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AN ATTEMPT TO ANALYSE COLOUR RECEPTION BY ELECTROPHYSIOLOGY

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SUMMARY

1. The problem of colour reception is that we do not know the action spectra of the visual pigments involved, the nature of the signals generated nor the interaction between these signals. We only know the incident light and the electric results of interaction.

2. In Part 1 we show that S-potentials from red/green (R/G) units saturated with deep red light show this property: added green light pulls down the ceiling of depolarization, but more added red had no power to raise it again. Thus lights that depress the deep red ceiling equally stimulate the green pigment equally. From this the action spectrum of the green pigment can be obtained.

3. If we assume that only two visual pigments are involved in the R/G unit, and that lights which do not pull down the deep red ceiling are below the threshold for green cones, then in this range only the red pigment is excited and we may obtain its action spectrum. Its maximum is at 680 nm where no visual pigment so far has been found.

4. In Part 2 we consider the following mathematical problem: 'Is it possible that two pigments of given action spectra could combine their outputs in such a way that the resultant would be identical with the output of a third pigment of given action spectrum, for every intensity of every monochromatic light?' The solution shows that this is always mathematically possible, and the necessary interaction function is deduced.

5. It is shown further that if the log action spectra are the 'visual parabolas' that resemble Dartnall's nomogram, then the interaction function is simply a linear transform such as Hartline & Ratliff (1957) have found with lateral inhibition in *Limulus* and Donner & Rushton (1959) with silent substitution in the frog.

6. An interaction that matches a single pigment to perfection for all monochromatic lights will not match it for certain mixtures. By this criterion the 680 nm excitability is a pigment and not the resultant of two other pigments, i.e. pigments more excitable in other spectral regions.

7. In Part 3 monochromatic lights are matched by red + green mixtures that give identical responses. From this the action spectrum of the red pigment may be obtained without involving nerve organization (except as a null detector). The result, which has one arbitrary constant, is given by the curves of Fig. 10, the continuous curve *R* or one of the dotted curves. Of these only curve *R* is acceptable.

8. Knowing the action spectra for red and green cones we may consider what signals are generated and how they interact to give the records. Figure 11 suggests a model that will account for the size and sign of S-potentials as function of the quantum catch by the two pigments. It does not embrace the time or space parameters which can be very complex.

INTRODUCTION

The fascinating study of colour vision in animals has commanded the attention of many electrophysiologists of the highest skill, and their work has enlarged very greatly our knowledge of the patterns of neural response that arise in various parts of the visual nervous system consequent upon procedures with coloured lights. But in electrophysiology it is much easier to obtain records than to analyse them, and usually the colour situation is complex. In general, any coloured light is absorbed by two or more unknown visual pigments each of which generates a signal of unknown magnitude and nature. These signals interact in some unknown manner and the result is an electrical change that can be recorded. The problem is to discover what we can about the unknowns.

The ideal starting place for this analysis of course is to begin at the beginning, and record from single cones, but this we have not been able to do. The earliest records so far available for detailed analysis are the S-potentials of Svaetichin (1953). These are obtained from retinal regions definitely external to the ganglion cells, and thus they probably arise earlier in the chain of 'processing'. They also look simpler for they still monitor the time course of the light fairly faithfully. But they clearly receive signals from more than one pigment system. The *R/G* units that we study in this paper exhibit the helpful characteristic that they have two directions of response; the red system depolarizes, the green system hyperpolarizes. We have measured the S-potential waves in a variety of conditions so chosen that from the results we can argue something definite. But before proceeding to a description of this work we must mention a fundamental principle that we believe to hold without exception, for our arguments rest upon it.

Principle of Univariance

It is not accurate to say that every quantum absorbed by a visual pigment has the same effect irrespective of its associated wave-length. For

light of 280 nm though much more strongly absorbed by rhodopsin than is light of 500 nm has negligible effect on bleaching or on vision (even in aphakic eyes). A better statement is, 'For each pigment system, every quantum that is effectively absorbed makes an equal contribution towards vision'. *Effective* absorption is measured not by densitometry (which has nothing to say about effects), but by determining the action spectrum—the reciprocal of the light at each wave-length that is required for some response to reach a critical size. According to the Principle, every kind of visual response from a single pigment system must give the same action spectrum.

We can only examine the univariance of pigment systems when they are isolated; twilight vision is the most familiar example. So long as only rhodospin is involved we experience a sense of brightness but not of colour and all lights are equivalent if scaled in scotopic units. In the red-green range, both protanopes and deuteranopes have no colour sense but only a univariance based on chlorolabe or erythrolabe respectively. The same is found in normal eyes in the conditions of Brindley's (1953) monochromacies.

Upon more general consideration, the Principle of Univariance is not only acceptable because we are familiar with the idea in the output of photocells, and are not familiar with nerves transmitting more than one dimension of message, but also because any other interpretation seems to make nonsense of the trichromacy of our colour vision. Trichromacy is a gross limitation in colour perception—as though the ear could only detect three different notes— but it is inevitable if (*a*) colour is detected by cones, (*b*) there are only three kinds of cone (Marks, Dobelle & MacNichol, 1964) and (*c*) each cone is univariant in output. If, on the other hand, one type of cone was *di*variant in output giving a signal compounded *independently* of intensity and colour, then this type alone should be able to perform a complete spectrum analysis, and our colour discrimination would be far more acute than it is without the help of any other type of cone. Our undoubted limitation to trichromacy could then only be attributed to a retinal organization so incompetent that it lost nearly all the information that divariance had gathered. We prefer to accept the Principle of Univariance.

Note. References to the preceding paper Naka & Rushton (1966*a*) will be quoted for short as (NR).

METHOD

The fish used were usually tench (*Tinca*), kept and prepared as described in the preceding paper (NR). The eye was removed from the dead fish, but the retina left *in situ* in the optic cup with as little disturbance as possible. The 300 comparable records from a single *R*/*G* unit studied in Part 3 of this paper constitute evidence that retinal survival can be adequate with this technique.

The S-potentials were obtained from glass micropipettes filled with potassium citrate, and

were recorded with horizontal shift that represented sometimes the time and sometimes the log intensity of flash.

The optical arrangement is shown in Fig. 1 of the preceding paper (NR). In the present paper no steady backgrounds were used. Spectral and other lights were mixed in the beam splitting cube *C*, and the mixture was presented in brief exposures by the electromagnetic shutter D. The energy of the spectral component of the mixture was monitored by the potentiometer *P* attached to the wedge W_1, and displayed either by *X*-shift of by the *Y*-shift of the second beam of the oscilloscope.

RESULTS

Part 1. *The Green Action Spectrum*

Figure 1 shows six sets of S-potentials from a *R*/*G* unit. Each is a continuous time tracing with the light flashed on and off in regular sequence. In each set a deep red light of wave-length 720 nm was increased by step from very dim to very bright. The increase was produced by moving (by hand) the wedge W_1 (NR, Fig. 1) during the dark periods between the flashes, and for each flash the intensity of the deep red light is shown by the monitoring staircase, whose steps are linked to log *I* by the scale shown below at the left of the figure. The flash of light presented, however, was not pure red; it was increasing red mixed with a fixed blue-green. An Ilford filter (no. 623) was placed at *F* (NR, Fig. 1) and the blue-green and red lights were mixed in the cube C and presented in the flashes together, darkness intervening. In each row of Fig. 1 the blue-green light is 4 times as strong as in the row above, since between each a 0·6 neutral filter was removed from the green optical path, as indicated by the numbers above each row.

It will be seen in the top line (where the green component was extremely weak) that all the waves are positive (depolarization). On the other hand when the green component is made 1000 times as strong (bottom line) all the waves are negative (hyperpolarization). The six rows of Fig. 2 have been aligned by sliding each sideways so that the 'staircase' cuts the vertical line across the figure at the step whose height is 1 log unit below the top. This displays an important and somewhat remarkable fact. It is that for each row, the S-waves, that continually change their height and shape as we go from left to right, freeze as they cross the line into a fixed shape and change no more. The deep red light that has been increasing goes on increasing a further 10-fold, but without effect. An increase in the green light on the other hand has a very great effect as may be seen by viewing the records right of the line in the order 3·6 down to 0·6. Green can pull the potential down and indeed reverse its sign, but the addition of more red (up to 10 times) has no action whatever in opposing this.

Figure 2(*a*) indicates the kind of organization to which we are led. The outputs from red cones (*R*) and green cones (*G*) converge upon the *R*/*G*

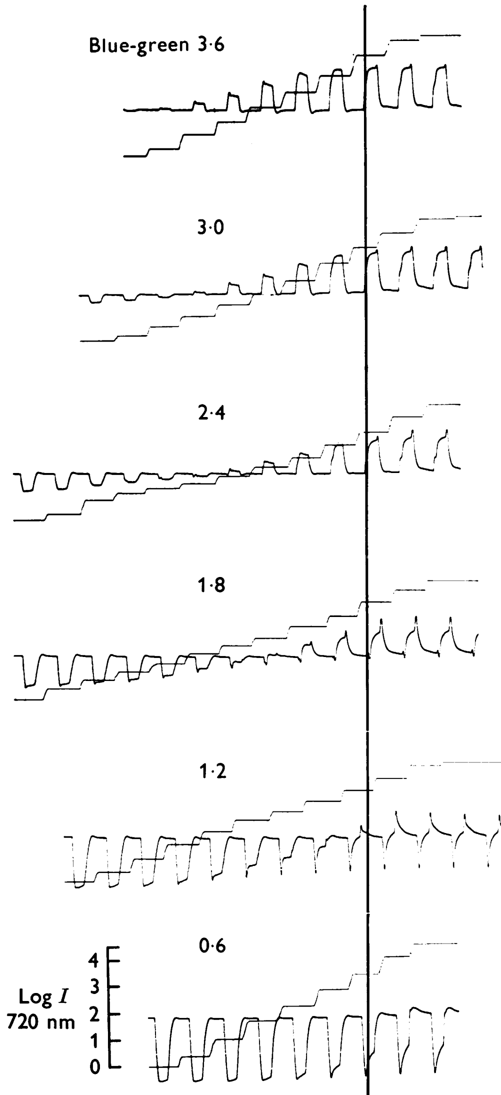

Fig. 1. Time records of the responses of a *R*/*G* unit to flashes of a mixture of blue-green light and deep red (720 mm). In each row the blue-green component was fixed in intensity by interposition of a neutral filter whose density is indicated by the number above the row. Throughout each row the red component increased in intensity at each flash, as monitored by the staircase. The level of each step is related to log intensity by the scale shown at bottom left of figure. The vertical line shows the place where the red light is 10 % of the maximum intensity available.

unit whose potential V is measured. From the records of Fig. 1 it is clear that increase in red light can only raise V to some fixed ceiling. This cannot be imposed by the resultant of the red/green interaction, otherwise when green had pulled down the potential from that ceiling, further red (which at lower intensity levels counteracts green) could counteract still and raise the level again. But this does not happen; further red has no effect at all. We must therefore conclude that saturation occurs before red and green have interacted. When the intensity of red light reaches the value corresponding to the vertical line (Fig. 1) the depolarization it can produce at the cell 'membrane' is the maximum possible and any further increase in red light is entirely without effect.

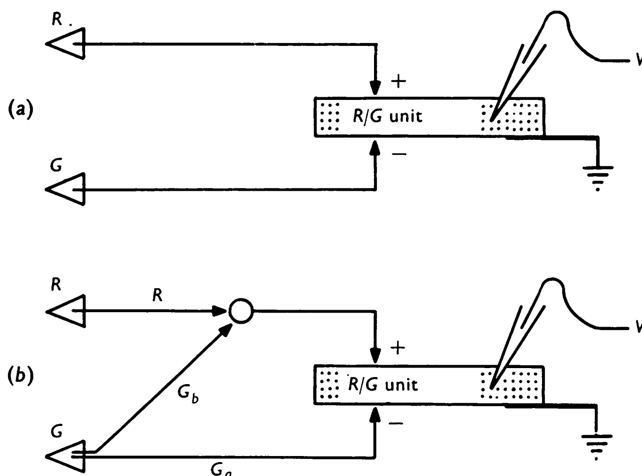

Fig. 2 (*a*). Scheme of R/G unit depolarized simply by red cones and hyperpolarized simply by green. (*b*) Scheme where hyperpolarization is still simply the result of green signals G_a, but where depolarization is the resultant of some interaction between R and G_b.

But this is precisely the situation we need to turn dichromatic vision into monochromatic vision where it can be analysed. If to a deep red light that saturates we add one that by itself excites both red and green cones, the red excitation will be entirely without action, and the effects observed must be due to the green pigment only. In that case all the consequences of its univariance should be observed.

Figure 3 shows records of a different kind. The time base of the oscilloscope was disconnected, and the X-shift driven by the potentiometer connected to the wedge W_1, so the displacement to the right is proportional to log I as shown by the scale, low on the right of the figure. The vertical lines are stationary records showing the maximal depolarization (up) or hyperpolarization (down) in response to lights of wave-length shown in

each row and of log intensity indicated by their horizontal position. In sets on the left (*a*) the spectral lights were presented alone, but in the sets on the right (*b*) a fixed saturating deep red light was mixed with the spectral light and added to the flash. This deep red was obtained by a gelatine filter that transmitted only light of wave-length greater than 740 nm; it was placed at *F* (NR, Fig. 1) and in the mixing cube *C* it joined the spectral light.

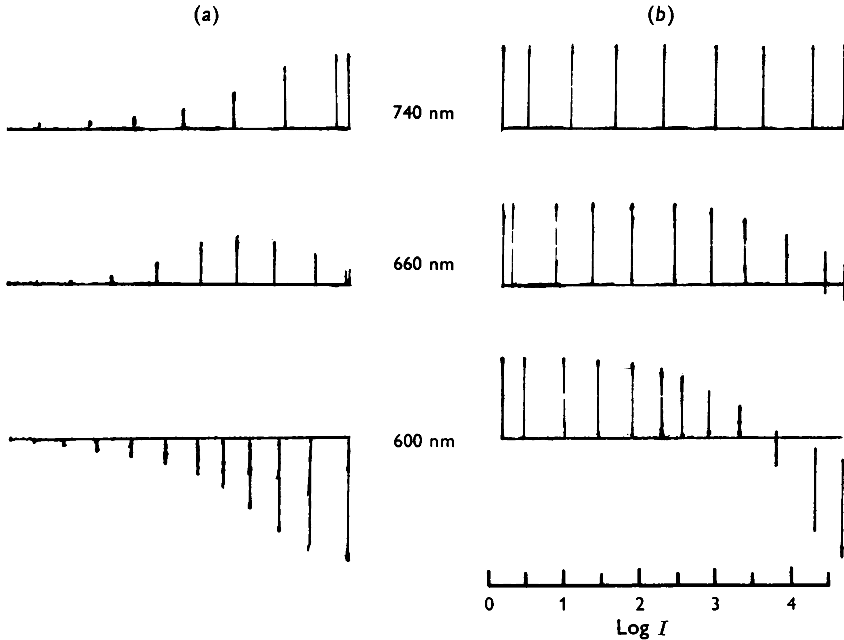

Fig. 3. S-potentials from *R*/*G* unit recorded upon a stationary time base. The *X*-shift was proportional to log intensity of light flash (scale bottom right). Records (*a*) (on left) when flash consists simply of the monochromatic light indicated. (*b*) (on right) when monochromatic light was mixed with saturating deep red light.

The top line of Fig. 3 shows essentially what was seen in the top line of Fig. 1. In (*a*) increasing intensity increases depolarization; in (*b*) when the ceiling was already reached by the deep red from the filter, there is no effect from the addition of more deep red light.

With 660 nm the situation is less simple. In (*a*) it appears that weak lights excite only the depolarizing red mechanism, but with rather strong lights the green hyperpolarizing mechanism is also excited and, with increasing intensity, depolarization is first arrested, and then reversed. In (*b*) this interpretation is supported, for so long as only red is excited there

is no effect upon the red ceiling, but when green begins to pull down the depolarization in (*a*), it also begins to pull down the ceiling in (*b*).

Figure 4 represents the results of Fig. 3 in a more condensed form. Instead of giving the vertical lines of Fig. 3 (*a*) only the upper limit of each line is plotted as a filled circle. In the same way only the upper limit of the

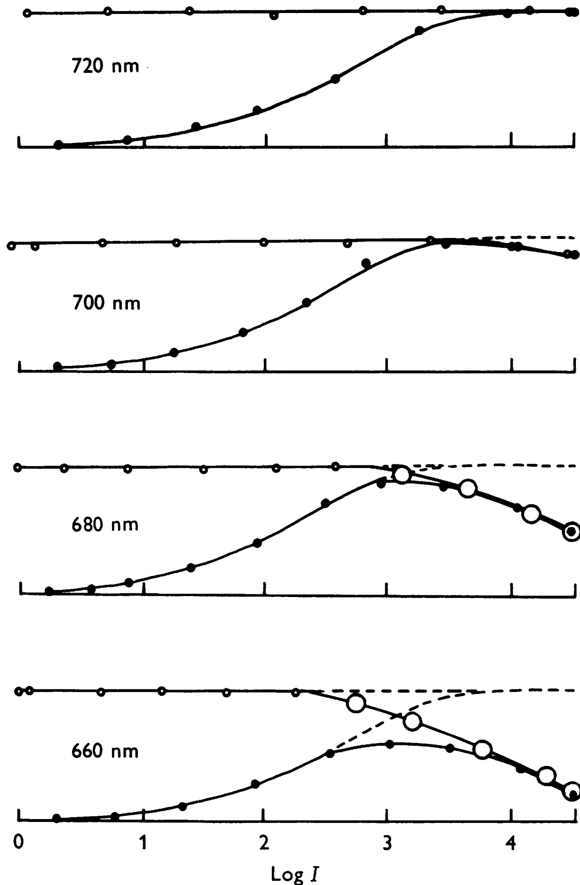

Fig. 4. Replot of records such as those in Fig. 3. Filled circles show upper ends of potential excursions from Fig. 3 (*a*); open circles show the same from Fig. 3 (*b*). Abscissae measure log intensity as before.

lines of Fig. 3 (*b*) is shown as an open circle. Displayed in this way the results of Fig. 3 may be more easily appreciated and it appears that when only the red component affects the filled circles there is no effect at all upon the open circles; when, however, a different pigment (green) is involved it pulls down the filled circles and the open circles together and nearly to the same extent.

Now if we assume that the R/G unit is dichromatic, and hence that the influence that pulls down the open circles (Fig. 4) from the ceiling is due to one pigment only, we may predict at once from the Principle of Univariance that the curve of downpull against log I for every wave-length must be identical in shape and be merely displaced laterally to an extent that depends upon the energy output of the spectrometer and the action spectrum of the green pigment.

In Fig. 5 (a) the downpull results are plotted against log I for nine wave-lengths (as indicated in nm), and a fixed template curve is drawn through them. With the exception of two points on the 660 nm curve and one on the 640, the prediction that all points should lie on a fixed curve is fulfilled.

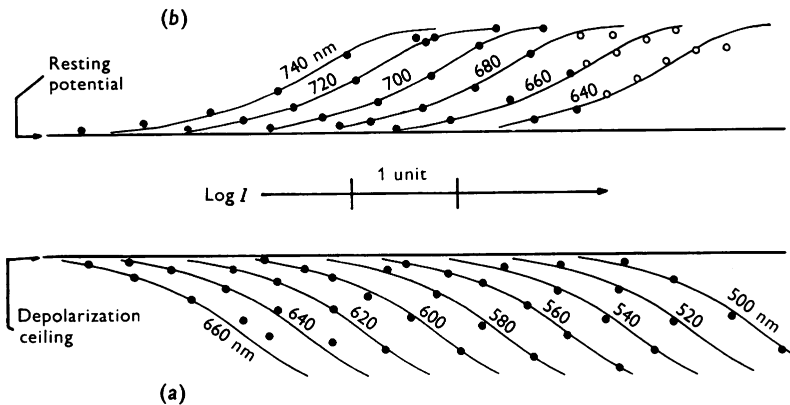

Fig. 5. In (a) each set of points corresponds to the set of open circles in Fig. 4 at the wave-length labelled, and includes many sets not displayed in Fig. 4. Each set of points is displaced sideways by a known arbitrary amount (for clearness) and a fixed template curve is drawn 'through' the points. In (b) the same is done for the filled circles of Fig. 4.

We have assumed that downpull from the ceiling is due only to one (green) pigment. If we assume further that lights that produce no downpull act only on one (red) pigment, we may apply the Principle of Univariance to *it* and predict that all the filled circles of Fig. 4 lie on the same curve (suitably displaced sideways) up to the point where the green pigment enters, as shown by the downpull of the open circles.

Figure 5 (b) shows these points (filled) with a fixed template curve drawn through them. In Fig. 4 we noted that the green pigment pulled down the open and the filled circles to about the same extent. Thus if to the height of the filled circles we add the downpull of the open circles we should obtain the course that the filled circles would have run had they not been depressed by action of the green pigment. In Fig. 5 (b) the open circles indicate these computed points, and it is plain that they too lie on the pattern of curves described by the fixed template.

As for the shape of the template itself, whenever the potential measured is due to one pigment only, it appears to fit very closely the relation

$$V = \frac{JU}{J+1} \tag{1}$$

where V is the recorded potential, U is the ceiling of depolarization (or hyperpolarization), and J is the effective quantum catch expressed in units of $I_{\frac{1}{2}}$, the effective catch (characteristic for the pigment) that brings V half way from the resting to the ceiling value (NR eq. 2).

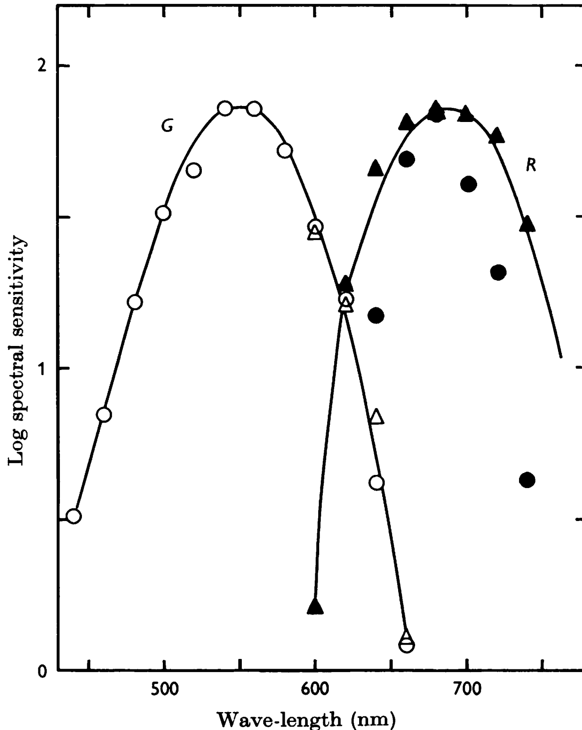

Fig. 6. Log action spectrum of green (G) and deep red (R) pigment. Circles give results from Fig. 5, triangles from Fig. 9.

From Fig. 5(*a*) and (*b*) we may deduce the action spectrum of the depolarizing (red) pigment and the hyperpolarizing (green) pigment. In that figure the points have been set out displaced by known amounts for clearness of presentation. But knowing the log quantum energy received from the spectrometer and the log displacement of the template curves for coincidence, we may plot the log quantum sensitivity of the pigments responsible for the curves in Fig. 5(*a*) and (*b*). They are shown in curves G and R (circles), Fig. 6. Curve G has its maximum at 550 nm and fits reasonably Marks's 530 nm pigment. Curve R has its maximum at

680 nm as found in the previous paper (NR); it does not fit the difference spectrum of any pigment hitherto described.

Summary of argument

As this completes the first and simpler part of the paper it may be useful to give in summary the principal steps that have led to the views so far put forward.

(1) *Assumption.* The Principle of Univariance holds for each pigment system.

(2) *Assumption.* Not more than two pigments are involved.

(3) *Observation (Fig. 1).* If the red component of a light is strong enough to depolarize to the 'ceiling', a green light can pull down the potential from that ceiling, but then the addition of yet more red has no effect whatever.

(4) *Deduction from* (1) (2) (3). Exactly two pigments which we label 'red' and 'green' are involved. Thus any light will excite these two pigments in some fixed proportion which depends upon the composition of the light but not upon its intensity.

(5) *Deduction from* (1, 4). Two lights (monochromatic or mixture) will 'match' when *each* pigment is equally affected by both of the two lights. Lights that match may in all circumstances be substituted one for another without any change in the form of the response.

(6) *Deduction.* Each monochromatic light is in general absorbed by the red and the green pigments and the recorded response depends upon the interaction between those pigment systems. If the light is mixed with deep red of saturating intensity, addition of more red has no effect (3), thus the downpull from the ceiling must be due to the output from the green pigment only. This is univariant (1), hence the downpull results of Fig. 5(*a*) should all fit the same template.

(7) *Observation, Fig.* 5(*a*). Prediction (6) is fulfilled.

(8) *Assumption.* We have deduced (6) that lights of wave-lengths and intensities that pull down the deep red ceiling do so entirely through the effect of the green system upon the response. Now we assume that wave-lengths and intensities that show no downpull have no green pigment effect upon the response.

(9) *Deduction.* In the region where the green pigment has no effect (8) the red pigment alone is effective, hence from univariance (1) the results of Fig. 5(*b*) should all fit the same template.

(10) *Observation, Fig.* 5(*b*). Prediction (9) is fulfilled.

(11) *Observation, Fig.* 5(*a, b*). The template curves fit the relation

$$V/U = J/(J+1),$$

where V is the S-potential, U its ceiling value and J the effective quantum catch expressed in units of the catch $I_{\frac{1}{2}}$ that makes $V = \frac{1}{2}U$.

(12) *Observation, Fig.* 6. Curves G and R show the action spectra of the two pigments obtained by plotting for each wave length the log quantum efficacy required to bring all the template curves of Fig. 5 (a, b) into coincidence.

(13) *Observation, Figs.* 3 *and* 4 (a). It is not far from true to say that the output of the red system simply adds to that of green. If this were true the white circles in Fig. 5 (b) should lie on the template curves, which they do.

Thus with three modest assumptions (1, 2 and 8) two simple laws of potential generation and combination (11, 13), the measured action spectra of the two pigments (Fig. 6) and knowledge of the appropriate unit of potential (U) and light ($I_{\frac{1}{2}}$) for each pigment, we can predict the height of the S-potential resulting from any specified light mixture and intensity. If the action spectra of the two pigments deduced from this analysis fitted adequately the difference spectra actually measured in isolated cones we might rest with some assurance that the assumptions were justified. The green pigment fits well enough, but the red pigment with maximum at 680 nm is too far from that found by Marks (1965) at 620 nm. As discussed in the preceding paper (NR) the discrepancy does not appear to be due to errors of various kinds and both 620 and 680 nm pigments appear together in potentials from L-units (see next paper Naka & Rushton 1966*b*). Thus we must consider more carefully the possibility that despite the simplicity and success of our three assumptions, one may be incorrect. We now take up the important question of how a combination of outputs from two pigments may mimic the output of a 'pseudo-pigment'—one that in fact does not exist, but if it did would have an output similar to that observed.

Part 2. *A Pseudo-Pigment*

It seemed worth while to follow up an alternative to our third Assumption (8). It was there assumed that deep red lights, whose effect was only to depolarize and which even at full strength caused no downpull from the ceiling, acted essentially only upon the red pigment; that action on the green pigment was negligible. Let us now consider the possibility that in the whole of this 'pure red' range, the red and the green cones are acting in organized conjunction. The idea is indicated in Fig. 2 (b), where the output of green cones G has two destinations. One (G_a) goes to the R/G unit and hyperpolarizes it as in Fig. 2 (a)— the way that we have been studying. The other (G_b) interacts with the output from red cones R in such a way that the result mimics the output of a pigment system different from those either of R or G.

The suggestion may sound ingeniously artificial but the eye appears full of ingenious artifices, and the scheme has something to recommend it. If it

were possible for the outputs of two pigments so to interact that the product imitated the output of a non-existent pigment it would give the eye some versatility. It could manufacture neurologically pseudo-pigments with action spectra where required, and their maxima need not lie in the range between the maxima of the two real pigments but might for instance fall further into the deep red than the maximum of the real red pigment.

Our own primary psychological sensation of full redness is elicited by wave-lengths longer than 630 nm. If the physiological basis of 'redness' could be isolated and its activity recorded, no doubt the action spectrum would show a maximum at about 650 nm. But the action spectrum of the red *pigment* system has its maximum at about 570 nm as Stiles (1949) has shown by increment thresholds, and Brindley (1953) by extremes of adaptation. And the red pigment erythrolabe itself has its peak action

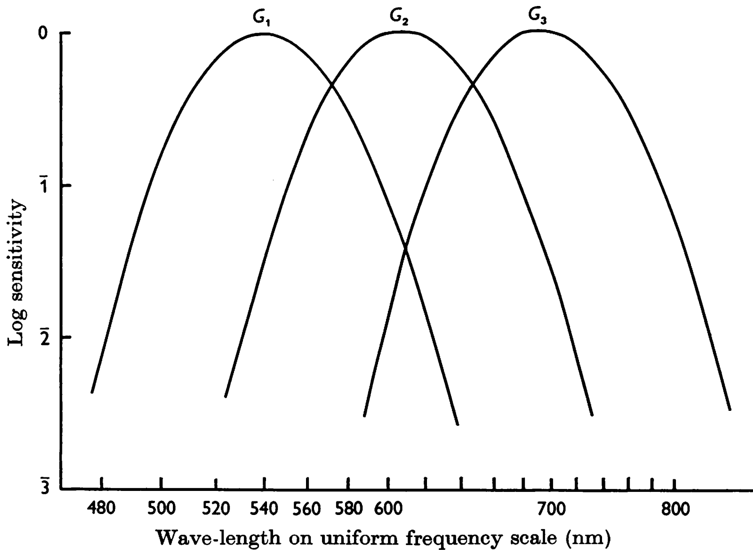

Fig. 7. Dartnall's nomogram for a visual pigment is a curve of fixed shape when plotted as log sensitivity against light frequency. The curve resembles a parabola. G_1, G_2, G_3 are three such 'visual parabolas'.

(Rushton, 1965) and peak absorption (Marks *et al.* 1964) also at about 570 nm. So here in our sense of redness we have the result of interaction between the erythrolabe cones (570 nm) and the chlorolabe cones (540 nm) that imitates some effects of a pseudo-pigment with maximum at 650 nm.

It therefore becomes interesting to ask what *are* the conditions of interaction between the outputs of two real pigment systems so that the result imitates the output of a pseudo-pigment system?

If this problem is posed precisely it will admit of a precise and interesting solution.

The mathematical problem

Given three univariant pigment systems whose log action spectra are G_1, G_2 and G_3 (Fig. 7) what is the necessary and sufficient interaction between the outputs of systems G_1 and G_2 such that for every intensity and wave-length of incident monochromatic light, the result of the interaction is identical with the output from system G_3?

Let y = log quantum energy of monochromatic incident light, $G_1(\lambda)$, $G_2(\lambda)$, $G_3(\lambda)$ be the given log action spectra.
Then effective log absorption

$$\left.\begin{aligned} \text{in } G_1 \text{ is } p_1 &= y+G_1(\lambda), \\ \text{in } G_2 \text{ is } p_2 &= y+G_2(\lambda), \\ \text{in } G_3 \text{ is } p_3 &= y+G_3(\lambda). \end{aligned}\right\} \tag{2}$$

Now the output from G_3 is some monotonic function μ of p_3, and since this is to be identical with resultant of the interactions between the G_1 and G_2 systems

$$\mu\,(p_3) = \mu\,[f(p_1, p_2)],$$

where $\mu[f]$ is some function of p_1 and p_2 to be determined. Since μ is monotonic the arguments of the two functions must be equal, hence

$$f(p_1, p_2) = p_3 = y+G_3(\lambda). \tag{3}$$

Now we change variable putting

$$\left.\begin{aligned} p_1+p_2 &= \alpha, \\ p_1-p_2 &= \beta. \end{aligned}\right\} \tag{4}$$

Thus $f(p_1, p_2)$ becomes some function

$$\psi(\alpha, \beta) \tag{5}$$

and

$$\frac{\partial f(p_1, p_2)}{\partial y} = \frac{\partial \psi(\alpha, \beta)}{\partial \alpha}\frac{\partial \alpha}{\partial y}+\frac{\partial \psi(\alpha, \beta)}{\partial \beta}\frac{\partial \beta}{\partial y}. \tag{6}$$

Now since from (3) $\quad f(p_1, p_2) = G_3(\lambda)+y,$

and from (2) and (4) $\quad \alpha = G_1(\lambda)+G_2(\lambda)+2y,$

$$\beta = G_1(\lambda)-G_2(\lambda)$$

and since each $G(\lambda)$ is independent of y eqn. (6) becomes

$$1 = 2\frac{\partial \psi(\alpha, \beta)}{\partial \alpha}. \tag{7}$$

Therefore

$$\psi(\alpha, \beta) = \tfrac{1}{2}a+\phi(\beta), \tag{8}$$

where $\phi(\beta)$ is the integration constant from the partial differential equa-

tion (7). By substitution from eqn. (5), (3) and (4) we may rewrite eqn. (8) as

$$p_3 = f(p_1, p_2) = \tfrac{1}{2}(p_1+p_2)+\phi(p_1-p_2). \tag{9}$$

Or again from eqn. (3) and (2)

$$G_3 = \tfrac{1}{2}(G_1+G_2)+\phi(G_1-G_2). \tag{10}$$

Since we are given the three log action spectra G_1, G_2, G_3 it is easy to evaluate the function ϕ. For we may calculate $[G_3-\tfrac{1}{2}(G_1+G_2)]$ and also (G_1-G_2) for every wave-length λ, and plotting one against the other gives from eqn. 10 the curve $\phi(G_1-G_2)$ as a function of (G_1-G_2). Knowing ϕ, eqn. (9) solves our problem. We asked what interaction (if any) between the effective log absorption p_1, p_2 of two given pigments would generate a resultant identical with p_3 (the effective log absorption of any given third pigment) for every intensity of every monochromatic light. Equation (9) shows that p_3 is always equal to $f(p_1, p_2)$ where $f(p_1, p_2)$ has the value defined on the right.

We conclude that it is always mathematically possible for *any* two real pigments so to interact that they mimic exactly any third given pigment in the response to every intensity of every monochromatic light.

The physiological situation

The mathematics have shown that, whatever the shapes of the log action spectra G_1, G_2 and G_3 may be, the outputs of G_1 and G_2 may combine so that the result mimics the output of G_3. Now we ask, 'Granted that G_1, G_2, G_3 have the actual shapes of log action spectra, is the kind of interaction required for mimicry physiologically plausible'. The answer is, 'Highly plausible'.

The actual shapes of log action spectra that fit Dartnall's nomogram are very nearly parabolas of fixed shape (upon an axis of light frequency)—they may be called 'visual parabolas'. It is a simple geometrical fact that the difference between two visual parabolas is a straight line.
Thus writing eqn. (10) as

$$\tfrac{1}{2}(G_3-G_1)+\tfrac{1}{2}(G_3-G_2) = \phi(G_1-G_2) \tag{10A}$$

we see that the left side is a linear function of light frequency, and so on the the right is (G_1-G_2) consequently $\phi(G_1-G_2)$ is simply a linear function $a(G_1-G_2)+b$. Thus eqn. (9) becomes

$$p_3 = (a+\tfrac{1}{2})p_1-(a-\tfrac{1}{2})p_2+b, \tag{9A}$$

where p is the effective log absorption.

Now in *Limulus* where records of nerve discharges may be obtained close to the primary receptors, there is a good linear relation between p and n the discharge frequency (MacNichol, 1958; Fuortes, 1958). Moreover, as

Hartline and Ratliff (1957) have shown in lateral inhibition, there is also a linear relation in the interaction between the two discharge frequencies. Consequently eqn. (9*A*), which describes what interaction two colour systems must have if it is to mimic a third, turns out to be almost exactly that actually found between two ommatidia in *Limulus*.

In the vertebrate we cannot record impulse trains from the receptors, nor expect ganglia to conform to the linear pattern, nevertheless evidence for a pseudo-pigment on the above lines has been obtained from the frog (Donner & Rushton, 1959). Ganglion cells were found in which the output from rods and cones combined to constitute a pseudo-pigment whose log sensitivity was the weighted mean of that of the rods and cones contributing (Rushton, 1959). This is precisely the interaction described in eqn. (9*A*). Thus in analysing colour systems by electrophysiology or psychophysics we must beware of concluding that a univariant output necessarily represents a single pigment.

Now our pigment at 680 nm has just the properties of a pseudo-pigment—too narrow an action spectrum (because interaction was not quite linear), too far in the red to be comfortable, and not found by Marks (1963, 1965) or by Liebmann & Entine (1964).

But if we must beware of concluding that a univariant output necessarily represents a single pigment, we must also hesitate to dub as 'pseudo' a pigment because still undetected and unfamiliar. Electrophysiologists need not wait upon absorption analysis, nor bend the knee to a difference spectrum. They may settle the matter by electrophysiology.

Part 3. *Colour Matching*

The foregoing proof that two pigments can combine to mimic a third for all strengths of all monochromatic lights might incline us to abandon hope of ever being able to tell by electrophysiology whether the appearance of a pigment was real or spurious. But such despair is premature; an organization that mimics monochromatic lights to perfection may fail with a mixture. Suppose that Fig. 7 represents G_1, the 540 nm pigment, and G_2, the 620 nm pigment, combining to give the appearance of G_3, the 680 nm pigment. As we have seen, the green output must subtract from the red output, thus if to light of 680 nm we add a very little light of 520 nm, it will be absorbed so much more readily by G_1 than by G_2 that it will act mainly by subtraction and hence *diminish* the 680 nm output. But naturally with a real 680 nm pigment every added light must *increase* the response, though the increase from light at 520 nm would be very small. This is one of the ways in which we can show that there is a real pigment at 680 nm.

The most comprehensive of our experiments is that from which the

records of Figs. 8 and 9 were obtained. The *R/G* unit remained in good condition for about 45 min and potential waves from some 300 different kinds of light flash were recorded in rapid succession. In Figs. 8 and 9 they are selected and displayed so as to lead to definite conclusions.

Figure 8 (*a*). The results here are similar to those of Fig. 3 (*b*). The flash always consisted of a mixture of deep red saturating light from the filter in path 2 (Fig. 1, NR) together with a monochromatic light of variable wave-length and intensity delivered from path 1 and mixing in the cube *C*. But whereas in Fig. 3 (*b*) the records were on a stationary base and gave only height as a function of log intensity, in Fig. 8 (*a*) the record was displayed on a time base and shows the whole shape of the wave.

The log intensity of the monochromatic light was controlled by the wedge W_1 (NR, Fig. 1) and its value was monitored on the second beam of the oscilloscope through the attached potentiometer *P*. Thus for each record in Fig. 8 (*a*) we know both the wave-length (λ) and the log intensity (log *I*) of the spectral component of the exciting flash. The arrangement of the records in Fig. 8 (*a*) is important. It was reached by two stages.

Stage 1. All the records corresponding to one wave-length λ_1 were cut out from the print and mounted on a horizontal strip of paper with the scale of log *I* drawn upon it (as at the top of Fig. 8 (*a*)). The instant when the record first left the base line was made to coincide with the scale reading that corresponded to the log *I* value of that record. Thus all the records where the flash contained λ_1 were mounted on the strip displaced laterally (as they are in Fig. 3 (*b*)) by an amount proportional to log *I*. In fact each row of Fig. 8 (*a*) is like a row of Fig. 3 (*b*) but the vertical lines have been expanded sideways (as it were) to display the time structure. Similar horizontal strips were constructed holding the records for each of the wave-lengths used, 720–600 nm. The lowest strip in Fig. 8 (*a*) will be mentioned later.

Stage 2. If it is correct to suppose that all the records of Fig. 8 (*a*) are obtained in a state of univariance, it must follow that every strip contains the same series of transitions. Hence it should be possible to displace each strip horizontally relative to its neighbour to reach a position where both show the same series of waves, one vertically above the other. In Fig. 8 (*a*) this sliding of the strips has been done, and the result is a rather striking demonstration of the univariance of the whole domain. A straight line placed vertically across the figure in any place crosses the rows each corresponding to a different wave-length. In every row the shape of the recorded wave where the line crosses it is always the same. Moving from right to left in general causes a marked change in shape; moving up and down causes none. Thus there is only one degree of freedom—univariance. In the lowest row of Fig. 8 (*a*) the flash was a mixture of saturating light

(720 nm from the spectrometer at full output strength) and a blue-green light obtained from Ilford filter no. 623. The blue-green light (but not the red) was attenuated by interposing neutral filters of known density, and hence the records may be placed with proper lateral shift upon a horizontal strip. This in turn has been suitably displaced in Fig. 8 (*a*) and shows a series of waves very nearly the same as those above. Thus univariance extends to about 500 nm. This lowest row was taken in fact from the records of Fig. 1 to the right of the vertical line, and includes some waves in addition to those shown in that figure. A vertical line has been drawn across Fig. 8 (*a*) through the zero of the scale of log I. From the calibration of the spectrometer we know the relative quantum output at each wave-length, i.e. the relative energy of the monochromatic component in the first flash of each row. But reading where this first record stands against the scale of log I allows us to deduce at once what is the energy level corresponding to the zero of the scale—where the records cut the vertical line across the figure. The logarithm of this relative energy is the number at the left of each row. These numbers, of course, are *minus* the log action spectrum of the green pigment (between 720 and 600 nm), and the results are plotted as filled triangles in Fig. 6, curve G.

It will be observed that the top row of Fig. 8 (*a*) exhibits a small change in wave form which means that the *green* pigment is slightly excited by light of 720 nm. The numbers show that at that wave-length the pigment sensitivity is 4·4 log units less than at 600 nm and hence about 4·7 log units less than at 540 nm.

Figure 8 (*b*). The records in Fig. 8 (*b*) are similar to those in 8 (*a*) and similarly positioned but they differ in shape because the spectral light was presented alone, unmixed with the deep red saturating component, in the flash that generated them. The records of Fig. 8 (*b*) were mounted upon horizontal strips in positions proportional to log I as before. But these strips were not displaced arbitrarily; each strip is displaced exactly as much as the corresponding strip (same λ) in Fig. 8 (*a*). The reader may place a piece of paper so that the edge passes through the zeros of the scales of Fig. 8 (*a*) and (*b*), and mark with arrows A and B the positions of those zeros on the edge. Then if with edge horizontal across the page arrow A points to a record of Fig. 8 (*a*), arrow B will show what form that record takes when the red saturating component of the light stimulus is removed.

In Fig. 8 (*a*) all the waves on any vertical line represented equal activity of the green mechanism and since in the presence of red saturation this was the only mechanism active, all the waves were of the same shape. In Fig. 8 (*b*) the waves on any vertical line still represent equal activity of the green mechanism, but, as we ascend the vertical, we note that depolarization increases. This is because each row higher represents stimula-

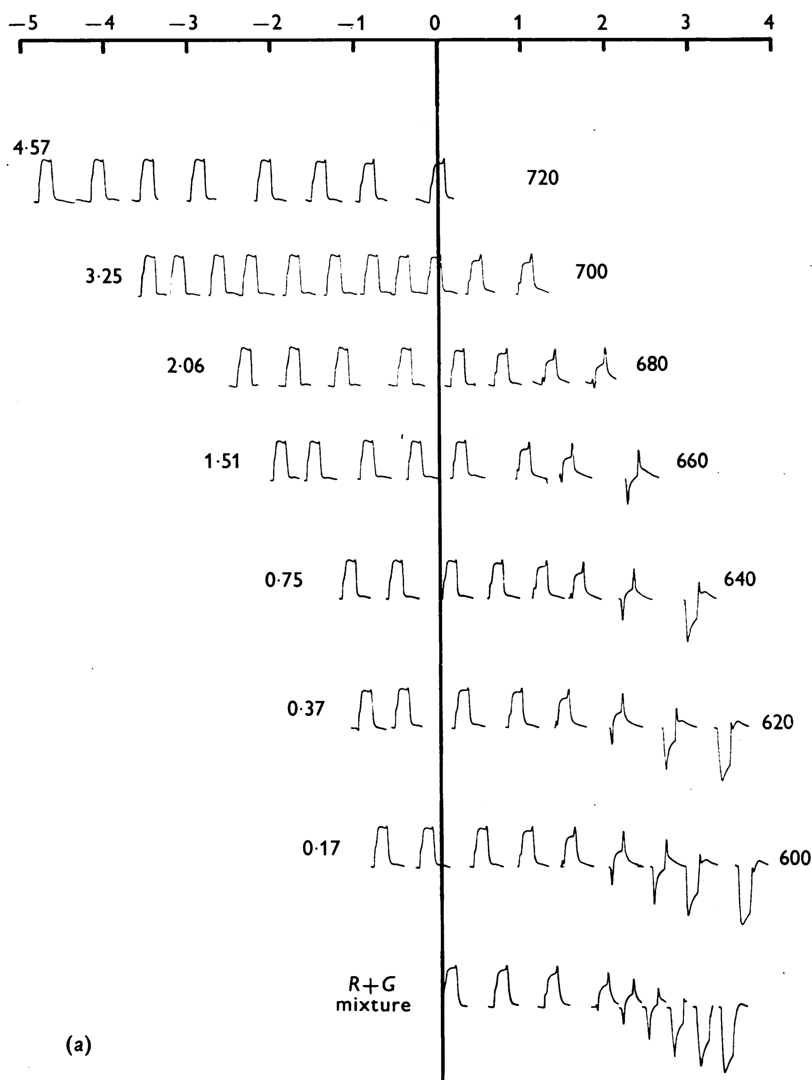

Fig. 8(*a*). Responses to flashes consisting of mixtures of monochromatic light+saturating deep red light. Wave-length (nm) of monochromatic light indicated by number at right of row, log intensity by position in row (scale above); log quantum energy at vertical zero line given by number at left of row. Bottom row, blue-green light (filter) mixed with saturating deep red from Fig. 1. Each row of records has been shifted sideways so that any vertical line passes through waves all of the same shape. (*b*) Monochromatic lights as in (*a*) but without deep red saturation. Corresponding wave-lengths and intensities have corresponding co-ordinates in (*a*) and (*b*).

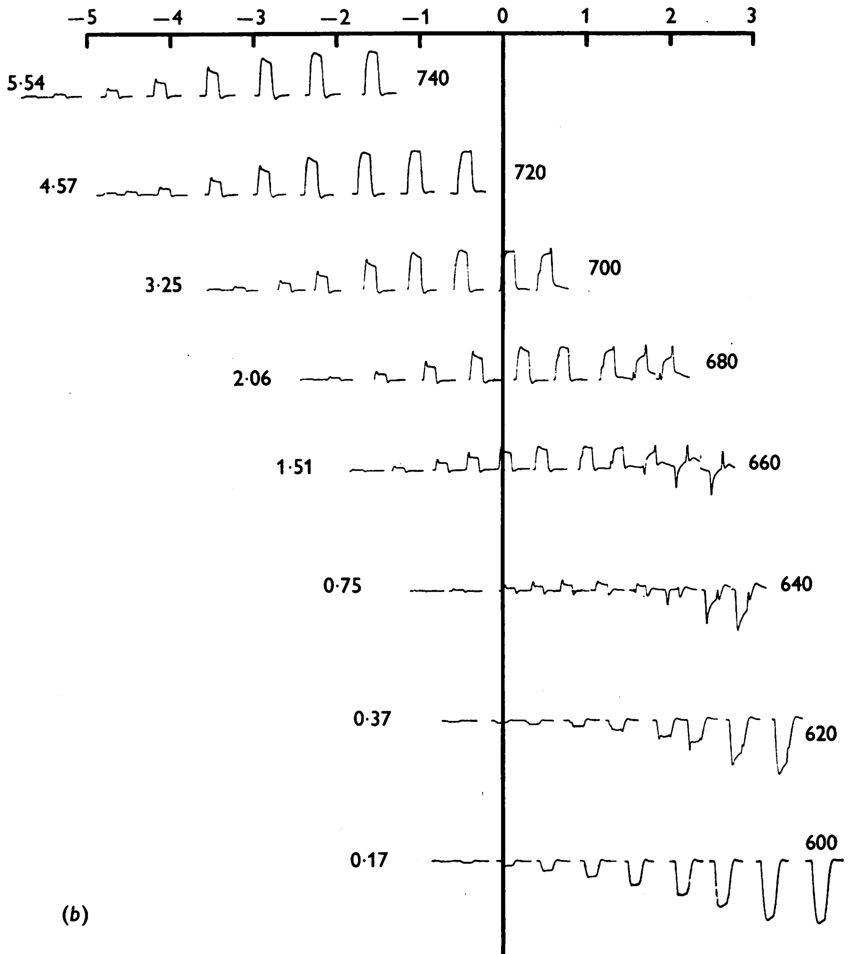

Fig. 8(*b*). For legend see opposite page.

tion by light of wave-length 20 nm greater, and upon the vertical line, where energies are scaled for constant *green* action, longer wave-lengths involve energies with stronger red action. Thus as we ascend any vertical in 8(*b*) we observe a transition in which the stimulus to the green pigment remains constant but that to the red pigment is continually increased. Now if we are in a dichromatic range this condition should resemble precisely that shown in Fig. 1, where in going from left to right along any row the green component remains constant and the red component continually increases. In Fig. 9 we examine how precise the resemblance is.

Figure 9(*a*). The records of Fig. 1 (together with others of the series not shown there) provide a good range of mixtures of blue-green light and

spectral light at 720 nm. In order to display these records so that they may be compared to those of Fig. 8 (*b*) we must re-arrange them. As in Fig. 8 (*a*) each wave is cut out and mounted in a position corresponding to log I, but in the present case the strip is vertical, the vertical scaling of log I being the same as the horizontal scaling in Fig. 8. For example, all the records of the top row in Fig. 1 were first labelled with their log I values as monitored by the corresponding step height of the staircase. Then they were cut from the print and mounted one vertically above the other with the base line of each opposite the proper log I value on the vertical scale. When this was done with all the records, the vertical strips laid side by side contained at the upper ends identically the series of records shown in the lowest line of Fig. 8 (*a*) (and some intermediate values). All these were adjusted against the horizontal scale exactly as in Fig. 8 (*a*), with the zeros of all strips (at first) on one horizontal line. Now the right hand strip (0·3) remains fixed, and each other is raised vertically by a distance equal to its distance to the left of the 0·3, so that the zeros now lie upon the $-45°$ line. This (except for one detail) is the position shown in Fig. 9 (*a*). If the 45° shift had not been made, as we ran through the array horizontally from left to right we should have seen the effect of increasing green and keeping red constant; with the 45° shift the horizontal sequence shows the effect of increasing green and red in some fixed proportion. But one fixed proportion of red and green stimulation is precisely the action of one monochromatic light of varying intensity, consequently each row of Fig. 8 (*b*) might be expected to match some horizontal line in Fig. 9 (*a*).

One detail must be added. The placing of the records in Fig. 9 (*a*) would be correct if it were true that light of 720 nm did not excite the green mechanism at all. The vertical zero line across Fig. 8 (*a*) shows that at maximum it excites the green about as much as the blue-green light with 3·6 density interposed. Thus in the left vertical strip of Fig. 9 (*a*), at bottom the green cones are being excited by the blue-green light with 3·6 alone, at top by full 720 nm as well which doubles the green cone excitation. This strip has therefore been mounted not vertically but with the top record 0·3 log units to the right of the bottom record. The next strip was more vertical (displaced by 0·1 log unit) and thereafter the effect is negligible.

Figure 9 (*b*). The strips in Fig. 9 (*b*) are copies of those in Fig. 8 (*b*) (with 740 nm added). Each represents one wave-length and hence each stimulated red and green in some fixed proportion but with increasing intensity according to the position along the log scale. Thus it should be possible to super-impose each horizontal strip upon Fig. 9 (*a*) in such a position that every record in the strip corresponds with the subjacent record in Fig. 9 (*a*). In finding the proper position no horizontal movement of strips is permissible for the horizontal position is already defined by the vertical

zero line across the records that makes them all equivalent for green. If they are also to be equivalent for red this must be obtained by vertical shifting only. Figure 9(*b*) shows the best vertical position for the strips of 8(*b*).

The reader may satisfy himself as to the extent of correspondence in the records of Fig. 9(*a*) and (*b*) by using a horizontal edge marked with arrows *A* and *B* corresponding to the distance apart of the two zeros in Fig. 9(*a*) and (*b*). In any horizontal position the two arrows point to a pair of records that should be identical.

There is no doubt that in general the correspondence is pretty good. Where it fails in detail, a vertical adjustment will not remedy the failure. Such discrepancies are only seen where the opposing potentials of red and green nearly cancel, and some unbalanced transient complex alone remains. There are three possible explanations. (i) The wave forms in question are changing fast with change in position on the figure, perhaps the required intermediate form has escaped being recorded. We do not think this an adequate explanation. (ii) Perhaps the range is not strictly dichromatic; for instance, the blue-green light in Fig. 9(*a*) may have excited *blue* cones more than any monochromatic light (of 600 nm wave-length or greater) in Fig. 9(*b*). If so the blue cone influence is only just detectable. (iii) It is not easy to keep S-units in such perfect equilibrium after penetration but that small changes such as these discrepancies may occur. In the course of the experiment of Figs. 8 and 9 the records of Fig. 8(*a*) and (*b*) were obtained first and in fact 177 waves were recorded from which Fig. 8(*a*) and (*b*) were constructed before starting on the (red + blue-green) mixtures that constitute Fig. 9(*a*). It would not be surprising if in the course of obtaining all these records for Fig. 8 (half of which involved very strong lights) there was some slight change in the potential transients. This is the interpretation that we shall adopt. The strip 740 nm at the top of 9(*b*) was placed so that when slid down the -1 gradient it would coincide with the records on strip 720 nm.

The Visual Pigments

For the purpose of the present analysis we define two lights as 'matching' when each pigment involved in the response is excited as much by the first light as by that which matches it. From the Principle of Univariance it follows that matching lights generate identical records. Now the records of Fig. 9(*a*) and (*b*) are identical in pairs, thus one possibility is that the corresponding pairs of lights are matching lights. The other possibility is that two non-matching lights can generate identical records. If this explanation applies to one pair in Fig. 9 it must apply to the whole of the 2 horizontal rows which we may label N_a in Fig. 9(*a*), N_b in Fig. 9(*b*), that

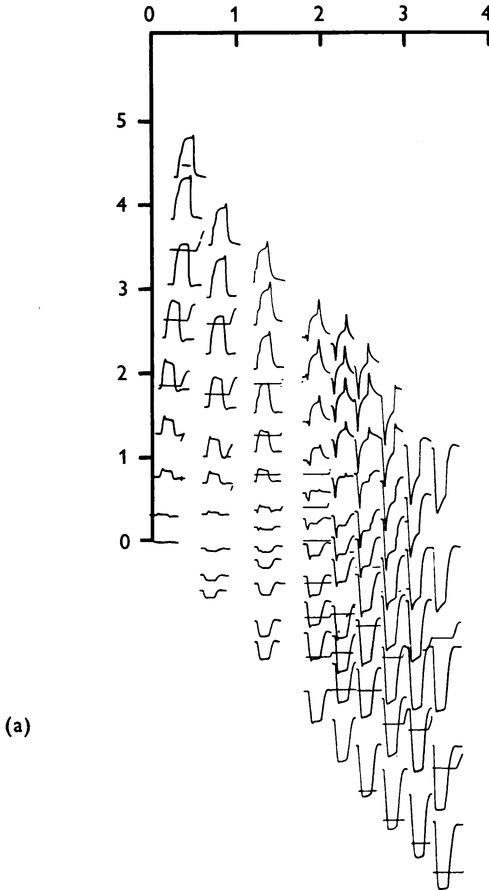

Fig. 9 (*a*). Records from Fig. 1 re-arranged so that horizontal rows there are vertical here. Horizontal placing as in bottom row Fig. 8 (*a*) where lateral displacement is proportional to log of blue-green intensity (= log *G*). Vertical displacement of first column is proportional to log of 720 nm intensity (= log *R*). In general vertical displacement proportional to log *R* − log *G*. (*b*) Records from Fig. 8(*b*) without lateral shift but each row shifted vertically so as to correspond with the record in Fig. 9(*a*) that has the same co-ordinates.

contain this pair. Consequently the array in Fig. 9(*a*) must contain two identical rows, N_a that resembles N_b exactly without matching, and the row in Fig. 9 (*a*) where red and green are excited in the same ratio as in N_b, and hence must 'match' it. Similarly, row N_b itself must be reduplicated exactly elsewhere in the array of Fig. 9 (*b*). Since no such reduplications are found we conclude that whenever the rows are identical the lights 'match'.

Now the stimuli for Fig. 9 (*a*) consisted entirely of the mixtures of two fixed lights, thus the array of Fig. 9 (*b*) that matches is also dichromatic; thus only two visual pigments green and red are involved in the spectral

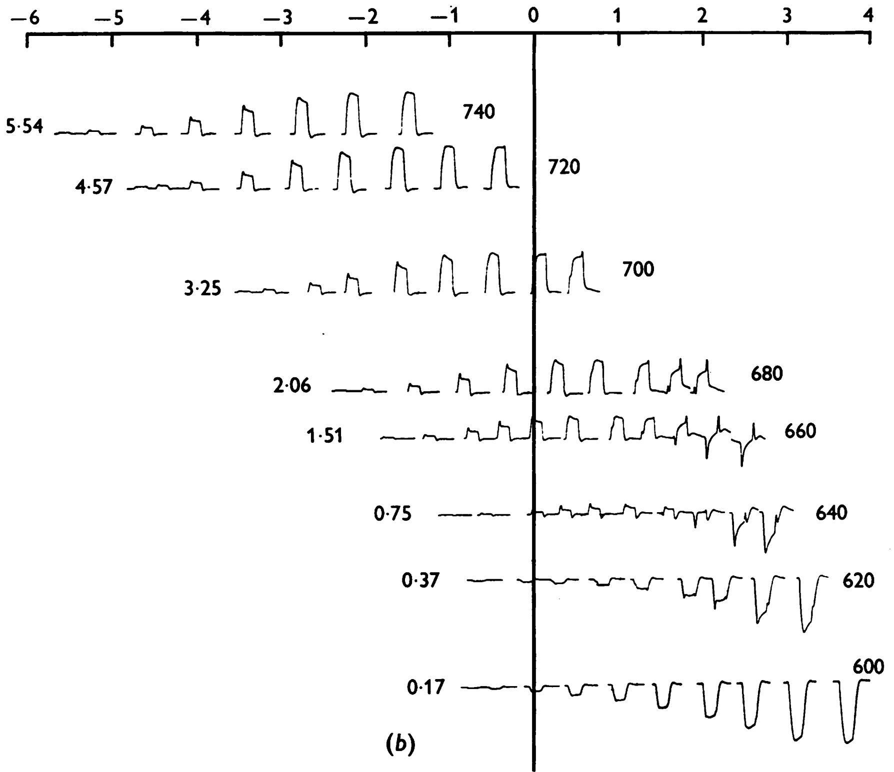

Fig. 9 (*b*). For legend see opposite page.

range 600–740 nm. The log action spectrum of the green pigment has been obtained isolated from the results with deep red saturation and is plotted in Fig. 6. We now extract the action spectrum of the red pigment.

From Fig. 9 (*a*) and (*b*) we know for each wave-length λ the energy E_λ that matches the mixture consisting of E_R units of 720 nm energy and E_G units of green energy. In this mixture the red makes a negligible contribution towards excitation of the green pigment, hence E_λ and E_G must excite green equally. Consequently $A(G)$ the action spectrum of the green pigment is given for each λ by

$$A(G) = E_G/E_\lambda. \tag{11}$$

Let us suppose that 1 unit of green light excites the red pigment as much as a units of 720 nm light. From matching we know that E_λ excites the red pigment as much as E_R of red + E_G of green, which is the same as $(E_R + aE_G)$ units of 720 nm light. Thus $A(R_a)$ the action spectrum for the red pigment is given by

$$A(R_a) = (E_R + aE_G)/E_\lambda. \tag{12}$$

If we suppose for a moment that $a = 0$, eqn. (12) will become

$$A(R_o) = E_R/E_\lambda \tag{13}$$

Now the action spectra $A(G)$ and $A(R_o)$ may be found at once from Fig. 9. Consider the records that lie on the vertical zero line; in 9(*b*) the number at the left of each row is in fact $\log E_\lambda$, and in 9(*a*), E_G is constant for all wave-lengths and $\log E_R$ at each level is simply read off the vertical scale of $\log I$. The two log action spectra are plotted as triangles in Fig. 6, and those curves *G*, *R* are replotted as linear action spectra *G*, *R* in Fig. 10.

From eqns. (11), (12), (13) we obtain

$$A(R_a) = A(R_o) + aA(G). \tag{14}$$

Thus the most general expression for the action spectrum of the red pigment is obtained by adding to $A(R_o)$ which is curve *R*, Fig. 10, some multiple a of $A(G)$ which is curve *G*.

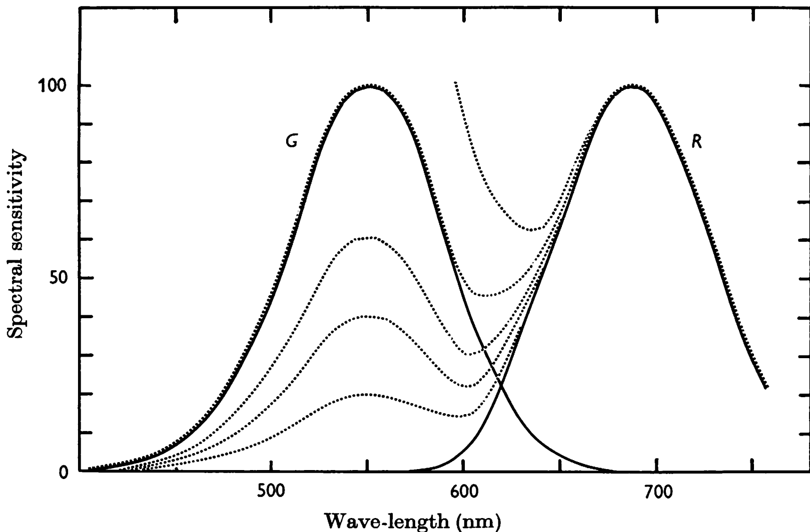

Fig. 10. Log spectral sensitivity curves of Fig. 6 replotted as (linear) spectral sensitivity curves *G* and *R*, continuous lines. Dotted curves are formed by adding to *R* some multiple a of *G*, where a is 0·2, 0·4, 0·6, 1·0 and 2·0. The only red sensitivity curve consistent with the results of Figs. 8 and 9 is of form $(R+aG)$ and the only acceptable shape is when $a = 0$. Thus *R* is the spectral sensitivity for the deep red pigment.

The dotted curves in Fig. 10 show the results of this addition for a range of a-values, and very improbable action spectra they look. No addition of this kind can make the ordinate at 620 nm a maximum since it lies at a region where both *G* and *R* are concave upwards and so all positive weighted means of the two curves must give a trough not a crest in this

region. And indeed unless a is zero or very nearly so the red action spectrum curve will show a double maximum one near 680 and one near 540 nm.

The only admissible answer is that a is zero and G and R of Fig. 10 represent the action spectra of the green and red pigments respectively.

DISCUSSION

In the complicated train of events that leads to colour vision, the input is always the effective quantum catch in various visual pigments, and we need to know it if we are to understand how colour is analysed. Strictly, *effective* catch can only be measured from physiological effects in the active eye, but we can hardly avoid some sense of confirmation when (as with our green pigment) the action spectrum is found to correspond closely to the difference spectrum determined by densitometry. That is because we have been infected with the faith of those who measure the spectra of pigments in solution (or otherwise) and hope that those observations may correspond to the action spectra in life. Though it is likely enough that in the effective spectral range each quantum caught produces an equal physiological action, yet apart from rhodopsin studies little evidence exists to support the idea.

In this paper we have not assumed *that*, but we have assumed for each pigment that there is a characteristic action spectrum (whether that corresponds to the difference spectrum or not), and that the output is univariant. We also assume at first that the output from the R/G unit studied depends upon two pigments only.

Part 1. By measuring the amplitudes (but not the time course of the waves), it was demonstrated that in the condition of red saturation, green light would hyperpolarize and that further red light would not oppose this (Fig. 1). Consequently the curves of potential downpull from the ceiling plotted against log I must depend upon the green pigment only. They should therefore all lie upon the same template curve whose lateral shift (taken in conjunction with the energy output of the spectrometer) should define the action spectrum of the green pigment. The curves of Fig. 5(a) fulfil the template prediction, and Fig. 6, curve G, shows that the action spectrum lies close to 530 nm where Marks found the difference spectrum of a cone pigment.

Having proved that when the record is pulled down from the ceiling it is due to the green pigment, we now assume that when there is no downpull the green pigment is without appreciable action, and so define a region in the red part of the spectrum where only the red pigment acts. The curves relating depolarization to log I in this region should therefore exhibit the constant template curve of univariance and its lateral shift should define

the action spectrum of the red pigment. The curves of Fig. 5(*b*) fulfil this template prediction, and Fig. 6, curve *R*, shows that the action spectrum lies not where Marks found his red pigment (620 nm) but substantially further into the deep red at 680 nm.

Part 2. This calls in question our last assumption and we consider theoretically whether it is possible for two pigment systems so to interact that the resultant is indistinguishable from the output of a single pigment system. It was shown that a pseudo-pigment could be contrived that would mimic to perfection a real pigment for all intensities of all monochromatic lights. Moreover, if the action potentials involved all had the shape defined by Dartnall's nomogram, the kind of interaction required would resemble closely that found by Hartline & Ratliff (1957) to exist between the ommatidia in the eye of *Limulus*, and by Donner & Rushton (1959) in ganglia from the frog.

For instance, if Z_1, Z_2, the outputs of the 540, 620 nm pigments, are proportional to the effective log quantum catches then (from the mathematics of Part 2) the expression $(2Z_2 - Z_1)$ will approximately be proportional to the log quantum catch of a pigment at 700 nm, and we have to consider whether our 680 nm pigment is really a pseudo-pigment generated from Z_1 and Z_2 in some such way.

The proposed scheme is that of Fig. 2(*b*) where the output of *G*, the 540 nm pigment, is of two kinds. That along G_A hyperpolarizes, and if mixed with a strong deep red light (which by itself would depolarize to saturation) will pull down the potential. The signal along G_B is that which might interact with the red (620 nm) signal to result in the 680 pseudo-pigment. Since the '680 pigment' operates in a range where G_A produces no downpull from the deep red ceiling we must assume in Fig. 2(*b*) that the threshold for effective signals in G_A is much higher than for effective signals in G_B.

Consider this univariant range where only the '680 pigment' is operative and where G_A exerts no downpull. We can answer the question whether the recorded waves represent the output Z_3 of a true 680 pigment according to scheme 2(*a*), or are the result of $(2Z_2 - Z_1)$, the appropriate reaction for scheme 2(*b*). For if we add to the monochromatic light of 720 nm a little green at 500 nm, this will increase appreciably the G_B output $(= Z_1)$, but will affect the red output $(= Z_2)$ a hundred times less, and hence $(2Z_2 - Z_1)$ will be *negative*. We should therefore get a result entirely contrary to the Principle of Univariance, namely that the addition of light of one wavelength acts like the *subtraction* of light at another wave-length. This striking result we have looked for and never seen. In all the mixtures of 720 and 500 nm light that have been recorded, one pattern was always observed: so long as green light was too weak to pull down the deep red ceiling, it

was too weak to affect the mixed output $(2Z_2 - Z_1)$. The component Z_1 was always negligible: the path G_B inoperative. Evidently the scheme of Fig. 2(*a*) is nearer the truth than 2(*b*), and consequently the 680 nm pigment is real.

Part 3. The same conclusion is reached through colour matching by an argument that does not rest upon the analysis of Part 2. The nerves are now used simply as *null* detectors for the quantum catch of the pigments. The results are thus independent of the nature of the nerve organization, provided that the detector is sensitive enough—which is seen to be the case. In Fig. 9(*a*) and (*b*) are two arrays of time records that show considerable diversity in wave form. In Fig. 9(*a*) the stimulus was a mixture of light at 720 nm (I_R) and of light at 500 nm (I_G), and each record is 'plotted' on Fig. 9(*a*) with abscissa linear with log I_G, ordinate linear with (log I_R − log I_G). In Fig. 9(*b*) the stimulus for each row was I_λ, some fixed monochromatic light. The abscissa was linear with log I_λ, the ordinate an arbitrary vertical displacement of each row. The striking thing about Fig. 9(*a*) and (*b*) is that waves with identical co-ordinates have identical shapes.

The records in Fig. 9(*a*) that lie on the horizontal with fixed ordinate y_1 are responses to light mixtures with a fixed red/green ratio. This row of records is the same as the row in Fig. 9(*b*) with ordinate y_1 and with no other row. But this row is the response to monochromatic light λ_1. Hence λ_1 excites the two pigments in the same ratio that the red and green lights do.

Now the red light at 720 nm when added to deep red saturating light never pulls down the ceiling. Therefore it does not stimulate appreciably the green pigment mechanism. Thus in Fig. 9(*a*) the green mechanism will only be excited by the green light I_G. Hence in both Fig. 9(*a*) and (*b*) the abscissae give the amount in log units by which the green pigment is being excited. This is confirmed by adding a saturating red light to all stimuli as in Fig. 8(*a*) and observing that the records (without lateral shift from 9(*b*)) exhibit a fixed wave form for a fixed abscissa. From the Fig. 9(*b*) we know, then, the energies of each wave-length that give fixed green action, and thus can plot the action spectrum for green. However, since only wave-lengths from 600 nm upward are recorded in Fig. 9, a more satisfactory curve (G, Fig. 10) is plotted from Fig. 5(*a*).

If we assume that the green light I_G of Fig. 9(*a*) does not stimulate appreciably the red pigment mechanism, then red is entirely excited by the 720 nm component in the mixtures of Fig. 9(*a*). This is known for every record in Fig. 9(*a*) from its co-ordinates, and hence is also known for the similar record in Fig. 9(*b*) with the same co-ordinates. Thus we can plot the action spectrum for the red pigment mechanism just as we did for the green. The result is curve R, Fig. 10.

The dotted curves about curve R show what would result if the green light at 500 nm stimulated somewhat the red pigment. They are formed by adding to curve R some fraction of curve G. They all must consequently show a secondary maximum near 540 nm. This unlikely and unnecessary feature makes all such curves much less acceptable than the original curve R. But since the colour matching array of Fig. 9 (*a*) and (*b*) makes the dotted curves the only alternative, we must accept curves G and R, Fig. 10, as action spectra of the pigments that serve the R/G unit.

Photography has long made use of 'sensitizers' to extend the spectral range of AgBr whose chemical decomposition actually constitutes the photograph. It would not be at all surprising if the eye also could extend the range of visual pigments whose bleaching initiates nerve signals by coupling them with a 'sensitizer'. This would be capable of catching quanta outside the absorption range of the pigment and transferring its activation so as to bleach the visual pigment and elicit its signal.

If this were common we should find many photoreceptors whose action spectrum differed markedly from the difference spectrum of their pigment, since the action spectrum would be that of the sensitizer. Clearly this is not the case with the rods, but the comparison has seldom been well made elsewhere.

Now Marks has looked carefully but unsuccessfully for the difference spectrum of a 680 nm pigment in conditions that should have revealed it (see Discussion in Ciba Foundation Symposium, 1965, pp. 280–85). So it looks as though the 680 nm action spectrum that is so prominent in our records is an action not associated with the bleaching of the pigment that caught the quanta. If there were two kinds of cone that contain the 620 nm pigment, one of which—the red twin cone that is mated with green for instance (Marks, 1965, p. 24)—being associated with a deep red sensitizer, then we could understand why by difference spectrum there is only 620 nm but in the R/G units there is only 680 nm—the peak absorption of the unbleachable sensitizer.

This paper opened with the problem of discovering what we can about the unknowns in the statement, 'Light is absorbed by unknown pigments each of which generates signals of unknown nature interacting in an unknown manner'. We have been able to throw some light on these three unknowns. (i) In the R/G unit that we have studied, the colour matching analysis of Part 3 has shown that only two pigments are involved, green and deep red, whose action spectra are shown by curves G, R in Fig. 10. (ii) The amplitude of response as a function of light intensity for each single pigment system can thus be measured, for red by choosing a wave-length such as 720 nm that affects R 10^5 times as strongly as G. For green the simplest way is to measure the downpull from the ceiling of deep red

saturation. Both are shown in Fig. 5 and both relations are similar. (iii) The nature of the interaction is very nearly simple (algebraic) addition. This is demonstrated by the way that the open circles of Fig. 5 (*b*) lie upon the template curve that fits the filled circles. A very much more stringent test is provided by Figs. 8 and 9, and it is plain that the green and the red signals are subtracted with some accuracy and this accounts fairly well for the various wave forms observed.

The curves of Fig. 5 each fit the relation

$$V = U.I/(I+I_{\frac{1}{2}}), \tag{14}$$

where V is the recorded potential height, U its maximum value, I the energy of the light stimulus and $I_{\frac{1}{2}}$ the value of I that makes $V = U/2$.

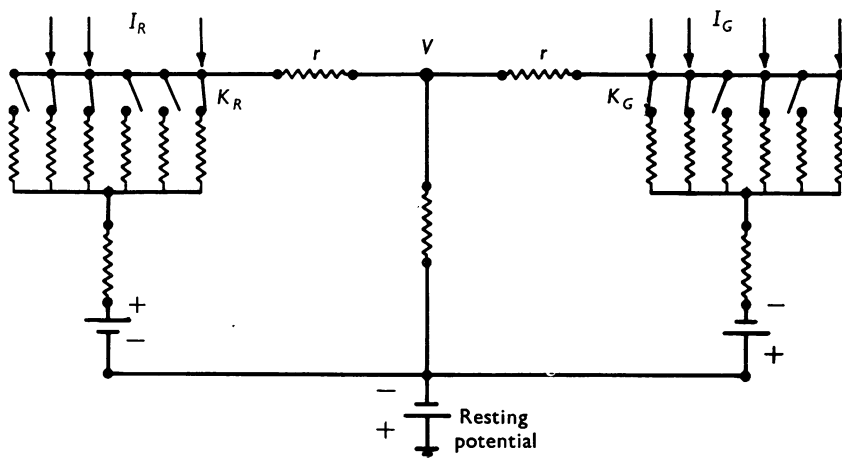

Fig. 11. Schematic model for R/G unit. Each signal I_R from a red cone momentarily shuts a key K_R. I_G and K_G in the green cone system act likewise. The two systems are isolated by large resistances *r*, *r*. The model gives the observed relation between the S-potential V and the quantum catches I_R and I_G.

In a later paper we hope to discuss the significance of this equation, but to anticipate, it is easy to see that it follows from the model of Fig. 11, that it is supposed to represent the membrane of a R/G unit. In the dark all the keys K_R and K_G are open and V has the resting dark value, that is the zero of our measurements. Suppose that every signal from a red cone increases for a short time the membrane permeability represented by closing briefly one of the very numerous micro-keys K_R. Then the conductivity of the K_R ensemble will be proportional to the rate of absorption of quanta I_R by the red pigment. Neglecting the right side of Fig. 11 it is easy to work out that the relation between V and I_R is given by eqn. (14). Similarly, on the right the same relation is supposed to hold with green cone signals.

But since the generator potential is here reversed, the effect of light is to add to the resting potential—to hyperpolarize. To explain the simple additivity observed between the potentials from red and green cones we must suppose that the K_R ensemble is electrically separated from K_G as by having large isolating resistances r.

This model gives a formal and quantitative picture of the red/green organization in the unit. It will doubtless need great modification to trim it to the facts of structure and electrochemistry.

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REFERENCES

BRINDLEY, G. S. (1953). The effects on colour vision of adaptation to very bright lights. *J. Physiol.* **122**, 332–350.

CIBA FOUNDATION SYMPOSIUM (1965). *Colour Vision*. London: Churchill.

DONNER, K. O. & RUSHTON, W. A. H. (1959). Retinal stimulation by light substitution. *J. Physiol.* **149**, 288–302.

FUORTES, M. G. F. (1958). Electric activity of cells in the eye of Limulus. *Am. J. Ophthal.* **46**, 210–223.

HARTLINE, H. K. & RATLIFF, F. (1957). Inhibitory interaction of receptor units in the eye of Limulus. *J. gen. Physiol.* **40**, 357–376.

LIEBMAN, P. A. & ENTINE, G. (1964). Sensitive low-light-level microspectrophotometer: detection of photosensitive pigments of retinal cones. *J. Opt. Soc. Am.* **54**, 1451–59.

MACNICHOL, E. F. (1958). Subthreshold excitatory processes in the eye of Limulus. *Expl Cell Res.* Suppl. **5**, 411–425.

MARKS, W. B. (1963). *Difference Spectra of the Visual Pigments in Single Gold Fish Cones*. Ph.D. Dissertation, Johns Hopkins University.

MARKS, W. B. (1965). Visual pigments of single gold fish cones. *J. Physiol.* **178**, 14–32.

MARKS, W. B., DOBELLE, W. H. & MACNICHOLL, E. F. (1964). Visual pigments of single primate cones. *Science, N.Y.*, **143**, 1181–1183.

NAKA, K. I. & RUSHTON, W. A. H. (1966*a*). S-potentials from colour units in the retina of fish (Cyprinidae). *J. Physiol.* **185**, 536–555.

NAKA, K. I. & RUSHTON, W. A. H. (1966*b*). S-potentials from luminosity units in the retina of fish (Cyprinidae). *J. Physiol.* **185**, 587–599.

RUSHTON, W. A. H. (1959). Excitation pools in the frog's retina. *J. Physiol.* **149**, 327–345.

RUSHTON, W. A. H. (1965). A foveal pigment in the deuteranope. *J. Physiol.* **176**, 24–37.

STILES, W. S. (1949). Increment thresholds and the mechanisms of colour vision. *Documenta ophth.* **3**, 163–183.

SVAETICHIN, G. (1953). The cone action potential. *Acta physiol. scand.* **29**, Suppl. 106, 565–600.