TABLE III

*Experimental measurements of the rate of activation of the G-protein per Rh**

Species	T (°C)	[GTP] (μM)	f_Φ	ν_{RG} ($G^*\ s^{-1}$ per Rh*)	Reference
(A) *Nucleotide binding*					
1. bovine	22	1.5	10^{-5}	12	[66, Figs. 4 and 5]
2. amphibian	–	125	10^{-5}	< 300	[120, Table I, Fig. 2]
3. frog	–	10	10^{-4}	26	[152]
4. frog	–	30–60	10^{-6}	24	[73, Fig. 6]
	–	30–60 [GTPγS]	$< 10^{-6}$	250	[73, Figs. 3–4]
(B) *Light scattering*					
5. frog	23	500	$< 10^{-3}$	1100	[188]
6. bovine	21	100	$< 10^{-3}$	> 3300	[189, Fig. 1]
7. bovine	21	(intact retina)	$< 10^{-4}$	2500	[142, Fig. 4]
8. bovine	20	(intact retina)	$< 10^{-4}$	800	[91]

The column 'ν_{RG}' gives the rate of G-protein activation (per Rh*), estimated by us from the results of each study. The column f_Φ gives the fraction of rhodopsin isomerized. –, indicates room temperature. We now give a brief summary of the methodology of each study, along with our derivation of the rate of activation. Our derivations of the rates are given, since in a number of studies the values are not estimated, and in others, the rates cited by the authors differ somewhat from those we obtain.

*Section A: Measurements of the rate of catalytic activation of $G_a^*\cdot NTP$ per Rh**

Study 1: incorporation of labelled GppNHp. The procedure employed 30 min of incubation, after which it was found that 0.022 mol GppNHp were bound per mol rhodopsin isomerized, when the fraction of rhodopsin isomerized was $f_\Phi = 10^{-5}$. In Fig. 4 of the paper it is shown that the time constant taken to reach equilibrium binding was approx. 3 min at 22°C and at this f_Φ. Thus the rate is $0.022/(10^{-5}\times 180\ s) = 12\ G^*\ s^{-1}$ per Rh*.

Study 2: binding of labelled GTP. The procedure employed a 1 min incubation time, and Table I of the paper shows that an average of 2650 [α-^{32}P]GTPs were bound per isomerized rhodopsin after 1 min, in the linear range of the response; i.e., for $f_\Phi = 10^{-5}$. However, the cited time course of about 10 s to reach steady-state binding, was determined at $f_\Phi = 10^{-3.4}$ (their Fig. 2) and not, apparently, in the linear response range. Thus the quoted time constant may have underestimated the actual time constant of the binding under the experimental conditions, since Fig. 1 of the paper shows that the total amount bound (at 1 min) was saturated at $f_\Phi = 10^{-3.4}$. In any case the estimated rate is not greater than $2650/10\ s = 270\ G^*\ s^{-1}$ per Rh*.

Study 3: binding of GTP. The report shows that 8.4 s after a flash producing a fractional isomerization of $f_\Phi = 10^{-4}$, about 0.022 mol GTP was bound per mol rhodopsin isomerized. Thus the estimated activation rate is $0.022/(10^{-4}\times 8.4\ s) = 26\ G^*\ s^{-1}$ per Rh*.

Study 4: binding of [^{3}H]GTP or GTPγ^{35}S. A suspension of rod outer segments was electropermeabilized, rather than mechanically disrupted. First line: at 20 s after a flash delivering 5000 isomerizations per outer segment, the amount of nucleotide bound was 0.8 mM GTP per mol rhodopsin. Taking the rhodopsin complement as 3×10^9 molecules, this corresponds to a rate of activation of $(3\cdot 10^9\cdot 0.8\times 10^{-3})/(5000\times 0\ s) = 24\ G^*\ s^{-1}$. Second line: 2.5 min after a flash producing 100–1000 isomerizations per outer segment 37000 GTPγ^{35}S per isomerization were found bound, yielding $37000/(150\ s) = 250\ G^*\ s^{-1}$ per Rh*.

Section B: Light-scattering measurements

(See review [182] for a general discussion of the light-scattering technique, its problems and the basis of the interpretations).

Study 5. Magnetically oriented, permeabilized frog rods were incubated in a medium containing 0.5 mM GTP at 23°C, and light scattering was monitored. A sensitive scattering signal was found, for which the initial rate of rise was proportional to flash intensity up to $f_\Phi = 10^{-3}$. On the assumption that the amplitude of the saturated signal corresponds to activation of all the G-protein, then the rate of activation of G-protein per Rh* is obtained as the normalized rate of rise of the signal divided by f_Φ and multiplied by the G-protein/rhodopsin ratio (assumed to be 1/10, see Table I). From their Fig. 2, with a normalized rate of rise of 3.7 s^{-1} at $f_\Phi = 3.4\cdot 10^{-4}$, the activation rate is calculated as $(3.7\ s^{-1}/3.4\cdot 10^{-4})\cdot 0.1 = 1100\ G^*\ s^{-1}$ per Rh*.

Study 6. Stacks of bovine discs were incubated in 100 μM GTP and 500 μM ATP, and the light scattering was monitored. Their Fig. 1 illustrates a scattering signal with a maximum of about 1% (at an angle of 24°) elicited within about 0.5 s, which appears to be linear with intensity up to a fractional bleach f_Φ of about 10^{-4}. At a just-saturating intensity of $f_\Phi = 269\cdot 10^{-6}$ the normalized rate of rise was about 6 s^{-1}. With the authors' G-protein/rhodopsin ratio of 0.15 (bovine), this corresponds to an activation rate of at least $(6\ s^{-1}/269\cdot 10^{-6})\cdot 0.15$, i.e., $> 3300\ G^*\ s^{-1}$ per Rh*.

Study 7. The scattering signal was measured from intact bovine retina at an angle of 6–10°. Their Fig. 4 illustrates the dependence of both the final level and the initial slope of the signal as a function of intensity. The amplitude saturated exponentially, and the rate of rise was linear with intensity up to $f_\Phi \approx 10^{-4}$. At $f_\Phi = 2\cdot 10^{-5}$ the normalized slope was 0.5 s^{-1}. Using the authors' G-protein/rhodopsin ratio of 0.1 (bovine), this corresponds to an activation rate of $(0.5\ s^{-1}/2\cdot 10^{-5})\cdot 0.1$, or $2500\ G^*\ s^{-1}$ per Rh*.

Study 8. The methods were similar to those of Study 7. Using the same approach as above, the authors estimated the rate of activation in their Fig. 7 to correspond to $800\ G^*\ s^{-1}$ per Rh*.