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THE PHOTOSENSITIVITIES OF VISUAL PIGMENTS IN THE PRESENCE OF HYDROXYLAMINE

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THE PHOTOSENSITIVITY of a substance that changes when it absorbs light of wavelength λ is defined (GOODEVE and WOOD, 1938) as the product of its extinction coefficient $(\alpha)_\lambda$ for the light and the quantum efficiency $(\gamma)_\lambda$ of the change.

The product $\alpha\gamma$ can be measured directly by the method of photometric curves—even in solutions where the absolute concentration of the photosensitive substance is unknown and other (inert) pigments are present throughout. In the method the transmission of the solution is recorded at frequent intervals as it bleaches (or darkens) in a constant light λ of known quantum intensity. When the appropriate function of the transmissivity is plotted against time, a straight line is obtained. The slope of this line is a measure of the photosensitivity at λ.

The method was devised (DARTNALL, GOODEVE and LYTHGOE, 1936, 1938) to measure the photosensitivity of frog rhodopsin in solution. It was later used (DARTNALL, 1958) to assess the photosensitivities of some other visual pigments relative to the frog pigment. Four additional pigments were studied, two being in the retinol (A_1) series and two in the 3-dehydroretinol (A_2) series. All five pigments were found to have photosensitivities of the same order, the values at the respective maxima ranging from 66 to 104 per cent of that for the frog pigment.

The present work is an extension of the 1958 investigation (to seven A_1 and five A_2 pigments) using the same apparatus and measuring techniques, but with the important addition that the photosensitivities were measured in the presence of hydroxylamine as well as without it.

In the absence of hydroxylamine some regeneration may occur, leading to a reduced overall quantum efficiency, and hence a reduced photosensitivity. When hydroxylamine is present, however, regeneration is inhibited. With hydroxylamine the photosensitivities are found to be significantly higher for some pigments (up to 31 per cent higher), though little affected for others. The use of hydroxylamine (presumably by removing the variable element of regeneration) has thus uncovered a well-defined two-class pattern of photosensitivities in the visual pigments.

MATERIALS AND METHODS

Visual pigments

Visual pigment extracts were prepared by conventional methods from the retinas of dark-adapted animals. In most cases whole retinas were used. These were either washed in several changes of buffer solution (usually pH 4-6) or were first dried, then extracted with petroleum ether, and then washed in the buffer solution. In two instances the photoreceptor outer segments were separated from the rest of the retina by flotation in sucrose and, in one case, then extracted with petroleum ether. After these preliminary

treatments (distinguished in Table 1 by the terms "washed retinas", "lyophilized retinas", and "outer segments") the material was extracted with 2–4 per cent digitonin solution. The extracts were made mildly alkaline by the addition of 10–20 per cent by volume of saturated sodium borate solution. In those cases where the photosensitivity measurements were to be made in the presence of hydroxylamine a neutral solution of this substance was added to the extract sample (making it 0·02–0·06 M in hydroxylamine—see Tables 3 and 4) shortly before the actual experiment.

Most of the extracts were prepared from fresh material, but in some cases frozen retinas from various sources were used. The dates given in Table 1 are those when preparation of the extracts was completed. The extracts were stored in a deep-freeze cabinet operating at about −20°C.

TABLE 1. DETAILS OF VISUAL PIGMENT EXTRACTS USED IN THE PHOTOSENSITIVITY EXPERIMENTS

Species	Extract[1]	Date of preparation	Material	Used in experiments
Conger conger	AA3 b, c, d	6.IV.65	Washed retinas (W.R.)	26 9 7, 27
Trigla cuculus	AA6 a b, c	18.VI.65	Lyophilized retinas (L.R.)	14 31
Galago crassicaudatus agisymbanus	W6[2]	28.VI.62	L.R.	6, 29
Rana temporaria	U11	25.I.61	W.R.	35
	V11	20.III.62	L.R.	34
	W9	11.X.62	W.R.	23, 25
	X2	2.1.63	W.R.	12
	AA1	18.II.65	W.R.	1, 2, 5, 10, 28
	AA7 a	7.VII.65	L.R.	15, 18
	CC1 a	22.III.66	Outer segments (O.S.)	36
Rana cancrivora	AA8 b	15.VII.65	W.R.	30
	AA9(2)a[3]	29.VII.65	O.S.	16
	BB7	26.X.65	W.R.	22
Salvelinus alpinus	AA5 c, d	3.III.65	W.R.	13
Coregonus clupeoides pennantii	AA2	17.III.65	W.R.	3, 4, 8
Cyprinus carpio	BB2 b	14.X.65	W.R.	20
Carassius carassius	BB3 a, b	19.X.65	W.R.	21
Coregonus clupeoides clupeoides	CC2 c	31.VIII.65	W.R.	17
Rutilus rutilus	T8	30.X.61	W.R.	24
	U8	5.XII.61	W.R.	33
	AA12	29.IX.65	W.R.	19
Osmerus eperlanus	AA4[4]	25.IV.65	W.R.	11, 32

[1] The letters a, b, etc., signify first, second, etc., extracts of a preparation. In other cases successive extracts were combined. In extract AA9(2)a, the (2) means that the extract was made from the second yield of outer segments. Some extracts were prepared from retinas that had been stored frozen from various times: AA2, since 16.II.65; AA3, since 1.IV.65; AA4, since 14.IV.65; AA5, since 28.XI.64; AA6, since 16.VI.65; BB7, since 15.VII.65 and CC2, since 8.VII.65.

[2] As used in previous work (DARTNALL *et al.*, 1965).

[3] As used in previous work (DARTNALL, 1967).

[4] A mixture of two extracts prepared on 21.IV.65 and 30.IV.65.

Some extracts were especially prepared for this investigation: others were remainders, from previous investigations, that had been stored frozen for various periods up to five years.

Extracts from twelve different species were used; one mammal, two amphibians, and nine fishes (three marine and six freshwater). Notes on the visual pigments present in these extracts follow. The names and attributions given to the species are those recommended by the British Museum (Natural History).

*The conger eel, **Conger conger** (Linnaeus)*

Extracts from conger eels are reported (WALKER, 1956) to contain a single A_1-based pigment of $\lambda_{max}=487$ nm. Recent measurements in this laboratory have given $\lambda_{max}=486$ nm.

*The red gurnard, **Trigla cuculus** Linnaeus*

Extracts from this species contain an A_1-based pigment of $\lambda_{max}=493$ nm (DARTNALL and LYTHGOE, 1965).

*The bush baby, **Galago crassicaudatus agisymbanus** (Coquerel)*

This extract contains an A_1-based pigment of $\lambda_{max}=501$ nm (DARTNALL *et al.*, 1965).

*The common frog, **Rana temporaria** Linnaeus, and the crab-eating frog, **Rana cancivora** Gravenhorst*

Extracts from frogs contain two A_1-based pigments, one derived from the red rods (rhodopsin, $\lambda_{max.}=502$ nm) and the other from the green rods ($\lambda_{max}=433$ nm) (DARTNALL, 1957, 1967; DONNER and REUTER, 1962). The green-rod pigment is destroyed by hydroxylamine.

*Willoughby's char, **Salvelinus alpinus** (Linnaeus)*

Extracts from the Lake Windermere char contain principally an A_1-based pigment of $\lambda_{max}=508$ nm, but also a small proportion of an A_2-based pigment of $\lambda_{max}=534$–545 nm (BRIDGES, 1967a). Bridges refers to this fish as *Salvelinus willughbii* (Günther).

*The gwyniad, **Coregonus clupeoides pennantii** Cuvier and Valenciennes*

Extracts from the gwyniad, which lives in Llyn Tegid (Lake Bala) in Wales contain a single, A_1-based pigment of $\lambda_{max}=520$ nm (BRIDGES, 1965).

*The common carp, **Cyprinus carpio** Linnaeus, and the crucian carp, **Carassius carassius** (Linnaeus)*

Extracts from the common carp (variety, mirror- or king-carp) contain a single A_2-based pigment of $\lambda_{max}=523$ nm (CRESCITELLI and DARTNALL, 1954). A pigment with the same properties is present also in the crucian carp (DARTNALL, 1962).

*The powan, **Coregonus clupeoides clupeoides** Lacépède, and the roach, **Rutilus rutilus** (Linnaeus)*

Extracts from the powan of Loch Lomond contain the single, A_2-based pigment of $\lambda_{max}=536$ nm (BRIDGES, 1967a, b). A pigment with the same properties is present in the roach also (DARTNALL, 1962).

*The smelt, **Osmerus eperlanus** (Linnaeus)*

Extracts from the smelt contain the single, A_2-based pigment of $\lambda_{max}=543$ nm (BRIDGES, 1967b).

Apparatus for bleaching experiments

The apparatus previously described (DARTNALL, 1958) was preserved as a unit, and was used again in the present bleaching experiments. Only in a minor particular was there any change in equipment; the two original glass optical cells (to contain the test solutions and water "control", respectively) had been broken, and two other cells were used instead. However, these were to the same size-specification as before and differed only in that the bodies of the cells were made of "black" instead of transparent glass.

The re-use of the previous apparatus permits a direct comparison to be made between the reaction rates obtained in the present and previous work. Thus the light intensities (which were not measured in absolute units) were monitored by the original galvanometer–photocell combination, and hence could be arithmetically related to those of the previous work. Proof that the sensitivity of the combination had not altered in the eight years that separate the two investigations was given by the fact that measurements on the same pigments under the same experimental conditions yielded the same reaction rates as before.

Procedure

The experiments were carried out as follows. One optical cell of the pair (always the same one) was filled with a sample of the visual pigment, and the other with distilled water. Since the cell holder had a limiting circular aperture in accurate correspondence with that of the cells care was taken to fill the "pigment" cell only to the base of its neck so that none of the extract would be unexposed to light. The cells were then placed in the cell holder so that the water-filled cell would be in the path of the monochromatic light beam (the other cell being occluded). The light was then switched on and, after a warming-up period, was adjusted by rheostat until the galvanometer reading (I) was slightly less than full scale.

The experiment was begun by moving the cell carrier smartly into its other position, so that the pigment solution now received the light beam. A reading of the initial transmission (I_o) was then taken. As the solution bleached, its transmission (I_t) increased, and readings were taken, on the minute, for thirty minutes or more. After this the pigment solution was left exposed to the bleaching light for a period (1–2 hr) sufficient to ensure that virtually complete bleaching had occurred, and that the transmission had reached its final value (I_f).

The intensities of the bleaching lights were not quite constant, there being slow fluctuations amounting to about ± 2 per cent of the mean value during the thirty-min measuring period. These changes were recorded in the course of each experiment by measuring, at two-min intervals, the galvanometer deflections caused by passing the light through the control cell. This was done immediately after alternate transmission readings, the operation of moving the control cell into the beam, taking the reading, and returning the pigment cell to the beam causing an interruption of about five seconds in the bleaching. Every transmission reading (I_t) was corrected by expressing it as a percentage of its appropriate I-reading. The relative intensity (I_m) for the whole experiment was taken as the mean of all I-readings expressed as a percentage of the full scale deflection.

There was no necessity to allow for the five-sec interruption in every two min of the bleaching since the procedure was followed in all cases, and only comparative values were required.

During the experiments water from a thermostat was circulated through the cell holder to maintain the temperature at 25 ± 0·2°C. At least five min was allowed for the temperature of the cell contents to equilibrate before starting the experiments. The bleachings were carried out with light of dominant wavelength 500 nm. The approximate band width of the light issuing from the exit slit of the monochromator was 15 nm.

THEORY

It has been shown (DARTNALL, GOODEVE and LYTHGOE, 1936, 1938; DARTNALL, 1958) that the equation

$$\log_{10} \frac{I_t}{I_f - I_t} = \phi \frac{\alpha \gamma I}{2{\cdot}303A} \cdot t + \text{constant} \qquad (1)$$

accurately describes the bleaching kinetics of visual pigment extracts under certain conditions. In this equation the symbols have the following meanings.

I, = the intensity of light (number of quanta per sec) incident on the front surface of a photosensitive solution contained in an optical cell having plane parallel faces at right angles to the light beam.

I_t, the intensity of light transmitted by the bleaching solution at a time t.

I_f, the intensity of light finally transmitted, that is after completion of bleaching.

t, time, in sec, from the initial exposure to light.

A, the exposed area (cm^2) of the solution.

γ, the quantum efficiency of the process, a ratio defined as

$$\frac{\text{number of chromophores destroyed}}{\text{number of quanta absorbed}}$$

α, the extinction coefficient (in cm^2) for a single chromophore as defined by the equation

$$\log_e \frac{I}{I_t} = \alpha c l$$

where c is the chromophore concentration (number per cm^3) and l is the length (cm) of light path through the solution.

The validity of equation (1) is not affected by the presence of light-absorbing impurities in the solution (provided they are stable) nor by the accumulation, as bleaching proceeds, of light-absorbing photoproducts. The reduction in reaction rate from either or both these causes is exactly allowed for by the slope-compensating factor, ϕ, in equation (1).

The function ϕ is given by

$$\phi = \frac{I_f}{I_f - I_t} \cdot \frac{I - I_t}{I} \cdot \frac{\log I_f/I_t}{\log I/I_t}$$

and hence, since it contains I_t, is a variable. However, the value of ϕ for each experiment decreases only very slowly as I_t increases from its initial value of I_o to its final value of I_f. In the present experiments this decrease averaged 0·4 per cent, the greatest decrease being 1·1 per cent. Thus the variation in ϕ is very small and a mean value (ϕ_m in Tables 2, 3 and 4) can be used without introducing significant error (DARTNALL, 1957).

RESULTS

Since, in any one experiment, ϕ is nearly constant, it follows from equation (1) that the values of the function $\log_{10} I_t/(I_f - I_t)$ should lie on a straight line when plotted against time. These plots are shown for the thirty-six experiments of the present work in Fig. 1, and all are acceptably linear. The measurement of time is virtually without error. Consequently the slopes of these lines have been calculated by the linear regression of $\log_{10} I_t/(I_f - I_t)$ on time (using data for the first thirty minutes of each experiment), and are listed in Tables 2, 3 and 4 ("crude" slopes).

From equation (1) the slopes, S, of the lines in Fig. 1 are given by

$$S = \frac{\phi \alpha \gamma I}{2{\cdot}303A} \tag{2}$$

from which, if I/A were known in absolute units (quanta per sec per cm^2) it would be possible to calculate the values for $\alpha\gamma$. As already mentioned, however, the light intensities were measured in relative, not absolute, units—though, since all experiments were done with the same bleaching light (500 nm), the intensities (I_m) in Tables 2, 3 and 4 are directly comparable one with another.

In order to put the results on a strictly comparable basis, the "crude" slopes, S, of Fig. 1 must be divided by the appropriate ϕ_m- and I_m- values.[1] The resulting "corrected" slopes in Tables 2, 3 and 4 are then measures, in relative units, of the photosensitivities at 500 nm. Finally, to relate these values to the respective maxima of the pigments concerned they must be multiplied by factors that give the ratio of the absorbance at the pigment λ_{max} to that at 500 nm. These factors have been obtained from data tabulated by WYSZECKI and STILES[2] (1967, p. 584) for A_1 pigments, and from the data of MUNZ and SCHWANZARA (1967) and of BRIDGES (1967b) for A_2 pigments. The factors are shown in Table 5.

[1] No allowance has been made for the light reflected back into the solutions from the rear surfaces of the optical cell and the surface of the photocell. This back-reflected light increases as the solution becomes more transmitting but this variation is partly offset by a corresponding decrease in ϕ. The amount of back reflection is small (*ca.* 5 per cent), and is much the same in most experiments, and hence can be neglected in these comparative experiments.

[2] These data are basically the same as those used in the construction of the visual pigment nomogram (DARTNALL, 1953) but are better since they are based on the mean values for a number of different A_1 pigments.

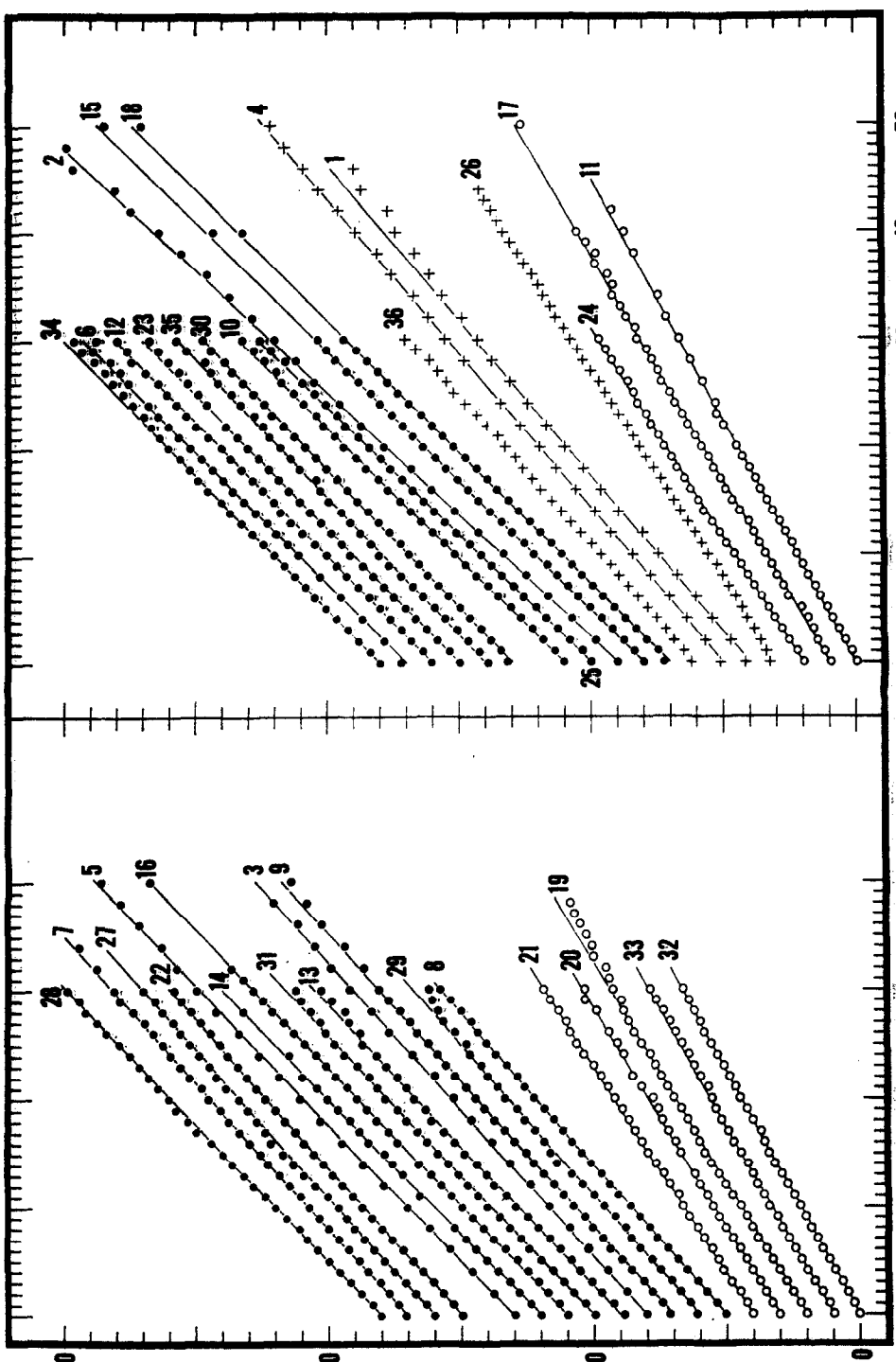

FIG. 1. The plots of $\log_{10} I_t/(I_f - I_t)$ against time for the thirty-six bleaching experiments at 500 nm, details of which are listed in Tables 2, 3 and 4. The filled and unfilled circles relate to experiments (done in the presence of hydroxylamine) with A_1- and A_2-based pigments respectively; the crosses to experiments (on A_1-based pigments) done in the absence of hydroxylamine. Note that all experiments yield linear plots. (To avoid overlapping, suitable arbitrary constants have been added to the ordinate values for each experiment.)

TABLE 2. DETAILS OF EXPERIMENTS ON A_1-BASED PIGMENTS IN THE ABSENCE OF HYDROXYLAMINE. $T=25°C$, BLEACHING WAVELENGTH = 500 nm. CRUDE SLOPE, THE SLOPE OF THE LINE, IN $sec^{-1} \times 10^{-4}$, OBTAINED BY PLOTTING $\log_{10} I_t/(I_f - I_t)$ AGAINST TIME (FIG. 1). CORRECTED SLOPE, THE CRUDE SLOPE $\times$ $100/(\phi_m I_m)$ WHERE ϕ_m IS THE MEAN VALUE OF ϕ, AND I_m THE MEAN BLEACHING INTENSITY IN ARBITRARY UNITS. THE CORRECTED SLOPES, AND THESE $\times$ THE RELEVANT ABSORBANCE FACTORS (TABLE 5) ARE MEASURES OF PHOTOSENSITIVITIES AT 500 nm AND AT λ_{max} RESPECTIVELY, RELATIVE TO THE CORRESPONDING VALUES FOR *Rana temporaria* RHODOPSIN

Experiment	Date	Extract	Age (days)	pH	Density at 500 nm Initial	Density at 500 nm Final	ϕ_m	I_m	Slope ($sec^{-1} \times 10^{-4}$) Crude	Slope ($sec^{-1} \times 10^{-4}$) Corrected	Corrected slope × absorbance factor ($sec^{-1} \times 10^{-4}$)
Conger conger											
26	12.XI.65	AA3b	220	7·9	0·732	0·108	0·903	98·7	4·27	4·78	5·12
Rana temporaria											
1	10.III.65	AA1	20	—	0·458	0·054	0·946	101·8	5·75	5·98	6·00 } 6·11
36	31.III.66	CC1a	9	8·6	0·310	0·019	0·979	98·7	6·00	6·20	6·22 }
Coregonus clupeoides pennantii											
4	19.III.65	AA2	2	—	0·536	0·051	0·950	99·5	5·82	6·17	6·81

TABLE 3. DETAILS OF EXPERIMENTS ON A_1-BASED PIGMENTS IN THE PRESENCE OF HYDROXYLAMINE. T=25°C, BLEACHING WAVELENGTH=500 nm. NOTES, AS FOR TABLE 2

Experiment	Date	Extract	Age (days)	pH	Hydroxylamine	Density at 500 nm Initial	Density at 500 nm Final	ϕ_m	I_m	Slope ($sec^{-1} \times 10^{-4}$) Crude	Slope ($sec^{-1} \times 10^{-4}$) Corrected	Corrected slope × absorbance factor ($sec^{-1} \times 10^{-4}$)	
Conger conger													
7	8.IV.65	AA3d	2	8·6	0·02M	0·518	0·014	0·986	99·5	6·20	6·32	6·76	6·71
9	14.IV.65	AA3c	8	8·0	0·02M	0·721	0·012	0·989	99·6	6·10	6·20	6·63	
27	16.XI.65	AA3d	224	8·3	0·02M	0·328	0·029	0·969	99·2	6·05[1]	6·30	6·74	
Trigla cuculus													
14	22.VI.65	AA6a	4	—	0·02M	0·240	0·037	0·961	98·4	6·67	7·05	7·16	7·12
31	29.XI.65	AA6b,c	11	6·5	0·02M	0·254	0·030	0·968	98·2	6·55[2]	6·97	7·07	
Galago crassicaudatus agisymbanus													
6	6.IV.65	W6	1013	8·3	0·02M	0·312	0·021	0·978	98·9	6·72	6·95	6·97	6·83
29	25.XI.65	W6	1246	7·8	0·02M	0·228	0·063	0·933	96·8	6·03	6·67	6·69	
Rana temporaria													
2	11.III.65	AA1	21	8·2	0·02M	0·419	0·052	0·945	105·8	7·30	7·30	7·32	7·08
5	26.III.65	AA1	36	—	0·02M	0·408	0·032	0·967	98·6	6·68	7·02	7·04	
10	30.IV.65	AA1	71	8·3	0·02M	0·409	0·029	0·969	99·6	6·80	7·05	7·07	
12	27.V.65	X2	876	8·3	0·02M	0·489	0·035	0·965	97·9	6·72	7·10	7·12	
15	9.VII.65	AA7a	2	8·4	0·04M	0·741	0·017	0·984	99·3	6·93	7·12	7·14	
18	17.IX.65	AA7a	10	8·3	0·04M	0·735	0·018	0·983	97·4	6·77	7·07	7·09	
23	8.XI.65	W9	1124	8·3	0·04M	0·416	0·019	0·981	99·6	6·68	6·83	6·85	
25	10.XI.65	W9	1126	8·5	0·04M	0·340	0·014	0·985	98·6	6·98	7·18	7·20	
28	17.XI.65	AA1	272	8·2	0·02M	0·402	0·028	0·971	96·4	6·67	7·12	7·14	
34	8.III.66	V11	1449	8·6	0·02M	0·290	0·018	0·981	98·4	6·72	6·95	6·97	
35	15.III.66	U11	1875	8·4	0·02M	0·412	0·036	0·963	98·9	6·58	6·90	6·92	
Rana cancrivora													
16	8.IX.65	AA9(2)a	41	8·6	0·04M	0·757	0·022	0·979	99·6	6·62	6·78	6·80	6·82
22	1.XI.65	BB7	6	8·5	0·06M	0·453	0·048	0·951	96·9	6·08	6·62	6·62	
30	26.XI.65	AA8b	134	8·3	0·02M	0·716	0·036	0·965	95·8	6·50	7·03	7·05	
Salvelinus alpinus													
13	10.VI.65	AA5c,d	99	8·7	0·04M	0·228	0·056	0·941	95·8	6·33	7·12	7·26	
Coregonus clupeoides pennantii													
3	18.III.65	AA2	1	8·2	0·02M	0·486	0·022	0·977	97·5	6·17	6·47	7·15	7·07
8	12.IV.65	AA2	25	—	0·02M	0·478	0·022	0·977	95·4	5·90	6·33	7·00	

[1] After subtracting 0·08 to allow for thermal decomposition.
[2] After subtracting 0·02 to allow for thermal decomposition.

TABLE 4. DETAILS OF EXPERIMENTS ON A_2-BASED PIGMENTS IN THE PRESENCE OF HYDROXYLAMINE. T=25°C, BLEACHING WAVELENGTH=500 nm. NOTES, AS FOR TABLE 2

Experiment	Date	Extract	Age (days)	pH	Hydroxylamine	Density at 500 nm Initial	Final	ϕ_m	I_m	Slope ($sec^{-1} \times 10^{-4}$) Crude	Corrected	Corrected slope × absorbance factor ($sec^{-1} \times 10^{-4}$)	
Cyprinus carpio													
20	15.X.65	BB2b	1	—	0·04M	0·288	0·063	0·934	98·7	4·07	4·42	4·80	
Carassius carassius													
21	27.X.65	BB3a,b	8	—	0·05M	0·244	0·031	0·967	98·9	4·35	4·55	4·95	
Coregonus clupeoides clupeoides													
17	15.IX.65	CC2c	15	8·4	0·04M	0·296	0·029	0·969	99·2	4·05	4·12	4·97	
Rutilus rutilus													
19	6.X.65	AA12	7	8·2	0·04M	0·370	0·036	0·963	98·9	4·05	4·27	5·15	
24	9.XI.65	T8	1471	8·5	0·04M	0·241	0·036	0·961	98·9	3·80[1]	4·00	4·83	4·91
33	3.XII.65	U8	1459	8·5	0·02M	0·285	0·070	0·928	96·9	3·53[2]	3·93	4·75	
Osmerus eperlanus													
11	4.V.65	AA4	9	8·6	0·03M	0·312	0·018	0·981	98·8	3·68	3·80	4·96	4·93
32	2.XII.65	AA4	221	8·5	0·03M	0·271	0·015	0·985	99·1	3·67	3·77	4·91	

[1] After subtracting 0·47 to allow for thermal decomposition.
[2] After subtracting 0·23 to allow for thermal decomposition.

TABLE 5. λ_{max} AND ABSORBANCE FACTORS (i.e. ABSORBANCE AT λ_{max} ÷ ABSORBANCE AT 500 nm) FOR THE PIGMENTS

Species	Pigment class	λ_{max} (nm)	Absorbance factor
Conger conger	A_1	486	1·070
Trigla cuculus	A_1	493	1·015
Galago crassicaudatus agisymbanus	A_1	501	1·003
Rana temporaria	A_1	502	1·003
Rana cancrivora	A_1	502	1·003
Salvelinus alpinus	A_1	508	1·020
Coregonus clupeoides pennantii	A_1	520	1·105
Cyprinus carpio	A_2	523	1·087
Carassius carassius	A_2	523	1·087
Coregonus clupeoides clupeoides	A_2	536	1·208
Rutilus rutilus	A_2	536	1·208
Osmerus eperlanus	A_2	543	1·304

The corrected slopes, and the products of these and the relevant absorbance factors, are listed with other experimental details in Tables 2, 3 and 4. The results are seen to be satisfactorily constant for any given species. For example, the eleven "slope × factor" values obtained for the *R. temporaria* pigment in hydroxylamine (Table 3) range only from 6·85 to 7·32 × 10^{-4} per sec (mean 7·078 ± 0·017 S.D.) and have no correlation with the ages of the extracts, which in this case varied from 2 to 1875 days. This reinforces the earlier conclusion (DARTNALL, 1958) that extract age is not important to the measurement of photosensitivity.

Most of the experiments were done with extracts containing hydroxylamine (0·02–0·06M). In some of the experiments on A_1 pigments, however, hydroxylamine was not used. A comparison between the mean "slope × factor" values obtained with and without it is made in Table 6 (mean data from Tables 2 and 3).

It is clear from the figures in Table 6 that the effect of hydroxylamine is to yield higher values for the "slope × factor" products (which are measures of $(\alpha\gamma)_{max}$, the photosensitivity maxima). Thus for the conger pigment the increase is 31 per cent (5·12 raised to 6·71), for the frog pigment it is 16 per cent, and for the gwyniad pigment only 4 per cent.

The question arises whether these increased photosensitivities might be spurious in being due merely to concurrent thermal breakdown of pigment induced by hydroxylamine. This substance is generally supposed to have no effect on the visual pigments[3], but some variation in this respect has been found in this laboratory with different samples of hydroxylamine and/or digitonin. It was thought advisable, therefore, to test this with the present materials. This was done by separate experiments in which the hydroxylamine

[3] A notable exception to this general rule is the behaviour of the green-rod pigment (λ_{max}=433 nm), which is present in small amount in the frog rhodopsin extracts. The addition of hydroxylamine causes rapid destruction of the 433_1 pigment without affecting the rhodopsin (DARTNALL, 1967). Since the 433_1 pigment does not appreciably absorb light of 500 nm wavelength, however, this reaction would not affect the photosensitivity measurements at 500 nm. It is interesting to note that the cone pigment iodopsin (λ_{max}=562 nm) is also destroyed by hydroxylamine, though rather more slowly (WALD, BROWN and SMITH, 1955).

solutions used were added to other samples of the relevant extracts and measurements of optical density made over periods of time. In most cases there was no detectable decomposition, but in four instances a slow loss of pigment density occurred according to the kinetics of monomolecular reactions. Even in these the rates were slow (almost negligible in two instances) in comparison with photolysis. They were allowed for by subtracting the velocity constants of the thermal decomposition from the "crude slopes" obtained in the bleaching experiments (see notes to Tables 3 and 4).

TABLE 6. COMPARISON OF RESULTS OBTAINED WITHOUT AND WITH THE ADDITION OF HYDROXYLAMINE

Species	Pigment λ_{max} (nm)	Mean corrected slope x absorbance factor ($sec^{-1} \times 10^{-4}$)	
		Without hydroxylamine	With hydroxylamine
Conger conger	486	5·12 (83·8)	6·71 (94·8)
Rana temporaria	502	6·11 (100·0)	7·08 (100·0)
Coregonus clupeoides pennantii	520	6·81 (111·5)	7·07 (99·9)

Thus hydroxylamine increases the photosensitivity, $\alpha\gamma$. The possible mechanism of this effect is considered in the Discussion. For the present, note only that the use of hydroxylamine leads to a greater consistency in the values. Thus in the absence of hydroxylamine the measures of $(\alpha\gamma)_{max}$ for the three pigments in Table 6 range from 84 to 111 per cent of the value for the frog pigment (arbitrarily taken as a standard) while with hydroxylamine the values are nearly constant (95–100 per cent). Because of this fact, fortunately noticed early in the investigation, most of the experiments were done with hydroxylamine.

In the present work two of the pigments studied in the absence of hydroxylamine, those of the conger eel and the common frog, had also been studied in the previous work with the same apparatus. The values for the corrected slopes ($x10^4$) for 500-nm bleaches in the earlier work were 4·65 and 6·07 respectively (DARTNALL, 1958). Multiplying these figures by the appropriate absorbance factors we obtain 4·98 and 6·09 as arbitrary measures of $(\alpha\gamma)_{max}$ for the conger and frog pigments. Within experimental error these figures are identical with those obtained in the present work, i.e. 5·12 and 6·11 (Table 6). This agreement shows that the sensitivity of the apparatus has not changed over the years, and justifies a direct comparison between all the results of the previous and present investigations.

This comparison is made in Fig. 2 in which the corrected slopes for 500-nm bleaches (i.e. measures of $(\alpha\gamma)_{500}$) are plotted as filled (A_1 pigments) or empty (A_2 pigments) circles. Absorbance curves relevant to each pigment are drawn through these circles, full-line curves for the A_1 pigments (one curve dotted to avoid confusion) and dashed-line curves for the A_2 pigments. On the left-hand side of Fig. 2 are shown the results of the previous investigation together with those of the present that were obtained without hydroxylamine. This part of Fig. 2 is similar to a previously-published figure (DARTNALL, 1958, Fig. 4) and shows again that in the absence of hydroxylamine the visual pigments have comparable photosensitivities, but does not reveal any special pattern. In contrast, the results obtained in the presence of hydroxylamine (right-hand side of Fig. 2) form a picture in which the A_1 and A_2 pigments are seen to fall into two crisply defined classes, the photosensitivity maxima of the A_2 pigments being about 70 per cent of those of the A_1 pigments.

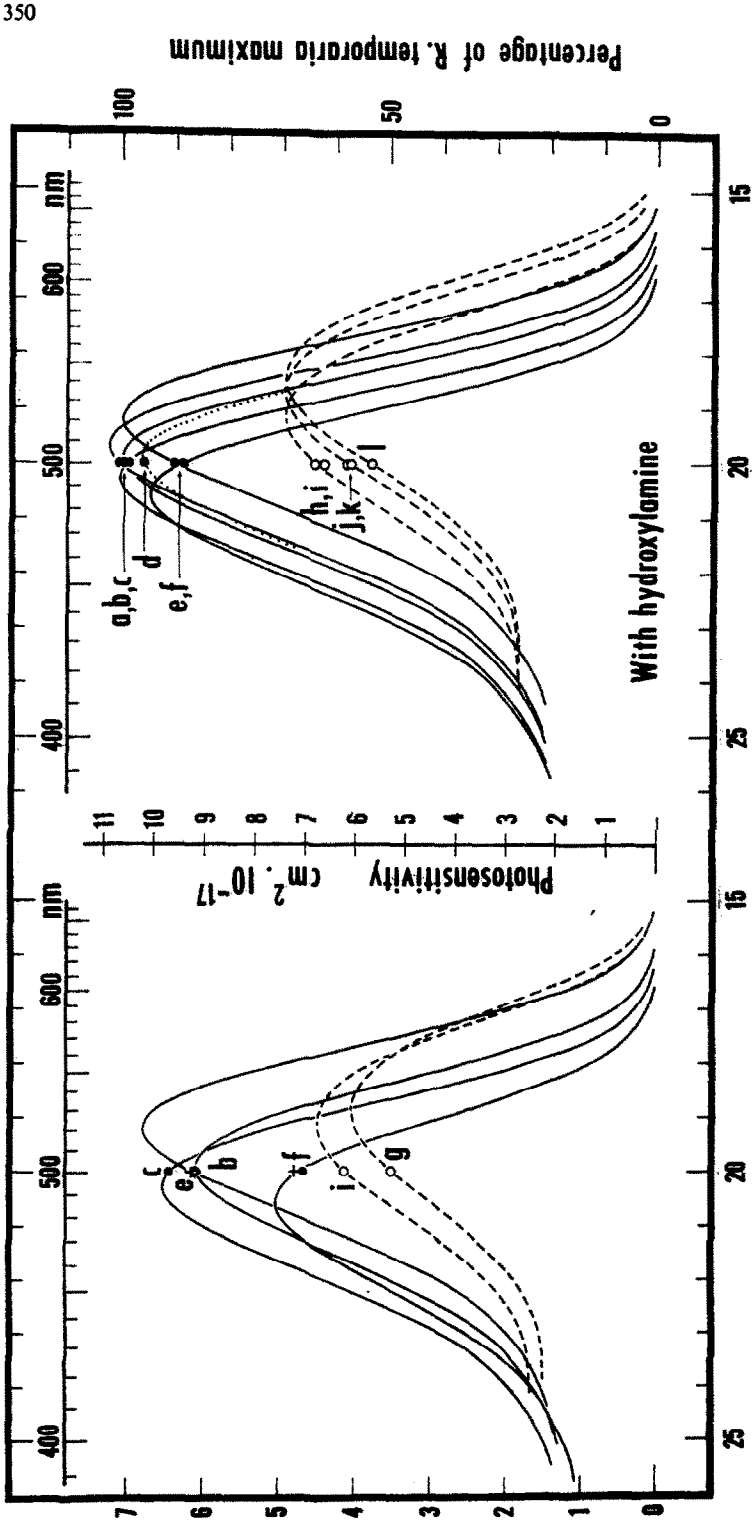

FIG. 2. The effect of hydroxylamine on the photosensitivities of visual pigments in digitonin solution, at 25°C. Filled circles give the photosensitivity values at 500 nm (20,000 wave-numbers) of A_1-based pigments; unfilled circles those of A_2-based pigments. The curves, full-line for A_1 pigments (dotted-line in one case to avoid confusion), and dashed-line for A_2 pigments are absorbance spectra scaled to pass through these values. The data on the L.H.S. (filled and unfilled circles) were obtained in a previous investigation (DARTNALL, 1958) and agree with those of the present work (crosses) that were also obtained without hydroxylamine. The data on the R.H.S. were obtained in the presence of hydroxylamine, and fall into two groups having maximum photosensitivities of either $10{\cdot}1$–$10{\cdot}9 \times 10^{-17}$ cm² (A_1 pigments) or $7{\cdot}4 \times 10^{-17}$ cm² (A_2 pigments). Key to letters as follows: a, *Salvelinus alpinus* (508_1); b, *Rana temporaria* (502_1); c, *Trigla cuculus* (493_1); d, *Rana cancrivora* and *Galago crassicaudatus agisymbanus* (502_1 and 501_1 respectively); e, *Coregonus clupeoides pennantii* (520_1); f, *Conger conger* (486_1); g, *Tinca tinca* (530_2); h, *Carassius carassius* (523_2); i, *Cyprinus carpio* (523_2); j, *Coregohus clupeoides clupeoides* (536_2); k, *Rutilus rutilus* (536_2); l, *Osmerus eperlanus* (543_2).

In Fig. 2 there are three ordinate scales. These are all measures of the same quantity—photosensitivity, $(\alpha\gamma)_\lambda$—in different units, and are interchangeable across the figure. The scale on the left expresses photosensitivity in terms of the corrected slope, $S \times 100/(\phi_m I_m)$. The slopes obtained experimentally at 500 nm are indicated by the symbols. The slopes for any other wavelength (as read off from the curves) are notional values that would be obtained with the present apparatus at light levels of equal quantum intensity to that of the 500-nm bleaches. The scale on the right gives the photosensitivities as percentages of the photosensitivity maximum for the frog (*R. temporaria*) pigment, i.e. $(\alpha\gamma)_{502}$, when determined in the presence of hydroxylamine. The middle scale expresses photosensitivity per chromophore in absolute units and was arrived at in the following way. In 1938, DARTNALL, GOODEVE and LYTHGOE obtained the value $9{\cdot}1 \times 10^{-17}$ cm^2 for the photosensitivity of the frog-rhodopsin chromophore at 506 nm. This indicates a value at λ_{max} (502 nm) of $9{\cdot}15 \times 10^{-17}$ cm^2, which was, in fact, subsequently obtained by SCHNEIDER, GOODEVE and LYTHGOE (1939). The middle scale of Fig. 2 has therefore been constructed so that the photosensitivity maximum of frog rhodopsin as determined in the present experiments *without* hydroxylamine corresponds to this value.

With this scaling the results in Fig. 2 show that (in hydroxylamine) the photosensitivity maxima $(\alpha\gamma)_{max}$ of the six retinol-based (A_1) pigments lie between $10{\cdot}1$ and $10{\cdot}9 \times 10^{-17}$ cm^2, and that those of the five 3-dehydroretinol (A_2) pigments are practically identical at $7{\cdot}4 \times 10^{-17}$cm^2.

In these values for $(\alpha\gamma)_{max}$, α is the extinction coefficient per single chromophore, and gives the actual area of the chromophore multiplied by the probability (which varies with wavelength) that a quantum falling within this area is absorbed. The molar extinction coefficient ϵ, on the other hand, is defined by the relation

$$\log_{10} \frac{I}{I_t} = \epsilon c l$$

where the concentration, c, is in number of gram-molecules per litre. Conversion to these more frequently used units is effected by multiplying α by $6{\cdot}023 \times 10^{23}$ (Avogadro's Number), by 0·4342 (natural to common logarithms) and by $c.10^{-3}$ (cubic centimetres to litres). If the maximum photosensitivities are expressed in these units we obtain (assuming each visual pigment molecule has one chromophore), $(\epsilon\gamma)_{max} = 26{,}300$–$28{,}500$ cm^2 for the A_1-based pigments (with a value of 27,800 for frog rhodopsin) and 19,300 cm^2 for the A_2-based pigments.

DISCUSSION

The spectral variation of photosensitivity

In the present comparison of the photosensitivities of visual pigments all measurements have been made at 500 nm, though the λ_{max} of the pigments studied range from 486 to 543 nm. In bringing the results to a comparable basis it has been implied that γ, the quantum efficiency, does not vary with wavelength. Thus the value of $(\alpha\gamma)_{max}$ for each pigment has been derived from the experimental value $(\alpha\gamma)_{500}$ by multiplying it by the relevant absorbance factor $\alpha_{max}/\alpha_{500}$.

SCHNEIDER, GOODEVE and LYTHGOE (1939) measured the photosensitivity of extracts of frog rhodopsin at various wavelenghts between 436 and 560 nm, and found that it varied with wavelength in practically the same way as did the absorbance α, thus indicating γ to be constant. However, better data are now available for the absorbance spectrum, and

a more critical comparison can be made. This is done in Fig. 3. In this figure Schneider, Goodeve and Lythgoe's data, plotted as percentages of the maximum value at 502 nm, are shown by the vertical crosses. The absorbance spectrum for frog rhodopsin is given by the continuous curve. Agreement is good on the long-wave side of the maximum, but there is a discrepancy, greatest at 430–440 nm, on the short-wave side. This discrepancy is confirmed by data of a quite different kind. Thus DARTNALL (1958) measured the photosensitivities of two A_1 pigments (conger and gurnard) and two A_2 pigments (carp and tench) relative to frog rhodopsin at a number of wavelengths between 440 and 580 nm. If we *provisionally* assume that the photosensitivities of the conger, gurnard, carp and tench pigments all vary with wavelength in the same way as their respective absorbances do (i.e. that γ for these pigments is indeed invariant) then we can obtain four estimates of the spectral variation of photosensitivity for the frog pigment. The means of these estimates are plotted as diagonal crosses in Fig. 3, and are seen to agree with Schneider, Goodeve and Lythgoe's results.

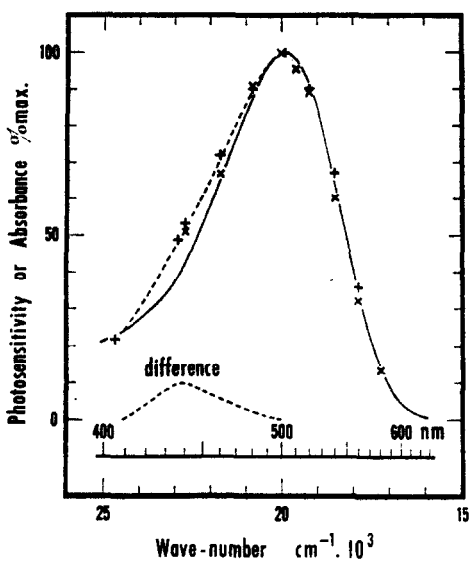

FIG. 3. Comparison between the absorbance spectrum of pure frog rhodopsin, $\lambda_{max}=502$ nm (full-line curve), and the photosensitivity spectrum as determined (in the absence of hydroxylamine) on retinal extracts (symbols). The vertical crosses are SCHNEIDER, GOODEVE and LYTHGOE's (1939) data and the diagonal crosses are derived from DARTNALL's (1958) data. Note the discrepancy (dashed portion of curve) between absorbance and photosensitivity, maximal at 430–440 nm, and separately plotted as a difference. This is probably due to the presence of the green-rod pigment, $\lambda_{max}=433$ nm, in the extracts.

The reason for the short-wave discrepancy in Fig. 3 is not hard to find. Both the 1939 and the 1958 data were obtained in the absence of hydroxylamine. We may presume, therefore, that the frog-rhodopsin extracts were "contaminated" by the photosensitive component of the green rods—an A_1-based pigment of $\lambda_{max} = 433$ nm (DARTNALL, 1967). In experiments with short-wave light the unsuspected presence of this pigment would lead to spuriously high values for the photosensitivity of rhodopsin.

Support for this idea can be extracted from a recent paper by KROPF (1967). He reports $(\epsilon\gamma)_{436}$ for frog (*R. pipiens*) rhodopsin as 11,400 cm^2 and compares this value with Schneider,

Goodeve and Lythgoe's of 11,800 cm² at 436 nm. But the results are not directly comparable, for Kropf's experiments were done in the presence of 0·01 M hydroxylamine (private communication from Dr. Kropf). Hydroxylamine has two effects—it destroys the green rod pigment (DONNER and REUTER, 1962; DARTNALL, 1967), and it increases the photosensitivity of rhodopsin by 16 per cent (this work). Thus in the absence of hydroxylamine Kropf's value for frog rhodopsin would be about 14 per cent less, i.e. 9,800 cm². This is now nearly 20 per cent lower than the Schneider, Goodeve and Lythgoe value (for rhodopsin plus green-rod pigment) and quantitatively accounts for the discrepancy at 436 nm in Fig. 3. Again, since the absorbance factor $\alpha_{502}/\alpha_{436}$ for rhodopsin is 2·58, Kropf's value indicates an $(\epsilon\gamma)_{max}$ of 29,400 cm², a result that is within 6 per cent of that for *R. temporaria* derived from the present experiments with hydroxylamine (27,800 cm²).

Thus the discrepancies at short waves between Schneider, Goodeve and Lythgoe's photosensitivity data and the absorbance of frog rhodopsin can be explained away. We can conclude that the wavelength variation of photosensitivity exactly matches that of absorbance, and hence that γ, the quantum efficiency, is invariant, at least for wavelengths >436 nm.

The rhodopsin chromophore has a secondary absorption band in the near ultra-violet (maximal at about 350 nm) but whether it has other bands in the further ultra-violet is not known for they would be obscured by the intense bands due to the protein part of the molecule. KROPF (1967) has shown that rhodopsin bleaches normally when exposed to ultra-violet radiation of wavelengths 280 nm and 254 nm and, on the implicit assumption that the chromophore has no absorption in this region, has concluded that energy absorbed by protein can be intramolecularly transferred to the prosthetic-group chromophore. This is a possible complication that need not concern us here, for the protein absorption bands are sharp, and do not tail into the visible part of the spectrum.

The quantum efficiency

The method of photometric curves yields values only for the photosensitivity, that is the product of the extinction coefficient (α or ϵ) and the quantum efficiency γ. In the original and early papers it was concluded (from the constancy of photosensitivity over ranges of temperature and pH, and from certain photochemical principles) that the quantum efficiency for the bleaching of frog rhodopsin "is equal to or not much less than unity" (DARTNALL, GOODEVE and LYTHGOE, 1936, 1938) and again "not less than 0·6" (SCHNEIDER, GOODEVE and LYTHGOE, 1939). No more precise statements than these seem possible unless the extinction coefficient is known.

Fortunately, measurements of extinction coefficients have since been made, both for A_1 and A_2 pigments. Thus cattle rhodopsin (λ_{max}=499 nm) has the value 40,600 cm² per retinene equivalent (WALD and BROWN, 1953) and chicken iodopsin (λ_{max}=562 nm) practically the same value (WALD, BROWN and SMITH, 1955). The only information on A_2-based pigments is the statement in BROWN, GIBBONS and WALD (1963) that "the molar extinction of porphyropsin is about 30,000[4] (WALD, BROWN and BROWN, unpublished observations)".

[4] Professor Wald has informed me that this measurement was made on a digitonin extract (to which hydroxylamine had been added) of the yellow perch, *Perca flavescens*, and that the result is correct to ± 500–1000. The molarity of the pigment was computed, as in the case of rhodopsin, by reference to the spectrum of the oxime formed after bleaching.

Although none of these species has been studied in the present work it is clear from published spectra that the absorption-band intensities of the visual pigments stand in nearly constant relations to those of their products after bleaching. This means that the ϵ_{max} values 40,600 cm² and 30,000 cm² are applicable (at least approximately) to all A_1 and A_2 pigments respectively. The quantum efficiencies can be obtained, therefore, by dividing the values of $(\epsilon\gamma)_{max}$ by the appropriate figure. The results of doing this are set out in Table 7, and show that in the presence of hydroxylamine the quantum efficiency is practically the same (2/3) for all visual pigments, whether A_1- or A_2-based.

The units of photosensitivity are cm² per mole of chromophore, and those of extinction are cm² per mole of retinene. In arriving at the values for γ listed in Table 7 we have assumed that the prosthetic group of the visual pigment molecule is based on one retinene equivalent. HUBBARD (1954) has shown that this is so for the cattle rhodopsin molecule, and in view of the marked similarity in properties of all the visual pigments (now extended to their quantum efficiencies) it is almost certainly true for all. The present results in fact give even further support to such a generalization. For if the prosthetic group were based on, say, two retinene equivalents then the molar extinctions would be twice 40,600 and 30,000 respectively and the values of γ would be half those shown in Table 7, i.e. about 1/3. Such a low value is not acceptable for, apart from other unfavorable considerations, it would suggest that there should be a greater discrepancy than may already exist between the number of absorbed quanta required for threshold vision (9–14) and the number of required "events" (5–8) as deduced from HECHT, SHLAER and PIRENNE's (1942) frequency-of-seeing curves (see also PIRENNE, 1962, p. 147). Thus we can conclude that γ is not 1/3, but 2/3 and, in turn, that the prosthetic group is based on one retinene.[5]

TABLE 7. THE PHOTOSENSITIVITIES OF THE VISUAL PIGMENTS AND THE QUANTUM EFFICIENCIES OF THEIR BLEACHING AT 25°C IN THE PRESENCE OF HYDROXYLAMINE

Species	Pigment	Photosensitivity: % frog pigment	Photosensitivity: $a_{max}\gamma$ ($cm^2 \times 10^{-17}$)	Photosensitivity: $\varepsilon_{max}\gamma$ ($cm^2 \times 10^4$)	Quantum efficiency γ
Conger conger	486_1	94·7	10·1	2·63	0·65
Trigla cuculus	493_1	100·6	10·7	2·80	0·69
Galago crassicaudatus agisymbanus	501_1	96·4	10·2	2·68	0·66
Rana temporaria	502_1	100·0	10·6	2·78	0·68
R. cancrivora	502_1	96·4	10·2	2·68	0·66
Salvelinus alpinus	508_1	102·6	10·9	2·85	0·70
Coregonus clupeoides pennantii	520_1	100·0	10·6	2·78	0·68
Mean for A_1-based pigments			10·5	2·74	0·67
Cyprinus carpio	523_2	67·9	7·2	1·89	0·63
Carassius carassius	523_2	69·8	7·4	1·94	0·65
Rutilus rutilus	536_2	69·4	7·4	1·93	0·64
Coregonus clupeoides clupeoides	536_2	70·3	7·5	1·95	0·65
Osmerus eperlanus	543_2	69·7	7·4	1·94	0·65
Mean for A_2-based pigments			7·4	1·93	0·64

[5] If the visual pigment molecule contained not one but n *independent* prosthetic groups, each based on one retinene, then the molar extinction and photosensitivity would both be n-times as great, and γ would remain, as before, at 2/3.

This work has shown that hydroxylamine enhances the photosensitivities of visual pigments by amounts that range from 4 to 31 per cent (depending on the pigment), and in so doing reveals a two-class system of photosensitivities (A_1 and A_2) that is not apparent from experiments done in its absence (Fig. 2).

The addition of hydroxylamine normally has no effect (apart from dilution) on the absorption spectra of extracts. This shows that hydroxylamine does not affect extinction. Hence it is the other term in photosensitivity—namely the quantum efficiency—that must be increased. It is not easy to see how hydroxylamine could have any direct effect on the quantum efficiency. Hydroxylamine could not act as a photosensitizer (for example) since it does not absorb the light used for bleaching. The most likely explanation of its action is that it inhibits regeneration, which otherwise, by opposing photolysis, leads to reduced overall quantum yields.

The initial photochemical event (absorption of a quantum by the chromophore) may be followed by a number of thermal changes to the molecule until a stable state, appropriate to the experimental conditions, is reached. These changes can be regarded as occurring in a series of well defined steps, as exemplified in the scheme proposed for rhodopsin by OSTROY *et al.* (1966) and reproduced in Fig. 4. In experiments carried out without hydroxylamine the "final" product is a mixture of NRO_{365} (the alkaline form of N-retinylidene opsin or "indicator yellow") and its hydrolysis product, $retinal_{387}$. At 500 nm (the wavelength of the bleaching light) both these substances absorb very feebly, so it is immaterial whether hydrolysis is completed. In the presence of hydroxylamine the end product is retinal oxime (λ_{max}=370 nm).

In the present experiments at 25°C straight lines were obtained by plotting $\log I_t/(I_f-I_t)$ against time, whether or not hydroxylamine was used (Fig. 1). This shows that disappearance of the intermediate products was always rapid in comparison with the present photolysis rates. Now the intermediates may vanish by two routes; by thermal decay to the end product according to the Fig. 4 scheme, and by regeneration to the parent pigment, e.g. by photoreversal (HUBBARD and ST. GEORGE, 1958, HUBBARD and KROPF, 1959; YOSHIZAWA and WALD, 1963, 1967).

Regeneration by photoreversal depends on the absorption of a second quantum by the molecule whilst it is in one or other of the intermediate forms. From the molar extinction of rhodopsin (40,600 cm^2) it follows that a cell-full (*ca.* 0·4 ml) of pigment of initial density 0·4 (per 0·5 cm path) contains about 5×10^{15} molecules of visual pigment. Since the light flux used in the bleaching experiments was about $1{\cdot}5 \times 10^{13}$ quanta per second, 60 per cent of which would, initially, be absorbed, only about one molecule in five hundred would receive a quantum in one second. Thus a molecule that had already absorbed a quantum (and had embarked on the downward path in Fig. 4) would have a chance (depending on its extinction relative to rhodopsin's at 500 nm) that was either somewhat greater, or very much less, than 1/500 of receiving another quantum in one second. Prelumirhodopsin, lumirhodopsin and $metarhodopsin_{478}$ (which have 500-nm extinctions comparable to that of rhodopsin) have such very short lives at 25°C that their chances of capturing a second quantum under these conditions can certainly be ignored. $Metarhodopsin_{380}$ is also short-lived at 25°C, and can be disregarded both on this account and because, like the relatively stable NRO_{365} and $retinal_{387}$, its extinction at 500 nm is negligible. This leaves $metarhodopsin_{465}$ (equivalent, according to ABRAHAMSON and OSTROY (1967), to LYTHGOE and QUILLIAM'S (1938) transient orange) as the most likely intermediate to pick up a second quantum. But even this substance has a short life at

25°C, and it is doubtful whether appreciable photoreversal could occur from it at the low intensity levels used. Moreover, if photoreversal were important one might expect some iso- (9-*cis*) pigment to appear, and this has not been observed at these intensity levels (DARTNALL, 1957).

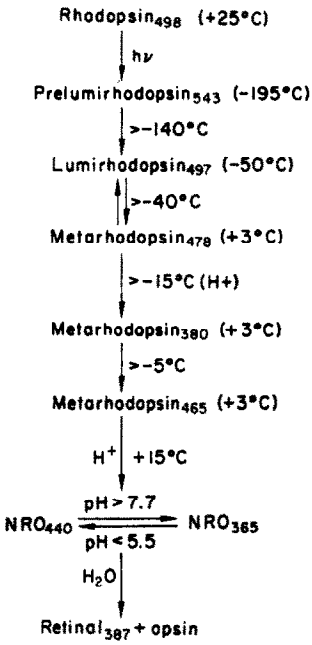

FIG. 4. The thermal reactions following the photolysis of rhodopsin, according to OSTROY, ERHARDT and ABRAHAMSON (1966). The subscript figures give the wavelengths in nanometers of maximum absorbance (at the bracketed temperatures) of the various intermediates. NRO = N-retinylidene opsin.

Thus photoreversal does not seem to be a very likely source of regeneration under the present conditions, and the possibility of spontaneous (i.e. thermal) regeneration from one or more intermediates cannot be ruled out. Clearly, further work is required to elucidate this point.

Regeneration, however it arises, must be less in the presence of hydroxylamine, for this substance (by capturing prosthetic-group retinene as oxime) greatly reduces the lifetimes of the intermediates (BRIDGES, 1962). In the absence of hydroxylamine the overall quantum yields vary from 0·49 (conger pigment) to 0·64 (gwyniad pigment), while in its presence the yield is practically constant (2/3) for all pigments. This constancy suggests that inhibition of regeneration is nearly complete in hydroxylamine, and that the quantum yield relates to the forward photolytic reaction alone, i.e. that about one-third of the visual pigment molecules that absorb a quantum do not bleach. This conclusion, if valid for the living retina, might account for the apparent discrepancy, noted above, between the number of quanta that have to be absorbed and the number of "events" that have to happen for threshold vision.

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Note added in Proof—The symbol in Fig. 3 for the photosensitivity at 405 nm is from GOODEVE, C. F., LYTHGOE, R. J. and SCHNEIDER, E. E. (1942). The photosensitivity of visual purple solutions of the scotopic sensitivity of the eye in the ultra-violet. *Proc. R. Soc.*, *B* **130**, 380–395.

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Abstract—The photosensitivities of six retinol-based (A_1) and five 3-dehydroretinol-based (A_2) visual pigments have been measured by using the pigment of the common frog as an actinometer.

The absorbance maxima of the pigments are located at wavelengths ranging from 486 to 520 nm (A_1 series) and from 523 to 543 nm (A_2 series). All photosensitivities were measured at 500 nm, the maximum values ($\epsilon_{max}\gamma$) being obtained by multiplying the results ($\epsilon_{500}\gamma$) by the appropriate absorbance ratio $\epsilon_{max}/\epsilon_{500}$.

In the presence of hydroxylamine all the A_1 pigments have nearly the same $\epsilon_{max}\gamma$ ($2{\cdot}63$–$2{\cdot}80\times10^4$ cm² per mole) and all the A_2 pigments about 70% of this value ($1{\cdot}89$–$1{\cdot}95\times10^4$ cm² per mole). In the absence of hydroxylamine regeneration occurs, to varying degrees with different pigments, and obscures this clear result.

From the values 40,600 cm² and 30,000 cm² respectively for the molar extinctions (ϵ_{max}) of A_1 and A_2 pigments it follows that the quantum efficiency γ (in hydroxylamine at 25°C) is about the same—2/3—for all visual pigments.

Résumé—On a mesure la photosensibilité de six pigments à base de rétinol$_1$ et de cinq pigments à base de 3-dehydrorétinol$_2$ en utilisant comme actinomètre le pigment de la grenouille commune.

Les maxima d'absorption des pigments se situent entre 486 et 520 nm pour la série A_1 et entre 523 et 543 pour A_2. Toutes les photosensibilités étaient mesurées à 500 nm, les valeurs maxima ($\epsilon_{max}\gamma$) étant obtenues en multipliant les résultats ($\epsilon_{500}\gamma$) par le rapport approprié des absorbances ($\epsilon_{max}/\epsilon_{500}$).

En présence d'hydroxylamine, tous les pigments A_1 ont sensiblement le même $\epsilon_{max}\gamma$ ($2{,}63$–$2{,}80\times10^4$ cm² par mole) et tous les pigments A_2 environ 70% de cette valeur ($1{,}89$–$1{,}95\times10^4$ cm² par mole). En absence d'hydroxylamine il se produit une régénération à un degré qui varie avec le pigment, ce qui obscurcit les résultats.

En admettant respectivement les valeurs 40600 cm² et 30000 cm² pour les extinctions molaires (ϵ_{max}) des pigments A_1 et A_2, on en déduit que l'efficacité quantique γ (dans l'hydroxylamine à 25°C) est à peu près la même—2/3—pour tous les pigments visuels.

Zusammenfassung—Die Lichtempfindlichkeiten von sechs Sehstoffen der Retinolgruppe (A_1) und von fünf der 3-Dehydroretinolgruppe (A_2) wurden gemessen, wobei das Pigment des gewöhnlichen Frosches als Aktinometer diente.

Die Absorptionsmaxima der Pigmente liegen bei Wellenlängen von 486 bis 520 nm (A_1 Gruppe) und bei 523 bis 543 nm (A_2 Gruppe). Alle Lichtempfindlichkeiten wurden bei 500 nm gemessen. Die Maximumwerte ($\epsilon_{max}\gamma$) wurden durch Multiplikation der Ergebnisse ($\epsilon_{500}\gamma$) mit den passenden Absorptionsverhältnissen $\epsilon_{max}/\epsilon_{500}$, gewonnen.

Bei Gegenwart von Hydroxylamin haben alle A_1 Pigmente nahezu dasselbe $\epsilon_{max}\gamma$ ($2{,}63$–$2{,}80\times10^4$ cm² pro mol) und alle A_2 Pigmente ungefähr 70% dieses Wertes ($1{,}89$–$1{,}95\times10^4$ cm² Pro mol). Bei Abwesenheit von Hydroxylamin tritt Regeneration auf, welche bei den verschiedenen Pigmenten unterschiedlich ausfällt und dieses klare Resultat verdeckt.

Aus den Werten 40,600 cm² und 30,000 für die molaren Extinktionen (ϵ_{max}) der A_1 und A_2 Pigmente folgt, daß die Quantenausbeute γ (in Hydroxylamin bei 25°C) bei allen Sehstoffen etwa die gleiche (2/3) ist.

Резюме — Была измерена световая чувствительность шести зрительных пигментов, в основе которых был ретиноль (A_1) и пяти пигментов в основе которых находился 3-дегидроретиноль (A_2), с использованием пигмента лягушки как актинометра.

Максимумы поглощения пигментов расположены в пределах длин волн от 486 до 520 нм (A_1 серия) и от 523 до 543 нм (A_2 серия). Для всех пигментов световая чувствительность была измерена на 500 нм, максимальное значение ($\epsilon_{max}\gamma$) было получено перемножением результатов $\epsilon_{500}\gamma$) на соответствующий коэффициент поглощения $\epsilon_{max}/\epsilon_{500}$.

В присутствии гидроксиламина все A_1 пигменты имели приблизительно одинаковый $\epsilon_{max}\gamma$ ($2.6 - 2.80 \times 10^4$ см² на моль, а все A_2 пигментв около 70% этой величины ($1.89 - 1.95 \times 10^4$ см² на моль). В отсутствии гидроксиламина регенерация происходит в различной степени для различных пигментов и затемняет этот ясный результат.

Для величин 40,600 см² и 30,000 см² для молярной экотинкции (ϵ_{max}) A_1 и A_2 – пигментов следует, что квантовая эффективность γ (в гидроксиламине при 25°C) приблизительно такая же — $\frac{2}{3}$ — для всех зрительных пигментов.