hydrogen impurity. Evacuation of the signal does not restore the loss in signal.

Ethylene surprisingly enough shows no decrease in the signal. This cannot be due to a molecular-sieve action of the pores in the Vycor glass since oxygen, nitric oxide, and methyl iodide readily diffuse to the sites of the methyl radicals.

Introduction of methyl iodide decreases the signal, but this loss in signal strength is recovered on evacuation. The disappearance of the signal may be due either to the gradual diffusion of the methyl iodide into the pores containing $CH_3$ radical and broadening the signal due to shortening of their lifetime as result of

$$CH_3 + CH_3I \longrightarrow CH_3I + CH_3$$

or to change in the $Q$ of the cavity, as the dielectric constant of $CH_3I$ is 7.

Methyl radicals do not react with ethane, normal butane, and toluene.

Attempts to produce and stabilize methyl radicals from acetone and dimethyl mercury, both good sources of methyl radicals, were unsuccessful both under static and flow conditions, in spite of substantial overall decomposition of these substances under our reaction conditions.

Similarly, no radicals were detected in subjecting the following substances to photolysis under our conditions: ethyl iodide, isopropyl iodide, toluene, and benzyl chloride.

Our study indicates the possibility of preparing and stabilizing methyl radicals for periods long enough to make them useful as organic reagents. Possibly other species such as H atoms, OH, $HO_2$, $NH_2$ can be prepared by proper choice of porous substances. Furthermore, the role of free radicals in surface catalysis can be evaluated.

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7. Supported by the Atomic Energy Commission.

3 March 1966

## Oxygen Dependence of Retinal S-Potential-Producing Cells

Abstract. *Changes in the membrane potential of the S-potential-producing cells (S-cells) in the isolated retina of fish (Gerridae) were correlated with changes in oxygen concentration. During brief hypoxia the changes in potential consisted of initial depolarization and subsequent hyperpolarization to near 70 millivolts. Depolarization occurred when oxygen concentration was reduced to a level of from 13 to 10 percent, and hyperpolarization occurred on reduction from 10 to 2.5 percent; there was variation from cell to cell. The recovery of S-cell function from anoxia was fast in oxygen but slow in air. The results show that the S-cell stops functioning in seconds without oxygen; hence this kind of cellular element in the nervous system is much more sensitive to oxygen deprivation than other cells studied thus far.*

Previous studies have established that the light-induced S-potentials originate in the horizontal and amacrine cells of the fish retina (*1*), and that $CO_2$, $NH_3$, temperature change (*2, 3*), alcohols, and volatile anesthetics (*3*) have immediate and drastic effects on the membrane potential of these S-cells. On the other hand, these particular agents and temperature change have much less effect on the neuronal membrane potential of ganglion cells of frog dorsal root (*3*). Further, the S-potential is graded in nature and is sustained as long as the light stimulus lasts, and the membrane potential of the S-cells is not electrically excitable (*2, 4*). These facts suggest that the S-cell membrane potential is maintained by a mechanism different from that of the neuronal membrane potential; the latter is exclusively dependent on transmembrane ionic gradient and gives rise to the all-or-none spike potential.

This paper deals with experiments on hypoxia in the S-cell membrane potential of the fish retina. The retina was dissected from a light-adapted fish (Gerridae) and placed, receptor side up, in a plastic chamber having a volume of 80 $cm^3$. The mixtures of gas were introduced into the chamber through valve-equipped inlets after they had been bubbled in water bottles; the gas circulated through the entire system at a flow rate of about 200 $cm^3$/min. Pure $O_2$ or air was used as the control gas medium; $O_2$ concentration was easily changed by introduction of other gases or gas mixtures through a by-pass valve system. In our gas exchange system, the total gas content in the chamber could be completely exchanged in 2 minutes. An oxygen macroelectrode (Beckman) mounted inside the chamber monitored the $O_2$ concentration changes for recording, while a micropipette electrode simultaneously recorded the S-cell membrane potential. An Ag–AgCl wick electrode lying beneath the retina was used as reference. When the tip of the microelectrode is located outside the S-cell, the potential difference between the microelectrode and the reference electrode, and the potential changes under various experimental conditions, are almost negligible in comparison with the S-cell membrane potential and its changes. If the microelectrode is placed on the receptor surface of the retina, a transretinal d-c potential of 3 to 5 mv can be recorded. This d-c potential has been observed to behave opposite in direction to S-cell membrane potential changes under certain conditions (*2, 3*). Identification of the S-cells is based on recent histological studies of various fish retinas in this department (*1*) and on other findings by an electrophoretic dye method for marking individual cells (*5*). Alternating blue (460 nm) and red (630 nm) light stimuli (300-msec flashes) were routinely employed to differentiate the types of S-potentials. The experiments were conducted at room temperature (20° to 22°C).

Typical examples of hypoxia experiments in the isolated fish retina are illustrated in Figs. 1 and 2. In Fig. 1, the continuous recording A–H was obtained from a stellate amacrine cell, which responds with the C-type S-po-

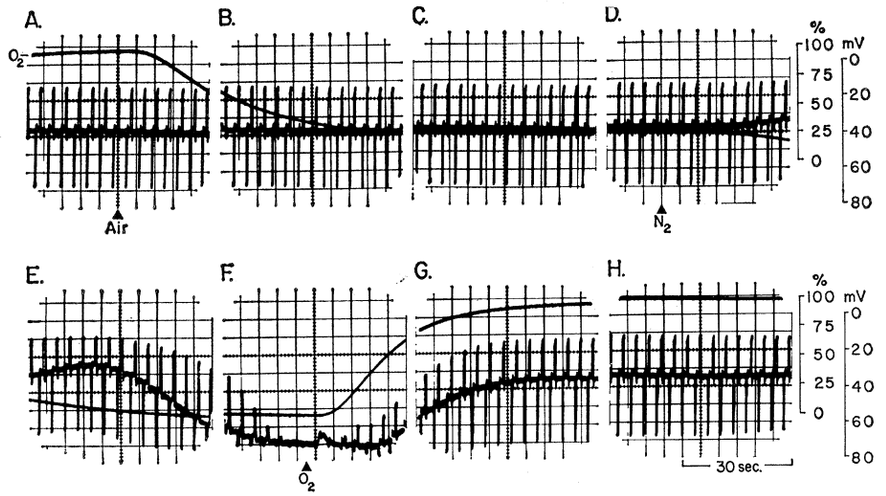


Fig. 1. Effects of hypoxia on S-cell membrane potential. The continuous recording (A–H) was obtained from a stellate amacrine cell. Pure $O_2$ was used as the control gas, and alternating blue and red light flash stimuli were employed. Hyperpolarizing (downward) potentials are responses to blue flashes and depolarizing (upward) potentials are responses to red flashes; $O_2$ concentration tracing is labeled "$O_2$" at record A; $O_2$ concentration scale in percentage and membrane potential scale in millivolts are shown to the right of records. The time scale of 30 seconds is given below record H.

tential; hyperpolarizing (downward) potentials and depolarizing (upward) potentials were responses to blue and red light flashes, respectively. Pure $O_2$ was used as the control gas; the $O_2$ concentration tracing is labeled "$O_2$" at record A. The $O_2$ concentration in the chamber was reduced first by introduction of air (Fig. 1, record A), and subsequently by introduction of pure $N_2$ (record D). Reduction of the $O_2$ concentration from 100 percent to about 20 percent during a period of about 3 minutes caused no change in the resting potential and the light-induced S-potentials (records A–D), whereas further reduction to 0 percent with $N_2$ during a period of about 2 minutes caused an initial depolarization and a subsequent hyperpolarization of the resting potential (records D–F), the latter accompanied by abolition of the light-induced S-potentials. When $O_2$ was reintroduced into the chamber (record F), the resting potential responded with a small depolarizing deflection, occurring faster than the reponse of the oxygen electrode to readmission of $O_2$. The recovery of S-cell function from anoxia was rapidly completed within 0.5 to 1 minute after $O_2$ readmission (records F–H).

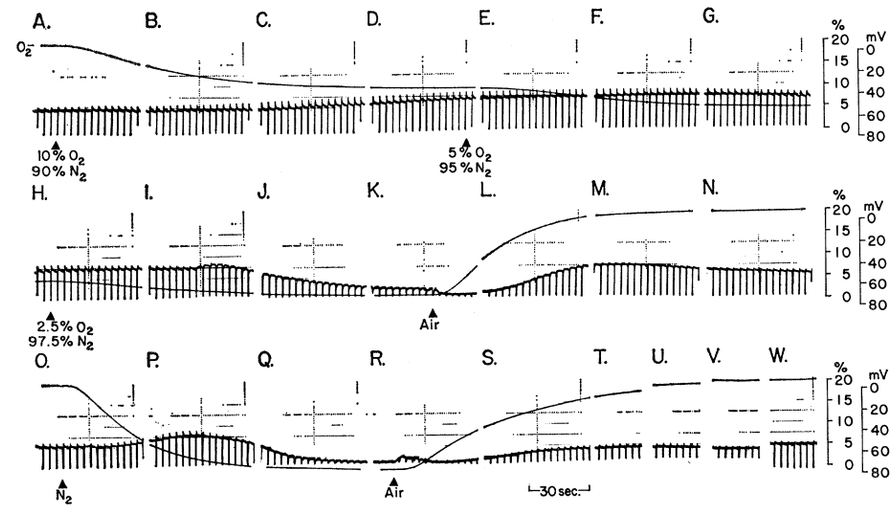


Fig. 2. Effects of successive reductions in $O_2$ concentration (10, 5, 2.5, and 0 percent) on S-cell membrane potential. Continuous recording (A–W) was taken from a horizontal cell. Air was used as the control gas and red light flash stimuli were employed. Elapsed time between records T and U, U and V, and V and W was 1, 2, and 15 minutes, respectively; $O_2$ concentration tracing is labeled "$O_2$" at record A, and the scale in percentage is shown to the right of the records. Membrane potential scale in millivolts is given to the right of records, and the time scale of 30 seconds is given below record S.

In an anoxia experiment illustrated in Fig. 2, air was used as the control gas medium. The continuous recording A–W was obtained from a horizontal cell producing the L-type S-potential—hyperpolarizing potentials in response to both blue and red light flash stimuli. In this case, however, since red light flashing was used without blue flashes, the hyperpolarizing potentials are responses to red light flashes alone. The $O_2$ concentration was gradually reduced by successive changes from air (about 20 percent $O_2$) to 10 percent $O_2$ in $N_2$ (Fig. 2, record A), 10 to 5 percent $O_2$ (record D) and 5 to 2.5 percent $O_2$ (record H). When air was replaced by the gas mixture of 10 percent $O_2$ in $N_2$, the resting potential of the horizontal cell was gradually depolarized from 50 to 40 mv, and the light-induced responses were enhanced (records A–D). During a reduction of the $O_2$ level from 10 to 5 percent (records D–H), the membrane potential remained unchanged, while further reduction from 5 to 2.5 percent $O_2$ (records H–K) caused a further slight depolarization and a subsequent hyperpolarization to near 70 mv, indicating that the S-cell function would be blocked. As the $O_2$ concentration was increased by readmission of air, the resting potential responded with a slight hyperpolarizing deflection (record K), followed by a transient depolarization during the recovery process (records L–N). When air was replaced by pure $N_2$, the initial depolarization and the subsequent hyperpolarization occurred rather rapidly (records O–R). In this anoxic state, the resting potential responded to readmission of air with a slight depolarizing deflection (record R), opposite in direction to the resting potential response to air readmission in the hypoxic state (as seen in record K). With air, the recovery of S-cell function from anoxia was slow, usually over a period of 10 to 30 minutes after readmission of air (records R–W). Elapsed time between records T and U, U and V, and V and W, respectively was 1, 2, and 15 minutes.

The critical and minimum $O_2$ concentration necessary for maintenance of S-cell function varied greatly, from 10 to 2.5 percent $O_2$ in $N_2$, between

individual cells in the same preparation and between cells in different preparations. In this connection, it should be noted that when the isolated retina was immersed in a thin layer (about 1 mm) of an oxygenated, phosphate-buffered Ringer solution, the S-cell function was blocked within a few minutes; it gradually recovered after the Ringer solution was withdrawn. This reaction is apparently due to hypoxia since the Ringer solution contains only about 2.5 percent $O_2$ in this procedure. It is known that only a small amount of $O_2$ can be carried in physical solution (*6*). Strong evidence is thus provided that S-cell function is much more dependent on aerobic metabolism than are nerve and muscle fibers, when studied in isolated preparations immersed in Ringer's solution. The great resistance of cat spinal motoneurons to $O_2$ deprivation was shown by Nelson and Frank (*7*), and we noted that the neuronal spike potential of frog dorsal root ganglion cells was evoked by sciatic nerve stimulation during a long term anoxia lasting from 30 to 60 minutes (*2, 3*). S-cell function never recovered with $O_2$ readmission after anoxia lasting 10 to 15 minutes, but the receptor function recovered under the same conditions.

It was also established in this study that the retinal S-cells require an $O_2$ concentration above 13 to 15 percent for normal functioning over a long period of time. Thus, S-cell function was maintained normally in both air and pure $O_2$ for several hours unless anoxia was employed. The recovery of S-cell function from the brief period of anoxia was rapid with $O_2$ (0.5 to 1 minute) and slow with air (10 to 30 minutes), due possibly to the different lengths of time that the retina was hypoxic or anoxic. Since complete replacement of gas in the chamber takes 2 minutes, the retina remains in a subcritical $O_2$ concentration for a longer period when air is used as the control gas medium than it does when pure $O_2$ is used.

A fundamental question arises as to whether the sensitivity of the photoreceptor to anoxia is reflected in the anoxic process of the S-cell membrane potential. Recent experiments in this department revealed that the function of the photoreceptor is not blocked by such a short term anoxia, and therefore the membrane potential changes observed during anoxia originate in the S-cell itself (*8*).

These experiments have indicated the strong dependence of S-cell function on oxidative metabolism, and they provide support for our earlier thesis that the S-cell membrane potential may reflect the rate of electron transfer in respiratory chains, forming structural components of the plasma membrane (*2, 3*).This concept is in general agreement with the redox battery membrane model proposed by Jahn (*9*). Quastel (*10*) observed that potassium ions stimulate (accelerate) the respiration of brain slices and that ethanol strongly inhibits the respiration so stimulated, whereas neither potassium nor ethanol affects the respiration of isolated brain mitochondria. He therefore suggested that both effects take place at "brain cell plasma membranes." In a recent publication, Chalazonitis (*11*) also discussed his studies on invertebrate neurons (Aplysia) in terms of plasma membrane respiration.

As demonstrated in earlier studies from this department (*2, 3*), a decrease in temperature from 20° to 15°C results in an immediate depolarization of the S-cell resting potential ($Q_{10} = 2$ to 3). The effect of cooling is similar to that of exposure of the retina to $NH_3$ in very low concentration, and of retinal dark-adaptation. Taking these observations into account, we can assume that the initial depolarization caused by hypoxia (13 to 10 percent $O_2$) is due to a deceleration of respiratory chain activity in the S-cell plasma membrane. As the $O_2$ concentration was further reduced (10 to 2.5 percent $O_2$), the S-cell membrane potential was rapidly hyperpolarized to near 70 mv. The hyperpolarized S-cell membrane potential during anoxia is almost completely insensitive to temperature change (*2, 3*), apparently because of the lack of respiration.

In regard to the classification of retinal S-cells, considered the horizontal cell, Cajal (*12*) a "short axon cell" analogous to the cortical short axon or Golgi II cells, whereas the amacrine cell is literally a cell without axon. However, recent light- and electron-microscope studies in the retina (*1, 13*) have revealed that the S-cells have more glial than neuronal characteristics, and electrophysiological experiments have shown that the membrane property of the S-cells also differs greatly from that of the neurons (*2, 3*). Therefore, we are in agreement with Gallego's suggestion that the S-cells represent an intermediate cellular element between typical neurons and glial cells (*14*). Considering the Golgi II cells as possible generators of the brain potentials, Jung (*15*) suggested that these cells may have an activity similar to the retinal S-potentials. Svaetichin *et al.* (*2, 3*) have also assumed that the Golgi II cells or Cajal's "short axon cells" in the brain are functionally equivalent to the retinal S-cells, all of which regulate the neuronal excitability level and inhibition in the nervous system; therefore, the same authors have named them "controller cells." If these findings in the fish retina may be extrapolated to brain cells, their strong dependence on oxygen may explain the well-known oxygen requirement of brain tissue.

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16. One of the authors (K.N.) expresses gratitude to W. R. Adey, R. T. Kado, and Mrs. F. Zetland in the Space Biology Laboratory, Brain Research Institute, University of California at Los Angeles, for help in preparing the manuscript. Supported by grants from the U.S. Air Force (AFOSR 619-64) and NIH (1 R01 NB 05770-01).

29 March 1966