J. Physiol. (1956) 134, 30–46

THE RHODOPSIN DENSITY IN THE HUMAN RODS

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(*Received* 20 *March* 1956)

The foregoing paper was concerned to measure in various conditions the change in rhodopsin density in that region of the human retina (15–20° parafoveal) where it is maximal.

The total rhodopsin density might be thought to follow at once from these measurements by noting the change between complete dark adaptation and full bleaching. But absorption in a complex structure such as the retina is not quite simple to appreciate, and in this paper an attempt is made to apply the proper corrections to the experimental observations.

The result gives a value for the density in the rods and for the mean density of this retinal region substantially higher than that commonly accepted, and former estimates are discussed. Finally the bearing of the new figure upon the quantum threshold for vision is indicated.

PART 1. THE IRREDUCIBLE LOWER LIMIT TO THE DENSITY OF HUMAN RHODOPSIN

The apparatus and experiments have been described in the foregoing paper (Rushton, 1956), where the results were used to substantiate the view that the density change measured was due to the bleaching of the rhodopsin in the rods. We now turn to the question of how much rhodopsin was thus bleached.

In that paper, Table 1, p. 19, it is seen that in the example quoted the balance point of the wedge had shifted 26 mm for light of wave-length about 505 mμ. The wedge density was calibrated in the red light which fell upon it and the results gave a linear change of 0·060 decadic density units per cm shift of wedge. Thus, in the example of Table 1, bleaching caused a decrease in retinal double-density of 0·156. The light passes twice through the retina, but only once through the wedge, so this figure should be halved to give the retinal density change for a single passage, which is what is meant in this paper by 'retinal density'. Now in Fig. 4 of the previous paper (Rushton, 1956) a difference spectrum was obtained which was found not to coincide with the

curve of human rhodopsin, but to be displaced towards the red. This was interpreted as due to the slow decomposition of an orange photoproduct which absorbs very appreciably at 505 mμ. If this is correct, the measured change in rhodopsin will fall short of the true amount by the density of this orange product. It is not easy in the living human subject to correct for this entirely, but two methods have been tried. In one the bleaching light was left shining for 20 min or more. The total wedge shift never quite came to a maximum but increased by about 1 mm for each doubling of the bleaching time. This gave a total density of 0·09 (sometimes slightly more) for about 20 min bleaching. Since regeneration and further orange photoproduction continues during the 20 min, the value of 0·09 is still below the full rhodopsin density.

The other method is to scale the curve of Fig. 4 to coincide with the rhodopsin curve of Crescitelli & Dartnall (1953) not at 505 mμ, but at some longer wave-length where it may be supposed the orange product does not absorb. If 530 mμ is chosen for coincidence, my points must be reduced in the ratio 7/8, so that the value of the maximum appears reduced by the orange product by this fraction. The true value then should be $0{\cdot}078 \times 8/7 = 0{\cdot}09$. Here again it may well be doubted whether the orange product is without effect at 530 mμ, but we may at least accept the density of 0·09 arrived at by both these methods as a *lower limit* for the rhodopsin of the human retina in the region where the rods are most numerous.

This figure, which represents 18% absorption of the light falling on the retina, is higher than has been usually accepted and it lies near the safe upper limit of Hecht, Shlaer & Pirenne (1942). Before proceeding further we may review for a moment some errors to which the measurements are subject in order to see if any can account for too high a figure.

Acting in the *reverse* sense are the following possibilities:

(*a*) The subjects may not have been fully dark-adapted, the initial measurements may themselves have produced a little bleaching, the main bleaching may not have been complete on account of regeneration, there did almost certainly remain a little orange product, and some dark regeneration of rhodopsin may have occurred before the final reading was taken. All these are small, all tend to make the figure higher and so will be neglected in forming the lower limit.

(*b*) It is assumed that the comparison red beam is unaffected by bleaching and that the only pigment bleached is rhodopsin. If the rhodopsin were not quite transparent for the red beam used it would produce a small effect in the same sense as group (*a*), and there is no evidence here or elsewhere of bleaching producing substantial *increase* in density in the red. It does not seem as though pigments other than rhodopsin and its photoproducts were involved, as was discussed in the previous paper.

(*c*) Suppose that the light returning through the retina after diffuse reflexion

at the choroid took a scattered path, passing and re-passing the rods. Perhaps the density measured by this return beam might be much higher than the density for the ingoing beam, so that a false estimate of the latter would be obtained by halving the density measured in the double passage. It is not difficult to imagine that rays obliquely reflected at the choroid might return after multiple internal reflexions (or otherwise), and leave the retina by a rhodopsin path perhaps twice as great as the rod length. What is less easy to suppose is that after this erratic course any large proportion of rays would end by travelling in the vitreous directly away from the illuminated retinal area in the direction parallel to the incoming rays. But only such rays could pass the retinal stop (Rushton, 1956; *S*, Fig. 1, p. 15) and enter the photocell. It seems hardly likely that these oblique rays can constitute the chief part of the signal, since they would be outweighed by the better reflected and less absorbed light returning directly and more or less axially up the rods. Moreover, even if the rods were so formed that a large proportion of oblique light emerged as parallel rays in the virtreous, then it must follow that a fair proportion of parallel ingoing light would take the same oblique path on entering and so the difference in density for entering and emerging light would not be expected to be great. Finally the effect of molecular orientation must not be overlooked, for oblique rays lose through dichroism much of what they gain through increased length of path. For small obliquities the two factors exactly compensate, and a ray returning by multiple reflexions at even 45° to the rod axis would only achieve a 6% increase in rhodopsin absorption for all its 40% increase in path length.

These expectations have been investigated experimentally by Lewis (1956) in the excised eyes of albino rats. The apparatus was similar to that used for human measurements, but the excised albino eye is so translucent that a photocell may also be placed behind the eye to measure the rhodopsin density by transmitted light. Wedge settings were obtained initially in the dark-adapted eye for both reflected and transmitted signals. Then, after bleaching, the two wedge settings were again read and the density change for the transmitted light was, as expected, about half that for reflected light. The actual ratio found was $0{\cdot}54 \pm 0{\cdot}03$ ($n^* = 10$), suggesting that the reflected light traversed rather less rhodopsin than that entering.

None of the factors considered tend to lower the figure of 0·09 for human rhodopsin density (though most of them could raise it), nor is it easy to think of any factor which will. This figure therefore seems to be the irreducible minimum.

PART 2. STRAY LIGHT

In the preceding considerations it was assumed that all the light received by the photocell had passed twice through the retinal rods. If some light has in fact come from elsewhere, this will dilute the change measured, so the true

value of that change must be greater than what appeared. It is easy to obtain a mathematical expression for the magnitude of this effect.

It will be remembered that the principle of the experimental measurements is to shine into the eye two lights in rapid succession. One is red and serves as an intensity standard; with the other, λ, the actual measurement is made. The photocell outputs for the two lights are equated before and after bleaching by moving a neutral wedge placed in the path of one of the lights. It does not matter which path contains the wedge; in the actual apparatus it was the red path, but in the following argument it is more direct to consider the wedge to have been in path λ, which would certainly have given the same results (with, of course, the opposite direction of wedge shift). We may thus simply consider that bleaching removes some pigment and thus increases the signal received by the photocell, and that the signal is returned to its former value by increasing the density of the wedge in the measuring beam.

Let I_0 = intensity of incident light (λ) upon the fully bleached retina,
I = intensity which gives the same photocell output in some partly bleached condition,
W = wedge setting calibrated in density units and measured from fully bleached position,
a = fraction of the light incident upon the eye which the photocell receives coming from the rods in the fully bleached state,
$a\alpha$ = fraction received from the partly bleached rods,
as = fraction received from every place except the rods;
thus s = stray light expressed in units equal to the signal from the fully bleached rods,
2ρ = rhodopsin density in rods for double passage
$= -\log_{10}\alpha$.

Now in the fully bleached state light received by the photocell is

$$(a+as)\,I_0.$$

After some regeneration the balance is restored when

$$(a\alpha+as)\,I=(a+as)\,I_0,$$

or

$$\log_{10}\frac{\alpha+s}{1+s}=\log_{10}\frac{I_0}{I_1}=-W. \tag{1}$$

From this equation it is easy to give to s values of 0, 0·2, 0·4, ..., 1·0 and in each case to work out the relation between W and α, or, making the substitution $2\rho=-\log_{10}\alpha$, the relation between the wedge reading W and the rhodopsin double density 2ρ. This relation is plotted in Fig. 1, whence it appears that if s is at all large, our wedge reading (W) of 0·18 is associated with a very much higher value of 2ρ, the double density in the rods themselves. It thus becomes

a matter of great importance to form some estimate of the actual magnitude of s, the stray light.

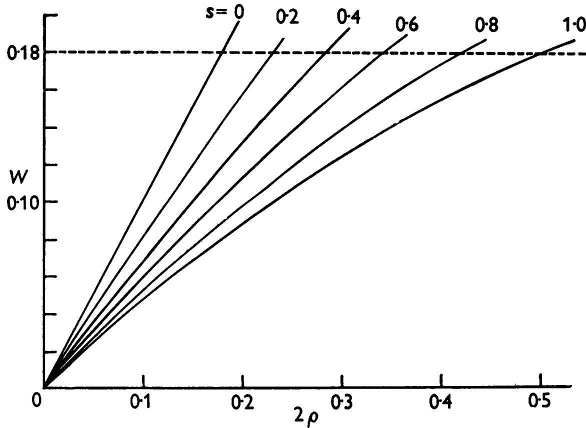

Fig. 1. Effect of stray light s upon the relation between wedge setting W, and true double density 2ρ.

Categories of light received

Light which reaches the photocell may be said to be of six classes:

(*a*) Light from the rods; this is the signal.

(*b*) Light from the room, etc.; this enters only as 'noise' and is almost entirely excluded by the phase-rectifier system.

(*c*) Light scattered from the cornea and anterior part of the eye.

(*d*) Light scattered from the retina anterior to the rods.

(*e*) Light returned from the spaces between the rods and cones.

(*f*) Light returned from the cones.

Light passing only once through the rods and entering or returning outside them will produce nearly the same effect as half this light in (*a*) and half in (*e*) and (*f*). Such light is therefore considered as embraced in those categories and is not separately treated. Categories (*c–f*) constitute the stray light. Of these (*c*) alone can be directly measured.

This was done by arranging the retinal image stop S_2 (Rushton, 1956, fig. 2, p. 16) so that instead of admitting light from the centre of the illuminated patch of retina it excluded this and accepted a neighbouring region just on the foveal side. Thus all the in-going light in front of the eye crossed the path of light from this dark patch of retina to the photocell, and scattered light would be received slightly better than in conditions where the signal was also received. On viewing the system from the position of the photocell a little light was now seen, probably scattered by the subject's lens at the edge of the field, and the stop S_4 had to be moved in a little to exclude this. The signal which

now consisted only of scattered light was so small that all the six spectral bands had to be shone simultaneously to get a measurable figure. Stray light type (*c*) from one band was about 5% of the normal total signal.

(*d*) In the discussion to their paper, Campbell & Rushton (1955, p. 144) conclude that where light falls upon the nerve fibres spreading out from the optic disk (10° nasal) the stray light dilutes the signal, so the wedge reading is half what it should be. If the signal can be halved there, it must be somewhat reduced everywhere. If we assume that 5% of the total light received at the photo-cell comes from this source, it still only means that a total of about 0·0005 of the light falling on the retina anterior to the rods is diffusely reflected in *all* directions.

(*e*) Campbell & Rushton (1955, fig. 4, p. 142) plot for various points on the retina the relative rhodopsin density measured and the rod density as determined by Østerberg (1935). The two relations were found to go hand in hand, but they certainly did not coincide on scaling. It was pointed out (p. 144) that only if the light were reflected as readily between the rods as through them would the curves be expected to coincide. The existing relation suggests that *reflected* light is brighter between the rods, which need not conflict with the observation of Schultze (1866) quoted earlier (p. 26) that *transmitted* light is brighter through them. For funnelling will clearly tend to concentrate light in one direction and to disperse it in the other.

But if the deviation between Østerberg's (1935) curve and our own is due to stray light, it might be possible to use the observed deviation to estimate this stray light, which is not easy to evaluate in any other way. We need to have information about the uniformity of the human rod outer segments in the region measured (5°–45° temporal). Mr E. H. Leach of Oxford has kindly made the necessary measurements for me upon human retinas which were normal in this region (e.g. removed after a perforating wound) and fixed in formol-saline or Bouin's fluid. He found that there was no appreciable variation in the dimensions of the rod outer segments, either in diameter or length, over the entire region considered. Let us further suppose that the rhodopsin content is also uniform, and that the number/mm^2 is given by Østerberg's measurements. Then where rods are less crowded the interspaces will be larger and hence contribute a greater amount of stray light which will reduce the signal (as we found). There should in consequence be a fixed (but not linear) relation between the concentration of the rods and the density of rhodopsin as measured. This is shown in Fig. 2 where the former data have been replotted in a different way, so that the ordinates give the apparent rhodopsin density of all the retinal points we measured, but instead of plotting this against retinal eccentricity, it is plotted against the rod density found by Østerberg at this eccentricity. Between 15° and 20° the rods and the rhodopsin are maximal, and moving towards the fovea gives the relation shown by the circles in Fig. 2; moving

peripherally gives the crosses. The assumptions which have been made require that circles and crosses lie on the same curve, and this is seen to be roughly the case. It has sometimes been thought that the retina changes progressively from fovea to periphery. Fig. 2 supports Leach's observation that such progressive change does not occur in rod structure over the range considered.

If then we can accept (though insecurely) the assumptions as to uniformity and also that Fig. 2 expresses simply the effect of stray light in diluting the rhodopsin signal, we may deduce something about the factors involved.

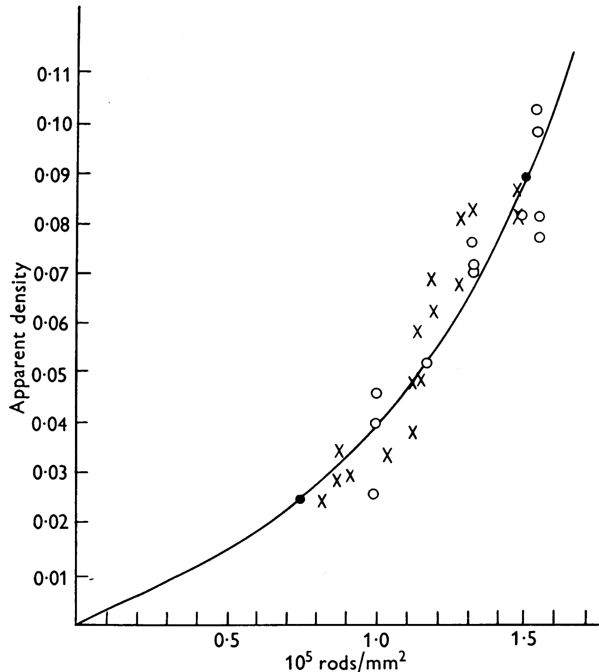

Fig. 2. Apparent rhodopsin density of various regions of the retina plotted against rod density of the region. o, regions less than 17° from the fovea; ×, more than 17°.

Let n = number of rods/mm² as given by Østerberg's count,

N = number of rods whose total area is 1 mm²,

∴ n/N = proportion of retinal area occupied by rods,

bn/N = total light from bleached rods,

$\alpha bn/N$ = total light from dark-adapted rods,

$\beta b(1-n/N)$ = total light from interspaces (including cones),

∴ β = average brightness of interspace in units of brightness of bleached rod,

γb = total light from elsewhere.

Then equation (1) may be rewritten replacing αa, the light received from the

unbleached rods, by $\alpha bn/N$ and as, the stray light by $\beta b(1-n/N)+\gamma b$ giving

$$\frac{\alpha n/N+\beta(1-n/N)+\gamma}{n/N+\beta(1-n/N)+\gamma}=10^{-W},$$

or

$$\frac{1-\alpha}{1+\beta\left(\frac{N}{n}-1\right)+\gamma N/n}=1-10^{-W}. \tag{2}$$

We have estimated that γb is at least 10% of the total light received in the bleached state. Let us assume that this holds for all positions on the retina; thus

$$10\gamma=n/N+\beta(1-n/N)+\gamma,$$

hence

$$\frac{0\cdot 9(1-\alpha)}{1+\beta\left(\frac{N}{n}-1\right)}=1-10^{-W}. \tag{3}$$

The three constants in this equation relating n and W are so grouped that they constitute only the two parameters $(1-\alpha)/(1-\beta)$ and $N\beta/(1-\beta)$; consequently if we introduce the condition that the curve must pass through the two black dots in Fig. 2 these parameters are determined and the curve shown results. This curve adequately describes the rather scattered experimental points, and we may turn to consider what value the constants must have consistent with these determined parameters. To do so let β assume values

TABLE 1. Deduced values of retinal quantities

β	2·0	2·2	2·4	2·6	2·8	3·0	3·2	3·4
$N\times 10^{-5}$	1·32	1·44	1·54	1·63	1·70	1·77	1·82	1·87
Rod fraction	1·17	1·07	1·00	0·95	0·91	0·87	0·84	0·82
α	0·688	0·625	0·562	0·500	0·438	0·375	0·311	0·250
ρ	0·08	0·10	0·125	0·150	0·180	0·214	0·254	0·300
Light absorbed (%)	17	20	25	29	34	39	43	50

2·0, 2·2, 2·4, ..., 3·4, and, for each, let us calculate the value of α from the first parameter and N from the second. The results are shown in Table 1. Now N is the number of rods whose total area is 1 mm². The maximum number/mm² observed by Østerberg was $1\cdot 55\times 10^5$, at about 17° from the fovea. The fraction of retina occupied by rods at this place is shown in the third row of Table 1 by dividing 1·55 by the second row. Since this fraction cannot be greater than unity, β cannot be less than 2·4 and the first two columns are inadmissible.

From the values of α we can at once obtain ρ the density, and also the fraction of light absorbed (both on single passage), for

$$2\rho=-\log\alpha,$$

$$\text{and the fraction absorbed}=1-10^{-\rho}=1-\sqrt{\alpha}. \tag{4}$$

Though these figures must be accepted with a good deal of caution, it seems safe to say that, in the region of maximum rod density, ρ cannot be less than 0·12; and, even so, only in the condition that no light at all is reflected from the receptor layer of the retina except through the rods. This raises the question of reflexion from the cones.

(*f*) *The cones.* As Denton & Pirenne (1954, p. 430) have pointed out, cones occupy about a quarter of the retinal area over most of the range we have been considering, so the contribution of light reflected from them becomes important. Clearly it must be small if the value of ρ is not to rise to an uncomfortable figure, but even if the 25% cone space only returned as much light as the 5% rod interspace, this would raise the value of ρ to 0·15.

We are now in a position to consider the figure we obtain for the diameter of the rods. If we assume that in 1 mm^2 of retina 0·25 is occupied by cones and that the remaining space is *completely* occupied by the 155,000 rods found by Østerberg, then the average rod diameter would be 2·5 μ as Denton & Pirenne (1954) have already calculated. This is substantially larger than the diameter given by Polyak (1941) which varies between 1 and 2 μ, and to accept even 2 μ would give a figure for stray light from the interspaces so large that it is outside the range given in Table 1.

We are therefore led to the conclusion that the receiving area of the rods corresponds to a diameter of 2·5 μ, though the absorbing area (outer segments) is only about half this. Now Mr Leach has kindly made measurements upon human retinas expressly to examine this point. He finds (after correcting for fixation shrinkage) that the inner segments of fresh rods are 2–2·5 μ in diameter, and that the outer segments are 1·4–1·7 μ. So the receiving area appears to be the inner segment of the rod and the absorbing area is in fact half of this. If the light then is funnelled from inner to outer segments with no loss, the photosensitivity would be twice that expected. In the foregoing paper (Rushton, 1956) the photosensitivity was measured: it was found to be about 1·7 that expected, and the present explanation was advanced to account for it.

Conclusion

The importance of estimating stray light despite the absence of direct measurements has forced this rather speculative treatment. Throughout, it has been borne in mind that the figure for rhodopsin density which emerges is so high that everything should be done to keep it down.

Light returning from places other than the receptor layer of the retina is assumed to total only 10% of the received signal (itself only 0·00005 of the light falling upon the cornea), and in the receptor layer it is assumed that, at the place of maximum rod density, interspaces and cones together only return the light that a 5% of interspace alone would do. These assumptions seem close to the very minimum admissible for stray light, and it already leads to a density

value of 0·15. A considerably higher figure would fit all the evidence so far considered, and with far less strain. But there are other considerations of quite a different kind which do not encourage an estimate much higher than 0·15, and these will now be discussed.

PART 3. SOME LOWER ESTIMATES

There are two other ways of estimating the density of rhodopsin: one is by extracting it and finding the density of the digitonin solution, the other is to compare in shape the rhodopsin spectral extinction curve with the curve of scotopic sensitivity.

Extraction

All the rhodopsin was extracted from a human eye by Koenig (1894) who found the amount to be such that if spread in an even layer over the entire retina, the density would have the value 0·018. This has recently been repeated under very good conditions by Crescitelli & Dartnall (1953) whose figure expressed in the same way was 0·016.

We now need to convert this into an estimate of the density in the outer segment of the rod itself. According to the measurements of Mr Leach the average diameter of the rod outer segment is 1–1·5 μ in a preparation shrunk 20–30 %. Thus the average area of an outer segment is 2 μ^2. The total number of retinal rods is 120 million, hence their total area will be 240 mm^2. Now the total retinal area over which Crescitelli & Dartnall spread their rhodopsin was 900 mm^2, so if they had spread it over 240 mm^2 the density would have amounted to 0·06. This figure does not take into account the orientation of the rhodopsin molecules in the rods (Schmidt, 1937; Denton, 1954) which would be expected to increase the probability of quanta being absorbed by a factor of 3/2, as Hagins (1954) found to be the case, bringing the rhodopsin density in the rods to 0·09. This still lies considerably below the figure of 0·15 obtained in Part 2, but it is not easy to know how completely the entire rhodopsin content of the retina can be extracted. On the one hand, Crescitelli & Dartnall certainly took great care to bring into solution all extractable rhodopsin, the digitonin solution when once formed is known to be very stable, and Arden's (1954) results where digitonin was added to rod suspensions suggest that very little of the rhodopsin is destroyed in the process of extraction. On the other hand, Denton & Wyllie (1955) measured the rhodopsin density in the rods of the frog *in situ* using a photographic method, and found a value some 30 % higher than that obtained by Wald (1938, bullfrog) and Dartnall (1953, *Rana temporaria*) in their extracts. And there seems to be the same order of discrepancy between the measurements *in situ* and by extraction in the case of the human eye here discussed.

We may conclude that the figures of Koenig and of Crescitelli & Dartnall are not incompatible with a rod density of 0·15 at maximum, but they do suggest that the density *in situ* is not likely to be very much higher.

Scotopic sensitivity

The estimated density of rhodopsin in the human rods at which we have arrived is not only somewhat higher than the figure usually accepted but it is higher even than the upper limit given by Hecht *et al.* (1942). It is therefore necessary to examine their reasoning which makes ingenious use of the following fact. If the density of a pigment is very low, each molecule has the same chance of absorbing a quantum and hence the absorption spectrum will coincide with the extinction spectrum. If, on the other hand, the density is considerable, the molecules deep in the solution will only receive light that has passed through a 'coloured filter' consisting of the more superficial part of the pigment solution. This will alter the shape of the absorption curve. The exact form of the curve may easily be calculated for, if ρ is the density at any wave-length, then

$$1-10^{-\rho}$$

will be the relative absorption at that wave-length. The relation is plotted as a family of spectral curves for various maximum values of ρ in Hecht *et al.* (1942, p. 830) and Weale (1955, p. 235).

Now it is generally held that the extinction curve of rhodopsin in the rods is the same as that in digitonin solution, and that the corrected curve for scotopic sensitivity represents the absorption curve of rhodopsin. Thus it should be possible by comparing these two curves to estimate the amount of self-screening and hence the corresponding rhodopsin density. Unfortunately the difference between extinction and absorption curves is very small (unless $\rho > 0{\cdot}4$), so the method depends upon exactness in the assumptions and great accuracy in the measurements. Hecht *et al.* found no significant difference between the two curves, and judged the measurements good enough to have revealed one if the density had been as high as 0·1. Crescitelli & Dartnall (1953, Fig. 2) have reached the same conclusion based upon the better measurements which are now available.

Stiles (1948) made a very careful comparison of the corrected scotopic sensitivity curve and the rhodopsin extinction curve (frog) and has drawn attention to the fact that they deviate in the direction *opposite* to that expected from self-screening. This is perhaps improved by using the extinction curve for human rhodopsin, but persists for wave-lengths longer than 550 mμ.

Fig. 3 is a simple way to display the relation under discussion. Vertically there is plotted (on tracing paper) the corrected log scotopic sensitivity corresponding to various wave-lengths: for each of these ordinates the abscissa is log extinction for that wave-length.

Now we know only the relative extinction and absorption, so the set of points will not give the correct absolute values read against the fixed axes of Fig. 3 unless the tracing paper has been suitably displaced (without rotation). To find the correct position we note that the quantities plotted are (on our assumptions) log ρ and log $(1-10^{-\rho})$ and hence must coincide with the mathematical curve shown in Fig. 3 which relates these quantities. The tracing paper can thus be moved parallel to itself until the points coincide with the curve, and we may then read off, corresponding to each point λ, the density and absorption at this wave-length.

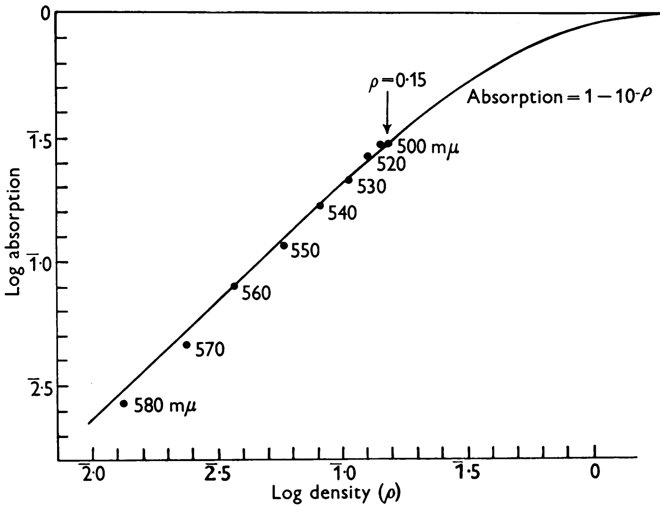

Fig. 3. Log scotopic sensitivity plotted against log rhodopsin density for various wave-lengths. The curve shows for any pigment the relation between the density and the proportion of incident light absorbed. The points show the experimental results assuming that density is 0·15 at λ 500 mμ.

The points in Fig. 3 are plotted from the following sources. The scotopic sensitivity is taken from Stiles (1948); it is a mean based on Wald's (1945) values by a threshold method and Crawford's (1949) values by a brightness-matching method. The measurements are expressed in quanta^{-1} and corrected for pre-retinal absorption based on Ludvigh & McCarthy (1938) and upon Wald's (1945) estimate of eye-lens absorption. The rhodopsin extinction figures were given me for the purpose by Dartnall. They referred to frog's rhodopsin in digitonin and have been converted into human figures by shifting 5 mμ towards the blue (at his suggestion). The figures fit exactly Wald & Brown's (1953) published curve for cattle rhodopsin, in so far as this can be measured from the linear plot.

Now what emerges from these results is that they fall close to the theoretical line but do not coincide with it, nor can they be made to coincide by any

movement of the tracing paper. It is largely a matter of taste where the 'best' position is, and that shown in Fig. 3 cannot obviously be rejected in favour of any other. This position corresponds to the density of 0·15 at 505 mμ which we have seen probably represents the actual rhodopsin density in the rods.

Dartnall has made two contributions to the comparison of the sensitivity and the extinction curves. In the first with Goodeve (Dartnall & Goodeve, 1937) he pointed out that energy should be expressed in quanta not ergs: this caused the maxima of the two curves to coincide exactly (but the lower part to diverge more). In the second with Crescitelli (Crescitelli & Dartnall, 1953) he pointed out that human rhodopsin had the maximum at 5 mμ shorter wavelength than frog's which had formerly been used for the comparison. This has almost exactly restored the original relationship.

The fact that the maximum is not quite the same for the two curves is most easily explained by assuming that the extinction curve of rhodopsin in the rods is not quite identical with that in digitonin extract as Denton & Wyllie's *in situ* measurements suggest (1955, Fig. 1). A shift of this kind in the highly refractive rods is to be expected from physical theory. But if this is held, it becomes a very delicate matter to argue about rhodopsin density from the exact shape of the scotopic sensitivity curve.

De Vries (1946) made an ingenious attempt to circumvent these difficulties in the following way. Since the absorption curve changes its shape with different densities, it follows that a blue-green and a yellow field which match at a high rhodopsin density level will not match if this is nearly all bleached. He therefore made the match in full dark-adaptation, and again as soon as scotopic vision was possible after complete bleaching. There was no detectable change in the match, and he concluded that there was less than a density of 0·05 at maximum.

But Campbell & Rushton (1955) found that the very bright lights needed to bleach away all the human rhodopsin left a period of 10 min or more (associated with a powerful after-image) before any scotopic vision was possible on the bleached spot. They also found that during this 10 min about 70% of the rhodopsin had regenerated. So de Vries was not distinguishing between the fully bleached and dark-adapted levels but between the 70% and the 100% regenerated levels, which for 0·15 maximum would hardly have been detectable.

Conclusion

The result of all these considerations is a somewhat uneasy compromise around a rod density of 0·15, for anything less is hard to reconcile with the retinal densitometer readings when corrected for a minimum of stray light, while anything more exaggerates the small discrepancies just reviewed.

PART 4. THE FRACTION OF INCIDENT LIGHT ABSORBED BY THE RODS

In 1942 Hecht *et al.* opened a new and important chapter in visual physiology with their masterly paper on Energy, Quanta and Vision. They showed, and van der Velden (1944) independently confirmed, that only a few quanta need be absorbed to obtain a visual sensation in optimal conditions. Hecht and his colleagues used two very different methods to evaluate the exact number of quanta required.

One was to argue from the random nature of quantum reactions and to deduce a verifiable relation between the number of quanta involved and the frequency of seeing. This has been developed in various directions by van der Velden, Bouman, Baumgardt, Pirenne and many others (see reviews by Weale, 1955; Pirenne, 1956). The difficulty in this line of approach is that some sort of assumption has to be made as to biological uniformity, neural organization, noise and significance levels, etc., which are not easy to substantiate independently. And in fact the various workers along these lines have reached figures varying between 2 and 8 for the minimum number of quanta required for vision. It is therefore satisfactory that there should be a totally independent way of arriving at the number of quanta absorbed. This is simply to measure the minimum visible light energy at the cornea and to estimate what fraction of it will be absorbed by the rods. It is assumed that in optimum conditions each quantum absorbed by rhodopsin contributes towards the visual act; though Hagins (1955) has suggested that half these quanta may be ineffective.

The measurement of light at the cornea is straightforward in principle (however tiresome in practice): and Ludvigh & McCarthy's (1938) figures for transmission loss through the eye will certainly give a fair approximation for the energy reaching the retina. So all that remains in order to obtain the correct number of quanta absorbed is to know the fraction of incident light which is absorbed by the rods at the region where the light flash fell (20° temporal in the experiments of Hecht *et al.* 1942).

If the density in the rods is 0·15 ($\lambda = 505$ mμ), then 30 % of the quanta falling upon the rods will be absorbed in a single passage. But at the optimal region of the retina about 25 % of the area is occupied by cones and about 5 % by rod interspace, so the chance of a quantum falling upon the rods is 70 %. Thus 20 % of the light falling upon the retina or 10 % of the light falling upon the cornea will be absorbed by retinal rhodopsin. This value is the same as that taken by Hecht *et al.* as an upper limit, and by Denton & Pirenne (1954) as a likely estimate, and it leads to the numbers 5–14 as the minimum quantum threshold for vision, if all the absorbed quanta co-operate in the visual act.

Now Weale has recently published a review (1955) based upon Crescitelli & Dartnall's (1953) extraction of human rhodopsin but interpreted in such a way as to give a figure five times as small as that above. Upon this estimate he

places sufficient confidence to press it in the face of some rather weighty objections. To obtain his rhodopsin value Weale simply took the actual figure of Crescitelli & Dartnall (expressed as absorption in a layer spread evenly over the whole retina) and assumed that this without much correction represented the proportion of light absorbed when the rhodopsin was orientated and concentrated in the rods at the optimum region of the retina, despite the fact that it led him to the value of *one* quantum as the threshold for vision. There are insuperable difficulties in accepting a single quantum for the threshold, as has been stressed by Baumgardt (1950) and Pirenne (1953). There is the same chance of one quantum being absorbed when a just-supra-threshold light is viewed for 0·1 sec as when a source 100 times as weak is viewed for 10 sec. So according to the one-quantum hypothesis these two lights should be equally visible: but everyone knows that the weaker light will not be seen. It is this and analogous spatial considerations which have made all the experimenters in this field agree that a one-quantum threshold is out of the question. We need not follow Weale further in his impossibly difficult task of reconciling the results of Hecht *et al.* with the assumption that only 3·5 % of the light falling upon the retina was absorbed, for as has been seen this figure is neither necessary nor likely. If reasonable assumptions are made as to the distribution of rhodopsin in the retina, the careful measurements of Crescitelli & Dartnall will certainly yield Hecht's 6 quanta and perhaps 10 is a more probable figure.

Barlow (1956), in an important contribution to the subject, has worked out some consequences of the hypothesis (verified in the cat) that light flashes are appreciated not as a nerve discharge appearing in a silent arena, but by a modification in the impulse traffic entering a rather busy forum. The signal not only has to arrive but it has to be detected against a good deal of 'random' activity. His treatment, which is both theoretical and experimental, goes a long way towards explaining how the different quantum figures can be reached by different workers using various techniques of observation and (especially) various significance levels for the detection of flashes against background noise. Naturally the number of quanta required by this treatment is higher than that deduced from matching the frequency of seeing curves with some appropriate Poisson distribution, since 'noise' lowers the precision of detection and that reduces the steepness of the curve. The same result follows from several other factors which may complicate the interpretation of frequency-of-seeing curves, as has been established by Pirenne & Marriott (1955). In all these cases the actual number of quanta absorbed must be greater than that expected from the simpler considerations. It is therefore satisfactory that the estimate of 5–14 quanta, which has been obtained in the present paper from density measurements in the living human eye, is able to meet the quantum requirements which have arisen from such a very different approach to visual function.

SUMMARY

1. A minimal value for the density of rhodopsin in human rods at 20° from the fovea is 0·09.

2. The inevitable presence of stray light reaching the measuring equipment from between the rods and elsewhere raises the figure to at least 0·15.

3. Published estimates based upon human rhodopsin extraction or the comparison of rhodopsin extinction and scotopic visibility curves do not encourage a figure very much higher than 0·15.

4. It is concluded that 10% green light entering the eye is absorbed by rhodopsin (20° parafoveal). And 5–14 quanta were absorbed in the threshold measurements of Hecht *et al.* (1942).

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