

## Measurements on the pH Dependence of Metarhodopsin Reactions in Sonicated Rod Outer Segments

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The kinetics of rhodopsin photolysis in sonicated suspensions of rod outer segments (ROS), unsonicated ROS suspensions and rhodopsin detergent solutions were compared. No significant kinetic differences were detected; however, the energy of activation for the metarhodopsin I to metarhodopsin II reaction was somewhat higher in sonicated ROS suspensions than in the unsonicated suspensions. While the sonicated preparations contained glycerol, it was not possible to show a chemical influence of glycerol on the reactions of the rhodopsin decay. An often assumed effect that the ROS outer membrane acts as a barrier toward diffusion of protons could not be confirmed.

Spectrophotometric measurements of suspensions of bovine rod outer segments (ROS) are hindered by a high degree of light scattering. When rhodopsin is solubilized by means of detergents such as digitonin or CTAB\*, the rhodopsin solution is almost transparent, eliminating light scattering problems; however, the conformation of rhodopsin may be changed by the neighbouring detergent molecules.

Photolytic measurements using sonicated ROS prepared by a modification of the procedure of Mason and Abrahamson<sup>1</sup> were made and compared with the results of v. Sengbusch<sup>2</sup> and Nöll<sup>3</sup> from unsonicated preparations and rhodopsin detergent solutions.

### Methods

Isolated bovine ROS were prepared according to the method of v. Sengbusch<sup>2</sup> and stored at 193 °K until use. Thawed suspensions were sonicated to varying degrees by means of an ultrasonic desintegrator (Branson Sonic Power Company, Md B-12, 20 kHz). The temperature of the suspension was maintained below 276 °K by using a cooling liquid of CaCl<sub>2</sub> in H<sub>2</sub>O (freezing point below 223 °K) to which dry ice was added continuously. Sonication was applied in one minute periods with intervals of 2–3 min between each sonication period.

The effect of different sonication times was monitored by optical transmission measurements using a Cary 17 Spectrophotometer and by electron micro-

scopy. Samples were prepared for microscopy by precipitation by centrifugation, staining with uranyl acetate and lead, embedding in Epon and finally sectioning.

Kinetic measurements were made with a 66% glycerol-ringer ROS suspension which was sonicated for 5 min (sum of sonication periods). The flash photometer used for the measurements has been previously described<sup>2</sup>. The transition reactions, lumirhodopsin to metarhodopsin I and metarhodopsin I to metarhodopsin II, were measured at various temperatures between 273 and 307 °K. The dependence of the absorption change resulting from the rhodopsin to meta II reaction on the pH value of the surrounding medium was measured between pH 4 and pH 12.5.

### Results

Fig. 1 shows the measurements of optical transmittance versus wavelength of the measuring light for different ROS suspensions. Sonication in normal Ringer's solution did not increase the transmittance considerably. However when glycerol was added to ROS suspended in Ringer's solution, there was a significant increase in transmission following sonication.

Figs 2\*\* and 3 show the corresponding electron micrographs. Electron micrographs show that the effect of sonication was the disruption of ROS membranes. The membrane fragments produced tended

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\* *N*-Cetyl-*N,N,N*-trimethyl-ammoniumbromid.

\*\* Figs 2 and 3 see Tables on pages 600 a and b.



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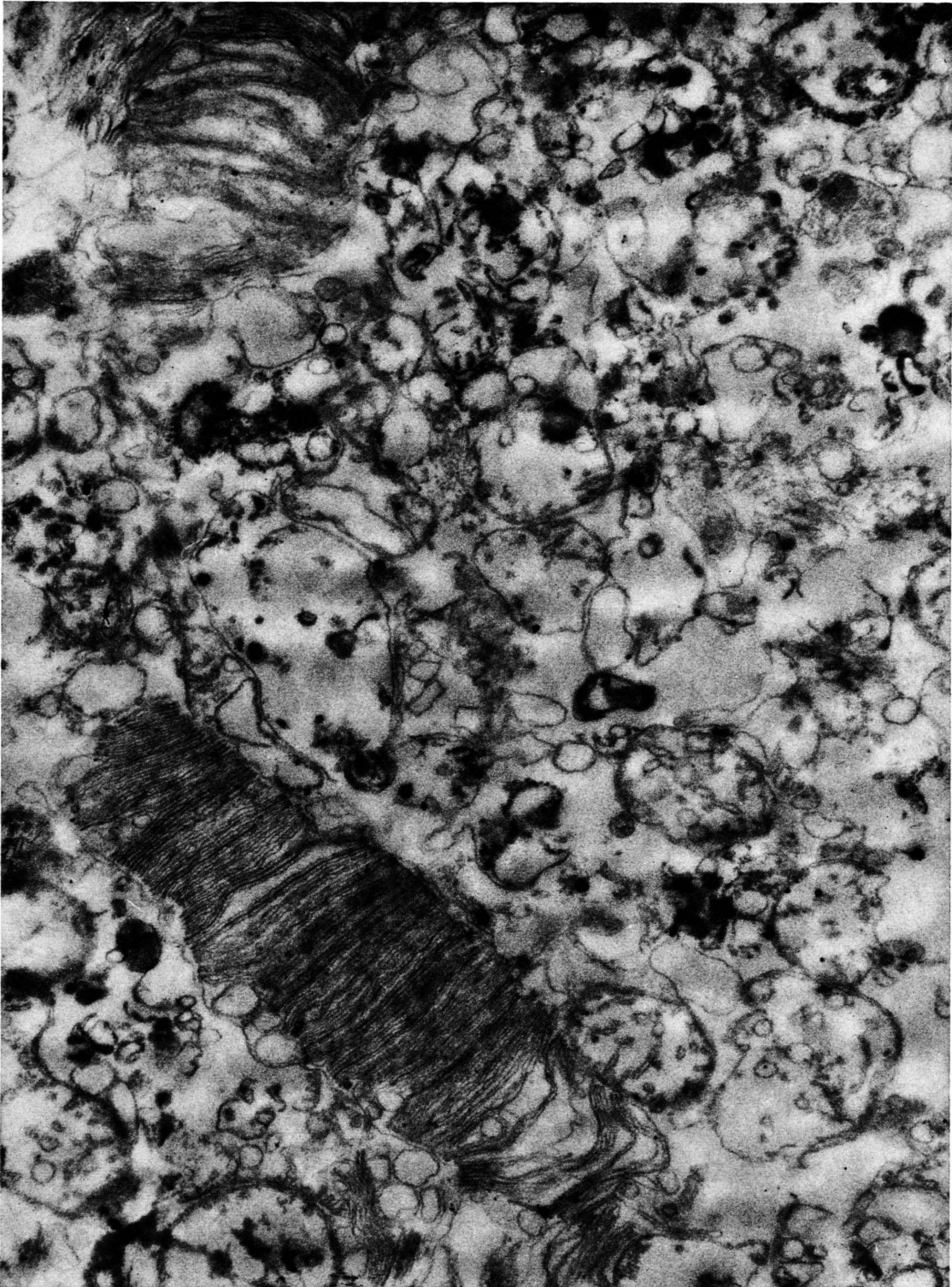


Fig. 2. Electron micrograph of a ROS suspension in Ringer's solution with addition of 66% glycerol before sonication.



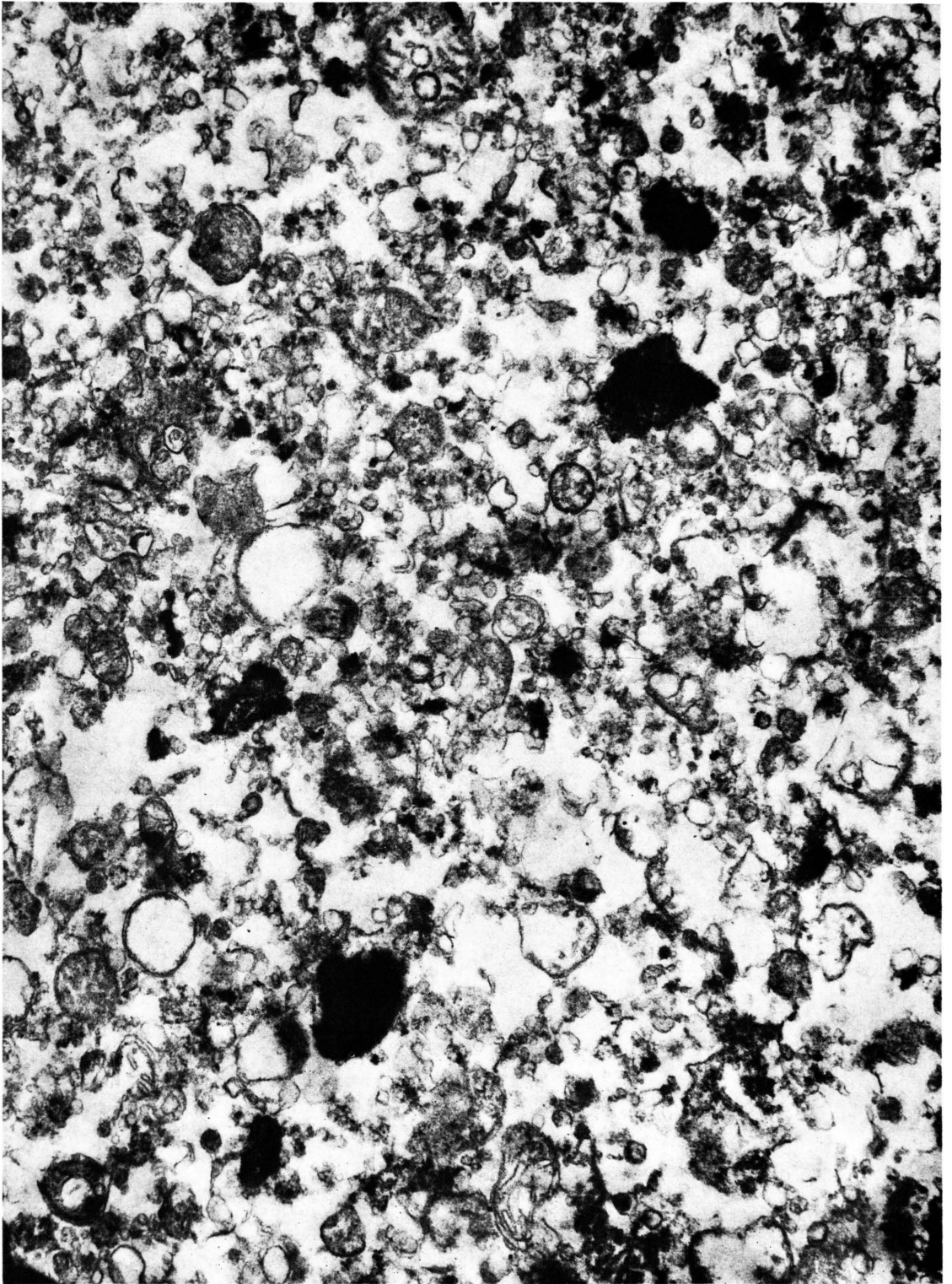


Fig. 3. Electron micrograph of a ROS suspension in Ringer's solution with addition of 66% glycerol after 2 min of sonication (sum of sonication periods).

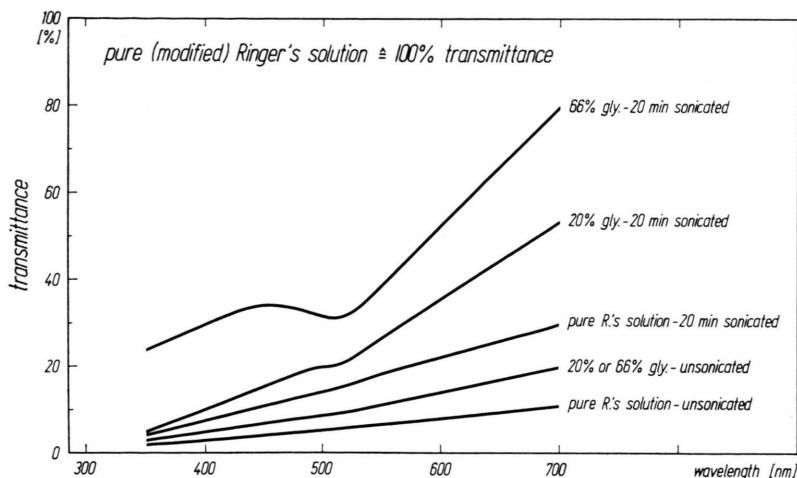


Fig. 1. Optical transmittance *versus* measuring light for different sonicated and unsonicated ROS suspensions at 293 °K.

to form vesicles of various sizes. This occurred in preparations with and without glycerol.

Increased duration of sonication of a ROS suspension in glycerol-Ringer's saline showed a close correlation between light transmittance and the size of the membrane fragments (Fig. 4).

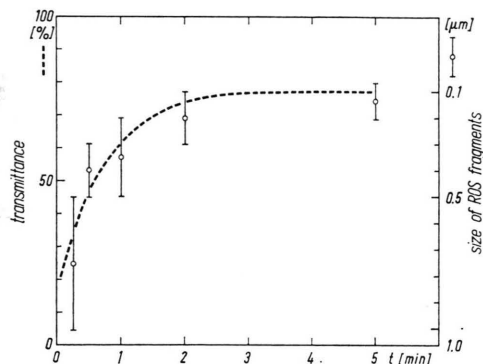


Fig. 4. Increase of optical transmittance at 750 nm (left ordinate, traced line) and the decay of average particle size (right ordinate, barks) in a ROS suspension in Ringer's solution with addition of 66% glycerol as a function of total sum of sonication periods.

Although sonication in normal Ringer's solution produces a considerable fragmentation of ROS membranes, the transmittance of light increases very little. It appears that glycerol acts to assimilate the indices of refraction in the cuvette, thus strongly reducing light scattering.

If two suspensions of equal concentration (with and without glycerol) are compared, an increase in the relative absorption based on meta I and meta II (other products not measured) can be seen after the same illumination. This phenomenon can be ex-

plained by the assumption that in the ROS suspension containing glycerol more rhodopsin is decayed. This could be the result of diminished light scattering and a consequent conservation of incident quanta. The measurements obtained indicate that in the ROS glycerol suspension the probability of a quanta bleaching a rhodopsin molecule is about two times higher than in the ROS suspension without glycerol.

In a series of measurements, the relaxation-time of the reaction meta I to meta II was monitored for possible effects of glycerol added to unsonicated ROS suspensions. The reciprocal relaxation-times obtained indicated no significant differences under the two conditions; reciprocal relaxation-time  $\tau^{-1}$  in unsonicated ROS suspensions at 283 °K with glycerol:  $3.0 \pm 0.2 \text{ sec}^{-1}$ . without glycerol:  $3.1 \pm 0.2 \text{ sec}^{-1}$  ( $n=5$ ).

The results of kinetic measurements are compared with those previously obtained from ROS suspensions and digitonin extracts in Table I and Fig. 6.

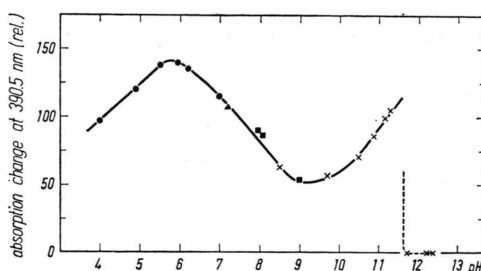


Fig. 5. Absorption change from rhodopsin to metarhodopsin II measured at 390.5 nm at 298 °K as a function of pH. ROS in 5 mM buffer solution. ● Citric acid/ $\text{Na}_2\text{HPO}_4$ -buffer; ▲  $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ -buffer; ■ Tris/HCl-buffer; × Glycine-NaCl/NaOH-buffer.

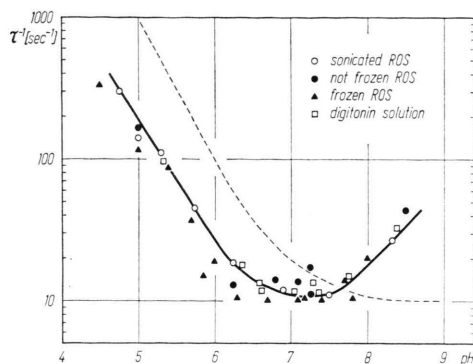


Fig. 6. pH Dependence of the reciprocal relaxation-time ( $\tau^{-1}$ ) of the reaction metarhodopsin I +  $nH^+$   $\rightleftharpoons$  metarhodopsin II at 283 °K. (— — —) Theoretical curve <sup>2</sup>.

Table I. Kinetic data of metarhodopsin I—metarhodopsin II reaction.

Material	Rhodopsin-digitonin-solution (v. Sengbusch <sup>2</sup> )	ROS suspension (v. Sengbusch <sup>2</sup> )	Sonicated ROS suspension
pH value	7.0	7.1	6.7
energy of activation $\Delta H$	$28.7 \pm 1.4$	$33.5 \pm 1.0$	$36.1 \pm 1.0$
[kcal/mole]	$28.1 \pm 1.4$	$33.1 \pm 1.0$	$35.5 \pm 1.0$
$\Delta F$ (293 °K)	15.1	15.0	13.9
[kcal/mole]			
$\Delta S$	$44 \pm 5$	$62 \pm 4$	$40 \pm 4$
[cal/degree]			
$\tau_{1/2}$ (310 °K)	$1.0 \pm 0.1$	$0.58 \pm 0.05$	$0.37 \pm 0.04$
[msec]			( $n = 7$ )

The pH dependence of meta I  $\rightleftharpoons$  meta II reaction is shown in Fig. 5. The curve is in general agreement with the data from v. Sengbusch<sup>2</sup> and Emrich<sup>4</sup> but deviates in certain aspects. Above pH 9 no inflection point was found in the present measurements. The reciprocal relaxation-time of the

meta I—meta II transformation steadily increases to pH 11.5. Above pH 11.5 rhodopsin decays in a dark reaction; the higher the pH value, the quicker the decay.

v. Sengbusch<sup>2</sup> found a difference between the measured data for the reciprocal relaxation-time of the transition reaction to meta II and the theoretical curve calculated for the reaction meta I +  $nH^+$   $\rightleftharpoons$  meta II (Fig. 6, dashed line). He explained the difference by the assumption that the protons necessary for the reaction are supplied primarily by the extracellular medium. In unsonicated ROS preparations this supply would be retarded by the diffusion barrier created by the plasma membrane. The present data do not support this hypothesis as the curves for sonicated suspensions and digitonin solutions, where no plasma membrane is present, do not differ significantly from the curve obtained for the unsonicated ROS suspension. This deviation from the theoretical curve is also not attributable to effects from freezing preparations in liquid nitrogen, as ROS suspensions which were not frozen give approximately the same values (Fig. 6). Thus it can be said that a diffusional barrier created by the plasma membrane has no influence on the pH-dependence of rhodopsin photolysis even if the assumptions on which the theoretical curve are based are inaccurate.

To summary, the results are similar to the measured values by v. Sengbusch<sup>2</sup> and Nöll<sup>3</sup> from unsonicated ROS and in rhodopsin solution. The differences in the data concerning the reaction meta I to meta II (Table I) as function of the temperature can be explained by the increase of the energy of activation in the experiments with sonicated ROS. Bargoot and Williams<sup>5</sup> have reported similar elevations in the energy of activation in harshly treated ROS preparations. Glycerol does not seem to influence the reactions measured here.

<sup>1</sup> W. T. Mason and E. W. Abrahamson, Personal communication.

<sup>2</sup> G. v. Sengbusch, Reaktionskinetische Untersuchungen an Stäbchen-Suspensionen und Rhodopsin-Lösungen mit der Blitzlichtmethode, Dissertation, Aachen 1970.

G. v. Sengbusch and H. Stieve, Z. Naturforsch. **26 b**, 488 [1971].

G. v. Sengbusch and H. Stieve, Z. Naturforsch. **26 b**, 861 [1971].

<sup>3</sup> G. Nöll, in preparation.

<sup>4</sup> H. M. Emrich, Z. Naturforsch. **26 b**, 352 [1971].

<sup>5</sup> F. G. Bargoot and T. P. Williams, Presented at Association for Research in Vision and Ophthalmology Conference, Sarasota/Florida 1973.