form, were investigated by means of a scanning electron microscope (SEM) (Cambridge Stereoscan) equipped with an energy-dispersive x-ray spectrometer. The following coal samples were studied by SEM: C-16030, C-16317, C-12062, C-16264, and C-15566; of these samples, the first three were from Herrin (No. 6) coal, the fourth was from Springfield (No. 5) coal, and the last was from Colchester (No. 2) coal, all members of the Carbondale formation (Pennsylvanian). Sphalerite was identified in each sample studied, and the identification was confirmed by x-ray diffraction. The presence of sphalerite in a sample of Herrin (No. 6) coal from northern Illinois had previously been identified by Zubovic (*11*). Cadmium was qualitatively determined by energy-dispersive x-ray spectrometry in the three samples on which analysis was attempted (C-16317, C-12062, and C-16264). An accelerating voltage of 8 kv was used and the $L\alpha_1$ (3.133-kev) x-ray emission peak of Cd was used to identify the element. A scanning electron photomicrograph of a sphalerite particle from sample C-16264 is shown in Fig. 1A; a portion of the x-ray spectrum of sphalerite is shown in Fig. 1B. Although this sample had less than one-tenth the Zn content of the three coals with the highest Zn contents, it had the lowest Zn : Cd ratio and therefore gave a clearer indication of the Cd content on the x-ray spectrogram.

Sphalerite samples were obtained from a fraction of high specific gravity from a LTA (sample C-16317) and from a fraction of similar specific gravity of an unashed coal (sample C-16264). The particles were individually picked from the high-specific-gravity fraction with a fine brush while the sample was under a microscope. The sphalerite was dissolved in nitric acid, and the solution was diluted with deionized water for analysis by atomic absorption. The sphalerite from coal C-16317 contained 0.55 percent Cd and 61.2 percent Zn, and that from coal C-16264 contained 0.88 percent Cd and 54.5 percent Zn. The Zn : Cd ratios of the sphalerite concentrates were similar to the Zn : Cd ratios of the whole coals from which they were obtained. For sample C-16317, the Zn : Cd ratio was 93 : 1 in the sphalerite and 97 : 1 in the whole coal. Sample C-16264 had a Zn : Cd ratio of 62 : 1 for the sphalerite and 60 : 1 for the whole coal.

The similarity of the Zn : Cd ratios of sphalerite and the Zn : Cd ratios of the whole coal from which the sphalerite was obtained suggests that most, if not all, of the Cd in the coal is within the sphalerite. Because no separate Cd-containing phase was observed, the Cd is thought to substitute for Zn in the sphalerite. The Cd contents we have reported (Table 1) for the sphalerites obtained from coals are not unusually large. As much as 4 to 5 percent Cd has been reported in other sphalerites (*12*).

The presence of sphalerite in discrete particles in the LTA and in the coal fractions with high specific gravity suggests that it occurs as an epigenetic mineral. We have observed sphalerite, associated with authigenic calcite, kaolinite, and pyrite, filling vertical fractures (cleats) in coals. Many coals are prepared by "washing" (various specific-gravity separation methods are normally used) prior to their utilization. A significant reduction in the Cd and Zn contents could likely be effected in some coals by such separation methods inasmuch as the high specific gravity of sphalerite (4.1) favors its removal during washing.

HAROLD J. GLUSKOTER
PETER C. LINDAHL
Illinois State Geological Survey, Urbana 61801

References and Notes

1. J. Liebig and H. Kopp, *Jahresbericht über die fortschritte der reinen, pharmaceutischen, und technischen Chemie, Physic, Mineralogie, und Geologie für 1847–1848* (Giessen, 1849), p. 1120; E. Jensch, *Chem. Ind. Berlin* **10**, 54 (1887); V. M. Goldschmidt, *J. Chem. Soc. London* **1937**, 655 (1937).
2. R. F. Abernethy, M. J. Peterson, F. H. Gibson, *U.S. Bur. Mines Rep. Invest. 7281* (1969).
3. J. V. Lagerwerff and A. W. Specht, in *Trace Substances in Environmental Health-IV*, D. D. Hemphill, Ed. (University of Missouri, Columbia, 1971), pp. 85–93.
4. V. E. Swanson, *Southwest Energy Study*, part 2 (U.S. Geological Survey, Washington, D.C., 1972).
5. T. Kessler, A. G. Sharkey, Jr., R. A. Friedel, *U.S. Bur. Mines Rep. Invest. 7714* (1973).
6. ———, *U.S. Bur. Mines Tech. Prog. Rep. 42* (1971); H. E. Goeller, E. C. Hise, H. B. Flora, in *Cadmium: The Dissipated Element*, W. Fulkerson and H. E. Goeller, Eds. [Oak Ridge National Laboratory (ORNL NSF-EP-21), Oak Ridge, Tenn., 1973], p. 85; P. J. Nord and E. Bingham, paper presented at the sixth annual conference on Trace Substances in Environmental Health, Columbia, Mo. 1972.
7. R. R. Ruch, H. J. Gluskoter, N. F. Shimp, *Ill. State Geol. Surv. Environ. Geol. Note 61* (1973).
8. H. J. Gluskoter, *Fuel* **44**, 285 (1965).
9. B. Bernas, *Anal. Chem.* **40**, 1682 (1968).
10. D. E. Buckley and R. E. Cranston, *Chem. Geol.* **7**, 273 (1971).
11. P. Zubovic, *Minor Element Content of Coal from Illinois Beds 5 and 6 and Their Correlatives in Indiana and Western Kentucky* (open file report, U.S. Geological Survey, Washington, D.C., 1960), p. 42.
12 W. A. Deer, R. A. Howie, J. Zussman, *Rock-Forming Minerals* (Wiley, New York, 1962), vol. 5, p. 168.
13. We thank Dr. P. Santiliquido for carrying out the Cd determinations by NAA and the staff of the Environmental Research Laboratory, University of Illinois, for conducting the Cd determinations by ASV. This research was supported, in part, by the U.S. Environmental Protection Agency under grant R800059 and contract 68-02-0246.

23 March 1973

Information Processing in the Retina: Importance of Chloride Ions

Abstract. *Electrophysiological recording methods were applied to the isolated, retina-eyecup preparation of the rabbit. Perfusion with a chloride-free solution has a selective, reversible effect on the retinal network. This study establishes chloride ions as an important requirement for certain "information channels" within the retina.*

Many recent advances in retinal function have relied on preparations that are artificially maintained after the eye is removed. Studies of the isolated retina have established the importance of sodium and potassium for the generation of both light- and dark-dependent responses (*1*, *2*). In comparison, the role of chloride ions in retinal function has received little attention (*3*). We used the isolated retina-eyecup preparation of the rabbit to examine electrophysiological properties of the retina under a variety of different ionic conditions. In the present study we report the selective, reversible effects that follow the introduction of perfusing medium in which chloride has been replaced by a large anion, usually sulfate. The effects of chloride-free solutions are apparent at many levels of retinal function, as revealed by recordings of mass ganglion cell discharge, the proximal negative response (PNR), the transretinal electroretinogram (ERG), and through intracellular recordings from cells presumed to be horizontal cells. Our results demonstrate the importance of chloride as a major ionic requirement for certain "information channels" within the retina.

The eye of a New Zealand White

rabbit anesthetized with urethane (0.6 g per kilogram of body weight) was surgically removed under dim red illumination. The iris, cornea, and lens were resected, and the remaining eyecup was everted and secured in a perfusion chamber (Fig. 1). Within 3 minutes after enucleation, the retina was perfused with control medium, composed of 120 m*M* NaCl, 5.0 m*M* KCl, 25 m*M* $NaHCO_3$, 10 m*M* glucose, 0.8 m*M* Na_2HPO_4, 0.1 m*M* NaH_2PO_4, 2 m*M* $CaCl_2$, 1.0 m*M* $MgSO_4$, and 2.5 percent horse serum. Chloride-free solutions usually had sulfate as a chloride substitute and consisted of 60 m*M* Na_2SO_4, 2.5 m*M* K_2SO_4, 25 m*M* $NaHCO_3$, 10 m*M* glucose, 0.8 m*M* Na_2HPO_4, 0.1 m*M* NaH_2PO_4, 62.5 m*M* sucrose, 1.0 m*M* $MgSO_4$, 8.0 m*M* $CaSO_4$, and 2.5 percent horse serum. A *p*H of 7.4 to 7.5 was maintained by constant aeration with 95 percent O_2 and 5 percent CO_2 in the main storage vessels. Temperature of the perfusing medium was monitored and maintained at 35°C. Fluid flow was gravitational and was maintained at 40 ml/min. A preliminary description of this technique has appeared (*4*).

The ERG was recorded (d-c) differentially between silver–silver chloride electrodes embedded in the upper and lower chamber halves. Summated ganglion cell discharge from a population of cells was recorded by amplifying the output of a tungsten electrode (tip diameter of 2 to 3 μm) or a platinum wire (diameter of 100 μm) placed on the retinal surface. In response to diffuse illumination, such records consist of impulse discharges from on, off, and on-off ganglion cells, although in the rabbit, the latter class tend to be directionally selective cells and respond poorly to diffuse illumination (*5*). Thus, the main discharge recorded in this study was probably weighted in favor of on-center and off-center ganglion cells. To ensure that this record was uncontaminated by the ERG, the recording bandwidth was restricted (—3-db cutoffs between 1 and 10 khz). The PNR was recorded from a fine (1-μm) tungsten electrode inserted into the inner retina, and evoked by a small (100-μm) spot of light centered over the electrode. Studies in the frog retina have suggested that the PNR is a reflection of amacrine cell activity (*6*); in both rabbit and frog this response consists of a transient negative deflection when light is switched on or off. Glass micropipettes with tip resistances of 50 to 300 megohms were used for intracellular recording and were usually filled with 2*M* potassium acetate. All responses were displayed on a Brush 260 rectilinear penwriter. White light stimuli were provided by shuttered tungsten iodine light sources. Light intensity was measured with a Fish-Schurman No. 6143 heat filter placed over a Hewlett-Packard thermopile. Except for the PNR studies, all light stimuli provided even illumination of the entire retina.

The top trace of Fig. 2 shows an ERG recorded from the isolated rabbit eyecup within a few minutes after perfusion was initiated with control solution. A negative a-wave is followed by a positive b-wave and a slow positive c-wave. This ERG is similar to one normally observed in a lightly anesthetized rabbit. In less than 1 hour, the c-wave begins to decline in amplitude and is not usually evident after 2 to 3 hours (ERG in left column of Fig. 2). During this period, however, a- and b-waves are relatively constant in amplitude, and ganglion cell threshold does not appreciably change. Thus these initial changes may be restricted to the pigment epithelium where the c-wave is generated (*7*).

Chloride-free solutions act rapidly in affecting the responses observed in this study. Changes in ganglion cell activity are evident almost instantaneously, and a steady state condition is usually observed in all responses within 3 minutes after the solution is introduced. A similar time course is seen for the

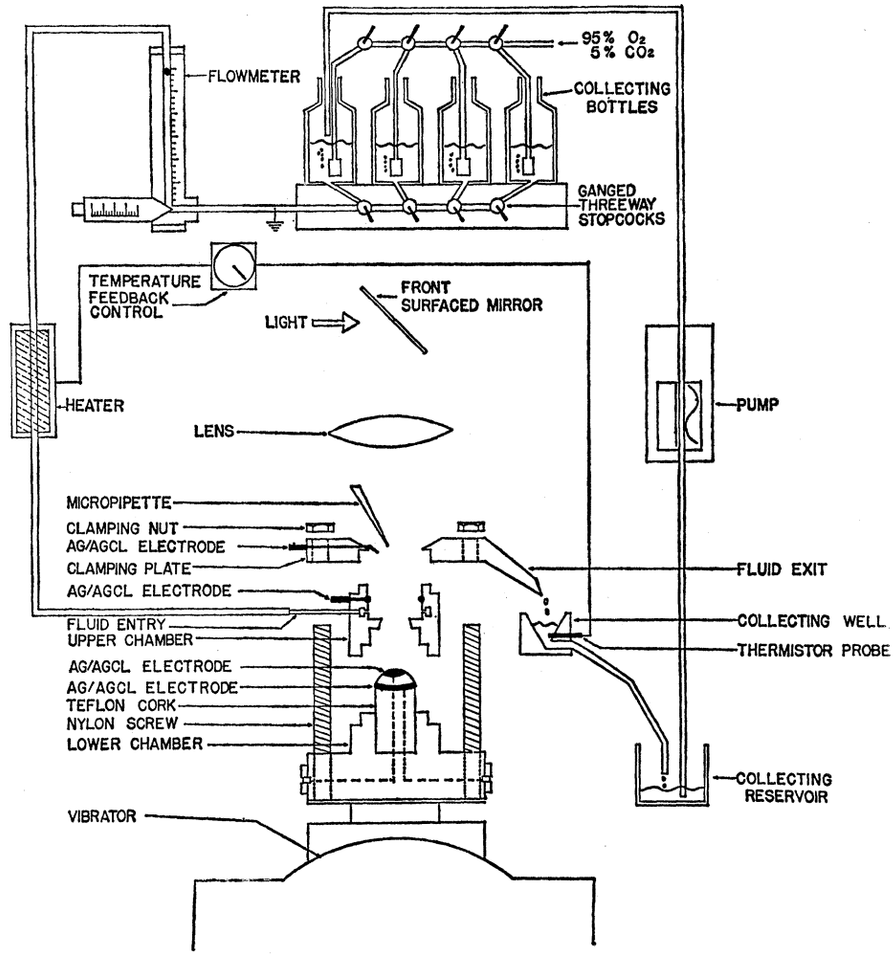

Fig. 1. The perfusion chamber, consisting of upper and lower portions and a clamping plate. The lower portion contains a Teflon plug over which the eyecup is everted. The eyecup is firmly secured by clamping the two chamber halves together. This is accomplished by tightening the nylon nuts over the clamping plate. Perfusion medium enters the upper portion, flows over the vitreal surface of the retina, and exits through the fluid exit channel. Medium is collected in a reservoir and returned to the main storage vessels by a pump. The system has a regulated heating element that maintains the temperature at 35°C and a flowmeter to maintain constant, gravitationally induced flow at 40 ml/min. Some design features of this chamber are similar to those of chambers reported for the frog and rabbit (*2*, *13*).

recovery after normal solution is reintroduced. The effects reported below are those that appear after about 3 minutes. However, on several occasions we recorded ganglion cell responses during long exposures (2 hours) to chloride-free solutions and found no significant difference between the long- and short-term effects.

The reversible effects of chloride-free solutions (Fig. 2) are as follows: (i) The b-wave of the ERG is abolished, and PIII is slightly enhanced. (ii) The ganglion cell on discharge is lost, leaving an enhanced and prolonged off discharge. (iii) The on PNR response is abolished, whereas the off response is enhanced; a slower positive-going response persists in chloride-free solutions (*8*). (iv) S-potentials (recorded intracellularly) are abolished (*9*). In summary, these results show that ganglion cells can respond with impulse discharge in chloride-free solution, and that the retinal network presynaptic to the ganglion cells is affected in a highly selective manner by the chloride-free environment.

The most intriguing effects of chloride-free solutions are observed at the level of the ganglion cell. It is here that one sees the final expression of the retinal network in transforming spatial and temporal information into impulse discharge patterns for central transmission. Additional insight into the chloride-replacement effects observed at this level has been provided by recordings from a number of ganglion cells studied as single units. These experiments have been restricted to cells in the peripheral retina that show antagonistic, concentric receptive field organization. Details will be published (*10*), but the main results are important here and can be summarized as follows: On-center cells show a loss of both center and surround mechanisms in chloride-free solutions. Conversely, off-center cells show a selective loss of the surround mechanism, but an enhancement of the off-center response. Thus, there appear to be two major defects in chloride-free solutions observed at the level of the ganglion cell.

Additional experiments on the ERG and mass ganglion cell discharge have been carried out to eliminate the possibility that the observations reported here result from specific effects of sulfate ions. We have used other large anions (methylsulfate and propionate) as chloride substitutes in the perfusing medium. These anions show effects similar to sulfate; therefore, it is the removal of chloride which is responsible for the observations of this study.

Considerable evidence has accumulated that the two types of simple ganglion cells (on-center and off-center) are subserved by different retinal channels. Not only can these two classes of cells be separated by the use of focal light stimulation, but each type of cell responds differentially to brief transretinal electrical stimulation, depending on the direction of current flow (*11*). Intracellular recording experiments in lower vertebrates have suggested that these retinal channels share similar receptors and that they first separate at the level of the bipolar cells, of which two different types have been observed: one that hyperpolarizes and a second that depolarizes in response to light stimulation (*12*). Our results suggest that chloride-free solutions result in a loss of the bipolar cell type that accounts for the on-center ganglion cell response. The remaining bipolar cells may be enhanced in chloride-free solutions, as evidenced by the enhanced off discharge and the large off response observed in the PNR recording. The loss of the horizontal cell response (S potential) accounts for the loss of the surround mechanism observed in the off-center ganglion cells. This view is consistent with conclusions based on intracellular recording experiments (*12*). Thus, the results in this study support and extend the notion of separate retinal channels by demonstrating that these channels have different ionic requirements. The use of chloride-free solutions provides a method of examining the off-center channel in isolation from its surround.

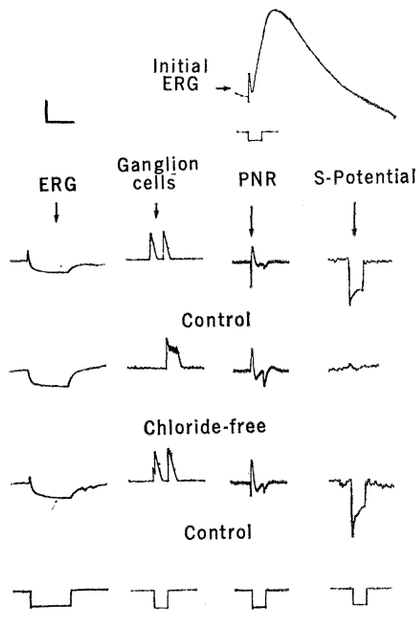

Fig. 2. Effects of chloride-free solutions. The top trace shows an ERG obtained early in the course of an experiment; a-, b- and c-waves are apparent. The four vertical columns, each obtained during separate experiments, show different retinal responses recorded in control solution, after a switch to a chloride-free solution, and after a return to control solution. Vertical calibrations: top trace and ERG column, 400 μv; ganglion cell column, uncalibrated; PNR column, 100 μv; and S-potential, 5 mv. Horizontal calibrations are 2 seconds for all responses. Positivity is indicated by an upward deflection. Light intensity: top trace, 8.25×10^{-4} watt/cm^2; ERG and ganglion cell, 8.25×10^{-7} watt/cm^2; PNR 8.25×10^{-6} watt/cm^2; and S-potential, 1.52×10^{-5} watt/cm^2.

ROBERT F. MILLER
RAMON F. DACHEUX
Neurosensory Laboratory, State University of New York, Buffalo 14214

References and Notes

1. I. D. Hamasaki, *J. Physiol. London* **173**, 449 (1964); *ibid.* **167**, 156 (19 → A. J. Sillman, H. Ito, T. Tomita, *Vision Res.* **9**, 1443 (1969); → W. A. Hagins, *Annu. Rev. Biophys. Bioeng.* **1**, 131 (1972); B. S. Winkler, *Vision Res.* **12** 1183 (1972).
2. R. F. Miller, *J. Neurophysiol.* **36**, 28 (1973).
3. I. Hanawa, K. Kuge, K. Matsumura, *Jap. J. Physiol.* **17**, 1 (1967); R. F. Miller and J. E. Dowling, *Int. Soc. Clin. Electroretinogr. Symp. 8th Pisa, 1970* (1972), p. 85.
4. R. F. Dacheux, M. Delmelle, R. F. Miller, W. K. Noell, *Fed. Proc.* **32**, 327 (1973).
5. W. R. Levick, *J. Physiol. London* **188**, 285 (1967).
6. D. A. Burkhardt, *J. Neurophysiol.* **33**, 405 (1970).
7. W. K. Noell, *Project 21-12-1-994, U.S. Air Force School of Aviation Medicine* (Government Printing Office, Washington, D.C., 1953); R. H. Steinberg, E. Schmidt, K. T. Brown, *Nature* **227**, 728 (1970).
8. The origin of the positive response that persists in the chloride-free solutions is not clear. It is likely that part of this response is slow PIII, which is very large and positive-going in the inner retina [D. S. Faber, thesis, State University of New York at Buffalo (1969)]. We have not tested for other possible contributions to this response.
9. S-potentials have been recorded from a variety of different species and have been localized to horizontal cells by intracellular staining techniques (*12*). In our experiments the loss of the S-potential was usually preceded by a slight (5- to 10-mv) hyperpolarization of the dark resting-membrane potential. We sometimes observed a slight reversal of the light-induced response to a small positive deflection. Also we frequently observed the S-potential to disappear or slightly reverse, and then to recover to a 1- to 3-mv response after several minutes. These latter effects are complex and irregular, and their explanation must await further experimentation.
10. R. F. Miller and R. F. Dacheux, in preparation.
11. D. Crapper and W. K. Noell, *J. Neurophysiol.* **26**, 924 (1963); S. Molotchnikoff, thesis, State University of New York at Buffalo (1972); S. Kuffler, *J. Neurophysiol.* **16**, 37 (1953).
12. F. S. Werblin and J. E. Dowling, *J. Neurophysiol.* **32**, 339 (1969); A. R. Kaneko, *J. Physiol. London* **207**, 623 (1970); ——— and M. Yamada, *ibid.* **227**, 261 (1972); R. H. Steinberg and R. Schmidt, *Vision Res.* **10**, 817 (1970).
13. A. Ames and D. A. Pollen, *J. Neurophysiol.* **32**, 424 (1969).

14. Supported by NIH grant EY-0084-02.

2 March 1973; revised 11 April 1973