

Transport and Phosphorylation of 2-Deoxy-D-Glucose by Amphibian Retina

Effects of Light and Darkness

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ABSTRACT We studied the uptake of 2-deoxy-D-glucose (2DG) and the synthesis of its phosphorylated product 2DG-6-phosphate (2DG-6P) by the retinas of the clawed frog (*Xenopus laevis*) and the bullfrog (*Rana catesbeiana*). Autoradiographs showed that most of the retinal 2DG uptake is by the photoreceptor layer. The 2DG accumulation by isolated *Xenopus* retinas was time and concentration dependent. The K_t for transport was 5.05 mM; V_{\max} was 6.99×10^{-10} mol·mg⁻¹ tissue wet weight min⁻¹. The K_m for 2DG-6P formation was estimated to be 2–3 mM and V_{\max} to be $\sim 4 \times 10^{-9}$ mol·mg⁻¹ min⁻¹. 2DG uptake was inhibited competitively by glucose with a K_i of 2.29 mM. Exposure to light reduced 2DG uptake by no more than 10% as compared with dark uptake. Low sodium or ouabain (10^{-4} – 10^{-7} M) treatment did not significantly alter 2DG uptake as compared with control retinas. In experiments upon intact, anesthetized bullfrogs, light reduced both the total amount of radioactivity acquired by the retina and the fraction of 2DG-6P present. The results are discussed in terms of the fraction of energy consumed by the retina required to maintain the photoreceptor dark current.

INTRODUCTION

The present report describes an attempt to relate the metabolic activity of retinal photoreceptors to their light-evoked electrical responses by using the glucose analogue 2-deoxy-D-glucose (2DG) (Sokoloff, 1977). The precise fraction of the total energy consumption by neurons devoted to nervous conduction or synaptic transmission relative to other cellular activities is not yet fully resolved and may vary markedly from system to system. For example, Kreutzberg and Emmert (1980) found that, in a study of 2DG incorporation during nerve regeneration in rat, there was a poor correlation between motoneuron electrical activity and the level of 2DG uptake. On the other hand, Yarowsky et al. (1979, 1980) report that 2DG accumulation was linearly related to nerve impulse frequency in superior cervical ganglion of rat. Studies of 2DG uptake

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by monkey visual cortex (Hubel et al., 1978) indicate that incorporation is heavy in certain cortical layers, whereas others, also containing abundant neuronal populations, label very lightly. In this context, an apparent advantage of the retina is that most of the 2DG transport and metabolism is by photoreceptors (Basinger et al., 1979; Morjaria and Voaden, 1979; Sperling et al., 1979; Witkovsky and Yang, 1982), cells whose light-evoked membrane responses are well characterized (reviewed in Kaneko, 1979).

Our antecedent study (Witkovsky and Yang, 1982) indicated that the 2DG content of photoreceptors could be divided into a soluble pool (a mixture of 2DG and 2-deoxyglucose-6-phosphate [2DG-6P], equivalent to about four-fifths of the total 2DG) and an insoluble pool of 2DG associated with glycogen. In the present report we relate the metabolism of the soluble 2DG pool in photoreceptors to their electrical activity. As a first step we characterized the kinetics of 2DG transport and phosphorylation, then measured the effects upon these processes of light or darkness, altered sodium gradient, or ouabain treatment, each of which affects receptor photovoltages in predictable ways (Penn and Hagins, 1969; Frank and Goldsmith, 1967). A preliminary account of this study has appeared (Yang and Witkovsky, 1981).

MATERIALS AND METHODS

African clawed frogs (*Xenopus laevis*) and bullfrogs (*Rana catesbeiana*) were obtained from commercial suppliers and maintained in aquaria on 10-h/14-h light/dark cycle. Anesthesia was induced by subcutaneous injection of 0.5–2.5 cm³ 4% (wt/vol) Tricaine methanesulfonate (Sigma Chemical Co., St. Louis, MO) in Ringer's solution. ³H-2DG (New England Nuclear, Boston, MA) was evaporated to dryness and resuspended in Ringer's solution, then used either as a bathing medium for isolated retinas or injected into the heart. For dark-adapted experiments, animals were kept in darkness overnight. The retina was dissected free under red light in initial experiments; in later studies an infrared image converter was used. In experiments upon intact animals, the head was wrapped in several layers of black cloth during surgery. After incubation in 2DG/Ringer, the retina was washed 30 min in three changes of ice-cold Ringer's solution, and in some cases, digested in Protosol and counted in a Packard scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, IL). (Counts per minute were converted to log moles 2DG min⁻¹ mg⁻¹ tissue wet weight by reference to the efficiency of the cocktail [~50%], the specific activity of the ³H-2DG [37.5–40 Ci/mM], and the weight of the retina. The latter value was derived in separate experiments by correlating retinal area with weight of blotted tissue [0.10 mg/mm²]; to avoid handling the tissue in experimental runs, the retinal diameter was measured and retinal weight was calculated.) Alternatively, the tissue was homogenized and spotted onto thin-layer chromatography (TLC) plates. Full details of the methods are provided in Witkovsky and Yang (1982), with two additions. First, Merck TLC plates (5506; MC/B Mfg. Chemists, Cincinnati, OH) gave more reliable separation of 2DG and 2DG-6P than those previously used. Second, we found that retinal homogenates left at room temperature gained 2DG at the expense of 2DG-6P, which indicates the presence of phosphatase activity (Holtzman et al., 1979). To avoid this degradation, we homogenized retinas by rapidly freezing and thawing three times, followed by a 15-min sonication at 4°C.

Electroretinographic traces were recorded DC by a Ringer/agar-filled capillary with a silver-silver chloride lead connected via a cathode follower (W-P Instruments,

Inc., New Haven, CT) to an oscillographic recorder (Hewlett-Packard Co., Palo Alto, CA). Light stimuli were provided by a two-channel photostimulator.

RESULTS

Uptake of 2DG by Isolated Retinas

The accumulation of label by isolated, dark-adapted *Xenopus* retinas was found to be both time and concentration dependent. Fig. 1 illustrates the effect of incubation time upon the retinal radioactivity, converted to log moles 2DG $\text{min}^{-1} \text{mg}^{-1}$ tissue wet weight, when the retina was incubated in $10 \mu\text{Ci}$

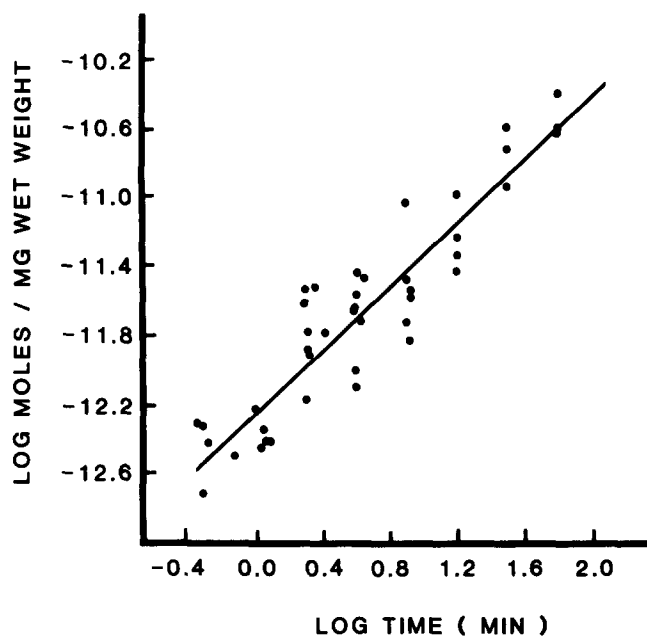


FIGURE 1. Uptake of 2DG by isolated *Xenopus* retinas in darkness as a function of time. Incubation temperature: 22–23°C. Each point represents an individual retina. 2DG concentration: $10 \mu\text{M}$. The molar uptake was estimated from counts per minute as noted in Methods. The line through the points was calculated by a least-squares regression analysis

2DG in glucose-free Ringer's solution. When plotted on log-log coordinates the uptake curve is well fit by a straight line, $\log y = \log a + n \log x$, i.e., a power function, with $n = 0.92$, as calculated by regression analysis. The correlation coefficient for the line was 0.93. Each point represents a separate retina; the set of points at a given incubation time illustrates the considerable variability encountered in 2DG uptake by identically treated retinas.

In Fig. 2, the results of varying bath concentrations of 2DG are shown. The line at the bottom of the graph is the regression line representing the data of Fig. 1. Note that a line of the same slope can be shifted vertically to fit at least

the initial portion of the uptake curves at each concentration tested. However, at higher concentrations, there is a tendency for the rate of uptake to diminish after 5–15 min incubation, which indicates that the transport mechanism is approaching zero net transport.

This tendency may be explained by the nature of the facilitated diffusion mechanism by which glucose is incorporated into many cells (Baldwin and Lienhard, 1981) in that the rate of transport is proportional to the concentra-

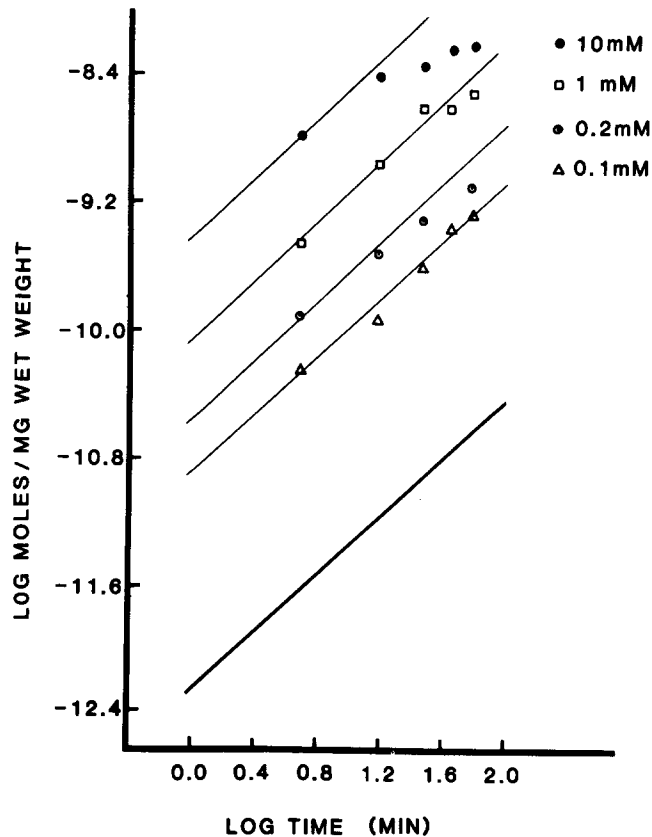


FIGURE 2. Uptake of 2DG by isolated *Xenopus* retinas in darkness as a function of 2DG concentration and time. The bath concentrations of 2DG used are indicated at upper right. The line without points at bottom is from Fig. 1. It has been shifted vertically by eye to fit the data points. Each point is a mean of four retinas. Molar uptake was calculated as for Fig. 1.

tion gradient. The gradient depends on the intracellular concentration of glucose, which in turn can be estimated by knowing the size of the retinal compartment for 2DG. Autoradiographs of 2DG incorporation by *Xenopus* retinal tissue (Fig. 3; also see Witkovsky and Yang, 1982) show that virtually all of the uptake is by the inner segments and synaptic bases of both rod and cone photoreceptors. Saxen (1954) found a rod/cone ratio of 1.24 in *Xenopus* and Engbretson and Witkovsky (1978) counted 7,000 rods/mm² in unfixed

Xenopus retinas. The average rod inner segment is $8\ \mu\text{m}$ wide by $30\ \mu\text{m}$ long; that of the cone, $8 \times 25\ \mu\text{m}$ (Witkovsky and Yang, 1982). Assuming cylindrical shapes and excluding nuclear volumes, we estimate the volume of the photoreceptor 2DG compartment to be 1.46×10^{-7} liters/ mm^2 retina. Considering

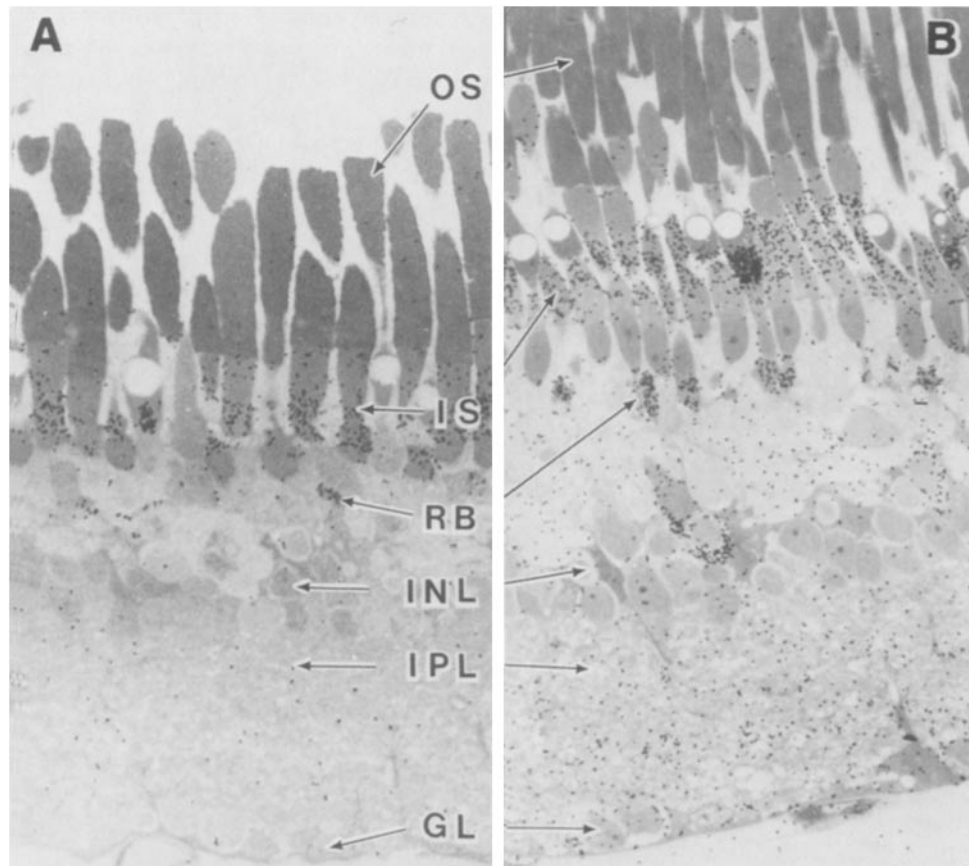


FIGURE 3. Autoradiographs of retinal tissue after incubation in darkness in ^3H -2DG. In A, $0.6\ \mu\text{Ci}$ 2DG was introduced into the vitreous, followed by 30 min incubation. In B, the isolated retina was immersed in $10\ \mu\text{Ci}$ ^3H -2DG for 60 min. Thereafter, both tissues were fixed in buffered aldehydes, followed by buffered osmium. Although fixation removes most of the label, it does not change its distribution in the retina (Witkovsky and Yang, 1982). Most of the uptake is by the inner segments and synaptic bases of the photoreceptors. GL, ganglion cell layer; INL, inner nucleus layer; IPL, inner plexiform layer; IS, receptor inner segments; OS, receptor outer segments; RB, receptor synaptic bases. $640\times$.

the highest bath concentration of 2DG used (10 mM), after 15 min, the retina had acquired approximately $-9.42\ \log\ \text{mol}/\text{mm}^2 = 3.8 \times 10^{-10}\ \text{mol}/\text{mm}^2$, equivalent to an intracellular concentration of $\approx 2.6\ \text{mM}$. After 60 min, the estimated intracellular concentration is 4.07 mM. These values are close to

the bath concentration of 10 mM and would account for a marked reduction in the rate of net transport compared with the rate seen initially.

By evaluating the uptake curves at an early time (1 min), one can estimate the initial rate of 2DG uptakes as a function of concentration. Fig. 4 graphs reciprocal velocity against reciprocal concentration in a Lineweaver-Burk plot. The points are fit by a straight line, calculated by regression analysis, with a correlation coefficient of 0.99. The x intercept provides a K_t value of 5.05 mM; the y intercept has a V_{\max} of 6.99×10^{-10} mol mg^{-1} min^{-1} . These values refer to the transport system that moves 2DG across the photoreceptor membrane. The kinetics of 2DG phosphorylation by hexokinase to 2DG-6-phosphate are considered later in this report.

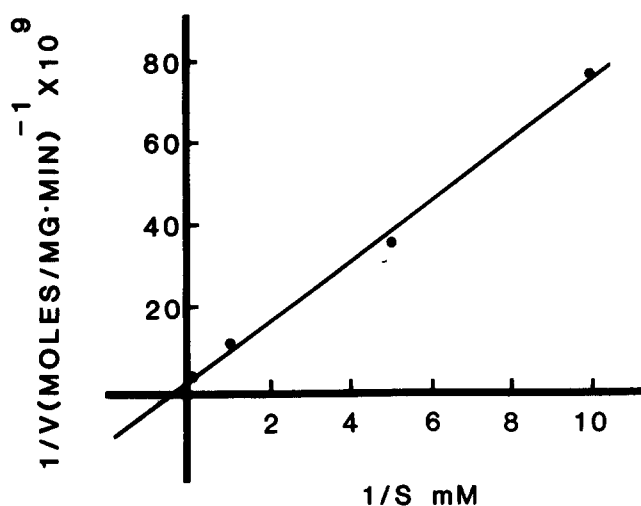


FIGURE 4. A Lineweaver-Burk plot of the initial rate of 2DG uptake ($1/V$) as a function of 2DG concentration in the bathing medium ($1/S$). The data points were derived from Fig. 3 (see text). The line (best fit to the data points) was calculated by regression analysis.

Competitive Inhibition by Glucose

The effect of glucose on 2DG transport was examined by comparing the uptake of ^3H -2DG in retinas incubated in 2DG alone (0.1–10 mM) with those incubated in the same series of 2DG concentrations to which 10 mM glucose was added. As expected for a competitive inhibition (Lehninger, 1975) the relative inhibition of 2DG uptake was in inverse proportion to the 2DG concentration. On a Lineweaver-Burk plot (Fig. 5), the inhibitory constant K_i was calculated to be 2.29 mM from the slope m of the inhibited reaction, and the expression $m = \frac{K_t}{V_{\max}} \left(1 + \frac{I}{K_i} \right)$. V_{\max} and K_t were taken from the data of Fig. 4; I is the glucose concentration (10 mM) used.

Kinetics of 2DG Phosphorylation

Studies of glucose metabolism document that the reaction $\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose 6-phosphate} + \text{ADP}$ is highly regulated. *A priori* we supposed that light/dark alteration of retinal glucose metabolism might work through the phosphorylation reaction. Accordingly, we sought to determine the rate of 2DG phosphorylation as a first step in analyzing the control mechanism. Isolated retinas were incubated in the dark in different concentrations of 2DG for different times. As before, total uptake of label was

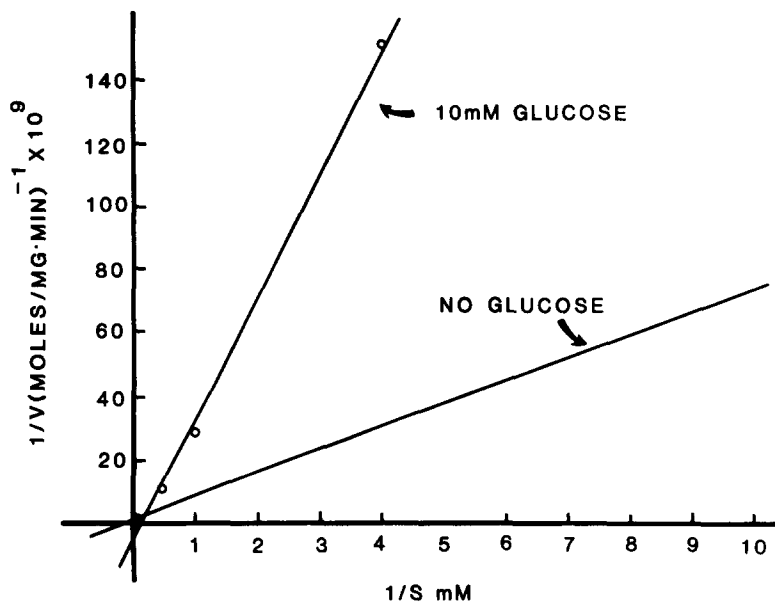


FIGURE 5. Competitive inhibition of 2DG uptake by glucose. Isolated retinas were bathed in Ringer containing 0.1–10 mM 2DG with or without 10 mM glucose. The reciprocal of the 2DG uptake at 1 min is plotted against the reciprocal of the 2DG concentration in a Lineweaver-Burk plot. The “no glucose” line is the data of Fig. 4.

estimated from scintillation counting and the rate of 2DG-6P production was estimated from thin-layer chromatography. Fig. 6 depicts moles per milligram of either 2DG or 2DG-6P at various times after incubation in 0.5, 2.0, or 5.0 mM 2DG. The rate of 2DG-6P production is seen to increase with increasing bath concentrations of 2DG. The data of Fig. 6 yield too few points to make a precise measurement of K_m but its value was estimated to be 2–3 mM. The value for V_{max} was $\sim 4 \times 10^{-9} \text{ mol min}^{-1} \text{ mg}^{-1}$ retina. Thus, as Fig. 10 indicates, the rate of 2DG phosphorylation is rapid and comparable to that of 2DG transmembrane transport.

Influence of Light/Dark, Low Sodium, and Ouabain on 2DG Uptake

If the transmembrane potential or the dark current of the photoreceptor plays a role in regulating its sugar transport, then light or dark might be expected to influence 2DG uptake, since light produces a sustained hyperpolarization of the photoreceptor, caused primarily by a reduction in the dark current (Korenbrodt and Cone, 1972; Kaneko, 1979). The data of Table I test this hypothesis. By reference to a previous study (Witkovsky et al., 1981), we used an orange (Wratten 22) light that raised the principal rod threshold >4 log units, and that of the cones 1.5 log units. In the first set of experiments we

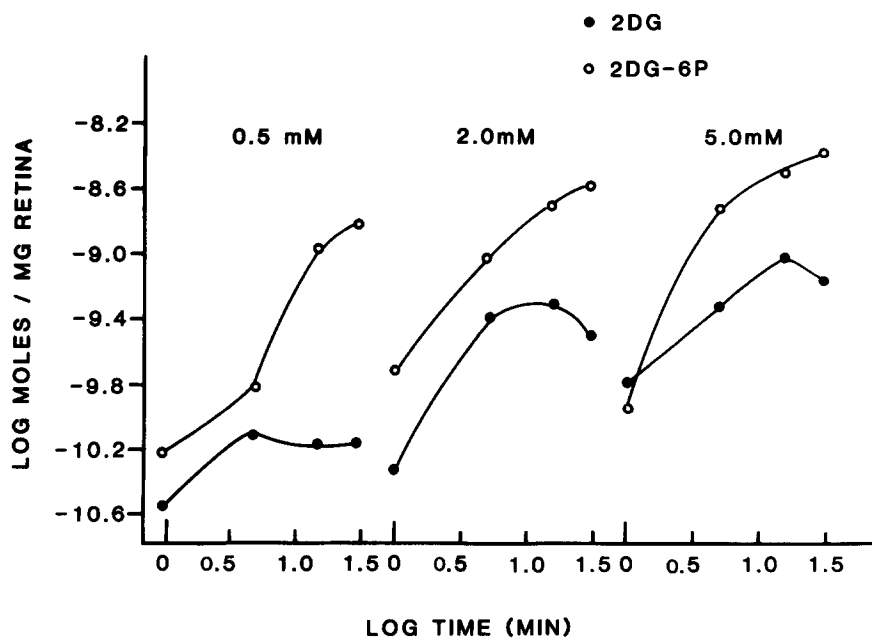


FIGURE 6. 2DG and 2DG-6P content of isolated *Xenopus* retinas after incubation in darkness in different bath concentrations of 2DG (0.5, 2.0, and 5.0 mM, as indicated). 2DG content was estimated from scintillation counts, that of 2DG-6P from a combination of thin-layer chromatography and scintillation counting (see Methods).

attempted to reduce the variability in uptake between identically treated retinas from different animals (Fig. 1) by dividing each test retina in half. Each half was incubated in $10 \mu\text{Ci}$ 2DG Ringer for 30 min, one in darkness, the other in constant yellow light, then rinsed in 5 ml ice-cold Ringer for an additional 30 min to remove extracellular label. As shown in Table I (unfixed retinas), there was no statistically significant difference in the mean 2DG content of light- or dark-incubated retinas. In a second set of experiments, we tested the hypothesis that light/dark influenced only the insoluble 2DG fraction (putative glycogen, Witkovsky and Yang, 1982). Accordingly, we used the same incubation procedure followed by fixation in buffered aldehydes

and post-fixation in buffered osmium. Table I (fixed retinas) shows that light-incubated, fixed retinas held $89 \pm 6\%$ of the 2DG contained by their dark-incubated, fixed counterparts.

Further consideration of the method used suggested that several uncontrolled variables may have affected the results of Table I just described. These were: (a) the dim red light used for dissection may have been sufficient to alter light-dependent glucose uptake; (b) insufficient time may have been allowed after enucleation for the stabilization of cyclic nucleotide levels (Mitzel et al., 1978), which may play a role in glucose uptake (see Discussion); (c) the glucose uptake of post-receptor cells could be altered by light in a direction opposite to that of the receptors; (d) handling or cutting the retina may have altered its glucose uptake capabilities; (e) the absence of glucose in the bathing medium may have resulted in a decline in the responsiveness of the preparation over the course of the experiment; and (f) the phosphate buffer system used might not promote oxidative metabolism as well as a full bicarbonate buffer (Winkler et al., 1977).

TABLE I
EFFECT OF LIGHT OR DARK INCUBATION ON RETINAL 2DG CONTENT

	Light/dark ratio of 2DG mg^{-1} retina	Number of animals
Unfixed retinas*	1.01 ± 0.18	6
Fixed retinas‡	0.89 ± 0.06	5
Eyecup§	0.89 ± 0.10	5

* Isolated retinas were incubated for 30 min in glucose-free phosphate-buffered Ringer containing $10 \mu\text{Ci}$ 2DG followed by a 30-min rinse in ice-cold Ringer.

‡ Isolated retinas were incubated as above, then fixed for 1 h in buffered glutaraldehyde and post-fixed in buffered osmium tetroxide.

§ Eyecups were incubated in darkness for 20 min in bicarbonate Ringer containing 10 mM Na aspartate and 2 mM glucose, then incubated in the same Ringer plus $10 \mu\text{Ci}$ 2DG for 30 min. The retina was dissected free in ice-cold Ringer and rinsed for 30 min.

A new set of experiments was designed to avoid these potential difficulties. The anesthetized animal was positioned under an infrared viewer. Both corneas and lenses were removed and the eyes were then enucleated and incubated for 20 min in bicarbonate Ringer bubbled with 95% oxygen/5% CO_2 , pH 7.3, containing 10 mM Na aspartate and 2 mM glucose. Thereafter, the eyes were transferred to a smaller chamber containing 2DG/Ringer for 30 min, either in light or darkness, then rinsed in ice-cold Ringer. After a 5-min rinse, the retinas were dissected free and rinsed for an additional 25 min. Table I (eyecup) shows that light-incubated retinas contained $89 \pm 10\%$ of the amount of 2DG held by dark-incubated retinas. The results of these experiments indicate that light retards, to a small degree, the 2DG uptake of the isolated retina.

The photovoltage of the photoreceptor depends on the sodium gradient across the outer segment (Korenbrot and Cone, 1972). Penn and Hagins (1969) showed that reduction of the Na concentration led to a reduction or

abolition of the photoresponse of the receptor layer in the rat retina. Accordingly, we incubated *Xenopus* retinas in Ringer solution in which the normal 100 mM NaCl was reduced to 10 mM with the remaining 100 mM NaCl replaced by an equimolar amount of choline chloride to which the photoreceptor is impermeable (Yau et al., 1981). Fig. 7 shows the mean and range of 2DG accumulation by normal vs. sodium-deficient retinas after a 15-min incubation period; it is apparent that the value of the sodium gradient did not influence 2DG uptake in a significant way.

Another method of altering the photovoltage is to expose the retina to the cardiac glycoside, ouabain, which blocks Na-K ATPase activity (Lehninger,

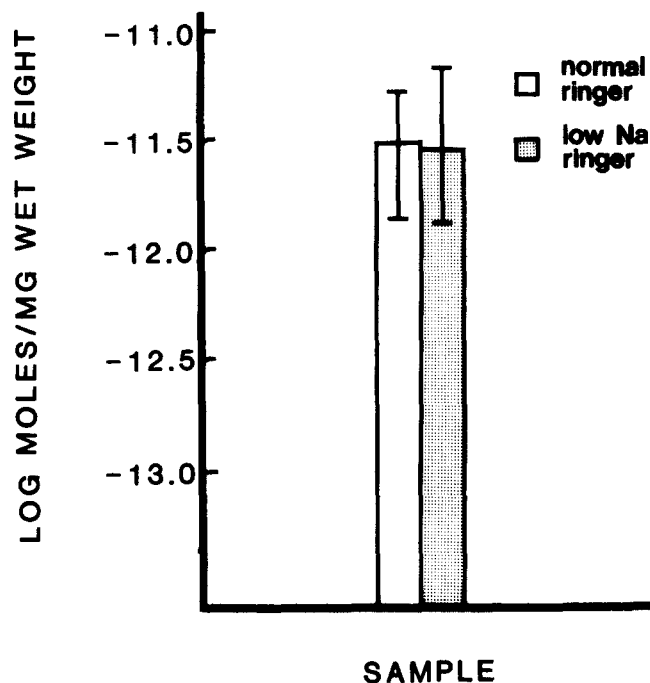


FIGURE 7. The mean and range ($N = 4$) of 2DG uptake by isolated *Xenopus* retinas, in darkness, when incubated for 15 min, either in normal Ringer or Ringer in which 100 mM NaCl was replaced by 100 mM choline chloride.

1975). Frank and Goldsmith (1967) demonstrated that exposure of the frog retina to 10^{-4} – 10^{-5} M ouabain led to a rapid abolition of the electroretinogram, a mass response of the retina to a light stimulus. We tested the effects of ouabain upon 2DG uptake by bathing retinas in Ringer solution to which ouabain in the concentration range 10^{-4} – 10^{-7} M was added. Fig. 8 shows that by reference to the regression line for the uptake function of control eyes (Fig. 1), ouabain was without apparent influence on 2DG uptake after either a 15- or 60-min incubation period. On the other hand, Fig. 8 (bottom) shows that application of 10^{-4} M ouabain led to a rapid disappearance of the electroretinogram.

In Vivo Experiments

We studied 2DG uptake by bullfrog or *Xenopus* retinas after an intracardiac injection of 50 μCi 2DG in 50 μl Ringer solution. Thereafter, the animal was maintained in light or darkness for periods of 5–120 min. Then the animal was decapitated, the eyes were enucleated, and the retinas were isolated, washed, and dissolved for scintillation counting. Fig. 9 depicts the 2DG

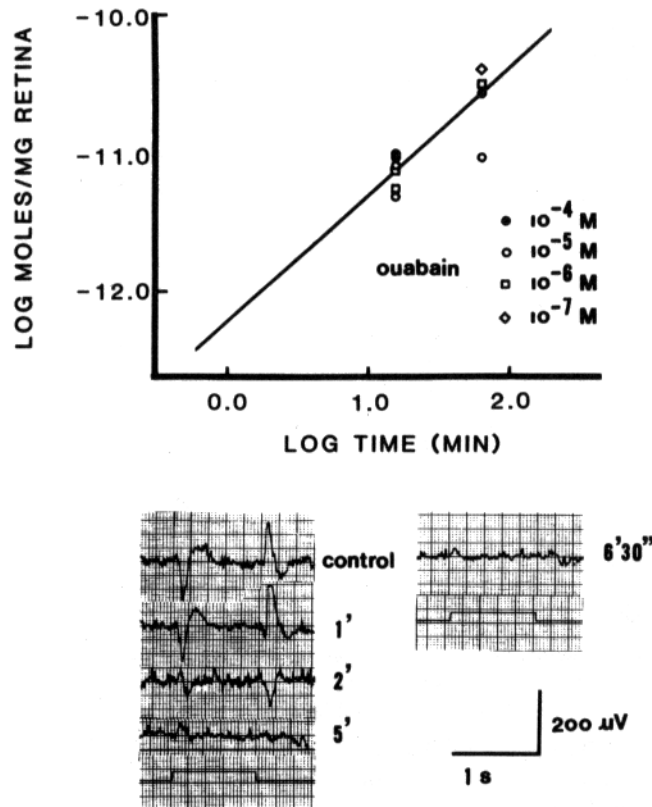


FIGURE 8. (Top) The effect of 10^{-4} – 10^{-7} M ouabain on the uptake in darkness of 2DG by isolated *Xenopus* retinas. The regression line is taken from Fig. 1. Each data point is one retina. (Bottom) Electoretinographic records from an isolated *Xenopus* retina before (control) and after exposure to Ringer containing 10^{-4} M ouabain. The numbers to the right of the traces indicate the time post-exposure to ouabain/Ringer. Flash duration is indicated below the traces.

accumulation by bullfrog retinas after an intracardiac injection of 50 μCi 2DG, followed by incubation in light or darkness for a variable period. Although no light/dark difference is apparent up to 15 min incubation, after 30 or 60 min there is a tendency for dark-adapted eyes to acquire more 2DG than comparably treated light-adapted ones, although the scatter of the data points is large. The adapting light in these experiments was a fiber optic lamp delivering $\sim 10^{-5}$ W cm^{-2} of white light at the cornea.

As a working hypothesis for the findings of Fig. 9 we supposed that light acts to reduce the rate at which 2DG is phosphorylated. Accordingly, after a pulse of tracer had dissipated in blood and extracellular space, free 2DG, but not its phosphorylated counterpart, could be transported out of the cell down the concentration gradient. In contrast, back transport would not be expected to occur in the isolated retina experiments described earlier, in which the bath concentration of 2DG remained essentially constant and higher than that of the retina throughout the incubation period.

Our experimental test of this hypothesis was to measure, via thin-layer chromatography (TLC), the relative fractions of free 2DG and 2DG-6P in

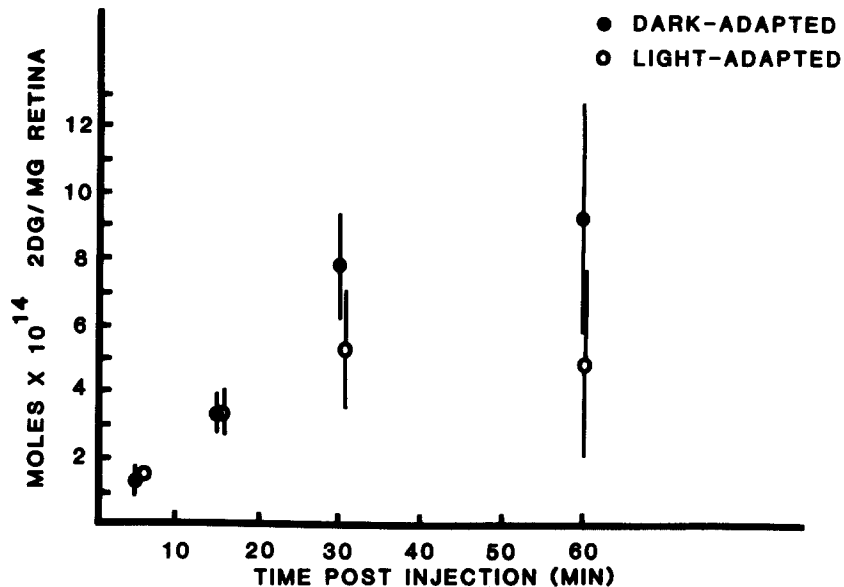


FIGURE 9. 2DG uptake by the intact retinas of the bullfrog *Rana catesbeiana*. At time zero a 50- μ Ci pulse of 2DG was injected into the ventricle. At various times post-injection light- or dark-adapted animals were decapitated, the eyes were enucleated and hemisected, and the retinas were removed and washed for 30 min in ice-cold Ringer. The mean and standard error ($N = 4$) are shown except for the light-adapted point at 5 min (single sample).

retinas subjected to the incubation conditions of Fig. 9. Fig. 10 is a graph of the results. Each point is the average of two chromatographic runs on a test animal, one from the homogenate of each three-quarters of a retina. The total molar uptake of 2DG was calculated from scintillation counting of the remaining one-quarter of a retina. 5 μ l of the homogenized fraction was spotted and co-chromatographed on TLC plates with cold, authentic 2DG and 2DG-6P. The radioactivity associated with 2DG and 2DG-6P fractions (from which a background level of 15 cpm was subtracted) was identified by reference to the positions of the nonradioactive authentic markers. The data show that after at least 15 min of dark incubation, ~80% of the 2DG content

of the retina was in the phosphorylated form, whereas in comparably treated light-adapted retinas, only ~50% 2DG was phosphorylated.

DISCUSSION

The present study provides a description of the kinetics of 2DG transport and phosphorylation in the amphibian retina. The accumulation of 2DG by the retina was found to be both time and concentration dependent. A previous study (Witkovsky and Yang, 1982) documented that most of the uptake was by photoreceptor cells. In the present report no evidence was obtained for more than one uptake compartment, e.g., a differential rate of 2DG accumulation by rods and cones. In this regard, however, the scatter of points in the 2DG uptake vs. time graph (Fig. 1) make our conclusion a tentative one.

It was found that modulation of the membrane potential and the dark current of the photoreceptors, by lowering the sodium concentration of the bathing medium or by adding ouabain to it, had, in experiments upon isolated retinas, an unmeasurably small effect upon the amount of 2DG incorporation. On the other hand, light-incubated retinas accumulated somewhat less ($\approx 10\%$) 2DG than their dark-incubated counterparts. In the intact animal experiments, light-adapted frogs had, on the average, a lower 2DG content and a lower percentage of 2DG-6P after 30 min incubation than did comparably treated dark-adapted animals.

Relation of Photoreceptor Dark Current to Photoreceptor Metabolism

An assessment of whether the rate of 2DG uptake and phosphorylation bears some relation to the electrical activity of the system depends on knowing precisely what the energy requirements of the electrical activity in question, namely the photoreceptor dark current, are.

There is some qualitative evidence that the dark current may not have a high energy requirement. For example, in his pioneering studies of the electroretinogram (ERG), Granit (1947) found that when the retina was subjected to anoxia, the most resistant of the ERG waves was the PIII or photoreceptor component. Similarly, Winkler (1972) has shown, in the isolated superfused rat retina, that the photoreceptor contribution to the ERG was unaltered by periods of anoxia up to 75 min. In a later study (Winkler, 1981), he found that reduction of the glucose concentration in the perfusate from 20 to 1 mM had little effect upon the receptor photovoltage.

Another approach to the effect of light and dark on retinal metabolism was taken by Kimble et al. (1980), who monitored oxygen consumption of the tissue. Compared with dark incubation, a strong light lowered oxygen consumption by only 0.6%. Moreover, blockage of the dark current with ouabain lowered oxygen consumption by just 3–4%. Comparable findings were obtained by Jaffe et al. (1975).

The dark current that underlies the receptor response has been measured directly in amphibian rods by Yau et al. (1981). In normal Ringer solution, each rod generates 15 pA of dark current. Measurements upon individual turtle cones (Schnapf and McBurney, 1980) indicate an average value of ~30 pA/receptor. By reference to the density of photoreceptors in the *Xenopus*

retina (12,500/mm²; Engbretson and Witkovsky, 1978) and the conversion factor, 1 A = 1.036×10^{-5} mol univalent charge/s, one obtains 2.77×10^{-11} mol univalent charge mg⁻¹ retina s⁻¹. Assuming that all the inward charge is caused by sodium (Korenbrod and Cone, 1972) and that the ATP-consuming Na-K exchange pump expels 3 Na/ATP (Lehninger, 1975), then 9.2×10^{-12} mol ATP mg⁻¹ s⁻¹ are required for the dark current.

Sickel (1972) showed that isolated frog retina incubated in 5 mM glucose incorporated 3.4×10^{-12} mol glucose mg⁻¹ s⁻¹ capable of generating 1.4×10^{-10} mol ATP mg⁻¹ s⁻¹. On this basis, the dark current would require 2.6% of the energy, but this may be an underestimate because not all ingested glucose is completely oxidized and the ATP generated by retinal cells other than photoreceptors is not considered. On the other hand, the respiration rate of rat retina (Jaffe et al., 1975) is unaffected by treatment with the synaptic blocker aspartate, which indicates that the photoreceptors account for most of the retinal respiration. Kimble et al. (1980) found that frog retina consumed 10.6 nM O₂/min. Taking into account the retinal area, the oxygen could produce ~1 nM ATP mg⁻¹ retina⁻¹, ~100 times the requirement of the dark current. Finally, Drujan et al. (1971) measured the oxygen consumption of single cones isolated from teleost retina. If used entirely in oxidative phosphorylation, the O₂ consumed would yield 7.2×10^{-14} M ATP cone⁻¹ s⁻¹, whereas the dark current requirements based on an estimate of 30 pA/cone (Schnapf and McBurney, 1980) would be 10^{-16} M ATP cone⁻¹ s⁻¹.

These diverse measures indicate that the high rate of energy consumption by retinal photoreceptors is directed primarily at activities other than maintenance of the dark current. The results of the present report showing that, in isolated retinas, bright light reduced 2DG uptake by no more than 10% from dark uptake levels is consistent with the conclusion that the energy requirement of the dark current is a small part of the total energy budget of the photoreceptor. It must be assumed further that either the activities accounting for most of the energy consumption are unaffected by light/dark or that little net change in energy use is seen because of acceleration of some activities and reduction of others caused by a change in illumination. The apparent failure to alter 2DG uptake by exposure of the retina either to ouabain or a low sodium gradient may be attributed to the small change expected and the inherent variability between preparations noted in Fig. 1.

Comparison of 2DG and Glucose Metabolism

The utility of 2DG as a probe of tissue metabolism depends on the degree to which its treatment by the tissue resembles that of glucose. In mammalian brain, Sokoloff (1977, 1981) and Lund-Anderson (1979) found that 2DG uses the glucose transport and phosphorylation mechanisms, although at different rates. The evidence of the present study is that the same resemblance holds for retina. The competitive inhibition of 2DG by glucose (Fig. 5) shows that 2DG is transported by the glucose carrier. The time and concentration dependence of 2DG uptake (Figs. 1 and 2) implicate a facilitated diffusion mechanisms because there is a tendency towards saturation when the intra-

cellular concentration of 2DG approaches that of the bathing medium. A facilitated diffusion system is found generally for glucose transport (Baldwin and Lienhard, 1981).

In a cell-free system derived from retina, we have found that the K_m for glucose phosphorylation is ~ 0.1 mM, whereas that for 2DG is 3–4 mM.¹ Moreover, glucose phosphorylation shows marked product inhibition by glu-

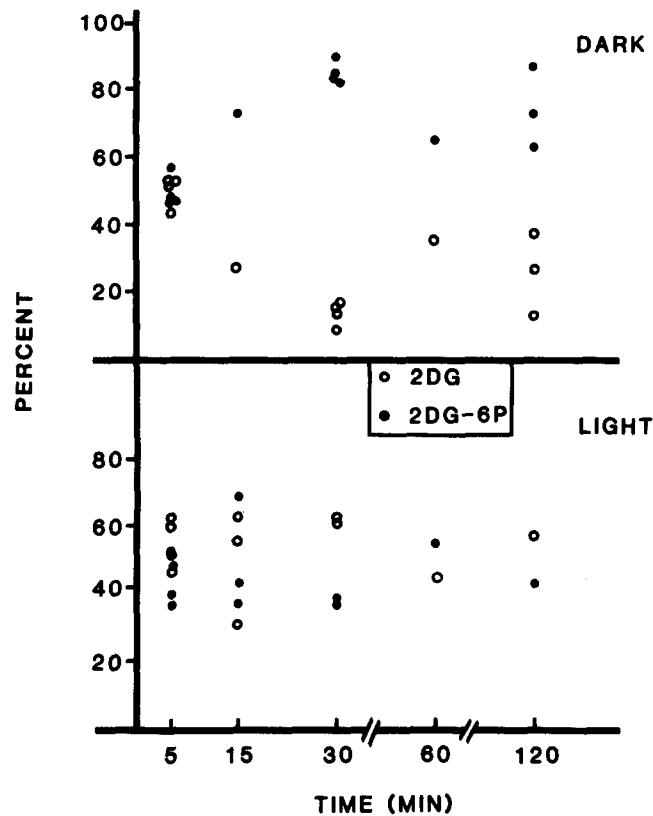


FIGURE 10. Relative fractions of 2DG and 2DG-6P present in bullfrog eyes after intracardiac injection of 50 μ Ci 2DG and incubation in light or darkness for variable times. Each point is the average of two chromatographic runs on a single retina.

cose-6-phosphate, whereas 2DG-6P inhibits 2DG phosphorylation to a much smaller degree. The latter point is particularly important in that 2DG metabolism mainly ends with 2DG-6P, which thus accumulates in the cell, although it has been shown that $\sim 20\%$ 2DG can be further metabolized to

¹ Liu, S. S., P. Witkovsky, and C. Y. Yang. Mitochondrial and cytosol hexokinase of retina: kinetic properties and the effect of cyclic 3'-5' adenosine monophosphate. Manuscript in preparation.

glycogen in retina (Witkovsky and Yang, 1982) and an invertebrate ganglion (Kaikai and Pentreath, 1981). In broad outlines, therefore, 2DG processing by retina appears to mimic the initial stages of glucose metabolism, although the rates differ. It is worth noting also that the retina appears to phosphorylate 2DG at a much faster rate than the brain does. Lund-Anderson and Kjelden (1977) studied 2DG transport and phosphorylation in rat brain slices. The rate of transport was found to exceed that of phosphorylation by an order of magnitude, such that after a 10-min exposure, the free 2DG concentration exceeded that of 2DG-6P. In amphibian retina (Fig. 6) after the same period of incubation, 2DG-6P concentration greatly exceeded that of 2DG.

Mechanism of the Light Effect upon 2DG Metabolism

Studies upon other systems show that the glucose phosphorylating enzyme hexokinase is capable of regulation by a number of factors, including substrate concentrations, product inhibition (Rose et al., 1974), ion concentrations, and a variety of metabolites, e.g., insulin (Knull et al., 1973). In the retina, obvious candidates for mediating a link between light level and hexokinase activity are the cyclic nucleotides 3'5'-guanosine monophosphate (cGMP) and 3'5'-adenosine monophosphate (cAMP) because, in the retina, both cAMP and cGMP are in high concentrations modifiable by light (reviewed in Lolley, 1980).

In the rabbit retina, Orr et al. (1976) found that the inner portion of the photoreceptor cell had higher dark than light levels of both cGMP and cAMP. In a cell-free system derived from frog retina we found¹ that micromolar concentrations of cAMP stimulated mitochondrial hexokinase activity 50–90%. Because light reduces cyclic nucleotide levels, the lower percentage of phosphorylated 2DG found in light-incubated bullfrog eyes (Fig. 10) could be accounted for by a reduction in the turnover rate of hexokinase. It should be noted that the difference in the 2DG-6P content of light vs. dark-incubated retinas may be underestimated in Fig. 10 in that light-incubated retinas had a lower total radioactivity (Fig. 9), possibly caused by back-transport of free 2DG from retina to medium.

We thank Drs. E. Smith and D. Krohn for the loan of equipment, Ms. V. Alones and Mr. J. Ahluwalia for excellent technical assistance, and Dr. S.-S. Liu for helpful advice. Supported by National Institutes of Health grant EY 03570 to P. W.

Received for publication 11 February 1982 and in revised form 18 May 1982.

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