Table 1. Effect of extracts of unlabeled HeLa cells on distribution of labeled lymphocyte material in the sucrose gradient. Released polypeptides, 0–45*S*; single ribosomes, 45–130*S*; polyribosomes, 130–300*S*.

HeLa cells added	Radioactivity in sucrose gradients (%)		
	0–45*S*	45–130*S*	130–300*S*
*Released polypeptides**			
0	99	1	0
+	98	2	0
Polypeptides attached to single ribosomes†			
0	18	80	2
+	10	82	8

* The labeled lymphocyte polypeptide chains were obtained from the top of a gradient in which labeled lymphocyte material had been run in the absence of HeLa cytoplasm. † Single ribosomes were obtained from the 60–80*S* portion of the aforesaid sucrose gradient.

the presence of unlabeled HeLa cell cytoplasm and analyzed (legend Fig. 3). Five hours of actinomycin treatment caused a 63-percent decrease in polyribosome-associated protein synthesis, whereas 7 hours of treatment resulted in an 83-percent reduction.

These experiments show that extracts of hyperimmune lymph node cells can cause the breakdown of HeLa cell polyribosomes, presumably by ribonuclease activity which has been detected in lymph node cell lysates. The presence of ribonuclease activity may explain the results of other investigators who have reported that protein synthesis in rabbit spleen cells is associated with single ribosomes and small aggregates (*6*).

When the breakdown was prevented by the addition of a large amount of HeLa cell cytoplasm, nascent C^{14}-labeled polypeptides in hyperimmune lymph node cells were associated with the polyribosome area of sucrose gradients. Two additional facts suggest that the observed radioactivity reflects polyribosome-mediated protein synthesis: (i) when exogenous ribonuclease is added to lymphocyte HeLa cell cytoplasm mixtures there is a shift of TCA-precipitable radioactivity from the polyribosome area of the sucrose gradient to the fractions occupied by the single ribosomes; this suggests that the lymphocyte single ribosomes are held together by a ribonuclease-sensitive structure. (ii) Addition of actinomycin D to cell suspensions causes a marked decrease in incorporation of amino acids in the material in the polyribosome region of the sucrose gradient. This corresponds to the decrease in total protein and γ-globulin synthesis in these cells after actinomycin treatment (*15*).

Since approximately 30 percent of the total protein made by the cells in these experiments is identifiable immunologically as γ-globulin (*15*) and since virtually all nascent protein is formed on polyribosomes, it can be concluded that γ-globulin synthesis occurs on polyribosomes. This was confirmed by immunological identification of γ-globulin polypeptides associated with ribosomes. Thus, the translation step of γ-globulin synthesis occurs on large aggregates of ribosomes held together by a ribonuclease-sensitive structure. This ribonuclease-sensitive structure is presumably mRNA, but this can only be proved by detailed studies of its synthesis and physical-chemical characteristics.

MATTHEW D. SCHARFF
Department of Cell Biology and Medicine, Albert Einstein College of Medicine, Bronx, New York
JONATHAN W. UHR
Irvington House Institute and *Department of Medicine, New York University School of Medicine, New York*

References and Notes

1. J. R. Warner, A. Rich, C. E. Hall, *Science* **138**, 1399 (1962); A. Gierer, *J. Mol. Biol.* **6**, 148 (1963); F. O. Wettstein, T. Staehelin, H. Noll, *Nature* **197**, 430 (1963); E. F. Zimmerman, *Biochem. Biophys. Res. Commun.* **11**, 301 (1963).
2. S. Penman, K. Scherrer, Y. Becker, J. E. Darnell, *Proc. Nat. Acad. Sci. U.S.* **49**, 654 (1963).
3. T. Staehelin, F. O. Wettstein, H. Oura, H. Noll, *Nature* **201**, 264 (1964).
4. J. W. Uhr, *Science* **142**, 1476 (1963); C. J. Wust, C. L. Gall, C. D. Novelli, *ibid.* **143**, 1401 (1964); J. D. Smiley, J. G. Heard, M. Ziff, *J. Exp. Med.* **119**, 881 (1964).
5. G. Acs, E. Reich, S. Vanju, *Biochim. Biophys. Acta* **76**, 68 (1963); M. Revel, H. H. Hiatt, J. P. Revel, *Science* **146**, 1311 (1964).
6. K. H. Stenzel, W. D. Phillips, D. D. Thompson, A. L. Rubin, *Proc. Nat. Acad. Sci. U.S.* **51**, 636 (1964).
7. Obtained from Microbiological Associates, Bethesda, Md.
8. H. Eagle, *Science* **130**, 432 (1959).
9. Algal hydrolyzate obtained from New England Nuclear Corp., Boston, Mass.
10. H. Harris and J. W. Watts, *Proc. Roy. Soc. London Ser. B* **156**, 109 (1962).
11. M. D. Scharff, H. J. Shatkin, L. Levintow, *Proc. Nat. Acad. Sci. U.S.* **50**, 686 (1963).
12. H. S. Slayter, S. R. Warner, A. Rich, C. E. Hall, *J. Mol. Biol.* **7**, 652 (1963).
13. Obtained from Schwarz BioResearch, Orangeburg, N.Y.
14. G. M. Edelman and J. A. Gally, *J. Exp. Med.* **116**, 207 (1962).
15. S. Tawde, M. D. Scharff, J. W. Uhr, in preparation.
16. Supported by PHS grant No. AI-05031-01-A1 and AI-01821-08, by NSF grant GB-984, by the Commission on Immunization of the Armed Forces Epidemiological Board, by the Office of the Surgeon General, Department of the Army, Washington, D.C., and by a PHS Career Development Award to Dr. Scharff. We thank Miss Hannah Goodman and Y. Chinn for technical assistance.

25 January 1965

Respiratory and Electrical Responses to Light Stimulation in the Retina of the Frog

Abstract. *Isolated retinas from frogs' eyes were preserved in a circulating medium; transretinal electrical potential and ultraviolet absorbancy were monitored. In response to visible stimulation, changes in absorbancy were observed which correlate with the c-wave of the electroretinogram. They are tentatively identified as cyclic oxidations of pyridine nucleotides reflecting the energy expenditure associated with evoked neuronal activity.*

The isolated retina in a perfusion system can be kept responsive to adequate stimulation and be subjected to various experimental procedures under stationary conditions for several hours (*1*). Specific requirements for optimum survival, tolerances toward controlled deviations, and changes in the perfusate effected by the retina pointed to the importance of energy yielding processes in the generation of the electroretinogram (ERG) (*2*). Therefore, an analysis for respiratory pigments seemed indicated on account of their role in intermediary metabolic reactions and of the speed of their spectroscopic detectability (*3*).

Diphosphopyridine nucleotide (DPN) was the first pigment sought. Its reduced form, DPNH, exhibits strong absorption at 340 nm with a resulting emission of bluish fluorescence. It was hoped that the analyzing procedure would not interfere with visual excitability (*4*). Preliminary experiments, however, showed that the isolated retina does respond electrically to flashes in the near ultraviolet region of the spectrum. Thus analyzing with beams of low intensities was mandatory. Because many substances in the retina are strongly fluorescent, giving a large background of steady fluorescence, it was not possible to detect changes in DPNH by changes in fluorescence. However, changes in ultraviolet absorption as a result of stimulation in the visible range could be measured easily.

In dim red illumination frog retinas were isolated from opened, submerged (*5*) eye cups and clamped at their peripheries to a tantalum grid which occupied the central opening of a black plastic support (Fig. 1, inset). The support also carried fluid and electrical connections into a modified tissue cul-

ture chamber and divided it into two compartments. Both halves were perfused in series at a rate of approximately 5 ml/min at room temperature. Silver ring electrodes in both compartments served to record the ERG from the bathing fluid.

In the optical system (Fig. 1) the retina was exposed to both an ultraviolet-analyzing and a visible-stimulating (and adapting) beam. From a deuterium arc driven by a stabilized power supply a suitable wavelength was chosen by means of a grating monochromator and directed through the retina onto and filling the head-on surface of a photomultiplier. A cover glass having a known absorption in the wavelength region of interest could be inserted for calibration. In the reverse direction (generally from the vitreous side) the retina could be stimulated and light-adapted. An ultraviolet transmitting filter in front of the photomultiplier and an ultraviolet rejecting filter in the combined path of the visible light prevented the stimulating light from being seen by the multiplier.

The photomultiplier was connected to a photometer (*6*) with highly stabilized high-voltage supply and feedback-amplifier. A compensating current balanced the photocurrent which resulted from the ultraviolet light transmitted during the resting state of the preparation. Hence, only changes in the amount of light received on stimulation appeared at the output.

Photometer output and amplified ERG were displayed on a dual channel, heated stylus recorder. Recorder operation and magnetic timing of stimulation were programmed by a series of wave-form generators. In general, single flashes of one to several seconds, or trains of 1-second stimuli with equal dark intervals were applied every 3 minutes. Stimulus intensities at 574 nm and the intensity of the analyzing beam at 340 nm at the retina were of the order of 10^{-8} watt/cm^2. The retinas were exposed to the ultraviolet light throughout the experimental periods.

Electrically and optically recorded responses to a 6-second light stimulus are shown in Fig. 2*a*. The ERG shows after a short latency a small (vitreous side) negative deflection (*a*-wave) immediately followed by a positive *b*-wave and at the end of the flash a renewed positive reaction (off-effect; *d*-wave). The polyphasic shape of the response can be analyzed into simple components: a delayed monophasic mirror image to the stimulus [negative at on, positive at off; P III according to Granit (*7*)] and positive transients (P II) following either P III-deflection. The proportions of the components are determined by the light situation, P II prevailing in dark adaptation. The adapting effect of the continual ultraviolet irradiation appears to be small. The simultaneously recorded optical response consists of transitory increases of the transmitted ultraviolet light both at onset and end of the stimulus light. Both types of response are graded according to the stimulus parameters and both saturate in unison. The proportions of the optical deflections at on and at off, respectively, appear to be equal to the electrical deflections, their peak values occurring at a later point.

Not reflected in the conventionally capacitor-coupled electrical recording of Fig. 2*a* (time constant: 1 second) are slow components, for example the positive *c*-wave (P I), which accompanies P II and is strongly marked in dark adaptation; P I outlasts short stimuli and exhibits summative behavior. In the experiment of Fig. 2*b* a train of stimuli was followed by an equal period of continuous illumination. The electrical response, recorded with an effective time constant of 10 seconds, displays a high initial b-wave followed by individual ERG's of smaller size superimposed on a positive potential change (P I). On cessation of flicker the electrical trace returns to the base line to show a renewed strong reaction at off. The optical response exhibits summation of transmission increments that occur as long as the flickering of the stimulus light is continued. Steady illumination allows the optical trace to return slowly toward the base line. The renewed reaction at off is dependent in its magnitude on how nearly complete this return is, as is any later evoked electrical or optical response.

Analogous absorption changes on induced physiological activity both in magnitude and direction have been observed in muscle (*8*) and have been interpreted as oxidation of DPNH, initiated by the utilization of adenosine triphosphate with resulting electron flow to oxygen and restoration of high-energy phosphates. Identification in the functioning retina of DPN as the substance undergoing changes in its oxi-

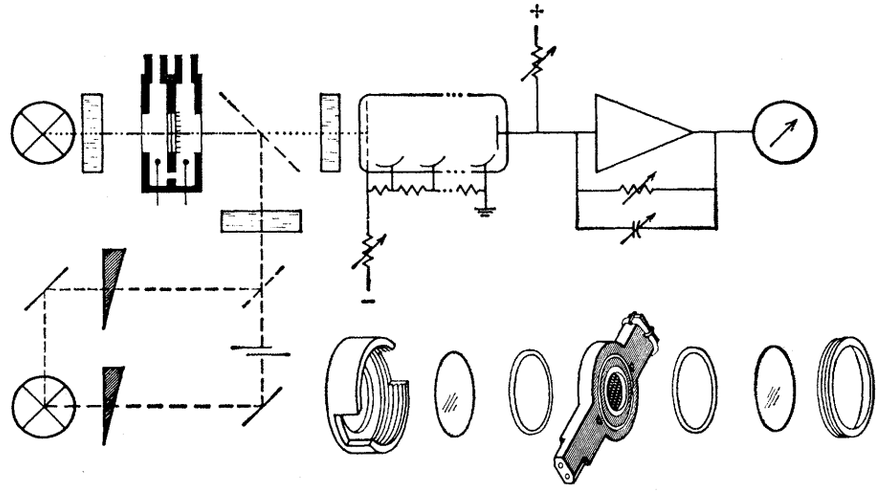

Fig. 1. The optical system. From a deuterium source (upper left, proceeding to the right) an ultraviolet beam after filtering (monochromator) traverses the perfusion chamber (expanded view shown as inset), a semitransparent 45° mirror, an ultraviolet transmitting filter and impinges upon the cathode of a multiplier tube, the photocurrent of which is balanced (positive supply); transients are further amplified and displayed on a recorder (top right). A tungsten filament lamp (lower left) supplies visible light for stimulating (magnetic shutter interposed) and adapting beams which are separately controlled in intensity and jointly band-limited (574 nm interference filter), and are reflected into the preparation. Inset: (from outside to center) tissue culture chamber sealed by cover glasses (25 mm in diameter) and rubber O-rings (1 mm working distance), modified to accommodate (center) plastic support. Centrally, in the plastic support, a surgical tantalum grid bears the circularly clamped retina, surrounded by silver wire electrode, which terminates—together with identical electrode of other side and ground connection—with a rubber cushion on the upper end of the support. Opposite bore holes are for inlet and outlet of perfusate; an overflow is located near upper end.

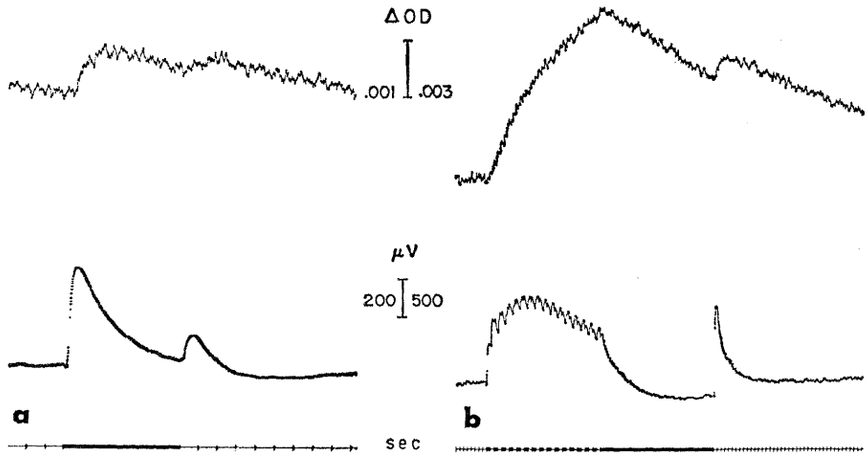

Fig. 2. Optical response (upper trace; decreased 350 nm absorption upward) and electrical response (vitreous side positive upward) of the frog's retina to single flash (*a*), and flicker and steady illumination (*b*). Stimulus 574 nm; 10^{-8} watt/cm^2; stimulation indicated by marks on time track. The electrical responses were recorded with a 1- and a 10-second time constant, respectively.

dation-reduction state was approached in the following ways.

1) Primary photochemical events in visual pigments of receptor cells can be excluded as possible causes for the observed phenomena. The optical effects occur (Fig. 2*a*) equally when the stimulus light is increased or decreased. Sustained visible illumination (Fig. 2*b*; twice as high in amount as in the preceding flicker period) causes the removal of the absorption changes. The triggered character, furthermore, of the optical as well as the positive electrical processes indicates that both take place at a more proximal site. Besides, similar absorption changes can be elicited electrically. Thus bleaching of photosensitive pigments does not account for the changes in optical density.

2) To exclude the possibility that the changes in transmitted light were caused by changes in scattering due to swelling or shrinkage of mitochondria (*9*) rather than by changes in absorption, the following experiment was performed. The light path between preparation and photomultiplier was altered in that it was enveloped in an ellipsoid-shaped mirror, the retina and the photosensitive surface of the photomultiplier tube occupying the focal points, and the directly transmitted ultraviolet light was excluded by a stop from falling onto the multiplier. In this way, stray light from some solid angle could be collected and funneled into the photocell. This was found to be similar in magnitude to the directly transmitted light. Changes in the scattered light that occurred on stimulation were smaller and equal in sign to those found directly. Thus scattering seems to play a minor role, if any.

3) A spectral characterization of the absorbancy changes was performed with single flashes or trains of stimuli being delivered every 3 minutes and the ultraviolet wavelength being changed every other time. Comparison was made by adjusting photomultiplier gain or analyzing beam intensity, or both, for equal resting output and additionally calibrating the evoked percentage changes in density against the spectrally known cover glass. Equal electrical response indicated equal amount of induced activity. The results obtained showed that the optical effects do peak near 340 nm; half-maximal values were found at 320 and 380 nm.

4) The following examples of a chemical characterization were selected from experiments in which the respective constituents had been added to or exchanged in the perfusate for a sufficient length of time to result in a stationary response behavior. (i) It had been noticed earlier that for the ERG an optimum potassium concentration exists. But, whereas the effect of excess K (known to remove the *b*-

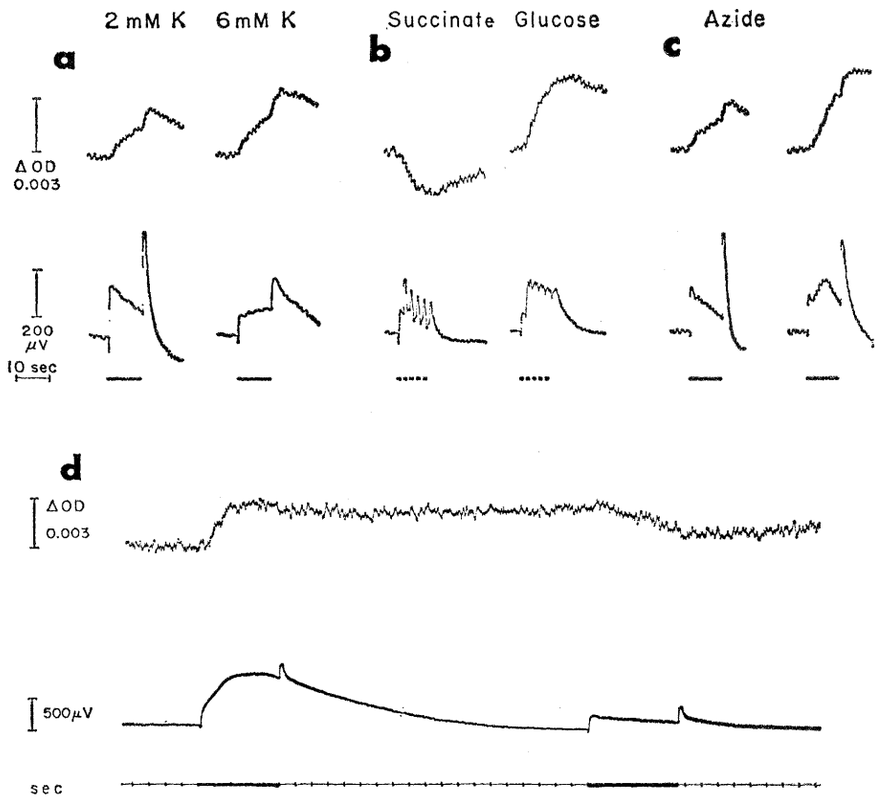

Fig. 3. (*a*, *b*, and *c*) Environmental influences on optical (upper trace) and electrical (middle trace) responses to light stimulation (bottom trace). (*a*) Normal and raised potassium concentration; (*b*) re-exchange of glucose (5 mmole/lit.) for succinate (7 mmole/lit.); (*c*) before and during administration of 10^{-1} mole/lit. sodium azide; (*d*) simultaneous exhaustion of the optical and electrical light response on double stimulation. The *c*-wave that follows the fast rising P II in this experiment was exaggerated by increased *p*H (8.1) of the perfusate.

wave and used to isolate P III) is readily reversible, that of lack of K is not. Impairment of function by excess K suggests a membrane effect; the minimum requirement points to its participation in some other limiting reaction, examples of which are the kinase reactions catalyzing the initial phosphorylations of glucose. The recording in Fig. 3*a* shows that a threefold increase in K clearly diminishes the *b*-wave, but it unmasks a subsequent positivity and even increases the optical change, suggesting the relief from a bottleneck above the system analyzed. (ii) Succinate as a substrate in some biochemical systems is known to bypass DPN and feed in electrons at a later stage of the respiratory chain with reduced yield in energy, part of which can be utilized to reduce DPN (*10*). On replacing glucose in the medium by succinate, much of the electrical response is maintained. The optical response, however, is completely lost and eventually reverses its sign, as if the system under study were driven backwards. These effects, too, are completely reversible (Fig. 3*b*). On the contrary, the resting absorption decreased markedly on adding succinate, with a concomitant change in the resting electrical potential difference. The transitory absorption increase on stimulation thus seems to require and trap energy when available. (iii) A frequently used means of enhancing spectroscopic effects in respiratory carriers is the addition of nonlimiting concentrations of azide, which slows down the flow of electrons just before they reach the oxygen (*11*). In the ERG, azide has been shown to augment the *c*-wave (*12*). Figure 3*c* shows the combined effects.

On the basis of these results I feel justified in tentatively identifying the stimulus-induced absorption changes as cyclic oxidations of pyridine nucleotides that parallel slow-wave activity of the retina. Specifically, it is the component P I of the ERG that most closely reflects the respiratory processes. This has been found in their quantitative correlation on graded stimulation, in their summating properties (Fig. 2*b*) under limiting (Fig. 3*a*) as well as facilitating (Fig. 3*c*) conditions, and can be demonstrated in their simultaneous exhaustion (Fig. 3*d*). The determining role on retinal excitability of slow electrical processes has long been recognized (*7*) and has recently been demonstrated in the isolated human retina (*13*). It seems substantiated by the measurements reported here.

This work appears to introduce knowledge on respiratory control mechanisms for the interpretation of bioelectrical slow-wave activity and demonstrates the manifestation in this preparation of oxidation-reduction states in measurable potential differences.

WERNER SICKEL*

Department of Biophysics,
Johns Hopkins University,
Baltimore, Maryland 21218

References and Notes

1. For method, see W. Sickel, in *The Visual System*, R. Jung and H. Kornhuber, Eds. (Springer, Berlin, 1961), p. 80; *Amer. J. Ophthalmol.*, in press. For sensitivity of preparation see C. Baumann, *Arch. Ges. Physiol.* **280**, 81 (1964). For application to human retina, see W. Sickel, H. G. Lippmann, W. Haschke, C. Baumann, *Ber. Deut. Ophthalmol. Ges.* **63**, 316 (1961). See also A. Ames, III, and B. S. Gurian, *Arch. Ophthalmol.* **70**, 837 (1963).
2. E. Bauereisen, H. G. Lippmann, E. Schubert, W. Sickel, *Arch. Ges. Physiol.* **267**, 636 (1958); C. Baumann, P. Dettmar, R. Hanitzsch, W. Sickel, *Acta Ophthalmol. Copenhagen Suppl.* **70**, 156 (1962); W. K. Noell, *J. Opt. Soc. Amer.* **53**, 36 (1963).
3. B. Chance and G. R. Williams, in *Advan. Enzymol.* **17**, 65 (1956).
4. T. Goldsmith, R. J. Barker, C. F. Cohen, *Science* **146**, 65 (1964) → B. Chance, *Proc. Natl. Acad. Sci. U.S.* **51**, 359 (1964).
5. The perfusate contained, in millimoles per liter, NaCl, 80; KCl, 2; $CaCl_2$, 0.1; $MgCl_2$, 0.1; phosphate buffer (*p*H 7.8), 15; it was saturated with room air.
6. Photomultiplier (EMI 6256 A)-photometer 53-PM 1B, designed by E. F. MacNichol, Jr., and described by A. M. Chase, in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1960), vol. 8, p. 61.
7. For review, see R. Granit, *Sensory Mechanisms of the Retina* (Oxford Univ. Press, Oxford, 1947).
8. C. M. Connelly and B. Chance, *Federation Proc.* **13**, 29 (1954); B. Chance, G. Mauriello, X. M. Aubert, in *Muscle as a Tissue*, K. Rodahl and S. M. Horvath, Eds. (McGraw-Hill, New York, 1962), p. 128.
9. B. Chance and L. Packer, *Biochem. J.* **68**, 295 (1958); A. L. Lehninger, *Physiol. Rev.* **42**, 467 (1962).
10. B. Chance, Ed., *Energy-Linked Functions of Mitochondria* (Academic Press, New York, 1963); G. V. D. van Rossum, *Biochim. Biophys. Acta* **86**, 198 (1964).
11. B. Chance and G. R. Williams, *J. Biol. Chem.* **221**, 477 (1956).
12. W. K. Noell, *Amer. J. Physiol.* **170**, 217 (1952).
13. R. Hanitzsch and A. L. Byzow, *Vision Res.* **3**, 207 (1963).
14. A preliminary report was published in *Federation Proc.* **23** (No. 2, pt. 1), abstr. 2492 (1964). I thank Dr. E. F. MacNichol, Jr., for providing the research facilities and his support, Dr. Britton Chance for the use of his double-beam spectrophotometer for some control experiments, and S. S. Easter, Jr., for help in preparing the manuscript. Mrs. Sickel assisted in performing the experiments. This work was supported by NSF grant G 18886 and NIH grant NB 03582.

* Present address: Physiologisches Institut der Universität, Koln, Germany.

25 January 1965

Electroretinogram in Newborn Human Infants

Abstract. *The electroretinogram of the newborn human shows the* x*-wave component which was demonstrated by Adrian and others to be a concomitant of photopic visual function in the adult. This finding may provide electrophysiological support for behavioral observations indicating that infants have some color vision and ability to resolve visual stimuli.*

Evidence has accumulated from several recent studies which suggests that the human visual system is more highly developed at birth than was once realized. In 1962 Fantz, Ordy, and Udelf (*1*) reported that infants under 1 month of age fix their eyes upon stripes subtending a visual angle of about 40 minutes of arc. Their results correspond roughly to visual acuity ratings in the newborn obtained by Gorman, Cogan, and Gellis (*2*) in their study of opticokinetic responses. Recent work by Dayton and his co-workers (*3*) indicates that some newborns show a well-developed fixation reflex and can resolve stripes subtending only 7.5 minutes of visual angle, the equivalent in Snellen notation of 20/150 vision. As early as 1932, Staples (*4*) demonstrated that 3-month-old infants tend to look at colored rather than gray papers equal in brightness. Also, Berlyne (*5*) and Fantz (*6*) have observed that infants fixate patterned stimuli longer than homogeneous fields.

Early work with the electroretinogram (ERG) of human infants has not confirmed the degree of visual development indicated by these behavioral studies. Zetterström (*7*) was the first to find electroretinograms in human infants. She reported scotopic *b*-waves of long latency and very low amplitude appearing within the first 3 days of life in full-term infants. By 8 weeks of age the waveforms and latencies became comparable to those of adults. Combined data on premature and full-term infants showed a positive correlation between birth weight and appearance of the ERG (*8*). In contrast to this, Horsten and Winkelman (*9*) found that both negative *a*-waves and positive *b*-waves of low amplitude but with adult characteristics were present in both premature