over, the dark terrain has an excess of craters smaller than 15 km in diameter as compared to the light terrain. In addition, the dark terrain has a higher total number of craters per unit area (Fig. 2); the ratio of the total crater density on the dark terrain to that on the light terrain is approximately 7:5.

The best interpretation for the fact that there is a higher density of larger craters on the light terrain as compared to the dark terrain probably is that the light terrain has a greater impact exposure age. The greater total number of craters per unit area on the dark terrain probably is a function of either more rapid erosion and filling of craters (particularly the smaller sizes) on the light terrain or greater production of small endogenetic craters in the dark terrain, or both. Polarimetric and photometric data for Mars were found to be consistent with mean particle sizes of 100 to 200 μm on the dark areas and 25 μm on the light areas, and the finer-sized material on the light terrain would be more easily transported by the surface and atmospheric processes hypothesized for Mars (*7*). It seems possible that the finer-sized particles might be more available to fill and erode the craters on the light terrain than would the coarser surface particles on the dark terrain. We propose that the lighter terrain is generally older than the dark terrain (frames 6N11, 6N13, and 6N19), but that the surface of the older light terrain may be covered with relatively young, mobile detritus.

The crater size frequency distributions of four wide-angle frames (6N11, 6N13, 6N19, and 7N25) coincide very closely (Fig. 4). These frames include a large total area and both light and dark terrains, and the average of these curves may approximate the size frequency distribution of craters of a large portion of the martian surface.

ALEXANDER WORONOW
ELBERT A. KING, JR.
Department of Geology, University of Houston, Houston, Texas 77004

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6. The mean diameters of the craters are as follows: light terrain, 27 km; dark terrain, 23 km. The standard deviation is 17 km for the light terrain and 11 km for the dark terrain. Maximum displacement between the two curves is approximately 11 km or 1 standard deviation at 85 cumulative percent. However, we should not expect differences of 2 or 3 standard deviations if the difference in age of the two surfaces is relatively small. Furthermore, there is an underlying assumption that the size and velocity frequency distribution of meteoroids near Mars did not change drastically during the interval between the age of the two surfaces.
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1 June 1971; revised 26 October 1971

Cyclic Nucleotide Phosphodiesterase: High Activity in a Mammalian Photoreceptor

Abstract. *Purified outer segments of bovine rods exhibit phosphodiesterase activity against adenosine and guanosine cyclic 3',5'-monophosphates (cyclic AMP and cyclic GMP). The enzyme hydrolyzed cyclic GMP more rapidly than cyclic AMP at low substrate concentrations. The presence of high phosphodiesterase activity in this highly specialized organelle suggests that this enzyme may function in control of cyclic nucleotide concentration during visual excitation or adaptation.*

The process by which the retina translates light energy into a nerve impulse is only partially understood. Much has been learned about both the bleaching of rhodopsin (*1*) and the ensuing electrophysiological changes which occur in the retina (*2*), but the mechanism linking these phenomena remains unknown. There would seem to be two criteria that this translating mechanism must fulfill. (i) It must achieve significant amplification, since bleaching of one rhodopsin molecule is sufficient to excite a receptor cell. (ii) This process must act over a distance, since in rods and primate cones the lamellae containing the rhodopsin are separated from the plasma membrane (*3*). Wald has proposed that the bleaching of rhodopsin might alter the activity of an enzyme (*4*). This suggestion has been given added impetus by the observation of Bitensky *et al.* (*5*) that outer segments of frog rods contain a light-inhibited adenylate cyclase. For this inhibition to significantly affect concentration of cyclic nucleotides within the short time in which visual excitation occurs, the receptor region of the retina must also contain high activity of phosphodiesterase, the enzyme which destroys cyclic AMP (*6*). As shown in Table 1, we have found this to be the case in outer segments of bovine rods.

Cattle eyes were obtained from a local slaughterhouse and dark-adapted in the cold overnight. Subsequent operations were carried out in dim light. The retinas were dissected out and swirled in 0.25*M* sucrose that contained 5 m*M* $MgCl_2$ and 10 m*M* tris(hydroxymethyl)-aminomethane (tris), *p*H 8.1. The suspension was filtered through a double layer of cheesecloth, and crude rod outer segments were obtained from the filtrate by centrifugation over a 41 percent sucrose cushion at 5000 rev/min in a Spinco SW-27 rotor (3200*g*) for 1 hour. This preparation was then centrifuged on a continuous sucrose density gradient (24 to 41 percent) for 12 hours at 25,000 rev/min (83,000*g*). A band containing pure rod outer segments, as determined by light and electron micros-

Table 1. Cyclic AMP phosphodiesterase. Enzyme activity is expressed as nanomoles of cyclic AMP hydrolyzed per minute per milligram protein. Samples of brain and retina from adult rats and 16-day chick embryos were homogenized in a solution containing 2 m*M* glycylglycine, 10 m*M* NaCl, and 10 m*M* KCl, *p*H 7.4. Rod outer segments were prepared as described in the text. Enzyme activity, measured as oxidation of reduced nicotinamide adenine dinucleotide (NADH), was monitored continuously by absorbance at 340 nm. The reaction mixture contained 0.1*M* tris buffer at *p*H 7.5, 5.5 m*M* α-ketoglutaric acid, 0.1 m*M* ethylenediaminetetraacetic acid, 0.1 m*M* NADH, 0.25 m*M* nicotinamide adenine dinucleotide, 1 m*M* $MgCl_2$, 2 m*M* cyclic AMP, *Escherichia coli* alkaline phosphatase (25 μg/ml), adenosine deaminase (0.02 μg/ml), and glutamic acid dehydrogenase (0.25 mg/ml) (*12*). Samples were assayed in duplicate under conditions in which reaction rate was a linear function of protein concentration. These values were corrected for the rate in the absence of cyclic AMP (which was negligible in the samples of rod outer segments). Protein was measured by the method of Lowry *et al.* (*13*).

Source	Enzyme activity
Rat brain	39
Rat retina	8.4
Chick brain	44
Chick retina	42
Bovine retina	15
Bovine rod outer segment	82

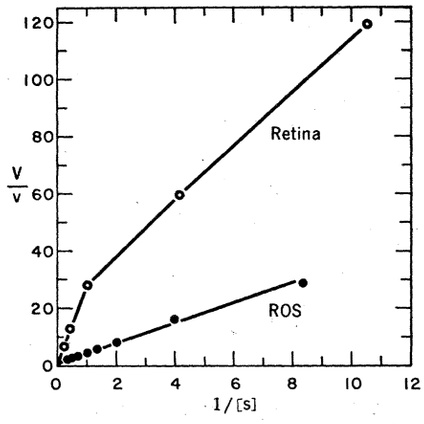

Fig. 1. Lineweaver-Burk (*11*) graph of the kinetics of phosphodiesterase activity in rod outer segments (*ROS*) and in a homogenate of whole retina. The abcissa is the reciprocal of substrate concentration (millimolar cyclic AMP). Enzyme activity was measured as described for Table 1. In the ordinate, V is maximum velocity and v is velocity at a given substrate concentration.

copy, formed near the top of the gradient. This preparation was used in the following experiments.

With 2 m*M* cyclic AMP as substrate, the specific activity of cyclic AMP phosphodiesterase in rod outer segments exceeded that in brain and in whole retina (Table 1). Our value for mammalian retina agrees well with that found for rabbit retina by Breckenridge and Johnston (*7*). The maximum reaction velocity of the preparation of rod outer segments was about four times the value listed in Table 1, because in contrast to other mammalian phosphodiesterases the enzyme in rod outer segments was not saturated at 2 m*M* cyclic AMP but exhibited a Michaelis constant (K_m) of about 7 m*M* cyclic AMP (Fig. 1). As seen in Fig. 1, there was no indication of a second phosphodiesterase with a lower K_m in this preparation. Homogenates of whole retina, on the other hand, showed complex kinetics similar to those observed in brain preparations (*8*). Thus, although high enzyme activity was present, maximal velocity could only be achieved at extremely high concentrations of cyclic AMP. This led us to ask if cyclic AMP was the preferred substrate for this enzyme.

Hydrolysis of the cyclic 3′,5′-monophosphates of adenosine, guanosine, cytidine, and uridine was measured in 100 μl of mixture containing 100 m*M* tris at *p*H 7.6, 5 m*M* $MgCl_2$, 0.25 percent Lubrol, and 5 m*M* cyclic nucleotide. Tubes were incubated for 30 minutes at 25°C, and the reaction was stopped by boiling for 10 minutes. Cyclic nucleotides were separated from the corresponding 5′-nucleotide products by thin-layer chromatography on polyethyleneimine-cellulose plates, which were developed stepwise with 0.2*M* LiCl followed by 1*M* LiCl. Preparations of rod outer segments were inactive against cyclic UMP or cyclic CMP but catalyzed the hydrolysis of cyclic GMP at a maximum velocity about one-tenth of that observed with cyclic AMP.

Nonionic detergents stimulated the phosphodiesterase activity of intact outer segments of rods. Magnesium ion was required for maximum activity; calcium ion would not substitute and inhibited when present in tenfold excess over magnesium ion. Phosphodiesterase was purified sixfold by sucrose density centrifugation of detergent-treated preparations. The band containing maximum phosphodiesterase activity contained very little rhodopsin, a result that indicates that these proteins are distinct. The ratio of activity against cyclic AMP to that against cyclic GMP was maintained through this purification, results that suggest that the same enzyme catalyzes both reactions.

Further evidence that the phosphodiesterases for cyclic AMP and cyclic GMP are identical is shown in Table 2. The K_m for hydrolysis of cyclic GMP is about equal to the inhibition constant (K_I) for inhibition by cyclic GMP of the hydrolysis of cyclic AMP. The low K_m observed for cyclic GMP indicates that the first-order rate constant for the rate of hydrolysis of cyclic GMP exceeds that for cyclic AMP by a factor of about 4.

Dibutryl cyclic AMP, caffeine, theophylline, and papaverine inhibited hydrolysis of cyclic AMP, with papaverine being the most effective (Table 2). Similar results were obtained for hydrolysis of cyclic GMP. Ebrey and Hood (*9*) observed that inhibitors of phosphodiesterase depress the amplitude of receptor potential in frogs. For these compounds, effectiveness of inhibition in that system parallels the effectiveness of inhibition of phosphodiesterase from rod outer segments (Table 2): caffeine is the least effective inhibitor, followed by theophylline and then papaverine.

Our observations are thus consistent with the conclusions reached by Bitensky *et al.* (*5*) and Ebrey and Hood (*9*) —cyclic nucleotides may play a role in the translation of light stimuli into a nerve impulse. However, cyclic GMP might be as likely an intermediate in this process as cyclic AMP. This suggestion is reinforced by our observations that outer segments of bovine rods contain high activities of guanylate cyclase as well as a protein kinase stimulated by both cyclic AMP and cyclic GMP (*10*).

Table 2. Kinetic constants of phosphodiesterase from rod outer segments. Cyclic AMP phosphodiesterase was measured as described for Table 1. Cyclic GMP phosphodiesterase was measured by the thin-layer chromatographic procedure described in the text; 3 nc of tritiated cyclic GMP was added to the reaction mixture. Spots corresponding to cyclic GMP and 5′GMP were eluted and their radioactivity measured in a scintillation counter. Inhibition of hyrolysis of cyclic AMP was determined by the method of Dixon and Webb (*14*).

Compound	K_m (*M*)	K_I (*M*)
Cyclic AMP	6.7×10^{-3}	
Cyclic GMP	1.8×10^{-4}	1.5×10^{-4}
Dibutryl cyclic AMP		8.0×10^{-4}
Caffeine		2.2×10^{-3}
Theophylline		3.6×10^{-4}
Papaverine		5.0×10^{-5}

R. G. Pannbacker
D. E. Fleischman
D. W. Reed

Charles F. Kettering Research Laboratory, Yellow Springs, Ohio 45387

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26 July 1971; revised 17 September 1971