Kinetics of Oxygen Consumption after a Single Flash of Light in Photoreceptors of the Drone (*Apis mellifera*)

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ABSTRACT   The time course of the rate of oxygen consumption (Q_{O_2}) after a single flash of light has been measured in 300-μm slices of drone retina at 22°C. To measure $\Delta Q_{O_2}(t)$, the change in Q_{O_2} from its level in darkness, the transients of the partial pressure of O_2 (P_{O_2}) were recorded with O_2 microelectrodes simultaneously in two sites in the slice and ΔQ_{O_2} was calculated by a computer using Fourier transforms. After a 40-ms flash of intense light, ΔQ_{O_2} reached a peak of 40 μl O_2/g·min and then declined exponentially to the baseline with a time constant $\tau_1 = 4.96 \pm 0.49$ s (SD, $n = 10$). The rising phase was characterized by a time constant $\tau_2 = 1.90 \pm 0.35$ s (SD, $n = 10$). The peak amplitude of ΔQ_{O_2} increased linearly with the log of the light intensity. Replacement of Na^+ by choline, known to decrease greatly the light-induced transmembrane current, caused a 63% decrease of ΔQ_{O_2}. With these changes, however, the kinetics of $\Delta Q_{O_2}(t)$ were unchanged. This suggested that the recovery phase is rate-limited by a single reaction with apparent first-order kinetics. Evidence is provided that suggests that this reaction may be the working of the sodium pump. Exposure of the retina to high concentrations of ouabain or strophanthidin (inhibitors of the sodium pump) reduced the peak amplitude of ΔQ_{O_2} by ~80% and increased τ_1. The increase of τ_1 was an exponential function of the time of exposure to the cardioactive steroids. Hence, it seems likely that the greatest part of ΔQ_{O_2} is used for the working of the pump, whose activity is the mechanism underlying the rate constant of the descending limb of $\Delta Q_{O_2}(t)$.

INTRODUCTION

The histology of the drone retina suggests that the metabolism is compartmented: the photoreceptors contain numerous mitochondria ranged at the periphery of the cell close to the cytoplasmic membrane; in contrast, the surrounding glial cells have very few mitochondria, but they do contain large quantities of glycogen particles (Perrelet, 1970). Possible metabolic interactions between these two classes of cells are under investigation (Coles and

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Tsacopoulos, 1981), but these observations already suggest that most of the aerobic metabolism of the drone retina is confined to the photoreceptors. There is some experimental evidence that suggests that the energy metabolism in the drone photoreceptors is predominantly oxidative (Baumann and Mauro, 1973; Tsacopoulos and Mauro, manuscript in preparation). Assuming that there is a tight coupling between ATP hydrolysis and its resynthesis via the oxidative metabolism, it appeared to us that a quantitative analysis of the kinetics of the oxygen consumption elicited by a single flash of light could give information about the way ATP is used and restored by the photoreceptor during and after its stimulation by light. Furthermore, we thought that the elucidation of these mechanisms could be of central interest in exploring the possibility that ATP is used in processes induced by light other than the expected increase of the working of the sodium pump.

The kinetics of a light-induced oxygen consumption $[\Delta Q_{O_2}(t)]$ in the retina do not appear to have been measured previously. The technique used here to measure $\Delta Q_{O_2}(t)$ from P_{O_2} changes elicited by light and recorded with O_2 microelectrodes is based on the mathematical method developed by Mahler (1978a) in his studies on the sartorius muscle, in which the Q_{O_2} and the intramuscular P_{O_2} are linked by the one-dimensional diffusion equation for oxygen (Mahler, 1978b). The use of this technique was possible because, as we have shown in a previous study (Tsacopoulos et al., 1981), the diffusion of O_2 in the superfused drone retina can also be satisfactorily described by the one-dimensional diffusion equation. On the basis of this equation, the steady state oxygen consumption (Q_{O_2}) and the coefficients of oxygen diffusion (D) and solubility (α) have been measured in the dark-adapted drone retina (Tsacopoulos et al., 1981). These values are now introduced in the diffusion equation for the calculation of $\Delta Q_{O_2}(t)$.

In this article it is shown that a single flash of light elicits a transient increase of Q_{O_2} from its basal dark level to a maximum. The amplitude of ΔQ_{O_2} is affected by both the intensity of the light flash and the ionic composition of the superfusate. The kinetics of the response, however, were invariable under these experimental changes. Inhibitors of the sodium pump greatly reduced the amplitude of ΔQ_{O_2} and also modified the kinetics of the response.

METHODS

Measurement of ΔP_{O_2}

The double-barreled oxygen microelectrode together with the associated electronic apparatus and their performance have been described in previous papers (Tsacopoulos and Lehmenkühler, 1977; Tsacopoulos et al., 1981). The only modification made for the needs of this study was the reduction of the length of the recess of the O_2-sensitive barrel to ~5 μm. This length was convenient because convection effects in the perfusate were still undetectable, and the response time to an abrupt change of P_{O_2} was reduced to 10 ms, sufficiently fast for the purpose of this study.

In some instances the light had a transitory effect on the electrode (Fig. 1). The amplitude of this effect, which will be called here a "photo-effect," was dependent on

the intensity of light but never exceeded 2 pA for electrodes having 0.10 pA/mm Hg. It appeared a few milliseconds after the onset of the light pulse and terminated before the onset of the light-induced transient drop of the P_{O_2}. We found no satisfactory explanation for the effect shown in Fig. 1: the pure Pt used in this study is not expected to give photocurrents of appreciable magnitude when it is immersed in ordinary physiological solutions (Gerischer, 1975). This photo-effect was present in 20–30% of the experiments and it was not related to the bioelectrical activity of the tissue because it could be recorded in dead or poisoned retinas. When it was present it persisted whether the electrode was in Ringer solution, physiological saline, or placed in the superfused tissue. It was increased by removal of the infrared filtering
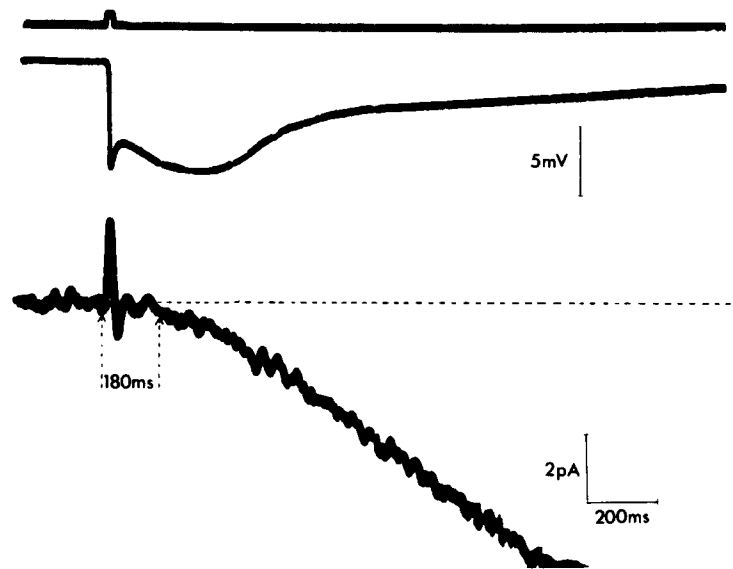

FIGURE 1.   Oscilloscope records of $\Delta Q_{O_2}(t)$ (lower trace) and extracellular receptor potential (middle trace), elicited by a flash of light (upper trace). Both quantities were recorded simultaneously by means of a double-barreled O_2-sensitive microelectrode (see Tsacopoulos and Lehmenkühler, 1977) positioned in the retina ~100 µm from the exposed retinal surface. A few milliseconds after the onset of the light pulse, a "photo-effect" (see text) was recorded by the O_2-sensitive barrel (upward deflection), whereas the P_{O_2} drop caused by the increase of Q_{O_2} was recorded later on. The dotted line is the baseline.

of the radiation of the xenon lamp and completely eliminated by turning the double-barreled electrode about its axis.

The main drop of P_{O_2} after a flash of light (see below) was not associated with the "photo-effect." The evidence for this is threefold: (a) the magnitude and the time course of the photo-effect were markedly different from the subsequently light-induced drop of the P_{O_2} (see Fig. 1); (b) when the tip of the microelectrode was placed >200 µm away from the exposed retinal surface (see Tsacopoulos et al., 1981), the photo-effect could be present but no light-induced $\Delta P_{O_2}(t)$ could be measured; (c) when the electrode was placed inside the tissue, the light-induced $\Delta P_{O_2}(t)$ could be completely and reversibly abolished by poisoning the retina with amytal, an inhibitor of the oxidative metabolism (Nishiki et al., 1979).

Preparation

Slices of drone retina 300 μm thick were used and the time course of ΔP_{O_2} elicited by a flash of light was measured simultaneously at the exposed retinal surface and inside the tissue. The rationale for this experimental arrangement was the following: (a) previously it has been shown that in the drone cut-head preparation superfused in darkness with oxygenated Ringer solution, ~450 μm of tissue is sufficiently oxygenated to maintain a constant O_2 consumption. Deeper, the tissue is anoxic (Tsacopoulos et al., 1981). Fig. 2 shows that when the photoreceptors were stimulated by a series of
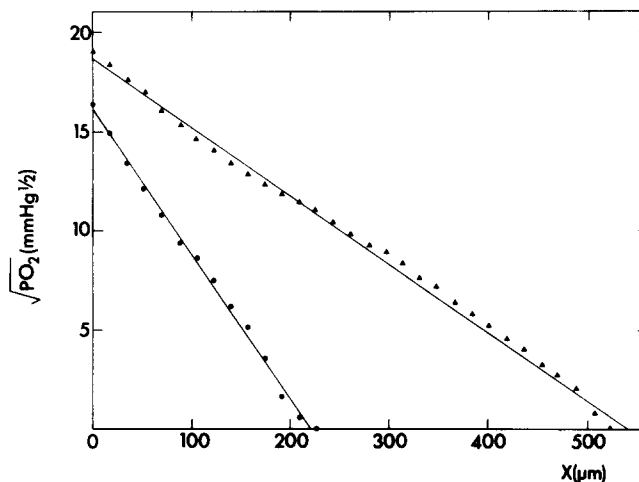

FIGURE 2.   A sample recording of P_{O_2} as a function of distance (x) made in the cut-head preparation (see details in Tsacopoulos et al., 1981). The position of the exposed retinal surface is indicated by zero. The P_{O_2} microelectrode was advanced by steps of 20 μm into the retina. The P_{O_2} decreased as a function of depth and reached zero at ~200 or ~500 μm, depending on whether the retina was repetitively stimulated by intense light flashes at 1 Hz (●) or was dark adapted (▲). Here $\sqrt{P_{O_2}}$ is plotted instead of P_{O_2} for clarity: when this plot is closely fit by a straight line, we can consider Q_{O_2} uniform inside the tissue (Tsacopoulos et al., 1981). This is the case in light and darkness down to very low P_{O_2}'s. The slope (γ) of these lines is $\gamma = -\sqrt{\dfrac{\bar{Q}}{2\alpha_R D_R}}$, where $\bar{Q} = $ mean Q_{O_2}, R signifies retina, α is oxygen solubility, and D is the oxygen diffusion coefficient; knowing $\alpha_B D_B$ (B signifies bath) and measuring $\Delta P_{O_2}/\Delta x$ above the exposed retinal surface, $\bar{Q}$ and $\alpha_R D_R$ could be estimated (see Eqs. 6 and 8 in Tsacopoulos et al., 1981). The results of 10 experiments are presented in Table I. From the ratio of $\gamma_{(light)}$ to $\gamma_{(dark)}$ it is calculated that $\bar{Q}$ under repetitive light stimulation is about threefold higher than in darkness.

flashes until a steady state of excitation was reached (Coles and Tsacopoulos, 1979), the oxygenated layer of tissue was reduced to about half of that in darkness. The results obtained from 10 retinas are presented in Table I together with the values of Q_{O_2} and αD under steady state light stimulation calculated by the method described in the previous paper (Tsacopoulos et al., 1981). Pilot experiments showed that stimulation of the retina by a single flash of light provokes a minor displacement of the anoxic core. Therefore, we decided to use retinal slices 250–300 μm thick placed

on an O_2-impermeable material (glass). Thus, under these conditions it was expected that everywhere in the tissue Q_{O_2} would be independent of P_{O_2} in both the dark steady state and also during transient stimulation with a single flash of light (see also Tsacopoulos et al., 1981). In addition, it was expected that, similarly to the cut-head preparation, in the superfused slice, O_2 should diffuse in one dimension perpendicular to the tissue-glass interface (Fig. 3). (*b*) Mahler (1978*a*) calculated the kinetics of Q_{O_2} in isolated frog muscle from the kinetics of P_{O_2} at one surface, via the one-dimensional diffusion equation. In Mahler's experiments one surface of the muscle was exposed directly to gas and he could reasonably assume that the P_{O_2} at this surface remained constant irrespective of the rate of consumption in the muscle. In this study, the retina was superfused by Ringer solution in which O_2 was dissolved. Consequently, there was a boundary layer of fluid of $\sim 100\ \mu m$ above the exposed retinal surface in which diffusion of O_2 dominated over convection (see Tsacopoulos et al., 1981). Thus, any increase of the metabolic rate of the retina was expected to induce changes of the P_{O_2} at the retinal surface exposed to the superfusate (see Fig. 2 and Results). Therefore, we decided to record P_{O_2} transients at the surface and at another location inside the

TABLE I

Experiment	Surface P_{O_2}	l	Q_{O_2}	$\alpha D \cdot 10^5$
	mm Hg	*μm*	*$\mu l\ O_2/g \cdot min$*	*ml $O_2/cm \cdot atm \cdot min$*
1	309	157	69	2.13
2	317	209	59	3.07
3	312	191	62	2.77
4	347	172	73	2.15
5	314	214	61	2.66
6	279	198	55	2.97
7	213	164	50	2.42
8	302	151	74	2.20
9	317	207	62	3.55
10	255	196	56	3.79
11	267	221	60	4.21
12	305	216	53	3.04
$\bar{x}$ SEM	294.7 ± 10.3	191.3 ± 7.0	61.2 ± 2.2	2.91 ± 0.18

Values of Q_{O_2}, αD, surface P_{O_2}, and oxygen-supplied depth (*l*) measured under steady state maximal light stimulation of the retina. The values of this table were used as a base for the choice of the experimental conditions.

tissue. In this way, the changes of the entering O_2 flux are taken into account in the calculations (see below). (*c*) The method of calculation of the time course of Q_{O_2} (see Mahler, 1978*a*, and below), is much simpler if the Q_{O_2} is uniform throughout the retina slice and thus varies only with time. In the drone retina, the glial cells that surround the photoreceptors contain a screening pigment that absorbs light. This pigment is predominantly concentrated near the cornea (Perrelet, 1970). Measurement of the amount of light transmitted through the retina slices showed that attenuation is relatively small down to a depth of 300 μm (<0.3 log units) except near the cornea (R. Muri, personal communication). However, to ensure the best possible homogeneity of light stimulation, we arranged for the slice to receive light from both sides (Fig. 3).

The preparation was tested as follows: (*a*) measurements in steady state of the P_{O_2} in the bath and inside the tissue as described in the legend of Fig. 2 and in Tsacopoulos et al. (1981) showed that diffusion of O_2 in the slice occurs as in the "cut-head preparation." It was also confirmed that the slice was fully oxygenated. (*b*) Using the usual electrophysiological techniques (Baumann, 1968), two photoreceptors were

impaled, one at a depth of ~50 μm from the exposed surface and another at ~200 μm. The receptor potentials were recorded at different light intensities from both cells and the potential measured at the peak of the transient of the receptor potential was plotted as a function of the log of the intensity. The intensity for the half-maximal response was the same in the two sites. This result indicates that the experimental arrangement provides fairly homogeneous light stimulation of the retina slice.

The experiments were done at 22°C.

Procedure

The retina slices were prepared as follows: the drone was decapitated, the antennae were cut off, and the head was inserted, with the posterior surface up, into a concave
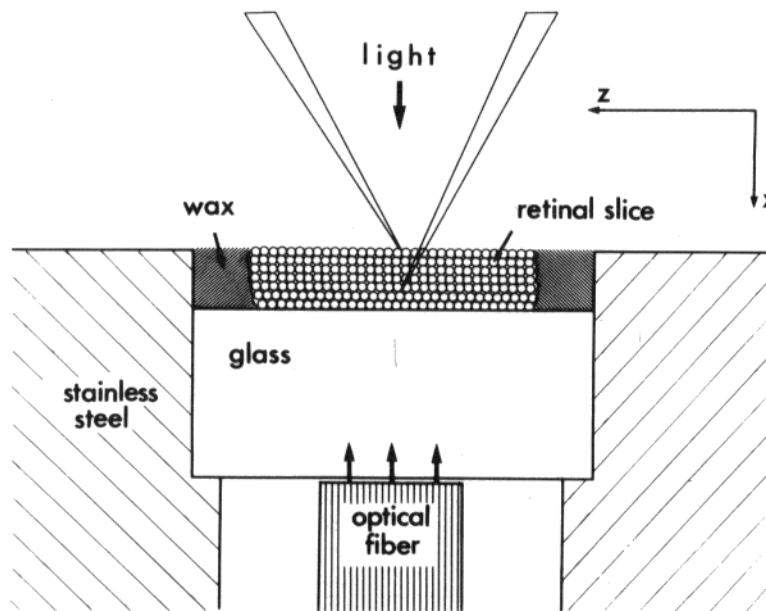

FIGURE 3.   Schematic section of the experimental arrangement. Two micro-electrodes are shown, one at the surface of the retinal slice, the other deep in the tissue. The 300-μm slice receives light from both sides. The oxygenated Ringer flows along the axis *z* and O_2 diffuses perpendicularly to the retina surface along the axis *x*.

well in a stainless steel head-holder maintained at 37°C; the well, whose depth was small compared with the size of the head (2.5 mm), was previously filled with low-melting-point (37–38°C) wax (Eicosan; Fluka, Buchs/Switzerland). The orientation of the head in the well was carefully adjusted so that the dorso-ventral axis of the head was parallel to the plane and about half of the head protruded above the edge. Then the preparation was cooled and a slice of chitinic tissue was cut from the dorsal part of the head with a new razor blade vibrating at 300 Hz to expose a layer of ommatidia of both eyes (Plate 1 of Bertrand et al., 1979, shows the preparation as it appears under the dissecting microscope). The cut was made parallel to the ommatidia and care was taken not to cut the axons at the level of the basal membrane. Then, by means of a microdrive whose displacement was calibrated by a dial comparator, the

head was displaced 300 μm vertically and the head was cut as before with a fresh part of the vibrating razor blade. The 300-μm slice thus obtained was fixed with Eicosan in the well of the slice-holder as shown in Fig. 3. The well of the slice holder was 300 μm deep so that the exposed layer of ommatidia lay flush with the floor of the chamber (see Tsacopoulos et al., 1981). The slice was superfused at 22°C in the perfusion chamber described in detail previously (Tsacopoulos et al., 1981) with oxygenated Ringer solution of the following composition: 273 mM NaCl; 10 mM KCl, 1.8 mM CaCl$_2$, 5 mM MgCl$_2$, 10 mM Tris-HCl, pH 7.4. No metabolic substrate was included in the Ringer solution. Pilot experiments showed that addition of 20 mM glucose, pyruvate, trehalose, or lactate did not modify the kinetics or the amplitude of the light-induced ΔQ_{O_2}. We did not explore this further but decided, because stable ΔQ_{O_2}'s could be recorded for >4 h of superfusion, that there was an efficient endogenous supply of metabolic substrate, presumably from the breakdown of glycogen, which is predominantly concentrated in the glial cells (Perrelet, 1970).

About 10 min after the onset of superfusion, the two microelectrodes were positioned under microscopic control as shown in Fig. 3 and as described previously (Tsacopoulos et al., 1981). The light was turned off and the retina was allowed to dark adapt. Usually, on turning off the light, P_{O_2} at both sites started increasing steadily because Q_{O_2} progressively declined to a lower dark steady state level (see Tsacopoulos et al., 1981). After ~30–40 min P_{O_2} reached a constant level. If the P_{O_2} did not become steady, the slice was discarded. If the P_{O_2} trace became level, the stimulation of the retina was started. After the last suitable record of $P_{O_2}(t)$ had been obtained, e.g., after a series of intensities (see Results), both microelectrodes were rapidly withdrawn from the retina to the bath and calibrated. During an experiment many calibrations were made even though the microelectrodes were generally satisfactorily stable for the whole time (see also Tsacopoulos et al., 1981).

Chemicals

Ouabain (Merk, Federal Republic of Germany) was dissolved in the Ringer. The solution of strophanthidin (Merk) was prepared as described by Deitmer and Ellis (1978): the strophanthidin was dissolved in ethanol to produce a stock solution of 100 mM. Thus 10^{-4} M and 5×10^{-5} M solutions in Ringer gave a final ethanol concentration of 0.1 and 0.05%. Pilot experiments demonstrated that 0.5% ethanol had no observable effect on $\Delta Q_{O_2}(t)$.

Illumination

The stimulating system was very similar to that described by Bertrand et al. (1979). The light source was a xenon lamp (XBO 15G of 150 W), whose unattenuated flux on the preparation was 6.53×10^{15} photons/mm$^2 \cdot$s. The light flux brought by the optical fiber (see Fig. 3) was 2.6×10^{14} photons/mm$^2 \cdot$s. The light beam was focused on the retina with lenses and a microscope objective. The diameter of the illuminated area was ~2.5 mm, thus including the whole exposed retina surface. The duration of the flash in all experiments was 40 ms.

Calculation of $\Delta Q_{O_2}(t)$ from $P_{O_2}(t)$

In the superfused drone retina and in steady state, the P_{O_2} is linked to the Q_{O_2} via the one-dimensional equation of diffusion (see above and Tsacopoulos et al., 1981). Because photostimulation was provided in such a way that it could be considered as roughly constant throughout the retina, it appeared reasonable to assume that the time course of the Q_{O_2} elicited by a single flash was uniform in the retina (i.e.,

independent of the position x). Besides, it was found in pilot experiments that the P_{O_2} drop after a flash was the same at different places on the exposed retinal surface, which confirms that the one-dimensional equation of diffusion is still valid under the present condition and that

$$Q(t) = \alpha D \frac{\partial^2}{\partial x^2} P(x,t) - \alpha \frac{\partial}{\partial t} P(x,t), \tag{1}$$

where P is the P_{O_2}, Q is the Q_{O_2}, x is the position in the tissue, and t is the time.

One way to solve this equation in the case of transient ΔP and ΔQ is to use the method of Fourier transforms (see Mahler, 1978a), which leads to the following result in our case (see Appendix):

$$\Delta Q(t) = \mathscr{F}_\omega^{-1}\{H_{12}(\omega) \cdot \mathscr{F}_t(\Delta P(x_1)) + H_{21}(\omega) \cdot \mathscr{F}_t(\Delta P(x_2))\} \tag{2}$$

with

$$H_jk(\omega) = \frac{i\alpha\omega \cosh \beta x_k}{\cosh \beta x_j - \cosh \beta x_k}$$

$$\beta = \sqrt{i\omega/D} \text{ and } i = \sqrt{-1}.$$

($\mathscr{F}_t$ is the Fourier transform with respect to t and $\mathscr{F}_\omega^{-1}$ is the inverse transform; x_1 and x_2 are the positions of the two electrodes.)

The analogue signals from the electrodes were sampled and converted to digital at a rate of 10 Hz. Then they were sent to a microcomputer (IMSAI 8080 microcomputer system, San Leonard, CA) through a microcircuit interface. The signals were stored on a magnetic disk and displayed on a Tektronix (Beaverton, OR) 4006 terminal. ΔQ_{O_2} was calculated from Eq. 2 using a Fast Fourier Transform routine (Brigham, 1974) with 256 points. One program was used to compute ΔQ_{O_2} and another was used to display the ΔQ_{O_2} or ΔP_{O_2} curves on a plotter.

RESULTS

Kinetics of ΔQ_{O_2} after a Single Flash of Light

In a dark-adapted retina, a 40-ms flash of bright light evokes a transient decrease of the P_{O_2} at the exposed retinal surface and inside the tissue. This effect is shown in Fig. 4. The two records were made simultaneously: trace A was recorded at the surface and trace B at an intraretinal site 250 μm from the surface. Both records show an initial decline along an S-shaped path, starting as soon as 200 ms after the onset of the flash (see Fig. 1), to a minimum level reached after ~10 s. The recovery was also S-shaped with a slower time course and, on the average, the P_{O_2} returned to the initial level in ~2 min. For technical reasons the sampling of data after a flash was limited to a maximum of 49.5 s. However, examination of the original polarographic trace presented in the inset of Fig. 4 shows that recovery was complete and perfectly stable. The more intense was the light, the greater was the final drop of P_{O_2}, but the time course of P_{O_2} showed little variation with the intensity of stimulus in a given retinal slice. Also, from one retina to another the ΔP_{O_2} showed relatively little variation. The sample records of Fig. 4 were obtained with the strongest light intensity. It is noticeable that the drop of P_{O_2} is greater and faster at 250 μm in the tissue (lower curve) than at its upper surface: this is to be expected

from the experimental situation. Of great importance in the context of the calculation of ΔQ_{O_2} was the observation that at the lowest level of this trace the P_{O_2} is well in excess of the reported "critical P_{O_2}" for this tissue (Tsacopoulos et al., 1981) and for muscle (Hill, 1948). Consequently, it appeared reasonable to infer that in the experiments the Q_{O_2} was not at all limited by O_2 delivery.

Fig. 5 shows an example of the time course of extra oxygen consumption $[\Delta Q_{O_2}(t)]$ calculated from the records of surface and intraretinal P_{O_2} by the computer. Samples of P_{O_2} were taken every 198 ms, but for convenience the points are connected by a continuous line. The conspicuous oscillations on the record are caused in part by the use of discrete rather than exact Fourier transforms in the calculation of $\Delta Q_{O_2}(t)$ (see also Mahler's [1978a] records in
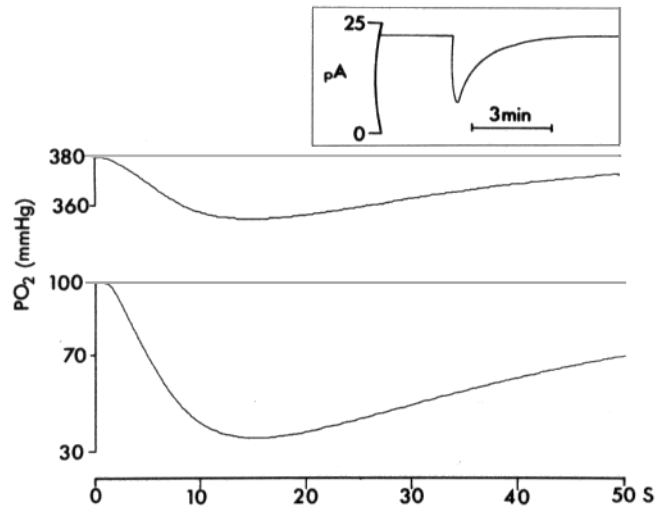

FIGURE 4.   A typical recording of the time course of P_{O_2} at the exposed retinal surface (upper trace) and at a site located 250 μm inside the tissue (lower trace). Both curves were traced by the computer, which calculated the P_{O_2}'s from the calibration curve of each microelectrode. Inset: original polarographic record made at the intraretinal site showing the complete recovery of P_{O_2} to its initial value.

muscles) and in part by the duration of the sampling: the longer the sampling, the less the amplitude of the oscillations (see Fig. 16A and comments in Appendix). From pilot experiments it was found that a sampling time of 49.5 s was in most cases sufficient. It was possible to smooth the $\Delta Q_{O_2}(t)$ curves without appreciably affecting the characteristics of the kinetics. This was done in each record by averaging every four values of ΔQ_{O_2} and placing the result at the corresponding average time. Records similar to that presented in Fig. 5 were obtained from 40 slices and basically all had similar time courses: after a flash of white light ΔQ_{O_2} reached a peak of 40 μl O_2/g·min ($\pm$ 7.3 SD, n = 40) and then declined to the steady dark level.

The declining phase of ΔQ_{O_2} in Fig. 5 is replotted on a semilogarithmic

scale in Fig. 6; the plot is reasonably well approximated by a single exponential. The straight line was fitted to the experimental points by eye. Similar plots were obtained using the computer (see also below). The time constant of this exponential decay (τ_1) was measured in 14 slices and found to be 4.96 s ($\pm$ 0.49 SD). The whole $\Delta Q_{O_2}(t)$ curve was fitted by a computer (CYBER 173; Control Data Corporation, Minneapolis, MN) using the method of least squares with curves of the form $Q_1(e^{-k_1 t} - e^{-k_2 t})$, where Q_1 (the intercept of the straight line of Fig. 6 at the origin $t = 0$), k_1, and k_2 were the variables to be adjusted. The fit was excellent and the calculated values were: $Q_1 = 105$ $\mu l\ O_2/g \cdot min$, $k_1 = 0.20\ s^{-1}$ ($\tau_1 = 5$ s), and $k_2 = 0.53\ s^{-1}$ ($\tau_2 = 1.90$ s). Both τ_1 and τ_2 were at least 30 times faster than those measured by Mahler (1978a) in the frog muscle at about the same temperature.

The validity and the precision of the mathematical and experimental methods used to measure $\Delta Q_{O_2}(t)$ were tested in experiments in which the P_{O_2}
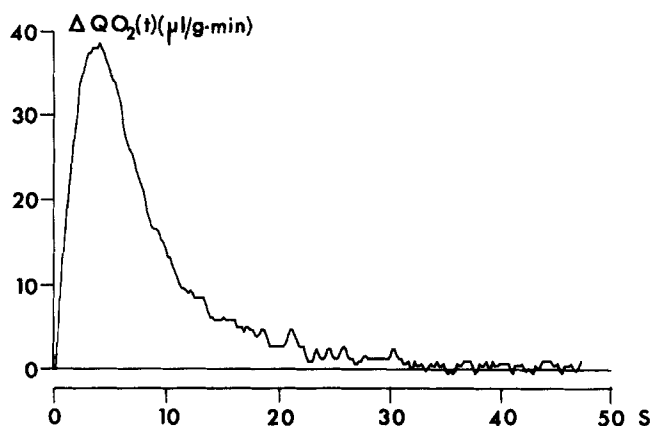

FIGURE 5.   Typical calculated time course of ΔQ_{O_2} after an unattenuated flash of light of 40 ms. The curve is derived from the P_{O_2} records shown in Fig. 4.

in the retina was changed without any change of Q_{O_2}. The experiments were done as follows: a flash was presented to the retina and $\Delta P_{O_2}(t)$ was recorded at two points as described before. Then the normal perfusate ($P_{O_2} \approx 700$ mm Hg) was replaced over 2 min by another in which the P_{O_2} was lower (≈ 600 mm Hg). This caused a drop of the P_{O_2} at the exposed surface of the slice and also deep in the tissue. Although the change from one solution to the other was fast (~ 100 ms, see Coles and Tsacopoulos, 1979, for a description of the apparatus used to make the rapid solution change), P_{O_2} changed more slowly than after a flash. At the intraretinal site (located 150 μm from the exposed surface) it took >40 s to reach its final level ($\Delta P_{O_2} \approx 44$ mm Hg). $\Delta Q_{O_2}(t)$ was calculated in the usual way from $\Delta P_{O_2}(t)$ values collected by the computer in both cases: after the flash and after the P_{O_2} change in the perfusate. The result was unambiguous: the light flash elicited a regular $\Delta Q_{O_2}(t)$ response; in contrast, the change of P_{O_2} in the perfusate did not lead to any deflection of

the calculated Q_{O_2} from its baseline. In this case, at least, the method proved capable of distinguishing a change in P_{O_2} associated with a change in consumption from a change in P_{O_2} with no change in consumption, and it seems reasonable to assume that the method is valid for all the other measurements we describe.

The kind of response shown in Fig. 5 could be stable for at least 3–4 h. For example, in the experiment presented in Fig. 7, record 1 was obtained ~90 min after the slice was made and perfusion was started and after a long period (40 min) of dark adaptation. Trace 2 was recorded ~60 min later than trace 1. During the time that elapsed between records 1 and 2 the microelectrodes were withdrawn to the bath, calibrated, and placed again in another position along the axis *z* of the retina (see Fig. 3 for the axis and Fig. 5 in Tsacopoulos
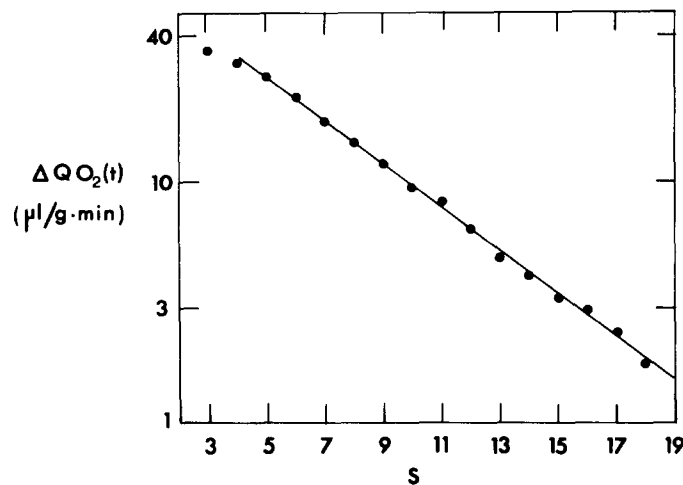

FIGURE 6. Semilogarithmic plot of the descending phase of the $\Delta Q_{O_2}(t)$ curve illustrated in Fig. 5. The values were measured on a record made on an expanded time scale. The straight line was fitted to the points by eye. The first point corresponds to the peak of ΔQ_{O_2}.

et al., 1981), again with one electrode at the exposed surface and the second in the tissue. The retina was then stimulated regularly with white flashes, one flash about every 2 min. Thus, the retina was partially light adapted when trace 2 was recorded. It can be seen clearly in Fig. 7, in which the two traces are superposed at the same scale, that they are very similar. This finding suggests several things. (*a*) There is a good metabolic stability of the tissue. In good preparations, deterioration usually started after 6–7 h of superfusion and it was manifested by a reduction of the amplitude of ΔQ_{O_2} and a slowing down of $\Delta Q_{O_2}(t)$. Most of the results presented in this article were obtained 1–3 h after the slice was made and superfusion started. (*b*) 2 min of dark adaptation were sufficient for $\Delta Q_{O_2}(t)$ to recover its dark-adapted amplitude and time course. (*c*) $\Delta Q_{O_2}(t)$ was uniform in the retinal slice, as assumed in the calculations. To confirm the last, similar experiments were done in 10 other

slices in which $\Delta Q_{O_2}(t)$ was measured in three different positions along the axis z of a given slice, at distances of ~500 μm. Generally Q_1, τ_1, and τ_2 agreed to within 10% for the three electrode locations, which confirmed that the amplitude and the shape of $\Delta Q_{O_2}(t)$ were independent of the position of the microelectrode.

Light Intensity and $\Delta Q_{O_2}(t)$

Fig. 8A illustrates the dependence of $\Delta Q_{O_2}(t)$ on light intensity. For clarity, only the responses elicited by three light intensities are plotted together on the same scale. The upper trace was elicited by an unattenuated flash (6.8 × 10^{15} photons/mm^2·s); the middle trace was elicited by a flash attenuated 16 times
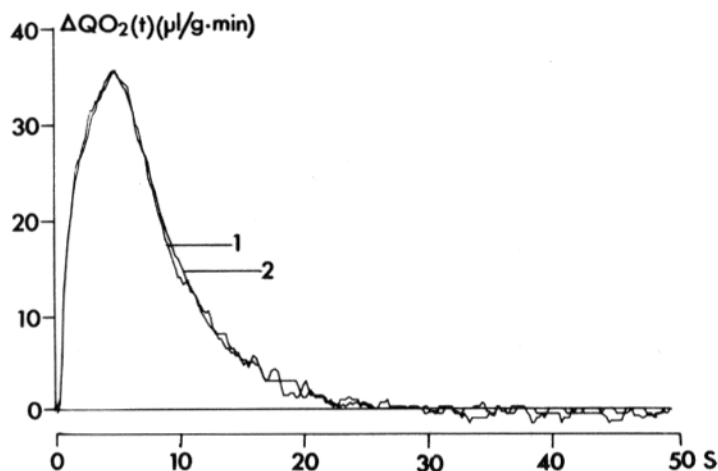

FIGURE 7.   Superimposition of two $\Delta Q_{O_2}(t)$ curves recorded in the same slice, but in two different sites along the z axis of the retina and under different conditions of dark adaptation: curve 1 was recorded after a prolonged dark adaptation, whereas curve 2 was recorded after only 2 min of dark adaptation (see details in the text).

and the lower trace was elicited by a flash attenuated 260 times. It can be clearly seen that the stronger the intensity is, the greater is the amplitude of the response of $\Delta Q_{O_2}(t)$. In Fig. 8B the three responses were matched in amplitude and superimposed on an expanded time scale. Because the curves can be superposed, the kinetics of $\Delta Q_{O_2}(t)$ are not dependent on light intensity and the extra quantity of O_2 consumed is proportional to the peak value of ΔQ_{O_2} (ΔQ_m). The dependence of ΔQ_m on the light intensity is shown in Fig. 9, where the results from 13 slices are plotted. It is clear that ΔQ_m increases linearly with the log of the intensity. A linear regression was made for each individual slice (a range of seven intensities) and the slope was calculated. The individual values are presented in Table II. The mean value of the slopes was 10.1 μl O_2/g·min·(decade increase in intensity).

Ionic Processes and $\Delta Q_{O_2}(t)$

The preceding section shows that the light-induced processes that give rise to the increase in oxygen consumption must have (in total) the same dependence on light intensity as $\Delta Q_{O_2}(t)$ itself. This provides a clue to the identification of these processes. As shown in Fig. 10, the amplitude of the receptor potential
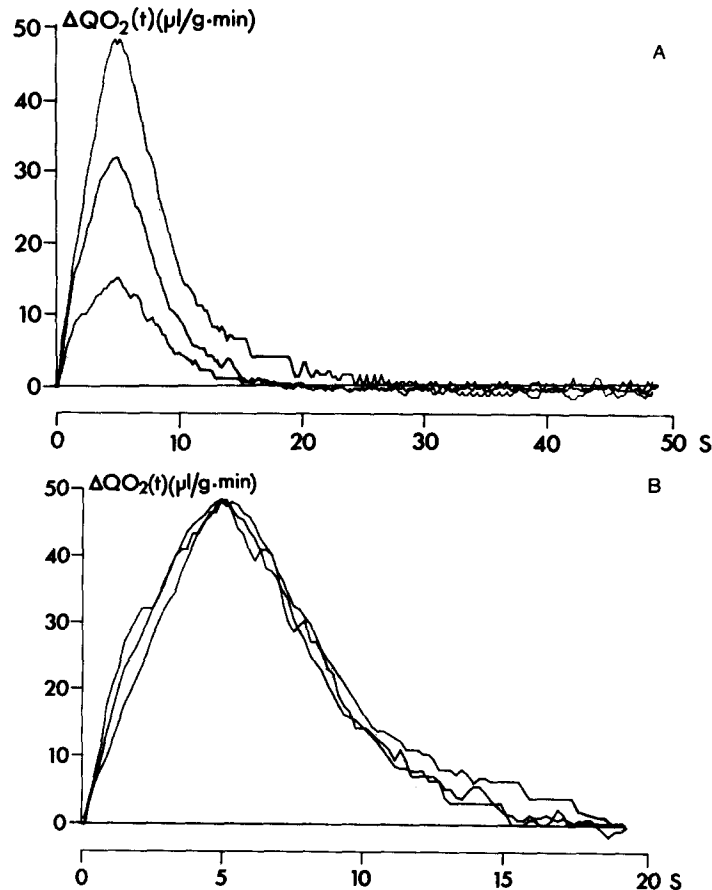

FIGURE 8.　A. Three $\Delta Q_{O_2}(t)$ curves elicited by flashes of various intensities, plotted on the same scales. B. The three curves are matched in amplitude by the computer and superimposed on an expanded time scale. The three responses, with different amplitudes, have the same time course.

can be only a minor determinant, because it saturates at an intensity at which ΔQ_m is still rising. Another quantity that may be of relevance is the flow of ions across the cell membrane. An indication of this flow is given by the measurement of changes in K^+ concentration in the extracellular space ($\Delta[K^+]_o$) with a potassium-sensitive microelectrode as described by Coles and Tsacopoulos (1979).

The amplitude of $\Delta[K^+]_o$ is plotted as a function of log I in Fig. 10 and compared with a ΔQ_m–log I curve, obtained under identical experimental conditions. Although the two curves are dissimilar at high and low intensities, there is a range (between -3.0 and -1.0 log units) in which $\Delta[K^+]_o$ and ΔQ_m are both increasing linearly with the log of intensity. Furthermore, it has been shown that in the glial cells of the drone retina, the amplitude of the depolarization (ΔV) after a single flash of light is a linear function of the log of the intensity (Bertrand, 1974). Assuming that the glial depolarization is related to the increase of the extracellular K^+ concentration (Bertrand, 1974; Coles and Tsacopoulos, 1979; Gardner-Medwin et al., 1981), this linear relation between ΔV and log I is an additional indication that the ionic charge movements elicited by a flash of light do not saturate at high intensities.

It has been shown that the light-induced current in arthropod photoreceptors is carried predominantly by sodium ions (Millecchia and Mauro, 1969;
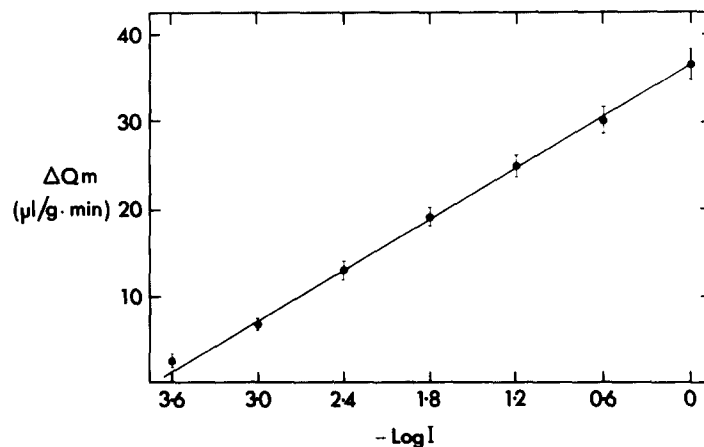

FIGURE 9. The relationship between the value of ΔQ_{O_2} at the peak of the amplitude and the logarithm of the intensity of light is shown: zero is the maximum available intensity.

Fulpius and Baumann, 1969). It therefore seemed interesting to make experiments in which the Na^+ in the Ringer solution was replaced by a relatively impermeant cation such as choline. This treatment is known to reduce the magnitude of the photocurrent in the voltage-clamped *Limulus* ventral eye by 90% (Millecchia and Mauro, 1969) and decrease the amplitude of the receptor potential of the drone photoreceptor by $\sim$60% (Fulpius and Baumann, 1969; M. Tsacopoulos, unpublished observations made in retinal slices). In a few minutes after the onset of superfusion with 0-Na^+ Ringer, the resting Q_{O_2} in the dark started decreasing and reached a steady state in 10–15 min (13 slices) in which the Q_{O_2} was $\sim$10% lower than in the control condition. This appeared in the records as an increase of the local P_{O_2} in both the exposed retinal surface and deep in the tissue. When the P_{O_2} trace became level, a flash was presented to the retina about every 5 min and $\Delta Q_{O_2}(t)$ under 0-Na^+ was recorded. For

the experiment of Fig. 11 the ΔQ_m decreased in the first 10–15 min by ~50% (average 37 ± 8% SD, $n = 11$) but it took on average an additional 20 min for ΔQ_m to reach a stable amplitude ~70% lower than that recorded under physiological Ringer (average 63 ± 4.0% SD, $n = 7$). When normal Ringer was reintroduced into the perfusion chamber, the amplitude of ΔQ_{O_2} rapidly increased to attain its initial value within ~15 min. Rarely (two slices) was a small transient overshoot of ΔQ_m observed. Thus recovery of the effect of 0-

TABLE II

LINEAR REGRESSIONS OF THE PEAK AMPLITUDE ΔQ_m vs. LOG *I*

Experiment	Intercept	Slope
	$\mu l\ O_2/g \cdot min$	$\mu l\ O_2/g \cdot min$ *(unit. opt. den.)*
1	30.0	7.34
	(0.46)	(0.20)
2	33.0	7.85
	(0.65)	(0.30)
3	41.57	10.47
	(0.92)	(0.42)
4	49.04	12.14
	(1.0)	(0.56)
5	37.71	11.14
	(0.48)	(0.26)
6	48.16	13.75
	(0.37)	(0.24)
7	39.90	11.60
	(1.36)	(0.62)
8	31.31	8.0
	(0.71)	(0.34)
9	33.43	9.8
	(0.81)	(0.48)
10	33.33	9.66
	(0.52)	(0.30)
11	33.57	8.38
	(1.2)	(0.67)
12	30.40	10.16
	(0.50)	(0.40)
13	40.05	10.47
	(0.76)	(0.48)
$\bar{x}$ SEM	37.03 ± 1.76	10.05 ± 0.40

Each experiment represents a different retinal slice. The numbers in parentheses are the values of the variance.

Na$^+$ Ringer was faster than its onset. Fulpius and Baumann (1969) found that after 10 min of exposure to sodium-free solution the recovery of the amplitude of the receptor potential in physiological solution followed a monotonic function and was faster than the diminution in sodium-free solution. They also found that replacement of Na$^+$ by either choline or Tris had very similar effects. In some experiments of the present study Na$^+$ was replaced by the amino acid arginine, taking care to maintain the same tonicity

and pH of the solution. The effects of arginine were very similar to those obtained with choline, except perhaps that the arginine had more effect on the junction potential of the reference electrode (see Tsacopoulos and Leh-menkühler, 1977).

In Fig. 12A three selected $\Delta Q_{O_2}(t)$ responses are presented on the same scale. The upper trace was recorded while the slice was superfused with normal Ringer. The general shape of the curve is as described above. The middle and lower traces were recorded after 15 and 30 min of superfusion with 0-Na^+ solution. It can be clearly seen that the amplitude of the response was diminished in 0-Na^+ solution. In Fig. 12B the three curves are superimposed
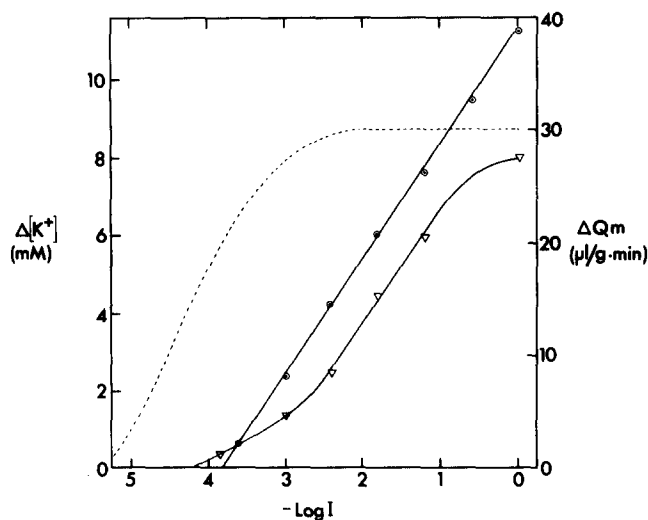

FIGURE 10.   Variation of three quantities with light intensity. ⊙: peak value of light-induced $\Delta Q_{O_2}(t)$, ΔQ_m (right-hand ordinate). ▽: peak light-induced increase in extracellular potassium concentration ($\Delta[K^+]_o$). The dotted line indicates the amplitude of the intracellular receptor potential: no vertical scale is given. Note that the amplitude of the receptor potential saturates while ΔQ_m and $\Delta[K^+]_o$ continue to increase. $\Delta Q_{O_2}(t)$, $\Delta[K^+]_o$ and receptor potential were recorded under identical experimental conditions. (This experiment was done with the collaboration of Dr. J. A. Coles.)

and matched at the peak on an expanded time scale. The good superposition of the three curves indicates that the kinetics of $\Delta Q_{O_2}(t)$ were negligibly affected by the diminution of the light-induced Na^+ current occurring when the retina is superfused with Na^+-free Ringer solution.

The persistence of a ΔQ_{O_2} even after prolonged replacement of Na^+ by choline does not appear to be explained by the persistence of a small amount of Na^+ in the preparation. If Na^+ diffused freely in the extracellular spaces, it is possible to calculate, making assumptions like those in the legend of Fig. 4 of Coles and Tsacopoulos (1981), that after 12.5 min of superfusion with 0-Na^+ Ringer the extracellular Na^+ concentration everywhere in the 300-μm

slice would be <1 mM. Moreover, extracellular measurements made with neutral-ligand Na^+-sensitive microelectrodes showed that after 10 min of superfusion with 0-Na^+ Ringer solution, $[Na^+]_o$ dropped close to zero (S. Levy, personal communication). It appears, therefore, that either tiny Na^+ currents are still present even after prolonged sodium replacement (see detailed discussion by Millecchia and Mauro, 1969; Fulpius and Baumann, 1969), or that some fraction of the ΔQ_{O_2} is not related to the sodium current via the work of the sodium pump (see Discussion), but is related to other ATP-consuming processes that can also be stimulated by light. In favor of the latter idea is the observation (Fig. 10) that small Na^+ currents induced by light flashes of intensities lower than −4.0 log units (equivalent, as far as the amplitude and the shape of the receptor potential are concerned, to the maximum effect of
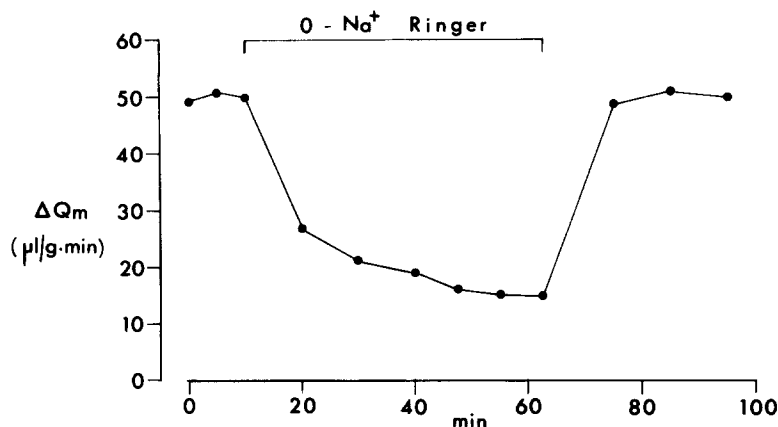

FIGURE 11.   The amplitude of ΔQ_m in sodium-free Ringer solution. The retina was stimulated by a flash every 4–5 min and $\Delta Q_{O_2}(t)$ was recorded. When the amplitude and the kinetics of the response were in a steady state, the solution was changed for the period indicated by the bar, and ΔQ_m was measured as a function of time. The reintroduction of physiological Ringer solution was followed by a rapid recovery of the response.

0-Na^+ Ringer) were not sufficient to trigger a measurable increase of Q_{O_2}. However, this hypothesis requires that the rate-limiting step of these processes and the Na pump have the same time constant; experiments are in progress aimed at resolving this question (see last paragraph of Results).

In addition, replacement of Ca^{2+} by Mg^{2+} did not further affect the residual ΔQ_{O_2}. In the presence of $[Na^+]_o$, 0-Ca^{2+} Ringer caused ΔQ_m to increase, typically from 40 $\mu l/g \cdot min$ to 60 $\mu l/g \cdot min$, without any detectable effect on τ_1 and τ_2. This result further confirms our interpretation of ΔQ_{O_2} as being caused mainly by a rise in internal Na^+, because Millecchia and Mauro (1969) have shown that superfusion of *Limulus* ventral photoreceptor with 0-Ca^{2+} artificial seawater causes an eightfold increase of the steady state light-induced current carried mainly by Na ions.

Effect of Cardioactive Steroids

Given that the major part of ΔQ_{O_2} is related to the light-induced ion fluxes, it appeared crucial to demonstrate that inhibition of the sodium pump by cardioactive steroids could affect $\Delta Q_{O_2}(t)$. First, the cardiac glycoside ouabain,
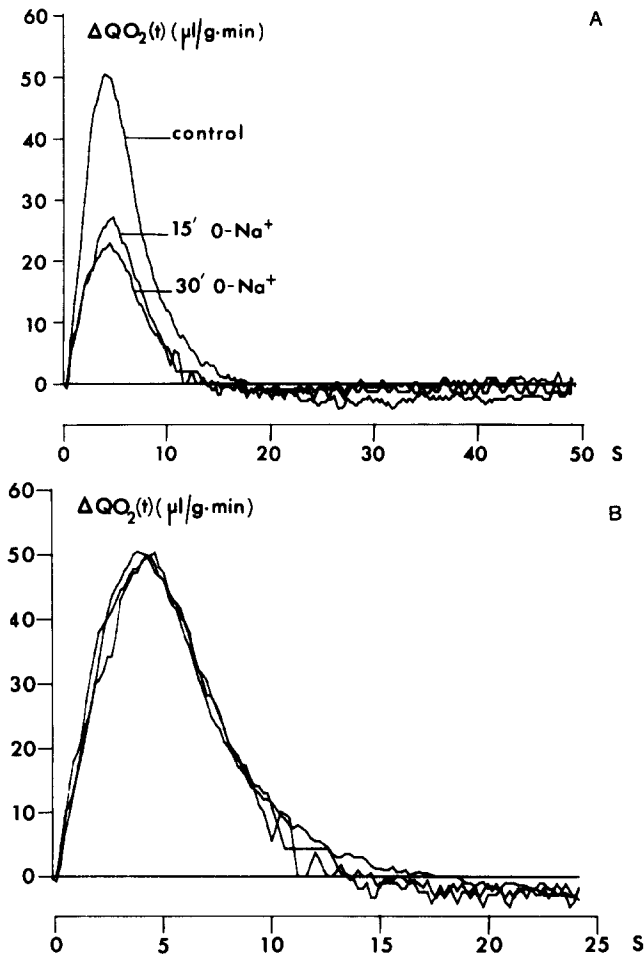

FIGURE 12.   A. Three selected $\Delta Q_{O_2}(t)$ responses from the experiment illustrated in Fig. 11 plotted on the same scale. Superfusion with $0\text{-}Na^+$ Ringer solution causes a reduction of the amplitude of ΔQ_{O_2}. B. The three responses of A are matched in amplitude by the computer and superimposed on an expanded time scale for better examination of the kinetics. The three responses have the same time course.

a potent inhibitor of the sodium pump, was used in a high concentration (5 × 10^{-4} M) (Abeles, 1969; Deitmer and Ellis, 1978). Even though the sensitivity of different animals and tissues to cardiac glycosides varies within wide limits (Skou, 1965), one can be confident that at this high concentration the activity

of any pump should be completely blocked. It was found that ouabain reduced by ~80% the amplitude of ΔQ_{O_2} after ~40 min of application (see Table III). The results from a typical experiment are shown in Fig. 13A, in which the control and other responses recorded during the 40-min period of ouabain application are shown. After 15 min of exposure, the amplitude of ΔQ_{O_2} was reduced by 18%. Whereas the resting Q_{O_2} was very little affected by ouabain, the steady state P_{O_2} increased slightly (~5%). Longer application of ouabain further reduced the amplitude of the response (see traces 20', 30', and 40'), but also slowed down the time constant of the recovery phase of $\Delta Q_{O_2}(t)$ (Figs. 13B and C). The time constant of the rising phase was not affected by this treatment. It is worth noticing that in this experiment, the extracellular receptor potential recorded simultaneously with the reference barrel of the P_{O_2} microelectrode (see Fig. 1 and Tsacopoulos and Lehmenkühler, 1977) was completely suppressed after 30 min of superfusion with ouabain. If Ca^{2+} was omitted from the medium, the receptor potential was not abolished (as observed previously by Brown and Lisman [1972] for *Limulus* ventral photo-
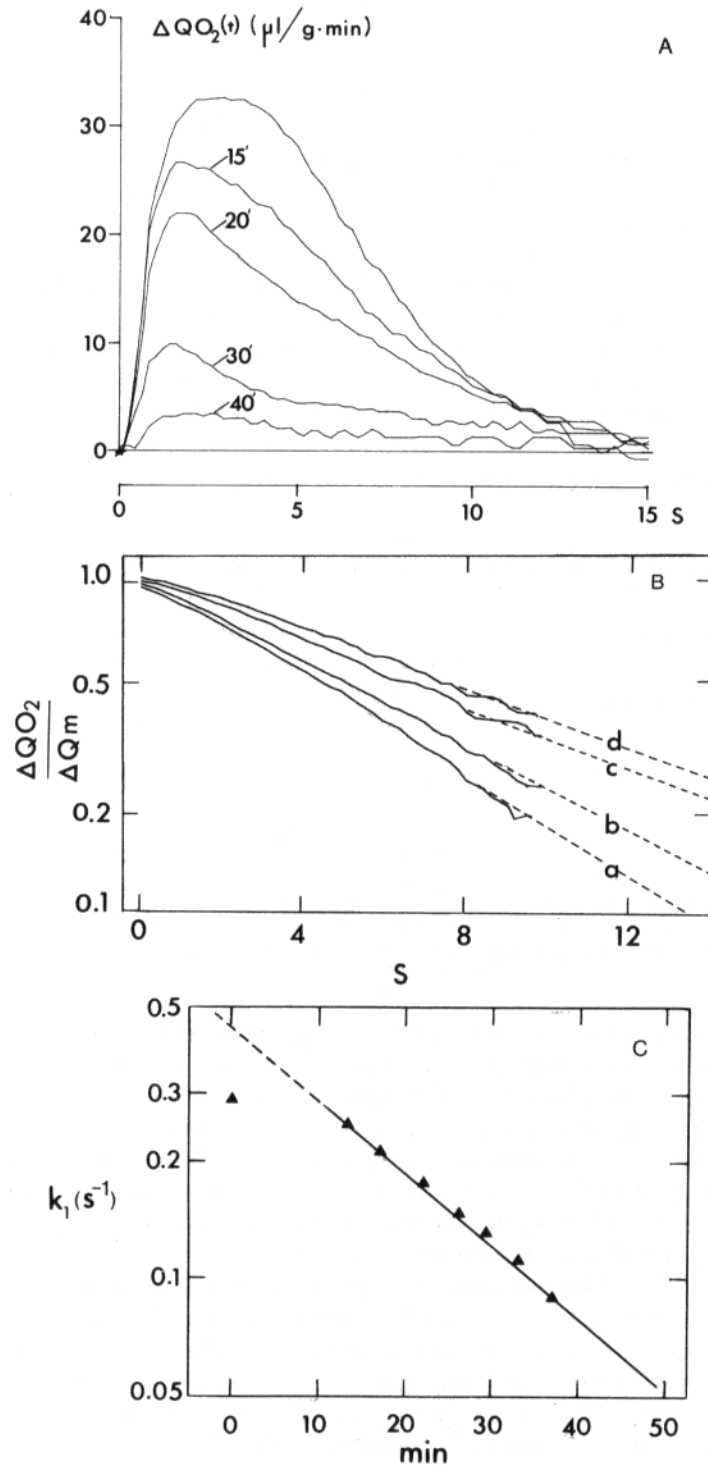
TABLE III

DEVELOPMENT OF INHIBITION BY CARDIOACTIVE STEROIDS

Experiment	Time of exposure			
	15 min	20 min	30 min	40 min
Ouabain $(5 \times 10^{-4}$ M)	21.5%	47.12%	66.0%	80.0%
$(n = 4)$	±2.7	±6.6	±2.23	±1.78
Strophanthidin $(10^{-4}$ M)	11.3%	31.1%	54.7%	75.2%
$(n = 9)$	±2.12	±4.2	±4.1	±4.7
Strophanthidin $(5 \times 10^{-5}$ M)	10.6%	28.0%	(45.0%)	
$(n = 11)$	±1.8	±1.7	(±2.1)	

Values are means ± SEM. The value of inhibition after 30 min of strophanthidin 5×10^{-5} M is in parentheses because in these experiments the drug was applied for only 20 min and then washed out (see text).

receptor). This suggests that the suppression of the receptor potential normally produced by ouabain may be caused mainly by an increase in intracellular Ca^{2+} concentration. However, in the absence of Ca^{2+}, ouabain still reduced ΔQ_{O_2} by 80%; it should be borne in mind that the amplitude of the receptor potential is a poor indication of the magnitude of the ion fluxes. Additional experiments showed that a five-times-lower concentration of ouabain $(10^{-4}$ M) had the same effect on the amplitude and the kinetics of $\Delta Q_{O_2}(t)$.

Because the effects of ouabain are effectively irreversible (Abeles, 1969; Erlij and Elisalde, 1974; Deitmer and Ellis, 1978), it was not clear what proportion of its effect on the retina was caused by the inhibition of the pump, and what proportion was caused by nonspecific actions on the membrane, leading to the death of the tissue. Thus it appeared more satisfactory to show a reversible effect of the inhibition of the pump on $\Delta Q_{O_2}(t)$. For this purpose we did experiments with strophanthidin (Abeles, 1969; Deitmer and Ellis, 1978). First, strophanthidin was applied on a high concentration $(10^{-4}$ M) known to

inhibit completely the sodium pumps of skeletal and cardiac muscles as well as brain axolemma (Abeles, 1969; Erlij and Elisalde, 1974; Deitmer and Ellis, 1978; Sweadner, 1979). Like ouabain, strophanthidin at 10^{-4} M inhibited ΔQ_m by ~75% (see Table III) and slowed down the recovery phase of the response. The maximum effect on the amplitude was observed after 40–60 min of exposure, depending on the freshness of the strophanthidin stock solution: the older the stock solution was (older than 10 d), the later the maximum effect occurred. The effect of 10^{-4} M strophanthidin was poorly reversible over a time span of 2 h. On the basis of many pilot experiments, the following experimental protocol was chosen that allowed us to record a clear, but reversible, effect of strophanthidin on $\Delta Q_{O_2}(t)$ in times compatible with the lifetime of the retinal slices: fresh 5×10^{-5} M solutions were used. After a stable $\Delta Q_{O_2}(t)$ response was recorded, the strophanthidin-containing Ringer solution was applied for 20 min. This provoked a reduction of the amplitude of ΔQ_{O_2} of ~40% and slowed down specifically the descending limb of the response (Fig. 14). Only a small diminution of the amplitude of the extracellular receptor potential was recorded. After removal of strophanthidin, the amplitude of ΔQ_{O_2} still dropped for some 10–15 min before it began to increase and reached the control level after another 50–60 min (Fig. 14). Even if there was full recovery of the amplitude, τ_1 was still slower than the control. Moreover, in ~50% of the experiments ($n = 11$) it was observed that 50–70 min after strophanthidin was washed out, the rising phase of the response was slightly slowed down so that the peak of the response was displaced to the right. For example, in the record shown in Fig. 14C, taken 60 min after the end of the 20-min application of strophanthidin, τ_2 is 2.08 s instead of the initial 1.95 s. We give no further attention in this article to this small and variable effect.

Variations of the Kinetics of $\Delta Q_{O_2}(t)$

In 40% of the experiments the first flash presented to the retina after the slice was made and superfusion began elicited $\Delta Q_{O_2}(t)$ responses in which the recovery phase transiently undershot the baseline by 1–3 μl/g·min. Fig. 15 shows a typical record of this phenomenon. The descending limb of the curve

FIGURE 13. A. The effect of ouabain (5×10^{-4} M) on $\Delta Q_{O_2}(t)$. The upper trace is the control. Four traces are shown recorded after 15, 20, 30, and 40 min of exposure to the drug. The maximum effect was recorded after 40 min of application. B. Semilogarithmic plots of the descending phase of $\Delta Q_{O_2}(t)$ vs. time made by the computer. The starting points of the plots are matched by displacement of the level of the baseline, for the purpose of better comparison of the slopes. The plots were followed for only 10 s because the traces towards the baseline are very noisy. For clarity only four plots are shown: $a = 15$ min, $b = 20$ min, $c = 25$ min, $d = 30$ min of exposure to ouabain. C. From the semilogarithmic plots the rate constant (k) was calculated (see text) and plotted semilogarithmically vs. the time of exposure to the ouabain. The dotted straight lines were fitted to the points by eye. The point at zero time is the control. The calculated time constant was 24 min.

crossed the baseline 12.5 s after the onset of the flash and returned slowly to the baseline. The transient character of the undershoot is also clearly visible in the $\Delta Q_{O_2}(t)$ curve shown in Fig. 16A of the Appendix, which was made from P_{O_2} values read by eye on the records of the polarographic currents sampled for >65 s. Two methods of calculation were used: the literal one of Mahler (1978a), and another one corrected for the use of discrete Fourier transforms (see Appendix). The transient undershoot appeared with both methods.

Careful examination of the experimental protocol of each experiment and inspection of the records did not reveal any clear information that could suggest why in some experiments a transient undershoot was present. For example, in all experiments the P_{O_2} was stable before the flash, and after the transient decrease it matched the value in the dark (inset of Fig. 15). Also,
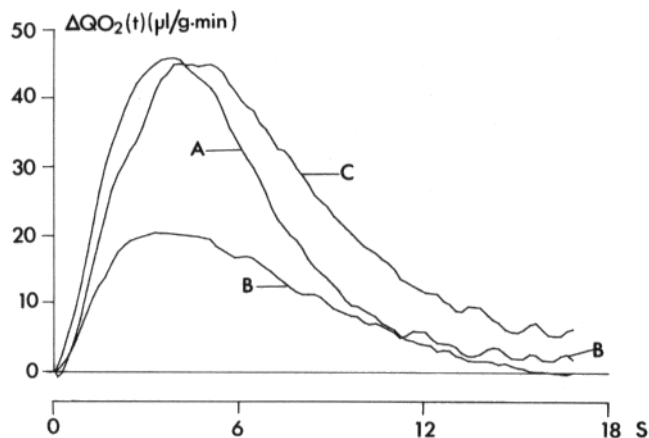

FIGURE 14. Reversible effect of strophanthidin (5×10^{-5} M) on ΔQ_{O_2}. Curve A is the control, B shows the maximum effect obtained after 20 min of exposure and 5 min of wash out. Curve C shows the extent of recovery after 60 min of superfusion with strophanthidin-free Ringer.

every experiment was performed under similar conditions of dark and light adaptation. Finally, when the undershoot was present, it persisted as long as the retina was stimulated by white flashes and it was present for all light intensities used in this study.

One possibility could be that this undershoot is caused by a transitory suppression of a process consuming metabolic energy in the dark. In a subsequent paper we will show that stimulation of the retina with colored lights, known to modify the state of the visual pigments, can prolong the period of increased consumption (increase τ_1) and abolish the undershoot.

DISCUSSION

In a dark-adapted drone retina, a bright flash of white light evoked a transient increase of the rate of oxygen consumption [$\Delta Q_{O_2}(t)$]. In most of the cases this

extra response had a fast-rising phase and after reaching a peak of about 40 μl/g·min declined exponentially to the baseline with a time constant of 4.96 s. Because the steady state Q_{O_2} in the fully dark-adapted drone retina was $\sim$18 μl/g·min (Tsacopoulos et al., 1981), a 40-ms flash could almost treble the Q_{O_2} in $\sim$3.0 s. This increase of Q_{O_2} is about the same as the maximum increase caused by a repetitive stimulation of the retina (see Table I). The question naturally arises as to what are the cellular processes triggered by light, which in turn stimulate the mitochondria and increase the Q_{O_2} of the photoreceptors.

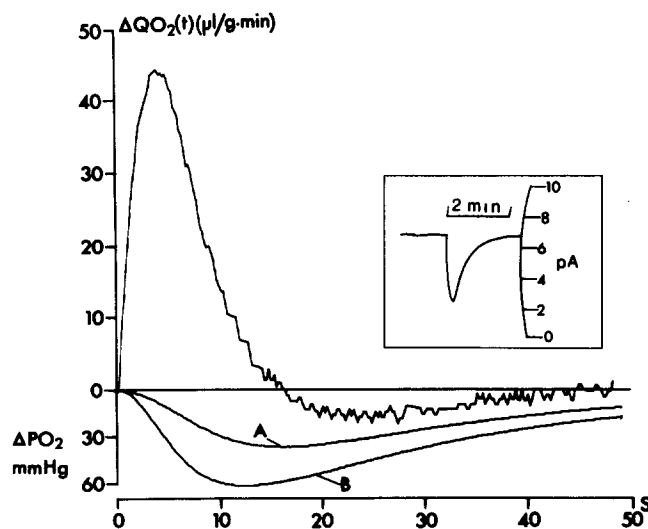From electrophysiological studies on photoreceptors of the drone and other

FIGURE 15.   Typical record of $\Delta Q_{O_2}(t)$ with a transitory undershoot. The lower traces are ΔP_{O_2}'s, from which the $\Delta Q_{O_2}(t)$ was calculated, recorded at the surface (A) and at an intraretinal site (B), located 120 μm from the exposed surface. Inset: original polarographic trace recorded with the intraretinal microelectrode showing the stability of P_{O_2} before the transient as well as the complete recovery to the baseline.

invertebrates it is known that the absorption of light by rhodopsin starts a chain of reactions that leads to the increase of the membrane conductance, mainly to sodium ions (Borsellino and Fuortes, 1968; Millecchia and Mauro, 1969; Fulpius and Baumann, 1969). This increase of intracellular sodium concentration will stimulate the activity of Na^+-K^+ ATPase and increase the pumping activity of the cell, resulting in a rapid increase of the hydrolysis of ATP to ADP and P_i (see, for example, De Weer, 1975). The consequence of such an increase of ADP concentration in the cytoplasm would be the stimulation of the mitochondrial oxidative metabolism and therefore the rate of consumption of oxygen (Chance and Connelly, 1957; Sacktor, 1975). Because in the drone retina only the photoreceptors contain significant
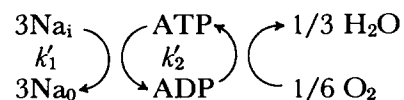
numbers of mitochondria, the observed extra oxygen consumption is associated mainly with their activity. The experimental evidence allowing us to believe that this basic concept is also applicable to the drone photoreceptor was mainly provided by the experiments in which the sodium ions were replaced in the Ringer's solution by choline, a cation that barely penetrates the light-activated channels (Brown and Mote, 1974). Thus, by reducing the electro-chemical gradient of sodium across the membrane, less sodium enters the cell per flash of light; consequently, there is less stimulation of the sodium pump and therefore, indirectly, less ΔQ_{O_2} (see Glynn and Karlish, 1975; De Weer, 1975). In addition, superfusion with $0\text{-}Ca^{2+}$ Ringer, expected to increase the light-induced Na^+ current (Millecchia and Mauro, 1969), caused an increase of ΔQ_{O_2}. The great diminution of the amplitude of ΔQ_{O_2} by the cardioactive steroids ouabain and strophanthidin gives further support to this hypothesis. It is known that these compounds bind to the pump molecule (identified as the enzyme $Na^+\text{-}K^+$ ATPase) on its site facing the extracellular space and block its activity. In the presence of an excess of cardioactive steroids, the amount of ATP hydrolyzed is proportional to the rate of disappearance of pumping sites (Schwartz et al., 1975). Therefore, it is possible to postulate that the reduction of the amplitude of ΔQ_{O_2} as a function of time of exposure to the cardioactive steroids observed in this study is caused by the blockage of pumping sites at the membrane of the photoreceptor by the drugs. Because exposure of the retina to 100 μM of ouabain or strophanthidin for ~1 h inhibited the ΔQ_m by >75%, it seems likely that the major part of the extra energy produced by the mitochondria after stimulation of the photoreceptors by a flash of light is used for pumping ions. A similar conclusion was drawn by other investigators from work on nerves (Baker and Connelly, 1965; Rang and Ritchie, 1968). In contrast, the resting Q_{O_2} was very little affected by the blockage of the pump by either replacement of Na^+ by choline or high concentrations of cardioactive steroids. This further supports the hypothesis that Q_{O_2} in the dark is mainly used for anabolic processes, such as protein synthesis (see Tsacopoulos et al., 1981).

In general, the time course of Q_{O_2} in any tissue will be determined by two factors: the time course of ATP splitting, and the processes that couple the splitting of ATP to its resynthesis in the mitochondria. In his analysis of the time course of ΔQ_{O_2} in frog muscle after a single tetanus, Mahler (1978a, 1979) presented evidence that post-tetanic suprabasal ATP splitting was small relative to the amount split during the contraction itself, so that on the time scale of recovery metabolism, suprabasal ATP splitting could be considered instantaneous. This implies that the time course of ΔQ_{O_2}, which had the same general form as that reported here for the drone retina, was determined by reactions subsequent to the hydrolysis of ATP. It is questionable whether this is also the case in the drone retina, because the activation and the recovery of ΔQ_{O_2} were >30 times faster than in muscle (see Mahler's records, 1978a) and the time course of ATP splitting presumably overlaps $\Delta Q_{O_2}(t)$. For example, the transient increase of the activity of the pump could not be considered

instantaneous relative to $\Delta Q_{O_2}(t)$: in fact, given that a single strong flash of light produces an increase of the intracellular sodium of at least 1 mM (Coles and Tsacopoulos, 1979, 1981), and assuming that the density of the sodium pumps is the same as in nerves, namely 750 pumps/μm^2 of membrane, and also that the maximum transport rate is 22 ions/s per pump site (Ritchie, 1973), it is easy to calculate that pumping (and therefore splitting of ATP) would last on the order of 20 s after a flash, almost the time required for the complete recovery of $\Delta Q_{O_2}(t)$ back to the basal dark level.[1] Thus, it appears that in the drone retina after a single light flash, unlike the case for frog muscle after a single contraction, the kinetics of ΔQ_{O_2} reflect the time course of ATP splitting as well as the reactions comprising respiratory control.

The data presented in this article show that the descending limb of $\Delta Q_{O_2}(t)$ can be fitted in the majority of the experiments by a single exponential, which suggests that the sequence of reactions initiated by the light flash, and ending with oxygen consumption, is dominated by a single relatively slow reaction with first-order kinetics. On the basis of the arguments presented above, the chemical reactions involved in the ΔQ_{O_2} process may be simply presented schematically as follows:

$$
\begin{array}{ccc}
3Na_i \searrow & \nearrow ATP \searrow & \nearrow 1/3\ H_2O \\
k'_1\ \Big) & \Big(\ k'_2\ \Big) & \Big(\\
3Na_o \nearrow & \searrow ADP \nearrow & \searrow 1/6\ O_2
\end{array}
$$

(The stoichiometries proposed by Harris et al., 1980, and Sacktor, 1975, are used here.) The first reaction is first order with respect to Na_i, which implies that ATP must be present in excess (at least 1 mM). Similarly, the second reaction is first order with respect to ADP, which implies that O_2 is present in excess.

The analytical solution of the linear differential equations describing these reactions gives for the expected changes of Q_{O_2} after a step increase of the Na^+ concentration ($\Delta[Na^+]_i$) the following equation (see Rodiguin and Rodiguina, 1964):

$$
\Delta Q_{O_2}(t) = \ell \frac{\Delta[Na^+]_i}{18} \frac{k'_1\ k'_2}{k'_2 - k'_1} [e^{-k'_1 t} - e^{-k'_2 t}]
\tag{3}
$$

where $\ell = 11.2\ \dfrac{\mu l\ STP}{g \cdot mM}$ is a constant used for the transformation of units and includes the fact that the photoreceptors occupy approximately half the volume of the retina. The simple model expressed by Eq. 3 implies that the

---

[1] We use the figures estimated by Coles and Tsacopoulos (1979), corrected for a misprint. The surface area of a photoreceptor is $3.8 \times 10^4\ \mu m^2$, so at a mean density of 750 pumps/μm^2 there are 2.85×10^7 pumps per cell and the maximum rate of pumping is 6.3×10^8 ions s^{-1}. The volume of the cell is $2.1 \times 10^4\ \mu m^3$. 1 mM of Na^+ corresponds to $6 \times 10^{23} \times 10^{-3} \times 2.1 \times 10^4 \times 10^{-15}$ ions $= 1.26 \times 10^{10}$. Hence if the pump functioned at its maximum rate until all the ions were excluded it would take ~ -20 s.

amplitude of ΔQ_{O_2} is proportional to the maximum concentration of intracellular Na^+, whereas the time dependence of ΔQ_{O_2} is governed by the two rate constants k_1' and k_2'.

The predictions proposed explicitly by Eq. 3 were to some extent confirmed experimentally. (a) The light-induced entry of Na^+ into the photoreceptor is associated with an efflux of K^+ (see Coles and Tsacopoulos, 1981). Hence, the measurements of changes in K^+ concentration in the extracellular space, $\Delta[K^+]_o$, probably give some indication of the way $\Delta[Na^+]_i$ increases with light intensity. As shown in Fig. 10, ΔQ_m increases in parallel with $\Delta[K^+]_o$ over about two log units of intensity, which supports the idea that $\Delta Q_m \propto \Delta[Na^+]_i$. At intensities below about -4.0 log units, neither ΔQ_{O_2} nor $\Delta[K^+]_o$ was detectable. At this intensity the receptor potential was already ~ 40 mV, not far from its maximum amplitude. The ion movement necessary simply to depolarize the capacitance of the surface membrane can be calculated from the surface-to-volume ratio of the drone photoreceptor. The value estimated by Coles and Tsacopoulos (1979) (correcting for misprint) is $3.8/2.1 \ \mu m^{-1} = 1.8 \ \mu m^{-1}$. Taking the specific membrane capacitance to have its usual value of $1 \ \mu F \cdot cm^{-2}$ (see Cole, 1968), and using Faraday's constant of 9.65×10^4 C·mol^{-1} we calculate that a potential change of 40 mV could be brought about by a $\Delta[Na^+]_i$ of 7.5 $\mu mol/liter$. Experimentally, we are at present unable to detect either a concentration change this small or the ΔQ_{O_2} that would be necessary to pump out the ions. At high light intensities, $\Delta[K^+]_o$ appears to saturate but ΔQ_{O_2} continues to increase: perhaps $\Delta[K^+]_o$ is held down by the entry of K^+ into the glial cells that is known to occur (Coles and Tsacopoulos, 1979). (b) Because the first-order nature of Na^+ pumping (see below) and the rate constant k_1' of the scheme should remain fixed under different conditions, the model predicts that for different $\Delta[Na^+]_i$ the descending limb of $\Delta Q_{O_2}(t)$ should remain monoexponential with fixed time (rate) constant. This is what is observed: when light intensity or extracellular Na^+ and Ca^{2+} concentrations are changed, the amplitude of ΔQ_{O_2} changed but not the kinetics. It is of interest that Gadsby and Cranefield (1979) have presented experimental evidence showing that, in voltage-clamped Purkinje fibers at constant $[K^+]_o$, the decline of $[Na^+]_i$ appeared to follow first-order kinetics with a rate constant that was independent of $[Na^+]_i$. This means that, as in the snail neurone (see Thomas, 1969, 1972), the rate constant of the pump is independent of the amplitude of the stimulus that activates it.

The electrical response of a drone photoreceptor to a short pulse of light consists of a depolarization followed by a slower repolarization whose duration depends on the intensity of the flash and the state of light or dark adaptation of the retina, i.e., after dark adaptation the repolarization is slower than when the retina is rather light adapted (Baumann, 1968). Under the experimental conditions of the present work, a bright flash evoked an electrical response in the dark-adapted retina that took on average 5 s to repolarize completely. Hence, the electrical response overlaps the rising phase of $\Delta Q_{O_2}(t)$, but apparently does not affect it because, as shown in Results, neither dark adaptation nor the intensity of the light significantly affected the kinetics of

the ΔQ_{O_2} response. Consequently, it appears that the intracellular increase of Na^+ occurs mainly at the beginning of the receptor potential and therefore, when examined on the same time scale as the ΔQ_{O_2} response, could be considered as an impulse change.

In such a case it is possible to calculate through Eq. 3 an upper limit for the magnitude of the $[Na^+]_i$ change after a bright flash of light by assuming that all the extra oxygen consumption elicited by this flash was used to re-establish the electrochemical gradient of Na^+. The comparison of Eq. 3 with the functions chosen for double exponential fit gives

$$Q_1 = \ell \frac{\Delta[Na^+]_i}{18} \left| \frac{k_1' \, k_2'}{k_1' - k_2'} \right|. \tag{4}$$

Taking the experimental values given in the results, a value of $\Delta[Na^+]_i = 8.64$ mM was calculated. Because in fact some of ΔQ_{O_2} may be used for work other than pumping of Na^+, this value is an upper limit. Measurements with intracellular K^+-sensitive microelectrodes give an estimate for a lower limit of $\Delta[Na^+]_i$ of ~1 mM (Coles and Tsacopoulos, 1979, 1981; J. A. Coles and R. K. Orkand, manuscript in preparation). In the absence of a more direct comparison of ΔQ_{O_2} and $\Delta[Na^+]_i$ we can at least say that there seems to be ample ATP to sustain the observed ion fluxes.

It is important to note that in calculating $\Delta[Na^+]_i$ we assumed implicitly that k_1 and k_2 have the same values as k_1' and k_2' of Eqs. 3 and 4. However, it is not possible to decide *a priori* whether $k_1' = k_1$ or $k_1' = k_2$. Consequently, it cannot be assumed, for example, that the declining phase of $\Delta Q_{O_2}(t)$ is predominantly influenced by the work of the sodium pump whose rate constant is defined as k_1', even though it was predicted above that its activity would overlap $\Delta Q_{O_2}(t)$ for >10 s. To overcome this difficulty we examined the $\Delta Q_{O_2}(t)$ response under the effect of cardioactive steroids that bind to the enzyme Na^+-K^+ ATPase and block its activity (Schwartz et al., 1975). An enzyme-steroid complex corresponds to the disappearance of a pumping site. It has been shown that in the presence of excessive free steroid the disappearance of pumping sites is an exponential function of length of exposure to the drug (see Schwartz et al., 1975). It is shown that exposure of the drone retina to these compounds caused a progressive increase of the time constant of the descending limb of the $\Delta Q_{O_2}(t)$ response, which is equivalent to a decrease of the rate constant (k) of the reaction. A semilogarithmic plot of the individual values of k_1 as a function of length of exposure to cardioactive steroids gave a straight line with a time constant of 24 min (Fig. 13C). It appears, therefore, that the change of the kinetics of $\Delta Q_{O_2}(t)$ under the effect of excess of ouabain or strophanthidin may be directly related to the decrease of the pump sites on the membrane of the photoreceptors and consequently to the decrease of the rate constant of pumping. On this basis, we have concluded that the descending phase of $\Delta Q_{O_2}(t)$ is dominated by the rate constant of the work of the pump. Additional evidence supporting this conclusion is provided by electrophysiological data. After a receptor potential, the photoreceptor membrane

often undergoes a transient hyperpolarization. The amplitude of this hyperpolarization is increased when Cl^- in the Ringer solution is replaced by a larger anion such as isethionate (F. Baumann, personal communication) and is abolished by cardiac glycosides. Simultaneously with the hyperpolarization, the extracellular K^+ concentration as measured with a K^+-sensitive microelectrode transiently undershoots the baseline (Coles and Tsacopoulos, 1979, and unpublished observations). These two observations can be accounted for if the photoreceptors pump out sodium in exchange for potassium by the electrogenic pump for several seconds after a receptor potential. The point relating to the present argument is that the duration of this process is roughly the same (on the order of 10 s) as the descending phase of $\Delta Q_{O_2}(t)$.

Mahler (1978a) concluded that in frog muscle the kinetics of the sequence of reactions linking ATP hydrolysis to oxygen consumption are primarily determined by a single relatively slow reaction with apparent first-order kinetics ($k \sim 0.3$ min^{-1} at 20°C), and has postulated that this reaction is the one catalyzed by mitochondrial creatine kinase (Mahler, 1980). The scheme presented here suggests that in the drone retina the reactions comprising respiratory control can, to a first approximation, be described as a system with rate constant $k_2 = 0.53$ s^{-1}. The 100-fold difference in rate constant might possibly be accounted for by a fundamental difference in the mechanism of control of respiration, since we were unable to detect the presence of creatine phosphate in the retina.[2]

A complication appeared when the recovery of the response after exposure of the retina for 20 min to 50 μM of strophanthidin was examined; even though the recovery of the amplitude was complete after 50–60 min, the time constant of the descending phase was still slower than before strophanthidin. Because both the amplitude of the response and the time constant of the descending limb of $\Delta Q_{O_2}(t)$ depend on the amount of active pumping sites present in the membrane, this discrepancy in the recovery of the two events is striking. We have no satisfactory explanation for this phenomenon. The possibility that during these long-lasting experiments changes of the kinetics may occur for reasons independent of the effect of the cardioactive drugs was considered but eliminated because in pilot experiments in which only ethanol (0.05%) was added to the Ringer's solution, such changes of the kinetics were not observed. In addition, amytal (Na$^+$ amobarbital), a reversible blocker of the mitochondrial respiratory chain (Nishiki et al., 1979), suppresses $\Delta Q_{O_2}(t)$ and dramatically modifies the kinetics of the response. However, the recovery of both the amplitude and the kinetics is complete in $\sim$30 min (S. Dimitracos and M. Tsacopoulos, manuscript in preparation). If we assume that the quantity of sodium that enters per flash is the same as before, then this result

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[2] The levels of ATP and creatine phosphate were measured in the same samples of drone retina using a fluorometric method (Lowry and Passonneau, 1972). Although the concentration of ATP was in the range found in other tissues (1.5 nmol/mg wt/wt), no creatine phosphate was detected by this method. The value of the ATP concentration was confirmed by the luciferin-luciferase method (V. Evêquoz and M. Tsacopoulos, unpublished observations).

implies that more ATP is consumed and that sodium is pumped out more slowly, i.e., some or all of the pump molecules have become less efficient. It is known that the strophanthidin-pump complex can be internalized in HeLa cells (Cook and Brake, 1978); perhaps newly formed pump molecules function inefficiently.

Beyond these complications, however, we conclude that on present evidence the hypothesis that the amplitude of ΔQ_{O_2} and the descending phase of $\Delta Q_{O_2}(t)$ are dominated by the requirements of the sodium pump is satisfactory. But apparently there is a surplus of extra oxygen consumption that might be required to synthetize ATP used in earlier stages of the phototransduction, such as a phosphorylation of the kind described for vertebrate rods by Liebman and Pugh (1979).

APPENDIX

Derivation of Eq. 2

The rate of O_2 consumption, Q, and the partial pressure of O_2, P, are related by the one-dimensional equation of diffusion:

$$Q(t) = \alpha D \frac{\partial^2 P}{\partial x^2}(x,t) - \alpha \frac{\partial P}{\partial t}(x,t) \tag{A1}$$

where α and D are the local solubility and diffusion coefficients of O_2. α and D are assumed to be constant throughout the retina. The space and time coordinates, x and t, are chosen so that $x = 0$ at the sealed surface of the retina and $t = 0$ at the onset of the photostimulation. There is evidence presented in Methods that shows that Q is independent of x.

Our intention is to replace the partial derivatives on the right-hand side of Eq. A1 by an expression that can be readily computed from the measured values of P. To do this, we must use the initial and boundary conditions corresponding to the experimental situation and solve Eq. A1 for P.

This study is concerned with the transient departure of Q and P from their steady state values, Q_{ss} and P_{ss} (x), after a flash of light. Thus, the initial conditions are:

$$\left. \begin{array}{l} Q(t) = Q_{ss} \\ P(x,t) = P_{ss}(x) \end{array} \right\} \, t \leq 0. \tag{A2}$$

In addition, because the diffusion flux of O_2 must vanish at the sealed surface, we have the boundary condition:

$$\frac{\partial P}{\partial x}(0,t) = 0 \text{ for all } t. \tag{A3}$$

For simplicity, we introduce the quantities:

$$\Delta Q(t) = Q(t) - Q_{ss}; \tag{A4}$$
$$\Delta P(x,t) = P(x,t) - P_{ss}(x).$$

With these quantities, we can rewrite Eqs. A1–A3 as:

$$\Delta Q(t) = \alpha D \frac{\partial^2 \Delta P}{\partial x^2}(x,t) - \alpha \frac{\partial \Delta P}{\partial t}(x,t); \tag{A5}$$

$$\Delta Q(t) = 0 \text{ and } \Delta P(x,t) = 0 \quad t \le 0; \tag{A6}$$

$$\frac{\partial \Delta P}{\partial x}(0,t) = 0 \text{ for all } t. \tag{A7}$$

There are different ways of solving these equations. One way is to use Fourier transforms: if $\Delta P(x,t)$ and $\Delta Q(t)$ are only transiently different from zero, their Fourier transforms with respect to time, $\overline{\Delta P}(x,\omega)$ and $\overline{\Delta Q}(\omega)$, are well defined. This method is attractive for two practical reasons: (a) the calculation of Fourier transforms by computer is straightforward; (b) in our case, this method requires less time for the computation of ΔQ than other methods (e.g., integration) for a similar accuracy in the results.

In terms of Fourier transforms, Eqs. A5 and A7 read:

$$\overline{\Delta Q}(\omega) = \alpha D \frac{\partial^2 \overline{\Delta P}}{\partial x^2}(x,\omega) - i\alpha\omega\overline{\Delta P}(x,\omega) \tag{A8}$$

$$\frac{\partial \overline{\Delta P}}{\partial x}(0,\omega) = 0 \quad \text{for all } \omega \tag{A9}$$

where $i = \sqrt{-1}$.

Integrating Eq. A8 with respect to x and using condition A9, we obtain:

$$\overline{\Delta P}(x,\omega) = A(\omega)\cosh\left(\sqrt{\frac{i\omega}{D}}\,x\right) - \frac{\overline{\Delta Q}(\omega)}{i\alpha\omega}. \tag{A10}$$

$A(\omega)$ is an unspecified function of ω that appears here as a result of the fact that only one boundary condition is available in our case; because of its presence, $\Delta P(x,t)$ has to be measured at two different depths in the retina (see Methods). If we denote by x_1 and x_2 the two places where the value of $\Delta P(x,t)$ is measured, we have:

$$A(\omega) = \frac{\overline{\Delta P}(x_1, \omega) - \overline{\Delta P}(x_2, \omega)}{\cosh\left(\sqrt{\frac{i\omega}{D}}\,x_1\right) - \cosh\left(\sqrt{\frac{i\omega}{D}}\,x_2\right)} \tag{A11}$$

and

$$\overline{\Delta Q}(\omega) = \frac{i\alpha\omega}{2}\left\{A(\omega)\left\{\cosh\left(\sqrt{\frac{i\omega}{D}}\,x_1\right) + \cosh\left(\sqrt{\frac{i\omega}{D}}\,x_2\right)\right\} \right.$$
$$\left. - \overline{\Delta P}(x_1, \omega) - \overline{\Delta P}(x_2, \omega)\right\}. \tag{A12}$$

Introducing A11 in A12, we obtain then for $\overline{\Delta Q}(\omega)$:

$$\overline{\Delta Q}(\omega) = i\alpha\omega \frac{\left\{\overline{\Delta P}(x_1,\omega)\cosh\left(\sqrt{\frac{i\omega}{D}}\,x_2\right) - \overline{\Delta P}(x_2,\omega)\cosh\left(\sqrt{\frac{i\omega}{D}}\,x_1\right)\right\}}{\cosh\left(\sqrt{\frac{i\omega}{D}}\,x_1\right) - \cosh\left(\sqrt{\frac{i\omega}{D}}\,x_2\right)} \quad (A13)$$

so that $\Delta Q(t)$ is equal to the inverse Fourier transform of the right-hand side of Eq. A13, as expressed by Eq. 2.

It may be useful to discuss here a technical point related to the use of discrete Fourier transforms in the computation of text Eq. 2. Let $f(t)$ be a function whose values are sampled during the period $0 \leq t \leq T$; its discrete Fourier transform with N points is defined as:

$$F_D\{f\}(\omega_k) = \frac{T}{N} \sum_{n=1}^{N} f(t_n)\exp(-i\omega_k t_n) \quad (A14)$$

where

$$\omega_k = \frac{2\pi(k-1)}{T}, \ t_n = \frac{(n-1)T}{N}, \text{ and } k \text{ and } n \text{ are integers.}$$

Because the values $F_D\{f\}(\omega_k)$ give a good approximation of the actual Fourier transform of $f(t), \overline{f}(\omega)$, in the interval $\omega_{-N/2} \leq \omega \leq \omega_{N/2}$ (see Brigham, 1974), the computation of Eq. 2 can be performed by using discrete transforms in the place of the Fourier transforms and simultaneously replacing the value of ω, whenever it appears explicitly, by the expression for ω_k given in Eq. A14. However, because computer routines usually give the values $F_D\{f\}(\omega_k)$ from $k = 1$ to $k = N$ and since $F_D\{f\}(\omega_k) = F_D\{f\}(\omega_{k-N})$, the actual values ω'_k used in the computation must be chosen sequentially as follows:

$$\omega'_k = \begin{cases} \dfrac{2\pi(k-1)}{T} & \text{if } 1 \leq k \leq N/2 \\[3mm] \dfrac{2\pi(k-N-1)}{T} & \text{if } N/2 \leq k \leq N \end{cases} \quad (A15)$$

(M. Mahler, personal communication).

Besides this literal method, there exists another way of using the discrete transforms in the computation of text Eq. 2. If the function $f(t)$ is smooth enough to allow the use of the approximation:

$$\frac{df}{dt}(t_n) = \frac{N}{T} \cdot \frac{f(t_{n+1}) - f(t_{n-1})}{2} \quad (A16)$$

we can write:

$$F_D\left\{\frac{df}{dt}\right\}(\omega_k) = \sum_{n=1}^{N} \frac{f(t_{n+1}) - f(t_{n-1})}{2} \exp(-i\omega_k t_n). \tag{A17}$$

Furthermore, if $f(t_1)$ has a value close to that of $f(t_N)$, we can make the substitutions $f(t_0) = f(t_N)$ and $f(t_{N+1}) = f(t_1)$ without introducing significant errors in the evaluation of $F_D\left\{\frac{df}{dt}\right\}(\omega_k)$. Then, Eq. A17 becomes:

$$\begin{aligned}F_D\left\{\frac{df}{dt}\right\}(\omega_k) &\simeq \sum_{n=1}^{N} \frac{\exp\left(i\omega_k \cdot \frac{T}{N}\right) - \exp\left(-i\omega_k \cdot \frac{T}{N}\right)}{2} f(t_n)\exp(-i\omega_k t_n) \\ &= i\cdot\sin\left(\omega_k \cdot \frac{T}{N}\right) \cdot \sum_{n=1}^{N} f(t_n)\exp(-i\omega_k t_n)\end{aligned} \tag{A18}$$

and we obtain the relation:

$$F_D\left\{\frac{df}{dt}\right\}(\omega_k) \simeq i\cdot\frac{N}{T}\sin\left(\omega_k \cdot \frac{T}{N}\right)\cdot F_D\{f\}(\omega_k). \tag{A19}$$

Eq. A19 is the counterpart, for the discrete transforms, of the well-known relation for continuous Fourier transforms:

$$\overline{\frac{df}{dt}}(\omega) = i\omega\overline{f}(\omega). \tag{A20}$$

The explicit presence of ω in Eq. 2 stems from the use of relation A20 in its deduction. Therefore, it follows from Eq. A19 that the use of discrete transforms in the computation of Eq. 2 allows us to replace ω, whenever it appears explicitly in the calculation, by the value $\frac{N}{T}\sin\left(\omega_k \cdot \frac{T}{N}\right)$, provided that the values of $\Delta P(x_1,t)$ and $\Delta P(x_2,t)$ are sampled in such a way that the approximation A16 is applicable and that, for both of them, initial and final values do not differ dramatically.

The interest of this "sine" method lies in the following properties: (a) because $\sin\left(\omega_k \cdot \frac{T}{N}\right) = \sin\left(\omega_{k-N} \cdot \frac{T}{N}\right)$, there is no need here, if k ranges from 1 to N, to reorder the values of ω_k as in Eq. A15 for the literal method; (b) the additional time required for the calculation of the expression $\frac{N}{T}\sin\left(\omega_k \cdot \frac{T}{N}\right)$ instead of ω_k is more than compensated, in elaborate equations such as Eq. 2, by the fact that for even values of N, $\sin\left(\omega_k \cdot \frac{T}{N}\right) = \sin\left(\omega_{N/2-k+2} \cdot \frac{T}{N}\right)$; (c) for a given set of sampled values of $\Delta P(x_1,t)$ and
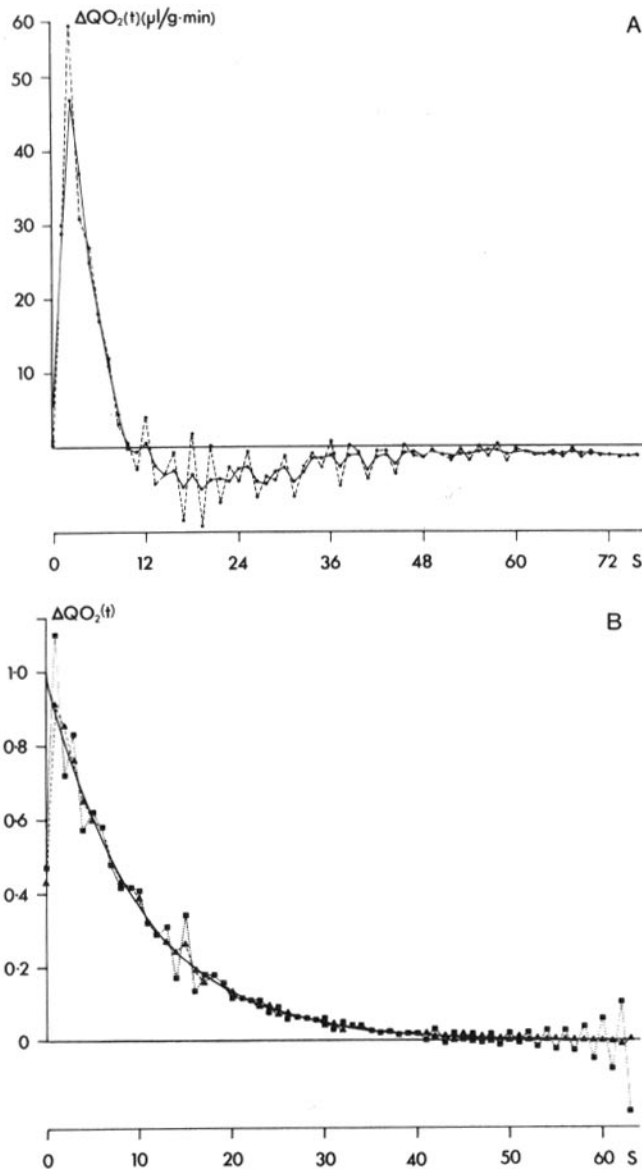
FIGURE 16. A. Comparison of the values of $\Delta Q_{O_2}(t)$ obtained with the literal method (dotted line) and with the "sine" method (solid line). The 64 pairs of respective values of $\Delta P_{O_2}(x_1, t)$ and $\Delta P_{O_2}(x_2, t)$ used in this test were measured from a chart recording. B. Test of the accuracy of the values of $\Delta Q(t)$ obtained with the literal method (■) and with the "sine" method (▲) in a simulated experiment. For this simulation, we proceeded as follows: (a) we considered the case where x_1 and x_2 are the positions at the exposed and at the sealed surface of a slice of retina 300 μm thick; (b) we assumed further that $\Delta P(x_1, t) = 0$ and

$$\Delta Q(t) = \frac{1\mu l\ O_2}{g \cdot min} \cdot \exp(-t/10\ s)$$ (solid line in the figure); (c) 64 values at 1-s

intervals were computed for $\Delta P(x_2, t)$ through the solution of the one-dimensional equation of diffusion corresponding to that case (see Mahler, 1978a, Appendix II); (d) the values of $\Delta P(x_1, t)$ and $\Delta P(x_2, t)$ were introduced into Eq. 2 in order to obtain the $\Delta Q(t)$ for each method.

$\Delta P(x_2,t)$, Eq. 2 provides more accurate values of ΔQ with this method than with the literal method (see Fig. 16); (d) if the initial and final values of $\Delta P(x_2,t)$ used in the computation of ΔQ should differ significantly one from the other, the main effect observed is (as with the literal method) an increase of the "noise" in the results without any significant alteration of the general
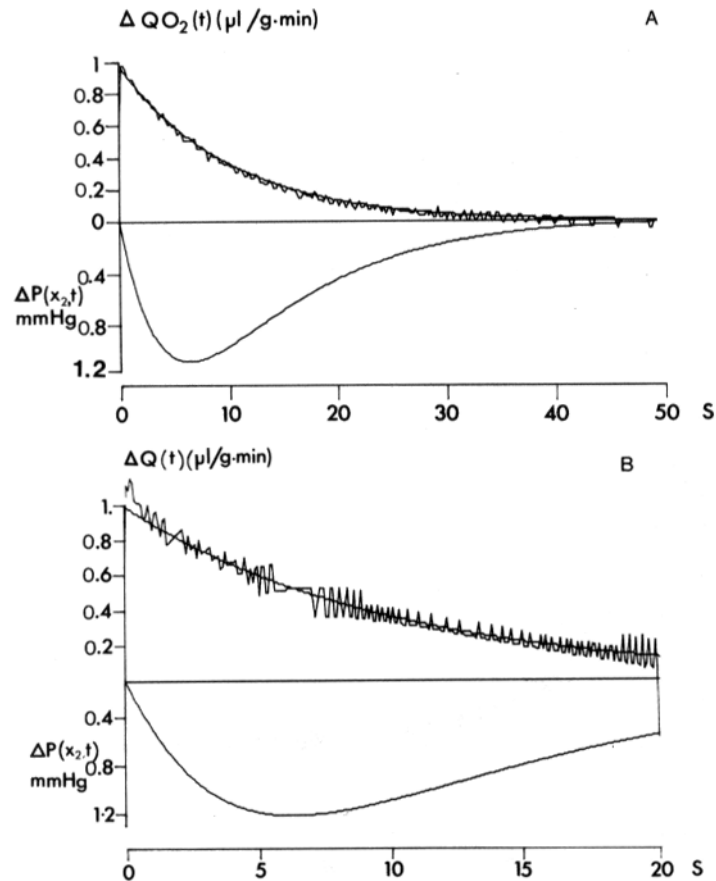

FIGURE 17. Test of the influence of the difference between initial and final values of $\Delta P(x_2, t)$. The procedure followed for this test was the same as that described in the legend of Fig. 16B, with the difference that 256 values of $\Delta P(x_2, t)$ were used in the computation of $\Delta Q(t)$ and that the sampling intervals were 0.2 s in (A) and 0.08 s in (B).

shape of the $\Delta Q(t)$ obtained (see Fig. 17). For all these reasons, we chose to use the "sine" method in our computations.

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