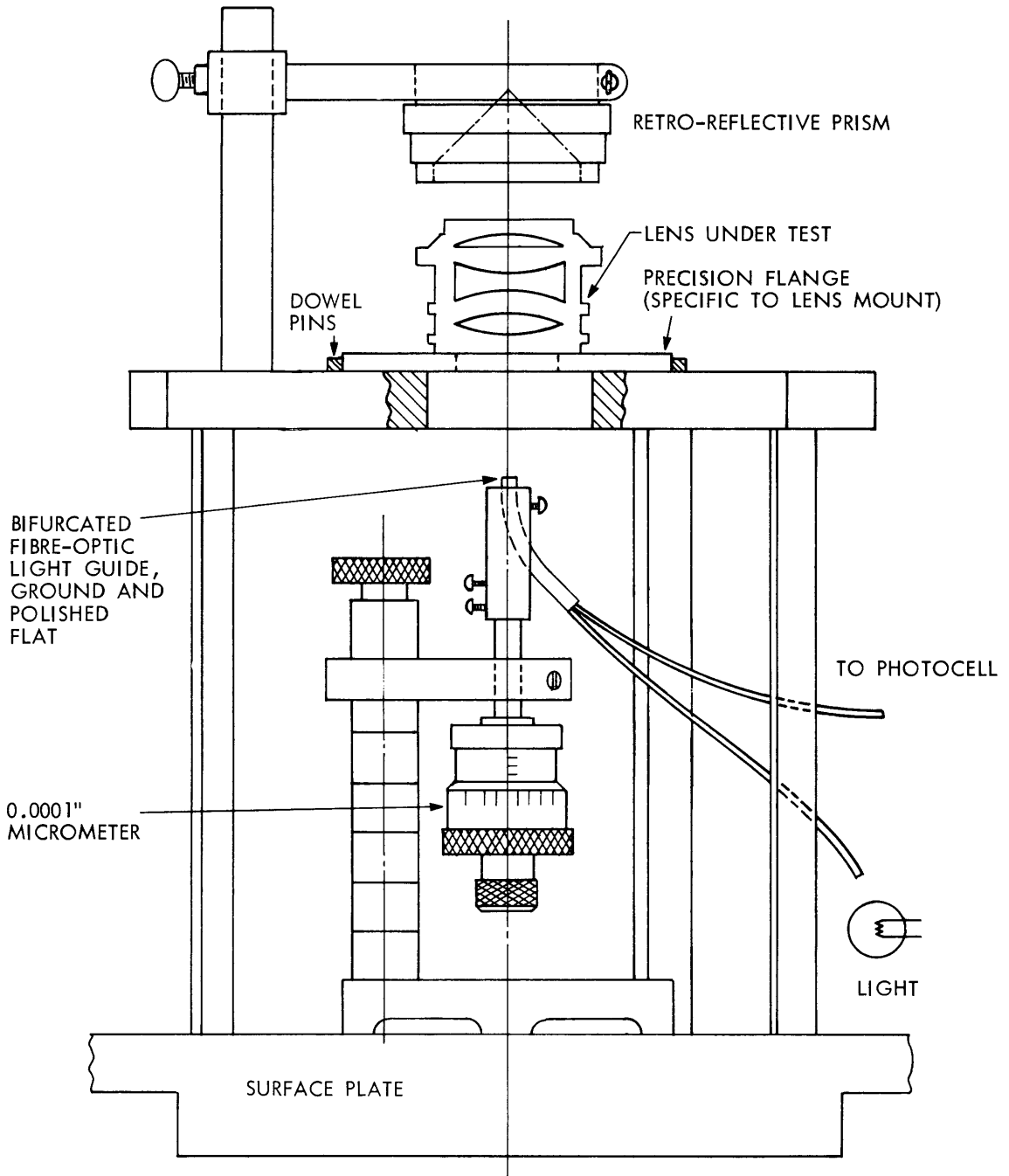
0.0001"
MICROMETER

LIGHT

SURFACE PLATE

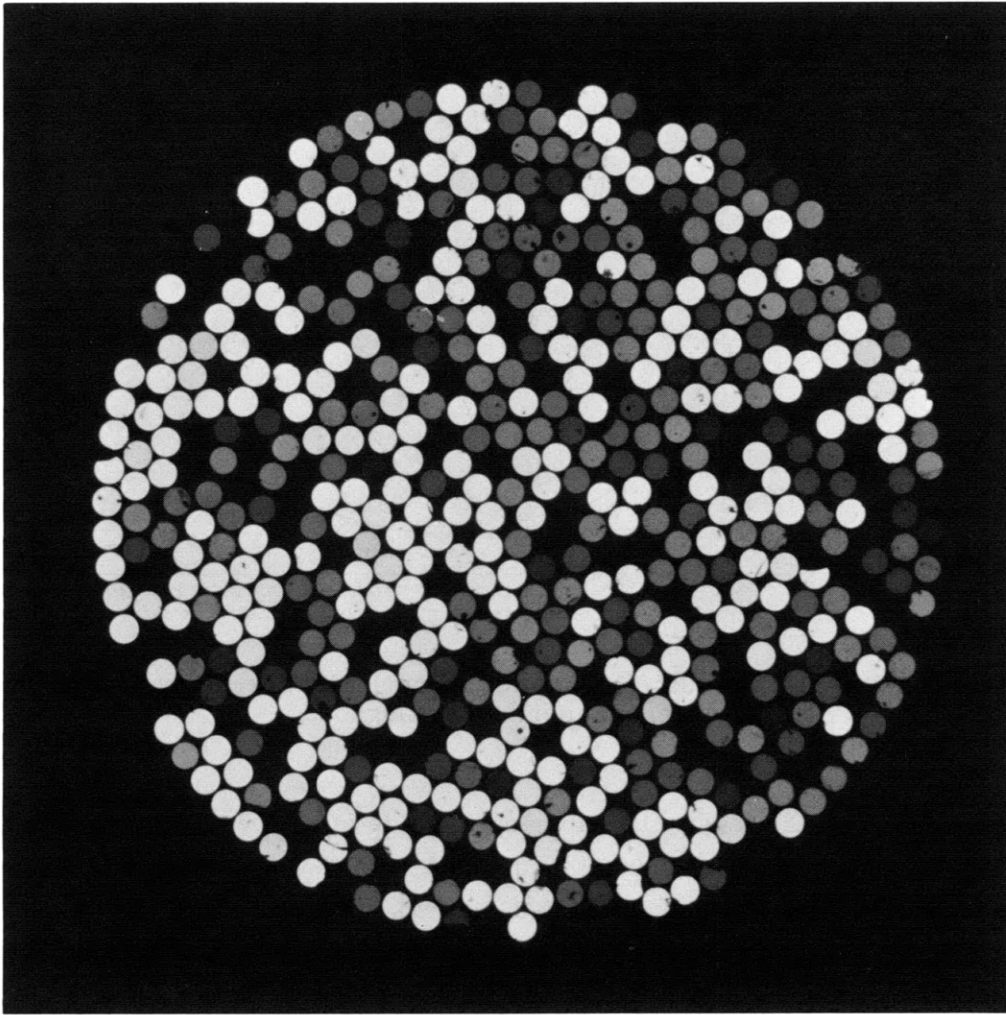Fig. XXVIII-9.   Apparatus for determining flange focal distance.

Fig. XXVIII-10.   Cross section of fiber optic bundle, illuminated to differentiate 2 sets of intermixed fibers (3-mil fibers).   White, "A".   Grey, "B".

or above the focal surface, respectively. As a consequence of the vertical displacement, light returned by the lens and retroreflector spreads into the second set of fibers, and is transmitted to the photomultiplier.

This action is illustrated in Fig. XXVIII-11, which shows the photomultiplier output plotted against vertical displacement for a 50 mm, f/1.2 lens of excellent quality and aspherically corrected.   The sharpness of the minimum in light output is seen to be an inverse function of the lens aperture setting; at full aperture the light output doubles with a 1.5-mil displacement.   Measurements of the minima of these curves could be repeated to 0.0001 inch (2.5 μ).

With the micrometer set for minimum light output, the distance from the plane of
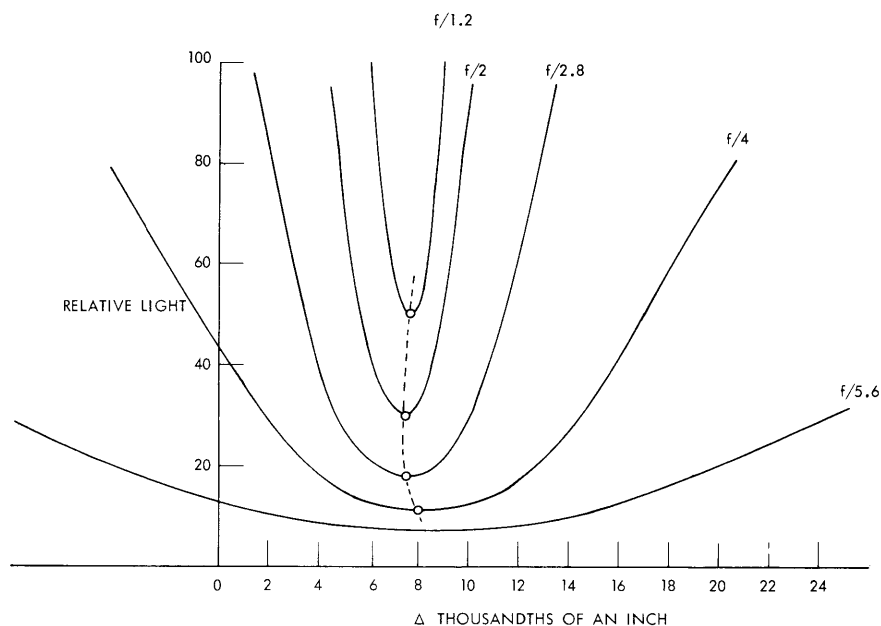
Fig. XXVIII-11. Results of test with aspherically corrected 50-mm lens at different aperture settings.

the flange supporting the lens to the fiber optic guide is easily measured with a precision height gauge to an accuracy of 0.0001 inch. The resultant uncertainty of flange focal distance is, therefore, of the order of 0.0002 inch, or 5 μ.

As expected, sharper minima were obtained with monochromatic illumination, by using interference filters interposed at the light source. With the aid of a set of such interference filters, we obtain directly the plot of axial chromatic aberration as a function of wavelength.

The test results of Fig. XXVIII-11 were measured with a lens that had 2 aspheric elements and was almost completely corrected for spherical aberration. Most photographic objectives of good quality are well corrected for this defect, and there will usually be minor ambiguity in the measurement of flange focal distance by this method. Lenses afflicted with appreciable amounts of spherical aberration will, of necessity, present problems in the location of the focal surface by this method; the curve of light output vs displacement will be asymmetric, and the position of the minimum will be affected by unevenness in light distribution over the aperture of the lens.

The accuracy of the elements of the apparatus (Fig. XXVIII-9) can be checked in an inspection department, save for the retroreflective prism. Defects in this element, for example, a spherical curvature of the front face, would affect the measurement of flange focal distance. The preferred method for checking corner-reflecting prisms, which was demonstrated to us at the Metrology Section of the National Bureau of Standards,

is by means of a Köster's prism, a form of white-light interferometer.

In a variation of the experimental arrangement, the beam emerging from the branch of the fiber optic light guide is projected onto an opal glass diffusing screen, located a few inches away. In this operating mode, the defects of the lens, including zonal and chromatic errors, can be qualitatively assessed — the apparatus acts as a projection microscope. This is a consequence of the geometry of optic fibers, by which the angle of emerging rays measured from the axis is equal to the angle of entering rays, again measured from the axis. In this form, the apparatus is suitable for classroom demonstration of axial lens aberrations.

Possible future applications of the method include the measurement of spherical aberration of microscope lenses, and of microscope lens systems. For this case, it will be desirable to have a more sensitive mechanism for moving the fiber bundle, and also a bundle of smaller fibers having a smaller total diameter. This last requirement suggests the possibility of fusing one of the present bundles of larger fibers, and then drawing it down to the requisite size by controlled heating. Another line of endeavor that would apply to these techniques would be development of means for preparing ordered arrays of intermixed fibers.

B. Howland, A. F. Proll

[Bradford Howland and Arthur F. Proll are Staff Members of Lincoln Laboratory, M.I.T.]

F. AN EFFECT OF LIGHT ON $^{42}K^+$ EFFLUX FROM LIMULUS VENTRAL EYE

We have begun a study of the ion fluxes underlying the receptor potential in the ventral eye of <u>Limulus</u> <u>polyphemus</u>.[1,2] Previous work on the physiology of this eye[1-3] has been done with microelectrode techniques that permit the measurement of membrane potential and membrane resistance. Such electrical measurements, however, do not afford unambiguous interpretation and quantification of movement of the various ionic species. These studies are intended to obtain information about the ionic fluxes responsible for the light responses of the photoreceptor cells.

In each experiment, a single ventral eye (or so-called lateral olfactory nerve[4]) was excised, desheathed, and mounted in a capillary tube. The tissue was incubated in artificial sea water,[5] which was drawn into the capillary tube; the sea water contained[35] $SO_4^=$ and either $^{42}K^+$ or $^{24}Na^+$. At the end of an incubation, the radioactive sea water was discharged from the capillary, and the tissue was perfused at approximately 1 ml/min with nonradioactive artificial sea water; the effluent was collected in a fraction collector.

Table XXVIII-1. Effect of pronase treatment on release of ^{35}S and ^{42}K.

Experiment	Time of fraction ending	cpm in fraction	
		^{35}S	^{42}K
1. No pronase	0. 6 min	283,830	141,200
	1. 0	79,780	39,410
	1. 5	47,000	19,050
	3. 0	29,920	9,526
	6. 0	4,056	3,956
	9. 3	1,134	3,048
2. Pronase treatment	3. 0 min	62,477	185,000
	6. 0	12	2,365
	9. 0	3	1,902
	12. 0	4	1,540

Experiment 1: The eye was incubated for 40 min in sea water containing 70 µC/mil $^{35}SO_4^=$ and 47 µC/ml $^{42}K^+$.

Experiment 2: The eye was incubated for 20 min in sea water containing 50 µC/ml $^{35}SO_4^=$ and 55 µC/ml $^{42}K^+$. Time zero is at the beginning of efflux.

The radioactivity in each sample was determined simultaneously for both isotopes on a liquid scintillation counter. The stimulating light was a focused, tungsten filament source; the unattenuated stimulus was 3×10^5 ergs cm^{-2} sec^{-1} between 380 nm and 750 nm. To produce flashing light, the source was shuttered by a sector wheel to produce periods of light and dark of equal duration at a frequency of 16 per sec.

The $^{35}SO_4^=$ content in the effluent samples decreased exponentially, with a half-time of less than 1.0 min (Table XXVIII-1). The $^{42}K^+$ content in the effluent drops as rapidly as that of the $^{35}SO_4^=$ at first, but then falls more slowly, with a half-time of approximately 20 min [Table XXVIII-1 (1)]. Since electrophysiological measurements [2,6] indicate that $SO_4^=$ does not cross the cell membrane of the photoreceptors, we assume that the $^{35}SO_4^=$ occupies only extracellular space in the tissue. The diffusion constants for $SO_4^=$ and K^+ in aqueous solutions are approximately equal; hence, we assume that the ratio of activities of $^{35}SO_4^=$ and $^{42}K^+$ in the extracellular space of the tissue are the same as in the labelled sea water. Knowing the amount of $^{35}SO_4^=$ in a given sample, we can calculate the contribution to that sample of $^{42}K^+$ eluted from the extracellular space. The results show that the $^{42}K^+$ eluted between 3 min and 6 min is approximately 30% extracellular. If, however, the eye is treated with pronase (15 mg/cc for 30-60 sec) before being incubated in sea water labelled with $^{42}K^+$ and $^{35}SO_4^=$, the rate of efflux of the extracellular label is markedly increased [Table XXVIII-1 (2)]. In this case, the amount of labelled $SO_4^=$ and K^+ held in the extracellular space is negligible after 3 min. That the final rate of outflux of $^{35}SO_4^=$ and $^{42}K^+$ can be made to differ so greatly by pronase treatment strongly supports the assumption that $SO_4^=$ does not enter the intracellular compartment of the tissue. Electrophysiological measurements show that such pronase treatment in no measureable way impairs the function of the photoreceptors.[6]

When the eye is illuminated with an unattenuated stimulus, the rate of $^{42}K^+$ release from the intracellular compartment increases abruptly by a factor of 1.8. This is shown by the increased content of $^{42}K^+$ in the first sample after the stimulus (Fig. XXVIII-12). The effect can be reproducibly obtained over a period of 10 h, and is unaffected by the pronase treatment. The increase is due to the visible light (380-750 nm); much higher total energy at wavelengths longer than 750 nm does not produce the effect. The effect is essentially the same if either steady or flashing light (at 16/sec) is used as the stimulus. Moreover, a response can be obtained with at least a 1000-fold decrease in illumination (Fig. XXVIII-12).

By varying the labelling period before beginning the efflux of $^{42}K^+$, we have found that a labelling period of approximately 40 min is saturating. The intracellular K^+ held in such an eye divided by an approximate tissue volume $(4 \times 10^{-8}$ l) gives an intracellular K^+ concentration of 360 mM. The figure is likely to be in error, because of the difficulty in estimating tissue volume precisely; however, it is in reasonable agreement with the K^+ concentration estimated electrophysiologically, and suggests that all of the cells
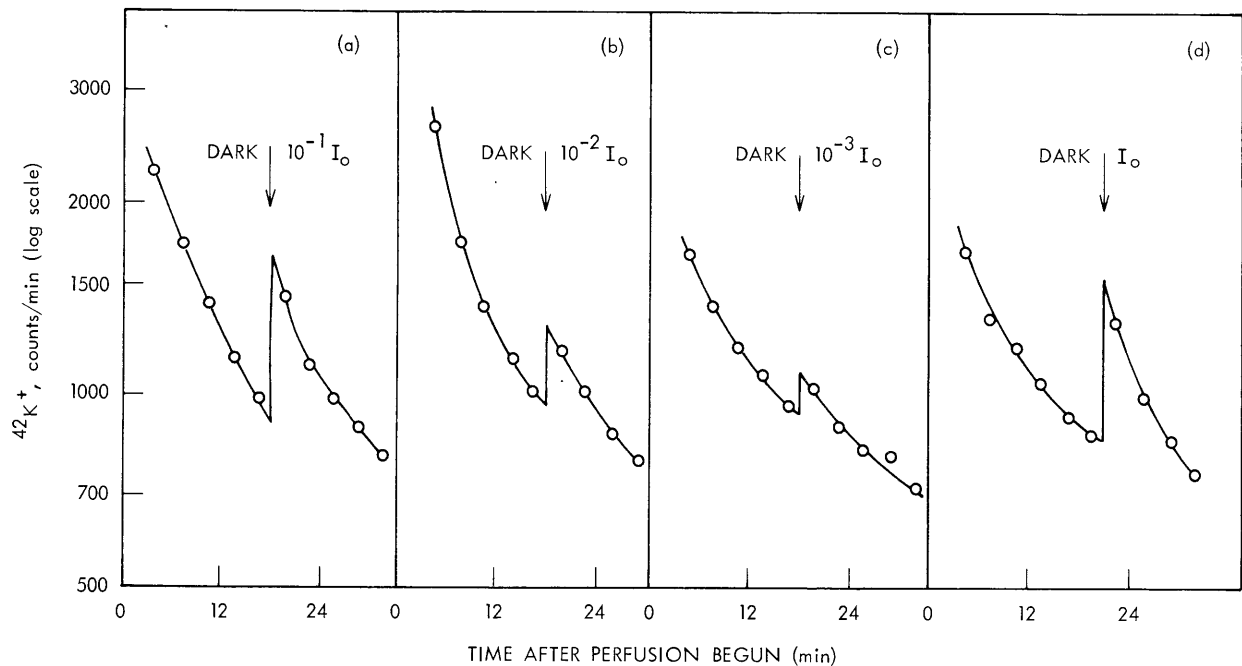
Fig. XXVIII-12.   Light-induced ^{42}K flux changes as a function of light intensity.   Each panel, (a)-(d), represents an experiment in which an eye was treated with pronase, labelled in $^{42}K^+$ sea water for 10 min and then rinsed with unlabelled sea water, initially in the dark and then in visible light of the intensities shown. Each point on the graph represents the radioactivity, plotted on a logarithmic scale, in the sea water collected during a 3-min interval. Arrows indicate the times when the light was turned on. Points are plotted at the center of the relevant time intervals. One eye was used for the series of experiments, which were performed in the order (a)-(d) as shown. The first fraction (not plotted) collected after each loading contains approximately 3×10^5 cpm of $^{42}K^+$. I_o is approximately 3×10^5 ergs cm^{-2} sec^{-1}. The radioactivity measurements have been corrected for decay.

are involved in the observed uptake.

We have found that the determination of the efflux of $^{24}Na^+$ by the technique employed above probably cannot be done without the use of pronase. With a large extracellular compartment that holds Na^+ at 10 or more times the concentration of the intracellular compartment, the intracellular contribution to efflux of Na^+ from the tissue is masked. Pronase may therefore reduce the ^{24}Na held extracellularly and permit a study of cellular sodium fluxes.

J. E. Brown, C. E. Holt III

[Professor Charles E. Holt III is in the Department of Biology, M. I. T.]

References

1. T. G. Smith, W. K. Stell, and J. E. Brown, "Conductance Changes Associated with Receptor Potentials in Limulus Photoreceptors," Science 162, 454 (1968).

2. T. G. Smith, W. K. Stell, J. E. Brown, J. A. Freeman, and G. C. Murray, "A Role for the Sodium Pump in Photoreception in Limulus," Science 162, 456 (1968).

3. R. Millecchia, J. Bradbury, and A. Mauro, "Simple Photoreceptors in Limulus polyphemus," Science 154, 1199 (1966).

4. W. Patten, The Evolution of Vertebrates and Their Kin (The Blakiston Company, Philadelphia, 1912).

5. Composition of artificial sea water: 423 mM NaCl; 9 mM KCl; 9.27 mM $CaCl_2$; 22.9 mM $MgCl_2$; 25.5 mM $MgSO_4$; 2.15 mM $NaHCO_3$.

6. J. E. Brown, J. A. Freeman, G. Murray, T. G. Smith, and W. K. Stell (unpublished results).