

The organization of the vertebrate retinal elements¹.

By

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With 28 Figures.

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Introduction.

In studying sense organs one of our ultimate aims is to learn to understand the mechanisms of sensory integration. For this reason it is necessary to use the appropriate adequate stimuli and combine an analysis of the sensory response in afferent nerves with parallel exploration of what takes place in the nervous centres. Every experimenter will realize that there can be no understanding of sensory integration before we know the specific attributes whereby one kind of response is discriminated from another, in fact, the fundamental general problem facing the investigator nearly always concerns

discrimination. The more important the sense organ, the better developed its discriminatory function. Sight, our most important channel of information, has brought discrimination to such a degree of perfection that a nervous centre behind the receptors immediately takes charge of the primary message for purposes of elaboration. This fact should always be borne in mind. It is a complication as well as an advantage providing us with unique opportunities for research.

What are those opportunities? They were perfectly understood already by PARINAUD (99) who emphasized «la nécessité de changer l'orientation qui a été donnée à l'étude de la vision, de porter la question sur son véritable terrain qui est celui même de la physiologie, de chercher l'explication des phénomènes visuels dans la structure de l'appareil visuel, dans les propriétés des éléments anatomiques.

Si l'on objecte que, pour expliquer les phénomènes visuels par les propriétés de structure de l'appareil visuel, il faudrait connaître cette structure, je répondrais: Il est possible que la question soit difficile, mais ce n'est pas en tournant le dos à la vérité qu'on la trouve, et s'il est vrai qu'une question bien posée est à moitié résolue, il importe avant tout de bien poser la question. Au surplus, la question n'est pas aussi difficile qu'il me semble au premier abord.»

The monumental work of RAMON Y CAJAL (104, 105) on the histology of the eye, by its analysis of the interneurons of the retinal nervous centre, had paved the way for a new physiology of the retina, provided that the physiologists could produce the tools necessary for their own advance. The body of knowledge from the earlier epoch of histology, in the hands of SCHULTZE (112), PARINAUD (99), KÖNIG (84) and VON KRIES (82), had led to the facts summarized in the duplicity theory. There was now available in the great work of SHERRINGTON and his collaborators (24, 113), a structure of concepts for the analysis of the central nervous system and ADRIANS (1) brilliant research on the electrical messages from sense organs, in particular his studies with R. MATTHEWS on the eel's optic nerve (4, 5, 6), clearly indicated the obvious course of development for the physiology of the retina. Elsewhere I have given a full account of the electrophysiological work on the retina up to 1945 (52) and this together with the more recent work should be studied against the background sketched above. I need not here rewrite my earlier summary in an abbreviated form. Since it was published very little has been done with the retinal action potential, the so-called electroretinogram, except human studies [ADRIAN (3), KARPE and TANSLEY (79)] and important clinical work [KARPE (76, 77, 78), MONNIER (90)], so that this field will not be further reviewed. I merely want to draw attention to papers by MOTOKAWA (93—97) on the human electroretinogram which during the war had escaped my notice. I shall restrict myself to recent studies on the organization of the retinal elements in vertebrates.

I. Histology of the retina.

The organization of the neural pathway.

POLYAKS (102) scholarly book on the histology of the retina deserves careful study by everyone interested in this field. It extended CAJALS analysis to the primate retina, confirmed his main findings and reported interesting new observations. Fig. 1 is taken from POLYAKS book and, though not complete, summarizes a number of essential facts. In this schematic picture the

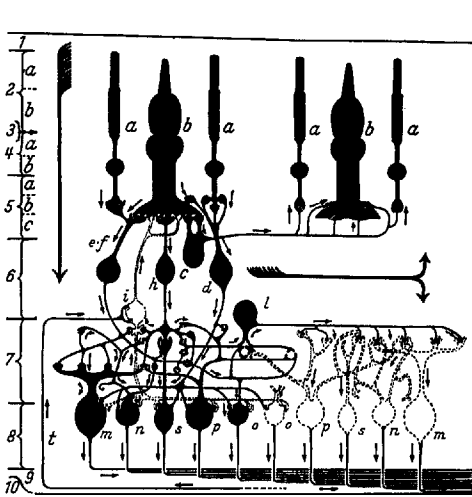


Fig. 1.

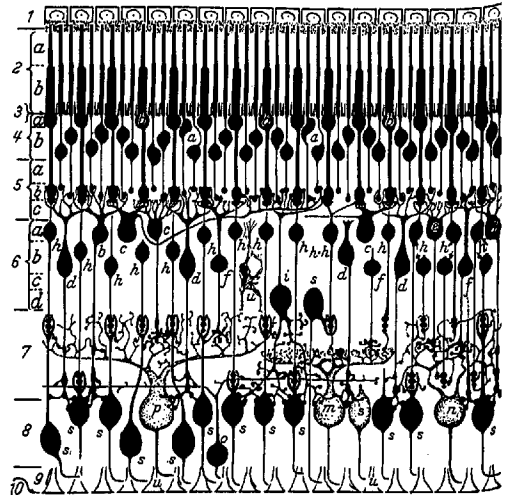


Fig. 2.

Fig. 1. The structure of the primate retina reduced to its essentials, including the synopsis of the propagation of the retinal impulses from the photoreceptors to other parts of the retina, to the brain, and from the brain back to the retina (direction indicated by the arrows). Labeling of the cells: (a, b) rods and cones, or the photoreceptors where the nervous impulses are generated by physical "light" (in the scheme only the left group of the photoreceptors is assumed to be stimulated by light); (c) horizontal cells by means of which the impulses are transmitted to the surrounding rods and cones; (d, e, f, h) centripetal bipolar cells of the mop, brush, flat and midget varieties, which "transmit" the impulses from the photoreceptors to the ganglion cells, the bipolars serving as "analyzers"; (i) centrifugal bipolar cell, a variety of the "amacrine cells", which probably receives the impulses from the centripetal bipolars, from the ganglion cells, and also from the brain by way of the centrifugal or efferent fibers (t) and transmits them back upon the photoreceptors (a, b); (l) an "amacrine cell" which possibly intercepts a part of the bipolar impulses and spreads them over the surrounding territory; (m, n, o, p, s) ganglion cells which receive impulses from the centripetal bipolars and transmit them to the brain along their axons called "optic nerve fibers." (POLYAK: Univ. Chicago Press 1941.)

Fig. 2. Scheme of the primate retina, showing the types of the neurons and their synaptical relationships, so far revealed by means of the method of GOLGI. (POLYAK: Univ. Chicago Press 1941.)

path through the retina resembles the reflex arc of the spinal cord. A receptor, an intercalated (bipolar) cell, a ganglion cell corresponding to the motor horn cell, form the main chain and around this main channel a complex system of interneurons is found. A horizontal cell (c) is seen to connect the foot of a cone (b) with adjacent rods (a) and cones. Adjacent paths are further joined by the amacrine cell (l) and very complicated interconnections are found at the level of the ganglion cells (m, n, s). There are several types of bipolar and ganglion cells. In fig. 2 some 'diffuse' bipolars are seen to range over a con-

siderable number of rods or a group of rods and cones, others, such as *h* in fig. 1 are individual and join a cone to a ganglion cell. The former type is probably identical with CAJAL's rod bipolar. POLYAK distinguishes between mop (*d*), brush (*e*) and flat (*f*) bipolars within the general category of diffuse ones (see fig. 2). POLYAK identifies the flat bipolars with CAJAL's cone bipolars. None of the diffuse bipolars seems to be exclusively a rod bipolar. All of them may be influenced by the cones too. The individual or midget bipolar, according to POLYAK, is related to cones alone which therefore have an additional or *alternative* individual path to the one in common with the rods. It would be a serious misinterpretation of the histological evidence to maintain that cones can only send their impulses along the path of the midget bipolar. The individual cone path should be described as a mechanism for giving additional information. It is a physiological problem to find out when, how, and to what extent cones can separate themselves from rods. Midget bipolars have not yet been described in mammals other than primates though they are seen in birds and lizards. In the light of the available evidence it is very doubtful whether they exist in the cat, which is the best mammalian preparation available for the physiologist.

Whatever the potential isolation of the message at the receptor and bipolar stage, additional mixing takes place at the ganglionic level, even in primates. In particular the giant ganglion cells, described by CAJAL, (types *m* and *p* of fig. 2) extend their dendritic network over not less and probably more, than 100—200 cone diameters. POLYAK mentions "probably more" than 350 μ . Their cell bodies are around 30 μ .

There cannot be much of a future now for the naive view that single cones only form simple straight line 1:1 relays to single cone equivalents in the central visual projection area. All known sense organs are characterized by peripheral and central overlap and the problem of discrimination of colour, brightness and form cannot be profitably developed without recognition of this fact. On the simple view the cone paths should also be the simplest structures in the retina whilst all the research on the retinal elements (see below) tends to show that the simplest possible path is a pure rod path, a convergence structure of the kind connecting to mop bipolars (*d*) in fig. 2 delivering a type of discharge comparable to that of stretch-receptors (see below, on-elements).

So far we have only considered a direction of travel from the receptors towards the ganglion cells forming the so-called optic nerve as well as a number of lateral interconnections. But CAJAL discovered centrifugal paths in the nerve and POLYAK describes a kind of centrifugal bipolar, the dotted structure in fig. 1. Its axon and dendrites are turned so as to suggest opposite functional polarity compared with that of the normal path towards the nerve. The centrifugal fibres of CAJAL are drawn to end on the body of this cell,

Thus, to sum up, the retina is a complex microcosm of a nervous centre. It will not be easy to translate structure into function and this task will require much time and patient work. On the other hand, as PARINAUD pointed out, this is no reason for turning ones back to the truth and restrict oneself to the kind of psychophysics or psychophotochemistry that neglects the existence of the neural network of the retina and the properties of the central nervous system.

II. Physiology.

1. General organization of the retinal elements.

Isolation of retinal elements. By an exquisite technique of microdissection HARTLINE (65, 66) succeeded in isolating fine strands of fibres from the optic

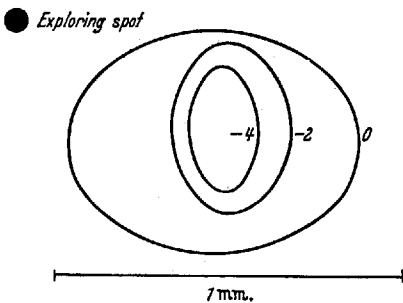


Fig. 3. Chart of the receptive field of a single optic nerve fibre of the frog. Each line encloses a retinal region within which the exploring spot light (relative size shown above left), of an intensity of which the log is given on the line, produced a response from the fibre. On each line the indicated intensity was the threshold; the set of curves constitutes a contour map of the distribution of the retinal sensitivity to light with reference to this particular fibre. [HARTLINE: *J. Opt. Soc. Amer.*, 30 (1940).]

nerve of the frog around the blind spot at the inside of the opened eye. The responses to illumination were what we have become accustomed to call single fibre discharges, brief spikes of the all-or-none type, varying in frequency with changes in illumination. (As to the criteria for single fibre discharges, see 52, p. 304).

Considering the convergence and internuncial network (figs. 1 and 2) the single spikes must always represent the integrated activity of a miniature nervous centre within the retinal microcosm of such miniature centres. By exploring the frog's retina with a minute light spot, 0,5 mm in diameter Hartline (68)

studied the 'receptive field' from which the single spike could be elicited by illumination. The main result of this work is illustrated in fig. 3, fully explained in its legend. The receptive field is most sensitive in the centre and its size can be increased by an increase in stimulus intensity. It is interesting to note that the width of the receptive field (1 mm) fairly well corresponds to the area within which ADRIAN and MATTHEWS (6) in their pioneer work had found interaction between adjacent and slightly separated spots of light projected onto the eel's retina. By using the flicker method for a corresponding analysis of interaction between 4 separated light spots on the human retina I (39) arrived at much the same figure and so did CAJAL (104) long ago in measuring the maximum distance of spread of the axon of the horizontal cells. The receptive field seems too large for a simple convergence

pattern attached to an optic nerve fibre. Within it excitatory and inhibitory effects are integrated in a complex fashion (52, 69).

Around 1937 FORBES and his collaborators (31) had developed a beautiful micro-electrode technique and recorded single spikes from the hippocampus area. The micro-electrode technique was later (87) taken up by LORENTE DE NÓ and developed in an interesting analysis of the properties of some cranial motoneurons. We have since seen it applied in a steadily increasing number of contributions to the study of the central nervous system, perhaps the most interesting ones by RENSCHAW (107—109) and BROOKS and ECCLES (19—22). A parallel development has taken place with the retina (52, 57, 136, 137). The advantage of this technique is, of course, that without much dissection any eye can be studied by placing the micro-electrode gently onto its retina after removal of lens and cornea. The original micro-electrodes (silver core pulled out by gently heating it together with a surrounding glass capillary, according to Svaetichin) were soon given up for another type which in my experience maintained the spikes better (41). This, which still is being used, consists of a 25 micra platinum wire, round which a glass capillary is fused in such a fashion that the tip forms a well rounded point that can be moved over the retina without causing any harm. Such micro-electrodes display condenser properties when tested by a rectangular shock through the recording system (41).

According to GERNANDT (33) two types of spike can be recorded by the platinum wire micro-electrode from the cat's eye; a small spike which need not concern us here because these spikes cannot be maintained well enough for the time necessary for analytical work, and a considerably larger spike. We have only used the large spike which ranges in size from 0.15—0.30 mV, never splits up in two, and in the best experiments could be maintained under the micro-electrode from 4—5 hours. Nowadays we only use the decerebrated cat for analytical work. [Added in proof. RUSHTON (*Nature*, 164, 743—744 (1949)), in this laboratory, has since obtained highly satisfactory evidence in favour of the view that the single spike in the cat's eye is delivered by a single ganglion cell of the diffuse type.]

The micro-electrode technique confirmed the fundamental finding by HARTLINE (66) on the frog's eye, namely that the discharges fall into three main types:

(I) *on-elements*, responding merely to onset of illumination and keeping up their discharge for a duration varying from case to case, apparently on the whole giving more lasting discharges in mammals than in the frog (to judge by HARTLINE's results on the latter).

(II) *off-elements*, merely responding to cessation of illumination, the off-discharge. These are inhibited by illumination, as may be seen when the element isolated is spontaneously active, or else, by repeating stimulation with light while the off-effect is in progress. This is then suppressed by pre-excitatory inhibition, so called because it attacks at 'on' and has a briefer latent period than excitation at 'on'.

(III) *on/off-elements*, combining these two properties.

So far very few types of eye have been surveyed from the point of view of the relative distribution of the three types of discharge. It would be important to have such measurements carried out on different animals and correlated with the histology of their retinae. In the frog's eye with about an equal number of rods and cones there are 50% on/off-fibres, 30% off-fibres and 20% on-fibres (66). The eye of the guinea pig, dominated by rods, seems to be dominated by pure on-elements (46). Of the cat's eye we have a very complete survey: there are 79% on/off-elements, 16% on-elements and only 5% pure off-elements (GRANIT and TANSLEY, 58), counted on a total of 164 elements without exercising selection by any other criterion than good isolation of the spike. The figure for the off-elements in cats is a maximum because the on-components seem to be more sensitive to interference and the definitions are limited by the strength of the spectrum used in this work as well as by prescribing dark adaptation. Some dark adapted pure off-elements may in light adaptation show an on-component (GERNANDT, 36). The cat's retina is very similar to our own periphery. According to WALLS (133) it has "a very respectable number of cones . . . about a third as many as we ourselves". In mammals the discharges, on the whole, tend to be of longer duration than in frogs. We do not know whether the micro-electrode isolates the same kind of spike as does micro-dissection but it seems probable that the micro-electrode picks up the so-called soma spike [LORENTE DE NÓ, (87)] of the ganglion. On the other hand, it should be remembered that well isolated spikes have been picked up by our technique from dorsal root filaments which shows that the micro-electrode need not necessarily be on a ganglion (52, *Ch. I*).

The method of backfiring into the optic nerve might be expected to solve this question. However, this technique is difficult to apply to an eye and recent evidence on backfiring into the ventral roots of the spinal cord (85, 21, 107—109) has demonstrated extraordinary and unexpected complications. Thus, for instance the antidromic impulse only seems to enter a certain fraction of the ventral horn cells [LOYD (85)] and in addition it sets up inhibitory effects [RENSHAW (107—109)] which tend to obscure the inferences from earlier work on antidromic stimulation.

The pure on-elements. These are the simplest structures in the retina in the sense that they reproduce the typical effect of all other well known sensory endorgans as well as the type of discharge obtained from the invertebrate retina of the horseshoe crab *Limulus polyphemus* [HARTLINE and GRAHAM (70)]. Their dominance in the retina of the guinea pig which contains very few cones (46) suggests that they are characteristic for rods. Whether they also would be present in pure cone retinae is unknown. They have been extensively analyzed in the cat's retina and there been found to reproduce the pure visual purple curve of spectral photosensitivity [DONNER and GRANIT (28)]. If an electrical current is passed through the retina between two electrodes on the sclera and the micro-electrode is placed just inside one of the electrodes the

pure on-elements are found to be stimulated by the cathode and inhibited by the anode differing in this respect from the opposite type, the pure off-element, which is stimulated by the anode and depressed by the cathode [GERNANDT and GRANIT (37)]. For this reason the pure on-elements have been called *cathodal*. The impulse frequency-log intensity curves of the on-elements generally rise less steeply than those of on/off-elements and soon reach a plateau at a relatively low maximum of around 100 impulses per sec. (49). If all wave-lengths in a spectrum are adjusted for equal bleaching of visual purple, then this relation for pure on-elements, in terms of impulse frequency is independent of wave-length at any constant multiple of the visual purple threshold (55). This fact shows that in the cat's eye on-elements hardly can play an important rôle in wave-length discrimination. The only complication noted in the on-elements is post-excitatory inhibition (49, 50), a kind of depression of their excitability at the end of a discharge. Post-excitatory inhibition, when present, increases, within limits, in duration with an increase in stimulus strength or stimulus duration (50). This phenomenon is also seen in the other types of element, Fig. 4.

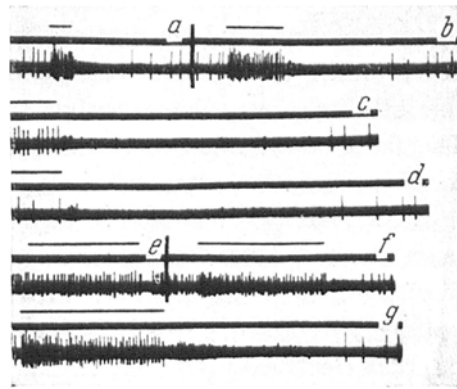


Fig. 4. Post-excitatory inhibition in eye of guinea pig. Micro-electrode. Records *a—d* show effect of duration of stimulus of 600 m.c., *a*, about 0.3 sec., *b*, 1 sec., *c*, end of exposure of 5 sec., *d*, end of exposure of 20 sec. Records *e—g*, effect of stimulus intensity. *e*, 20 m.c., *f*, 150 m.c., *g*, 500 m.c. [GRANIT: Vet. Akad. Ark. Zool. 36 (1945).]

In summarizing briefly the properties of the pure on-elements or, indeed, of any elements I would like, once and for all to draw attention to the statistical nature of all work with isolated units. Only experience, as it grows, can compensate for this factor. With this reservation I want to conclude that the pure on-element is a simple convergence structure of the type shown in POLYAKS fig. 2 (above), characteristic for rods and that its main task in vision is to integrate a general „scotopic white“ corresponding to the visual purple photosensitivity. At higher intensities it may also contribute somewhat to the background of photopic white.

The off-elements. The response to cessation of illumination is such a remarkable property of the retina that it is necessary to consider the theoretical aspect of it from the very beginning. In doing so we are faced with two possibilities: (I) the off-effect could be produced in the neural network of the retina in the manner of reflex rebound, well known from SHERRINGTONS (113) work on the spinal cord; (II) alternatively there may be special receptors inhibited by light and discharging to the removal of the stimulus.

Inhibition of the off-response by re-illumination was first demonstrated by GRANIT and THERMAN (59, 60) and soon afterwards confirmed by HARTLINE (65, 66) when, in the frog's retina, he isolated the pure off-elements. Since

we have every reason to assume the stimulus to influence the retina by way of changes of potential across the retinal structures one would, on this view, expect the off-receptors to set up potential differences of opposite sign to the changes of potential characterizing pure on-elements. By throwing in flashes of light timed to fall on top of the off-effect GRANIT and THERMAN (59, 60) actually showed that the heavy inhibition of the off-discharge, thus elicited, coincided with a potential change (component potential PIII of the ERG) of opposite sign to the one that had been shown to coincide with excitation [component potential PII of the ERG (40)]. This argument, that inhibition and excitation are correlated with opposite changes of potential in the retina, can be adapted to either theory of explaining the off-discharge, the receptor theory as well as the synaptic theory. The facts at the moment seem to be of greater importance than the theory to which they are attached.

HARTLINE (67) has studied an invertebrate retina, that of PECTEN, which possesses two layers of receptor cells. The one only responds with an on-discharge, the other one only with an off-discharge. A retinal response has not been obtained. This argument, too, is plastic since the receptors may interact electrically (cf., for instance, in the manner postulated by ECCLES [see BROOKS and ECCLES (19)] important theory for central inhibition), may be connected by synapses or may, on the other hand, act separately and possess receptors which are directly silenced or activated by light. In favour of a synaptic localization of the inhibitory process is the fact that in *Limulus*, on the distal side of the optic ganglion, on/off-responses are obtained instead of the pure on-responses from its retina (138).

There is a recent interesting paper by PARRY (100) on the ocellus of a locust (*Locusta migratoria migratoroides*). This organ responds to illumination by increased polarization that can be recorded as a positive electrotonic component from the ocellar nerves. At cessation of illumination this change is reversed, the ocellus becomes *depolarized* with an overswing, and a negative electrotonic component can be recorded from the ocellar nerves. Neither change was found to be accompanied by any impulse activity in the ocellar nerves but the polarization, due to the light and transmitted electrotonically to the next station in the ganglion cells, discharged a burst of impulses from the latter down the circum-oesophageal commissural path when the positive response reversed to negative, that is, at cessation of illumination. Here then is a clear case of polarization of a sense organ by illumination instead of depolarization. From what is known about the properties of nerve excitation this would be expected to silence an active ganglion (if it can reach it) whilst, similarly, the ensuing depolarization at 'off' would be expected to activate the ganglion. The distance between ocellus and ganglion, bridged by the exceptionally large (25μ) ocellar nerves, is only 1 mm.

PARRYs observation provides the first definite evidence for the existence of sense cells which are polarized by light. The receptors of LIMULUS (70) and DYTISCUS (10) are *depolarized* by illumination.

On the strength of PARRYs evidence it is conceivable that there might be two kinds of sense cells in the vertebrate retina and that the opposite component potentials PII and PIII of the electroretinogram which have been interpreted as truly opposite potential changes (56, 52) arise because of the existence of two systems of opposite polarity. On the other hand, this evidence refers to a photoreceptor of a very primitive type.

Inhibition is such a well-known property of the central nervous system, of which the retina is a part, that one must seriously consider synaptic inhibition in the eye. Even though the precise mechanism of synaptic inhibition is unknown — and it is impossible to review this problem here — the safest statement at present seems to be that the retinal types of inhibition, the pre-excitatory and probably also the post-excitatory form, belong to this class of phenomena. The existence of opposite component potentials of the electroretinogram, of which PII has been shown to be connected with excitation and PIII found to be dominant when the chief effect of light is inhibition (as when a flash is thrown in on top of the off-effect), suggests interesting correlations with the central nervous system which have been discussed at some length in my recent summary (52) as well as by ECCLES (19).

Finally the polarization test has given new information. Whilst the pure on-element is excited to discharge by cathodal polarization and is inhibited by anodal polarization, the pure off-element has opposite properties (37, 53). It is excited by the anode and inhibited by the cathode. Simultaneous tests with illumination have added to this distinction and it will be discussed in detail below when we have become acquainted with the on/off-elements. In several types of test their off-components behave like the pure off-discharges. Both with the off-component of the electroretinogram (52) as well as with that of the nervous discharge (66, 52) it has been observed that the light has to shine for some time in order to elicit an off-effect. The eye therefore seems to be somehow prepared by the inhibitory process and by it brought into such a state that it can respond at 'off'. However, in the cat's eye several pure off-elements have been found to respond to the very briefest flashes of light.

The on/off-elements. These together with the pure off-elements seem to be in the majority in any eye containing cones. It would be premature to conclude from this fact that off-components necessarily presuppose cones. Measurements carried out over several years have shown that even some pure off-elements merely reproduce the visual purple distribution of sensitivity in the dark adapted eye (28, 58) and, at least in the eye of the dark adapted cat, all on/off-elements show the influence of visual purple upon the distribution

of spectral sensitivity in the dark (28, 52). We have reason to believe that some on/off-elements actually consist of rods alone (28). On the other hand, off-components are more prominent in eyes with cones (58). We shall return to this question below. But it is clear that, since the mechanism of colour contrast must be organized by a system capable of responding at 'off', there are strong *a priori* grounds for assuming the cone system to be the one that particularly has specialized on the developmental possibilities inherent in an organization capable of signalling both onset and cessation of light. Such a system, utilizing both excitation and inhibition, must do what co-operation between similar processes of opposite sign are doing for fineness of function elsewhere in the central nervous system, *i. e.* further discrimination.

The on/off-elements respond to increasing stimulus intensity by frequency variations within a greater range than the pure on-elements (49). In guinea pigs the latter only reached a frequency maximum of 100 impulses per sec., counted per 1 second. (By measuring the least interval between two consecutive impulses at the top of the time-frequency curve one may, of course, reach higher values.) With on/off-elements I have measured (for 0.1 second) values above 300 impulses per sec. For 'white' light the frequency-log intensity curves differ a great deal from element to element in the cat, and most elements lose the main part of their on-component in the high intensity range as if, at 'on', inhibition took precedence over excitation. This was noted by ADRIAN too (2) in records from the whole optic nerve suggesting that this type of element actually is in the majority in cats. In the optic nerve of the monkey the on-components were better preserved at higher intensities (cf. the frog, 66). In the cat some elements tend to lose their off-components in the high intensity range and quite often one finds elements for which either component is suppressed at some intermediate light intensity and again reappears as stimulus intensity is increased. This suggests that receptors of different spectral sensitivity and threshold take part in the make-up of an element leading to variations in the balance of excitatory and inhibitory influences as the light increases in strength. We shall return to this question below.

The off/on-ratio. An interesting property of the on/off-elements is their off/on-ratio (58, 34, 35, 37); this is the ratio of off-sensitivity to on-sensitivity at the threshold of the fully dark adapted eye. (Since threshold energies are the reciprocals of the sensitivities the figures for the off/on-ratios give the on/off-ratio in terms of energy.) This is best understood by imagining a threshold measurement carried out with gradually increasing stimulus intensity. In some elements the on-component turns up first and the intensity has to be increased in order to evoke an off-component. Vice versa, there are elements for which the off-component is more sensitive and the on-component turns up at higher intensities. This range of variation is enormous

and, in a sense, one may say that the off/on-ratio is infinitely high with the pure off-elements and zero with the pure on-elements. But among the elements that clearly behave as on/off-elements some may be 1000 times more sensitive at 'on', others 1000 times more sensitive at 'off'. The largest collection of data has been assembled by GERNANDT (34) from whose work fig. 5 is taken. It refers to 196 elements tested with green (5200Å), 105 with red (6500Å) and 100 tested with blue (4600 Å) light. Of these 72 were tested with all three lights.

It was pointed out above that in the polarization test the pure on-elements were cathodal, the pure off-elements anodal. The on/off-elements were anodal or cathodal (37, 53) and in fig. 5 the white columns refer to anodal elements,

the black ones to cathodal elements. In some on/off-elements polarity can be changed by light adaptation (29) showing that these elements contain both anodal and cathodal components. It also is seen in fig. 5 that polarity to some extent is a function of wave-length. With the green test light the cathodal elements tend to group themselves among those of lower off/on-ratio and, similarly, though less markedly so, for

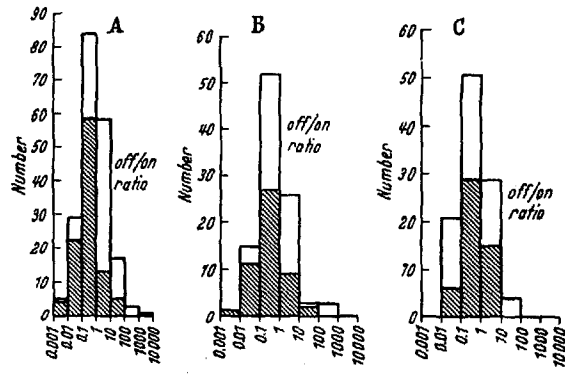


Fig. 5. The ordinates show the number of elements present within certain ranges of off/on-ratios, which are grouped in logarithmic units on the abscissae. A Green, B Blue, C Red. [GERNANDT: Acta Physiol. scand. (Stockh.) 15 (1948).]

the blue test light. With the red test light which (in 6500 Å) very little if at all stimulates visual purple receptors the off/on-ratio seems to be distributed in a more symmetrical fashion over anodal and cathodal elements. Considering that the pure on-element is cathodal, GERNANDT suggests that in dark adapted cats the correlation between cathodality and low off/on-ratio or high on-sensitivity depends upon the same factors that make the pure on-elements cathodal. Since these by several criteria are rods it is probably a greater contribution of rod-activity (the visual purple maximum being in the green) that tends to make certain on/off-elements of low off/on-ratio cathodal.

According to this view there must be some correlation between wave-length and off/on-ratio. In a large number of elements, measured at the threshold there is none. The off/on-ratio is practically constant in the spectrum of the dark adapted cat retina suggesting visual purple dominance. But it is quite common, too, to find elements that vary and then (most of them — though not all) tend to vary in the fashion shown by fig. 6 ([GERNANDT (35)]. If the off/on-ratio, instead of being measured on a threshold basis, is given as the ratio of impulse frequencies at some supra-threshold level of the dark

adapted eye, one often obtains the same type of curve (55) provided that all spectral energies are adjusted for equal visual purple absorption so as to keep this factor constant. Thus, towards the ends of the spectrum, very many elements tend to become more off-sensitive, more so in the red than in the violet. Since this takes place with the spectral energies adjusted for equal stimulation of visual purple it is clear that other receptors must have been activated at the two ends in the red and the violet. There is, to be sure, a general correlation for all colours in the sense that the general level of the off/on-ratio *at the threshold* is maintained throughout the spectrum. A highly off-sensitive element belongs to that category whatever the stimulating wave-length of light may be. The threshold variations with wave-length are on a smaller scale and take place within the range of the general effect. But at

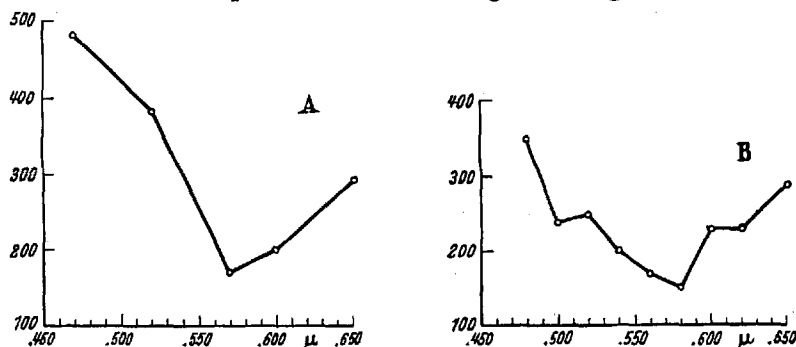


Fig. 6. Average off/on-ratios of 8 (above) and 7 (below) elements as a function of wave-length. For the individual curves averaged in A' and B the minimum off/on-ratio was given the value of 100 and for the other wave-lengths the values were given as multiples of 100 so as to make it possible to average off/on-ratios from different experiments. [GERNANDT: Acta Physiol. scand. (Stockh.) 17 (1949).]

higher levels of intensity the variations in off/on-ratio with wave-length become dominant showing, as so many other facts, that much as one would like to establish a physiology of the retina on the basis of the far more convenient experimentation with white light alone this is impossible and means deliberate neglect of one of the most important variables in the organization of the retinal elements. Sooner or later all types of experiment lead to the problem of wave-length reception.

It is being claimed by animal psychologists that our best experimental animal, the cat, is lacking colour vision. A wealth of experimental experience with the retinal elements, to be reviewed below, shows that cats definitely possess receptors with sensitivity optima located in three preferential regions far apart, so that they must have some of the fundamental peripheral mechanisms necessary for colour vision. Personally I fail to see how an experiment, based on animal behaviour, ever could exclude more than that the messages from certain types of receptors play a dominant rôle in an animal's pattern of behaviour. For such reasons an animal may refuse to co-operate in the experiments. Considering the complex nature of behaviouristic experiments it is curious that so much attention should be paid to a negative result. In far simpler experiments one is rightly cautious in drawing conclusions from negative answers.

Returning to the variation in the off/on-ratio at the absolute threshold of the dark adapted eye it is necessary to realize that it is impossible to ascribe

it merely to threshold variations in the receptors. The eye is fully dark adapted and in that state all elements (and by now a very large number has been measured in the course of various experiments) show the influence of the visual purple spectral distribution of sensitivity. Under such circumstances the range of variation in the off/on-ratio from element to element must be chiefly due to a variation caused by the interneurons of the retina [GRANIT and TANSLEY (58)]. The polarization technique to which we now will return has proved this view to be correct.

Polarity of the retinal elements. Polarization of the retina was used for analytical purposes already by WALLER (131, 132) in an early study of the electroretinogram and RENQVIST (106) showed that the slow phase of the retinal response could be removed by these means. Already 130 years ago PURKINJE (103) and others around the same period tried to investigate the effect of polarization of the eye upon our colour sensations and, indeed, found a general effect, the cathode tending to introduce phosphenes from the long wave-lengths, the anode from the short wave-lengths. A review of this work has been given by SCHAEFER (111). The most important and comprehensive study of the effect of polarizing currents on colour vision we owe to the eminent psychologist G. E. MUELLER (98). The differential effect of anode and cathode on short and long wave-lengths was confirmed. The most recent contribution to this aspect of the problem is due to KRAVKOV and GALOCHKINA (81) who found that the spectral sensitivity to short wave-lengths was increased by the anode, that to long wave-lengths by the cathode. From other points of view the effects of polarization on the human eye have been studied by a group of French workers (17, 18).

Our own work began in 1939 with a study of the effect of polarization on the electroretinogram (56). I tried, at that time, but did not succeed in recording by a micro-electrode from the frog's retina during polarization. Nor have I since found this possible. In this small eye the current upsets the baseline of the recording instrument. Not until the cat's eye became our standard preparation did it prove possible to study the response of single elements during polarization (51). In the meantime BERNHARD, GRANIT and SKOGLUND (11) had polarized the optic ganglion of the waterbeetle, *Dytiscus marginalis*, and noted that both the anode and the cathode had excitatory and inhibitory effects. SKOGLUND (115—117) then took up polarization of the vertebrate spinal cord and made the valuable observation that extensor reflexes were elicited more easily by the anode, flexor reflexes by the cathode. In pursuing this problem he found that LOEB (86) previously had made similar observations with invertebrates. GERNANDT and I (37) then undertook a systematic study of the effects of polarization on retinal elements of different type. We found, as pointed out above, that the on-elements were excited by the cathode and inhibited by the anode whilst the off-elements were excited by the anode and inhibited by the cathode. At cessation of polarization there was reversal of these effects. The on/off-elements were anodal or cathodal, as pointed out above. GERNANDT (32) first studied the effect of polarization upon the spectral thresholds of single elements and found the on-elements to be relatively insensitive whilst in the on/off-elements the thresholds for certain wave-lengths were selectively facilitated or inhibited by polarization. I shall return to this question in connection with the problems of colour vision (p. 62).

The polarity of the retinal elements (fig. 7) and, in particular, the fact that the on- and the off-elements are stimulated by opposite poles must reflect some fundamental properties of organization. Measurements of the light thresholds during polarization have given interesting information (53): we define as the rheobase the minimum current necessary for the element

to discharge in response to polarization. Then polarization strength can be given in multiples of the rheobasic current. Similarly the changes of the light threshold can be given in multiples of the threshold *before* polarization.

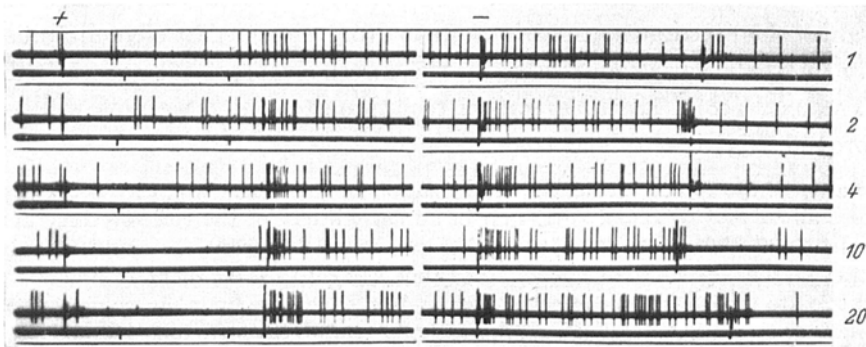


Fig. 7. The effect of currents of opposite direction on cathodal element. Increasing current strength (right) in terms of multiples of rheobase (uppermost record). Onset and cessation of electrical stimulation clearly shown by shock artefacts. One second marked by dots below each record. [GRANIT: J. Neurophysiol. 11 (1948).]

Fig. 8 shows what has happened to the light threshold (the off-component of an anodal on/off-element and a pure (anodal) off-element) during cathodal polarization which, as stated above, would be inhibitory for anodal elements.

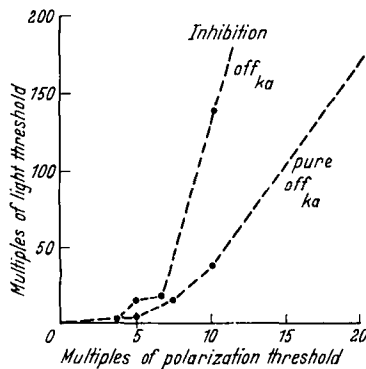


Fig. 8. Plot of increase of light threshold (inhibition) for wavelength 5200 Å against polarization strength for the off-component of an anodal on/off-element and for pure off-element, both polarized cathodally. [GRANIT: J. of Neurophysiol. 11 (1948).]

The light threshold is relatively little influenced by modest polarizing currents but above 5 rheobases the curves rise at a rapid rate demonstrating that the light threshold of the off-component has been greatly raised by the passage of current through the retina. This heavy suppression of the off-component by the polarizing current can be studied in the actual records of fig. 9. There is first, in record 1, the normal response of the element to onset and cessation of illumination, this time at a suprathreshold intensity. In record 2 the polarizing current (20 rheobases) has somewhat diminished the on-component but completely suppressed the off-response. Records 3 and 4 show respectively normal control and effect of

polarization for a 10 times stronger illumination. Facilitations are rarely as marked as inhibitions but fig. 10 shows a case of striking facilitation in a cathodal on/off-element.

It should now be realized that such effects of polarization upon the light threshold have not been obtained with the pure on-elements (53). There may be a small just measurable rise or fall of the threshold corresponding to the early part of the curve of fig. 8 but the large effects have not been found

with pure on-elements, neither by GERNANDT (34—36) nor by myself (53) working independently. Therefore the effects of polarization can hardly be directed towards the straight forward path from receptor over bipolar to

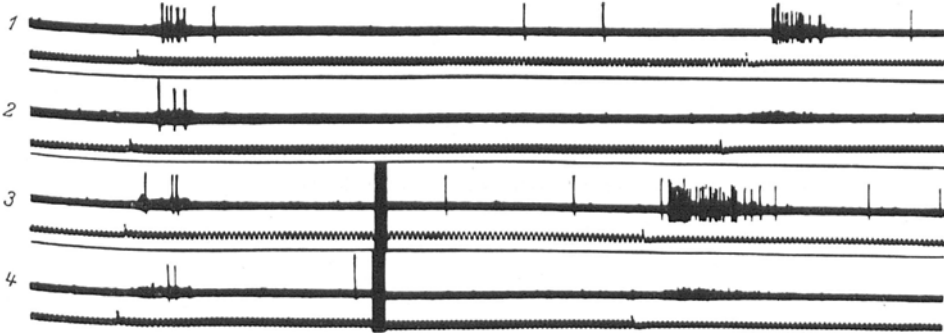


Fig. 9. Anodal element. 1, normal response to illumination; 2, same during cathodal stimulation; 3 and 4, similar pairs but for 10 times stronger illumination, as fully explained in text. About 1 second cut out in the middle of records 3 and 4. Period of 50/sec. marked on line below record. Beginning and end of stimulation with light as artefacts on time base. [GRANIT: J. of Neurophysiol. 11 (1948).]

ganglion cell. Several cathodal on/off-elements have behaved like the pure on-elements suggesting that these too must have been deficient in the specific structures necessary for an effect of polarizing currents upon the light thresholds. These structures must therefore be localized to interneurons. Furthermore, the effects upon the light thresholds are more often than not highly selective in the sense that sometimes the on- sometimes the off-component alone is affected. Nearly always there is great asymmetry in the effects on these two components of the light response. In particular the anodal elements (pure off- and on/off-elements) seem to contain an abundance of structures sensitive to polarization but there are some cathodal on/off-elements that behave in a similar fashion, merely displaying opposite effects on account of their opposite polarity, facilitation at the cathode and inhibition at the anode (note that the inhibition was cathodal in the anodal element of fig. 8).

From another point of view the structures, assumed to be interneurons, which asymmetrically influence the light thresholds of on/off-elements during polarization, may be called structures maintaining the off/on-ratio or at least largely responsible for this variation. The reason for this conclusion is the fact that polarization may be described as a method of changing the off/on-ratio of an element (see figs. 9 and 11). I have actually seen elements with a normal threshold off/on-ratio of 0.008 (in the dark) during polarization

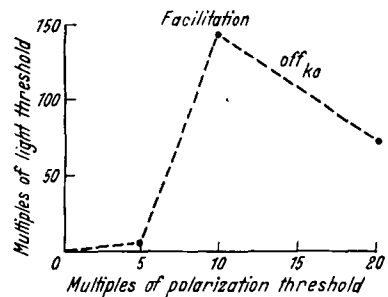


Fig. 10. Same as in fig. 8 but this time decrease of threshold (facilitation) for the off-component of a cathodal on/off-element during cathodal polarization. [GRANIT: J. of Neurophysiol. 11 (1948).]

acquire an off/on-ratio of 1.0. In view of such facts it would seem to be necessary to conclude that the off/on-ratio already at the threshold is

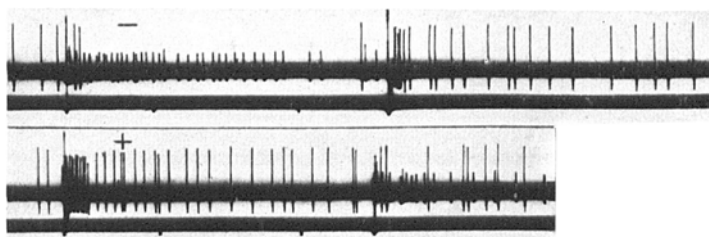


Fig. 11. A large and a small spike isolated by the same micro-electrode. Stimulation between the two clearly visible shock artefacts with nasal electrode cathode (above) and anode (below). One second marked on lower line in the middle of each period of stimulation. Note opposite responses of small and large spike during and after polarization. [GRANIT: J. of Neurophysiol. 11 (1948).]

maintained by some interaction pattern determined by internuncial neurones which, indeed, even may expend energy on the upkeep of a particular

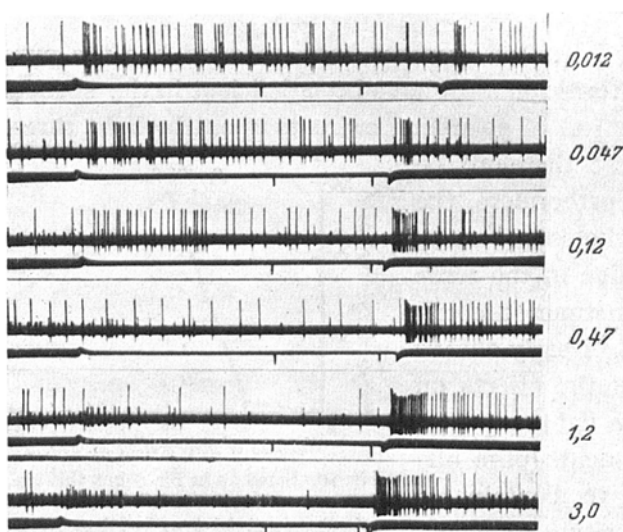


Fig. 12. The large and small spike of fig. 11 elicited by illumination with wave-length 4600 Å at the relative intensities indicated beside the records. Period of illumination marked by photocell and amplifier connected to second beam of cathode ray below the one recorded from. This beam also records the 50-period A.C. of the mains but, at this film speed, so compressed that the duration of 1 second has been indicated by separate marks below it. [GRANIT: J. of Neurophysiol. 11 (1948).]

state of balance between excitation and inhibition. The polarization experiments may therefore be said to have delivered the formal proof of the statement, made above, that the off/on-ratio is determined by the interneurons of the retina.

Just as polarization influences the light threshold so also does illumination at about 1000 time threshold sensitivity change the threshold for polarization effects (29, 51), generally by raising it. After illumination the polarization threshold

quickly returns to normal while it may last a very long time before the light sensitivity of a dark adapted eye has returned to its original value.

In order to summarize the effects of polarity on the on/off-elements and at the same time obtain some idea of the changes in off/on-ratio with stimulus intensity we can study fig. 11 and fig. 12. It is a case in which two spikes have been localized by the same micro-electrode. In the upper record of

fig. 11 the small *cathodal* spike is stimulated by the cathode and inhibited at 'off', the large *anodal* spike is inhibited by onset of polarization and activated at cessation of polarization. For the lower record the polarization current has been reversed. Now the large anodal spike is excited by onset of polarization and the small cathodal one inhibited. At 'off' the small spike is accelerated and the large one inhibited. The same pair of spikes in the same experiment was next tested by stimulation with *light* (fig. 12). The light intensity increases from record to record downwards in the figure. For both elements a complete shift of off/on-ratio takes place when the light is made stronger. The large anodal spike has a low off/on-ratio at the low stimulus intensity and an infinitely high off/on-ratio at the high intensity. It is now completely inhibited by the onset of illumination. At this high intensity the small cathodal spike takes over signalling to onset of illumination. It is as if the low light intensity in this particular case had represented an anodal stimulus, the high intensity a cathodal stimulus. The elements of opposite polarity seem to be part and parcel of a system organized for a definite purpose. The presence of slow opposite potentials in the retina adds significance to this fact. Perhaps, at this point, attention should be drawn to POLYAKS centrifugal bipolars as a possible explanation of the opposite polarity of the off-element.

It will probably require years of research before the full significance of all these facts in the organization of the retinal elements are fully understood. Already, however, it seems clear that a variation in off/on-ratio from the point of view of vision must enhance discrimination, representing, as it does, a remarkable device for changing pattern with stimulus intensity. In addition GERNANDT (35, 36) has found that, in general, the more sensitive component is the first to be attacked by adaptation to light. It is, so to speak, the reacting component of the element, a tentacle stretched out towards the energy variation called light. In studying colour thresholds he also found that there was a negative correlation between green-sensitivity in the on-component and red-sensitivity in the off-component signifying that the elements that were specialized on responding to red at 'on' did not in general combine this property with a response to green at 'off' whilst between red and blue or green and blue no such correlations were found.

2. The organization of colour reception.

Historical comments. The electrophysiological analysis of colour reception has a fairly brief history. I have reviewed it elsewhere (52) and pointed out that the early period of research did not succeed in advancing beyond the, as such, important conclusion that there were some differences in the electrical response of rods and cones, as recorded by the electroretinogram, and that, by this index, there was a PURKINJE shift in consequence of a change in the state of adaptation. Equal energy spectra were not used and for this reason the PURKINJE shift could not be quantitatively measured. Differences between rods and cones need not mean differential colour sensitivity.

In 1935 GRAHAM and HARTLINE (38) studied the horseshoe crab using the single fibre preparation and six colour filters. There was, to be sure, a slight variation between the fibres analyzed and the authors concluded that it would fit in with a colour theory along the lines of HECHT (72) in which all colour-primaries are assumed to have practically the same spectral distribution of sensitivity. Colour discrimination, on the theory, is the result of this slight variation around the mean. However, this is not the way colour reception is organized to judge by all our work on the vertebrate eye in which, in contradistinction to the results on the horseshoe crab, there are definite maxima in three regions of predilection rather far apart in the spectrum. Our results, to be reviewed below, have put HECHT'S theory out of court. This theory also has not found any support in the recent experiments on the human eye which rather uniformly support the view of sensitivity maxima in different regions of the spectrum [see e.g. STILES (118, 119), WRIGHT (139, 140), WALTERS (134), WALTERS and WRIGHT (135), THOMSON (122, 123), JUDD (75), HARTRIDGE (71)]. Not only is there no evidence in favour of HECHT'S theory but it also would seem to require central properties of unimaginable fineness to evaluate messages which hardly differ at all at the primary station of reception. *Mutatis mutandis* this holds good for the crab eye too.

Our own attempt to investigate colour vision began in 1937 with a study of the electroretinogram [GRANIT and WREDE (62)] in which it was established that the light adapted frog's eye in addition to visual purple and some photopic substance with maximum around 5600 Å, contained a special blue-sensitive substance, as confirmed by GRANIT, THERMAN and WREDE (61). I next tried selective adaptation on the electroretinogram and demonstrated that the red end of the spectrum was selectively depressed by red pre-adaptation whilst the green part of the spectrum maintained itself with green pre-adaptation. It was thus established that there must be some substance in the long wave-lengths in addition to the substance in the short wave-lengths and also something in the green region. The experiments on selective adaptation were published in a review written together with Dr. W. D. WRIGHT (141). All these experiments were carried out with the frog's eye in which rods and cones occur in about equal proportion. Next followed the experiments with micro-electrodes [GRANIT and SVAETICHIN (57)] on the frog eye confirming the conclusion that there are colour sensitive substances with maxima in different spectral regions. The final development of this work lasted several years and was carried out with a variety of animals utilizing both direct methods based on plotting of the curves as recorded by the micro-electrode technique as well as indirect methods such as selective adaptation and polarization. The micro-electrode technique extended the scope of analysis, particularly since, in the last four years, it was improved so as to make it possible in the best experiments with the cat's retina to maintain the same element under the electrode for 4—5 hours.

The scotopic dominator. Visual purple being the best known of all the substances in the retina it seemed reasonable to expect its absorption curve to be represented in the responses recorded by the micro-electrode from dark adapted retinae containing rods. The work of KÖNIG (84) and TRENDELENBURG (124) had made its absorption spectrum known. The first and most accurate measurements from recent times we owe to the late R. J. LYTHGOE (88). Somewhat later KRAUSE and SIDWELL (80), CHASE and HAIG (23), SAITO (110) and WALD (127) published their measurements and the values of some of these workers have been averaged by HECHT, SHLAER and PIRENNE (73) who, besides, have tried to arrive at some idea about the actual concentration of visual purple in the human retina. It should be noted that light absorption is a quantum affair whilst the spectra, as a rule, are calibrated for equal energy. Comparisons with actual photochemical data therefore

presuppose a minor correction for equal quantum intensity which, of course, can be derived exactly from the spectral energy data [DARTNALL and GOODEVE (26)].

All micro-electrode work has, in fact, shown with great uniformity that if an eye contains visual purple, then, in the state of dark adaptation, its absorption spectrum is reflected in the curves obtained (see e.g. summary 52). Besides, this has been demonstrated with the electroretinogram (52). In eyes containing visual violet, this substance takes the place of visual purple (44, 52). The question is now with what accuracy the curve for visual purple photosensitivity is reproduced by threshold measurements with isolated elements. Our curves are always measured in terms of reciprocal of quantum intensity necessary for an audible acceleration of impulse frequency. The pure on-elements are least likely to be influenced by retinal interaction and their great number in the rod-retinae of guinea pigs (46) suggests that such elements also are likely to give the purest visual purple-rod response obtainable.

It should be noted that in thus measuring the threshold of any element in different wave-lengths it is imperative to allow for any change in the general level of sensitivity. This may be done by measuring between every second or third reading the threshold for some fixed wave-length near the maximum of spectral sensitivity. Thresholds at each stage of the experiment are then expressed as multiples of the control threshold obtained at that moment. All our experiments on the spectral properties of retinal elements the earlier ones as well as the more recent ones, have been carried out with a calibration wave-length and they have all been threshold measurements. If these conditions are not adhered to, any sort of result may be obtained.

Our best individual curve (on-element of scotopic cat) is shown in fig. 13 and four curves are averaged in fig. 14, plotted on a logarithmic basis to show the base of the curve more clearly [DONNER and GRANIT (28)]. The small black dots represent the average curve for visual purple in solution (73). Some of our earlier curves were almost equally good (52).

These curves illustrate the spectral distribution of sensitivity of the so-called pure *scotopic dominator*. They are seen to be somewhat narrower than

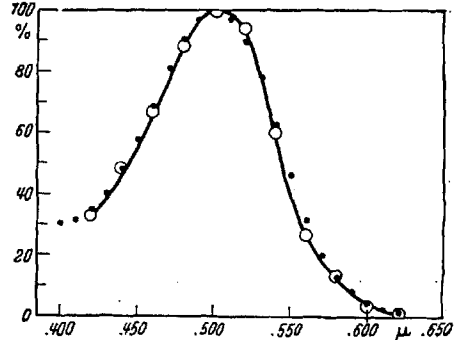


Fig. 13. Dots: in this and figures to follow, standard visual purple curve. Curve drawn through the readings: spectral distribution of scotopic sensitivity of pure on-element. In this and all other figures the spectrum is corrected for equal quantum intensity. [DONNER and GRANIT: Acta physiol. scand. (Stockh.) 17 (1949).]

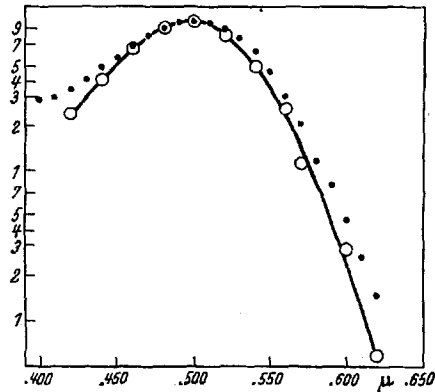


Fig. 14. Average distribution of scotopic sensitivity of four pure on-elements compared with the V.P. curve. Logarithmic ordinates. [DONNER and GRANIT: Acta physiol. scand. (Stockh.) 17 (1949).]

the visual purple curve. A very great number of measurements have now been made on various elements in the scotopic cat's eye. All of them show the influence of the scotopic dominator, a fact which is in agreement with

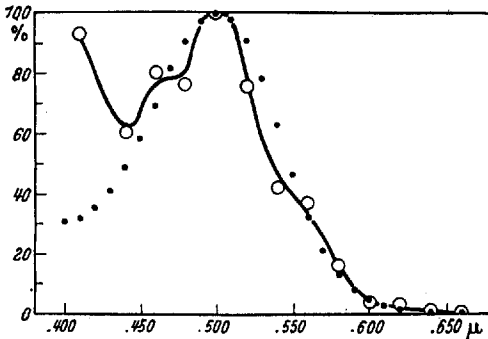


Fig. 15. Averaged scotopic values for on/off-components of an on/off-element. [DONNER and GRANIT: *Acta physiol. scand.* (Stockh.) 17 (1949).]

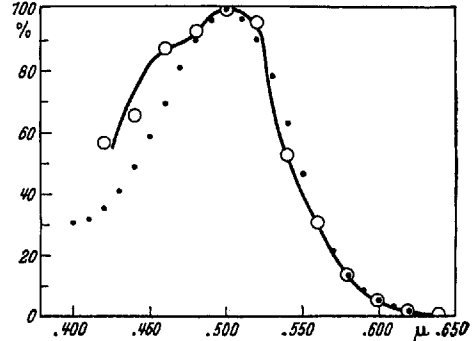


Fig. 16. Averaged scotopic values of on/off-components of a relatively off-sensitive element observed during $4\frac{1}{2}$ hours. [DONNER and GRANIT: *Acta physiol. scand.* (Stockh.) 17 (1949).]

the convergence of rods and cones upon the same ganglion cell as demonstrated by POLYAK (101). Several on/off-elements also give the curve of fig. 14 with the same degree of purity as the pure on-elements. Apparently then, these are also relatively pure rod-elements or, if they contain cones, these do not occur in sufficient number to cause any deviation from the scotopic dominator (28, 53).

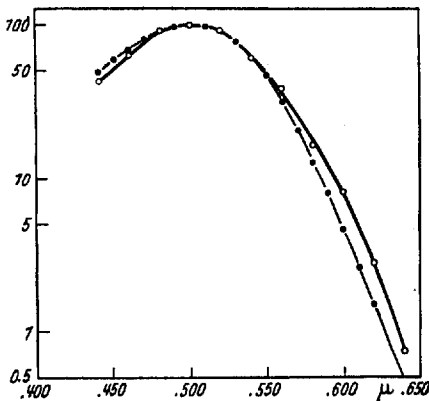


Fig. 17. Logarithmic plot of average scotopic curve from the work of GRANIT (1945). The thin line joins the dots representing the V.P. standard. [DONNER and GRANIT: *Acta physiol. scand.* (Stockh.) 17 (1949).]

However, a certain number of on/off-elements do not reproduce the scotopic dominator in the relatively pure state in which it is found in fig. 14. They very definitely show the influence of something else. Specimens are given in figs. 15, 16 and 17. There are humps in both the long and short wave-lengths, particularly interesting the hump that sometimes is seen as far out as at 4200 \AA . Humps in the red are quite common, as are also humps in the blue around 4600 \AA .

It is possible to show that these humps represent a differential sensitivity to light because modest light adaptation may make them more prominent than they were in the dark. Examples are given in the two curves of figs. 18 and 19, before (curves drawn in full) and after (curves in broken lines) adaptation to a neutral light of 800 m.c., diminished by a neutral filter of density 2.4.

What is the explanation of these humps? By labelling the differentially sensitive components (contributing to the convergence structure recorded from) cones we have merely given them a different name. The final explanation must always be in terms of photochemically sensitive compounds. The idea that breakdown products, formed by visual purple, by serving as internal filters may shift its spectral sensitivity owing to selective absorption [DARTNALL (25)] encounters the difficulty that the humps are seen at the absolute threshold in the state of complete dark adaptation without any breakdown of visual purple. There is the further difficulty that the humps occur in both the short and long wave-lengths making a theory based on decomposition products of visual purple not only improbable but also of questionable value as an aid to experimentation because it becomes necessary to invent additional mechanisms to explain the nice adjustment and maintenance of these internal filters at appropriate levels of density. Nevertheless DARTNALL's work is of value as a quantitative attempt to examine a definite set of ideas.

Since only one photochemically active compound, visual purple, is known in the retina it would seem to be fundamentally sound to enquire whether this compound is plastic enough to undergo the chemical transformations necessary for shifts in its absorption curve. The humps would then be due to actual variations in the chromophore or its linkage with the proteins of the receptors (7, 52). I shall end this summary by considering the evidence for this view. The conclusions will be easier to understand when some further facts have been presented.

The photopic dominator (fig. 20). If a retina contains a sufficient number of cones good light adaptation, neutral in colour, shifts the spectral sensitivity of most of its elements towards the red end and the maximum now occurs at 5600 Å. This, in terms of retinal elements, is the equivalent of the PURKINJE shift of the human luminosity curve. In the laboratory animals such as the cat it is quite clear that the same element, depending upon the state of

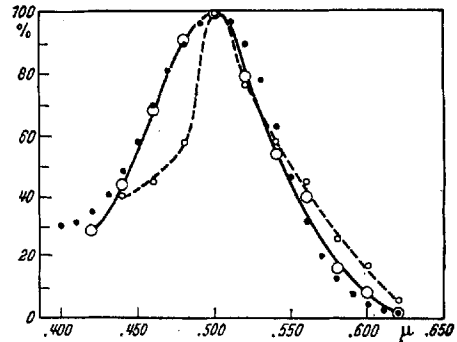


Fig. 18. The scotopic curve, (drawn in full, refers to the off-component of a slightly more off sensitive on/off-element. The dotted curve represents the properties of the same element after modest light adaptation, as explained in the text. [DONNER and GRANIT: Acta physiol. scand. (Stockh.) 17 (1949).]

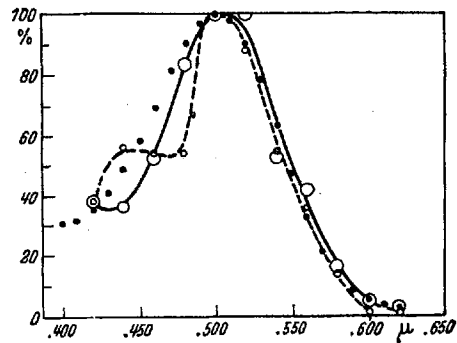


Fig. 19. Same experiment as in fig. 18 with another element. [DONNER and GRANIT: Acta physiol. scand. (Stockh.) 17, (1949).]

adaptation, can serve both as scotopic dominator with maximum at 5000 Å and as photopic dominator with maximum at 5600 Å. Photopic dominators are easily found in frogs (45) but not in the eyes of rats and guinea pigs (43, 46) which have a very modest number of cones. In the cat only 36% of the elements investigated gave the full shift to the photopic dominator (48). In the rest the number of cones probably was insufficient since the maximum only shifted to about 5200 Å with a secondary hump at 5600 Å, or it remained at 5000 Å.

This being so it would be of great value to find a pure cone eye. The snake retina proved to be useful. No scotopic dominator was ever found in

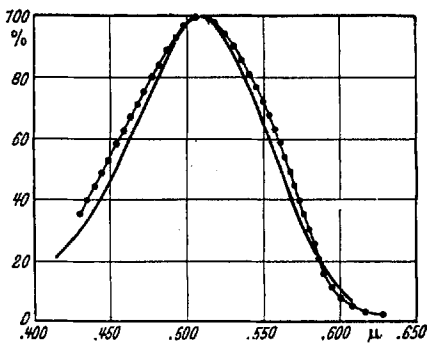


Fig. 20. Photopic dominator curves of frog and snake (-----). Equal quantum intensity spectrum. [GRANIT: Nature (Lond.) 151 (1943).]

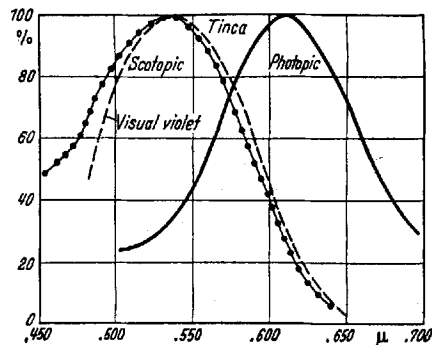


Fig. 21. Scotopic (left), photopic (right) spectral distribution of sensitivity for tench, the former compared with visual violet (see text). Spectra of equal quantum intensity.

this eye, but the photopic dominator was easy to demonstrate and found to be independent of state of adaptation (47). Photopic dominators are shown in fig. 20.

Recalling that visual violet (porphyropsin), discovered by KÜHNE and SEWALL (83), is a substance very similar to visual purple (rhodopsin) and that it occurs in certain fishes I studied a number of fish retinæ containing visual violet and also that of the fresh water eel which belongs to the ordinary visual purple type. In fig. 21 (left) the spectral absorption curve of visual violet (tench, *Tinca*), as determined by BAYLISS, LYTHGOE and TANSLEY (9), is compared with the electrically measured sensitivity curve and the agreement is seen to be good. The physiological curve is somewhat broader in the short wave-lengths. Whether this is due to fluorescent substances such as riboflavin [v. EULER and ADLER (30)], to impurities in the visual violet extracted by BAYLISS *et al.* or to some other cause is at present unknown.

Now GRUNDFEST (63, 64) had shown with the aid of behaviouristic tests that the sensitivity of the sunfish (*Lepomis*) after light adaptation undergoes a PURKINJE shift to the region around 6000 Å from its dark adapted porphyropsin maximum around 5300 Å. The spectra of the fish were somewhat distorted by what he held to be selective filters within the eye. An electro-

physiological analysis showed, indeed, that the sensitivity maximum of the visual violet system of fishes, after light adaptation, shifts towards the long wave-lengths by the same amount as that of the visual purple system so that the photopic dominator in porphyropsin eyes actually has its maximum around 6000—6100 Å (see fig. 21, right). The fresh water eel, a fish with its scotopic dominator around 5000 Å (visual purple), had a shift to only 5600 Å. Thus the photopic dominator, whether belonging to the rhodopsin or the porphyropsin eye, always occurs at a fixed spectral distance from the scotopic dominator. This being so, we may well ask whether, corresponding to the pure cone eye of the snake, there might not also exist pure cone eyes belonging to the visual violet system. I found the tortoise (*Testudo*) to be such a species (42, 44). Single elements were easily obtained in this eye and their maxima around 6000—6100 Å were independent of state of adaptation. It was, in fact, a curve identical with that of fig. 21 (right).

The results just mentioned are of particular interest for the theory of photo-reception because they very definitely suggest some chemical interrelationship between the substances responsible for the scotopic and photopic dominators. On the chemical side this fact is further emphasized by WALDS observation (125, 127, 128, 129) that visual purple, upon bleaching, ultimately is transformed into vitamin A₁ whilst visual violet is changed into vitamin A₂. Otherwise too the decomposition products of rhodopsin and porphyropsin at all stages are chemically similar and merely appear displaced symmetrically in the spectrum in various tests, the porphyropsin photoproducts, like vitamin A₂ and porphyropsin itself, being shifted towards the long wave-lengths.

In conclusion we may state that two photochemical systems are known in vertebrates, the rhodopsin and the porphyropsin systems, both represented in records from retinal elements by the micro-electrode technique with their respective scotopic and photopic dominators.

The modulators. In the eye of the light adapted frog (45) very much narrower curves than the photopic dominators were also obtained. Particularly definite was a narrow curve with maximum around 6000 Å and another one in the blue region around 4600 Å. Demonstration, in the frog's eye, of a narrow green curve with maximum around 5300 Å proved somewhat more difficult because incipient dark adaptation, tending to favour a change in the direction of the scotopic dominator, had to be avoided. These narrow curves were called *modulators*. With the intention of basing further analysis of cone eyes on simpler results with an eye in which rods would predominate I next took up the rat's eye with the micro-electrode technique (43). Greatly to my surprise, I found in this retina the red modulator at 6000 Å. In a certain number of elements good neutral light adaptation completely suppressed sensitivity in the rest of the spectrum (i. e. suppressed it below the instrumental threshold of the HILGER-TUTTON spectrometer then in use) so that

the red modulator remained alone in a practically isolated state. This red modulator, sometimes in combination with a narrow band in the region of visual purple, was the only indication of something that could not be referred to the rods which are assumed to respond by the rhodopsin distribution of sensitivity. At that time I had not yet made a systematical study of the sensitivity distribution of pure on-elements (see above) but the photopic curves with maximum at 5000 \AA were narrower than the visual purple curves of fully dark adapted rats. To prevent misunderstanding it should be realized that the early colour work with various eyes differs from the later experi-

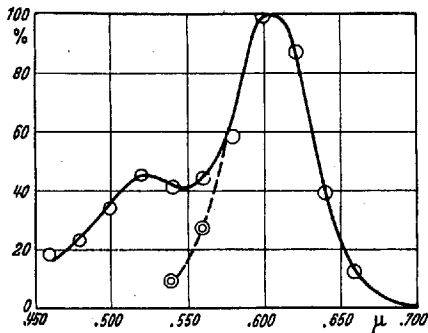


Fig. 22. Red modulator curve of snake. Averaged results of 7 series; (dotted line) averaged results in 2 series in which there was little or no hump in the green. Equal quantum intensity spectrum. [GRANIT: *Acta physiol. scand.* (Stockh.) 5 (1943).]

ments in not being limited to single spikes. This is clearly pointed out in my summary (52) as well as in the original papers.

This very definite evidence for a red modulator at 6000 \AA , occurring without a sign of a photopic dominator, was somewhat perplexing and suggested that the dominators could be compound curves in which several types of photopic receptors were thrown together into one element for activity in unison. Before investigating this point, however, it seemed necessary to obtain some information with the pure cone eye of the snake. This has high thresholds

(partly due to the urethane narcosis, possibly also due to the absence of visual purple) so that the blue end of the spectrum which in itself has a low energy content could not be included in the measurements. The red modulator at 6000 \AA was easily found. Generally it occurred together with a hump at 5200 \AA and there were all kinds of gradations in the purity of these two maxima, from practically pure red modulators, over red modulators with small and large humps in the green at 5200 up to photopic modulators with more or less well-marked humps at 6000 \AA . An example is shown in fig. 22, several others in the original paper (47).

Are the modulators at 6000 , 5200 and 4600 \AA the only ones found? By the direct technique, so far discussed, a yellow modulator at 5800 \AA , was seen in the frog's eye. The rat had a green modulator at 5000 \AA looking like a narrow visual purple curve whilst in the frog the green modulators did not appear at 5200 but somewhat further towards the red end at 5400 \AA . Quite clearly, however, the modulators were confined to three regions of predilection, one in the red, one in the green and one in the blue part of the spectrum. But within these regions there was some variation, difficult to ascribe to errors of measurement because of the use of a calibration wave-length and also

because the curves were so narrow. The latter circumstance tended to make the maxima at the top stand out rather clearly whatever the uncertainty at the base. In fig. 23 is given a summary of the modulators seen in various animals, all obtained by the technique of direct threshold measurement.

In a mammal such as the cat (48) the photopic dominator had a hump at 6000 Å but only once I obtained a curve that could be put down as a modulator. This curve had its maximum at 5200 Å. Here then it was necessary to employ some indirect technique which at the same time would show whether the photopic dominator was a compound curve as suggested by its hump at 6000 Å. This it shared, for instance, with the pure cone eye of the snake and by the rat's eye. The first indirect technique used was selective adaptation

(52) based on the idea that illumination of the eye with light selected from the red, green or blue regions of the spectrum would suppress the sensitivity of its own receptor and thus make other components visible. The ease with which single spikes can be isolated and maintained in the cat's eye made it a very suitable animal for this purpose.

Some elements did not respond selectively to selective adaptation and thus must have been pure visual purple elements. But others gave curves of the narrow modulator type, and, again, some variation was found within the three regions of predilection. The red modulator at 6000 Å was the

most common one but in addition a yellow modulator with a maximum at 5800 Å was seen twice. Green modulators were found at 5000, 5200 and 5400 Å, blue modulators at 4600; one was further out in the short wave-lengths, at 4400 Å. This violet modulator came as a surprise but the later analysis of the scotopic dominator by DONNER and myself (28) showed, indeed, humps as far out in the violet as at 4200 Å (cf. above fig. 15). The modulators obtained by selective adaptation are averaged in fig. 24 which thus illustrates the centres of gravity of the three regions of predilection. The three fundamental maxima are seen to be around 4500—4600, 5200—5300 and 6000 Å.

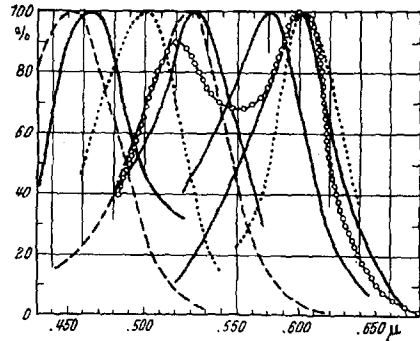


Fig. 23. Modulator curves. Dots: rats; broken lines: guinea pig; continuous line: frog; $- \cdot -$: snake. Equal quantum intensity spectrum. [GRANIT: *Nature* (Lond.) 151 (1943).]

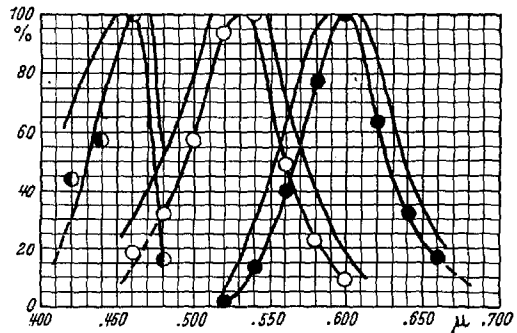


Fig. 24. Average modulator sensitivity curves of the cat obtained by selective adaptation. ● red; ○ green; ● blue. Outer curves indicate the dispersion of the results in each case. Equal energy spectrum. [GRANIT: *J. of Neurophysiol.* 7 (1945).]

(blue) were employed. After 5 min. continuous illumination through either of these filters the adapting light was switched off and the threshold re-determined for the test lights. It was generally found to have risen as a consequence of the adaptation and the 'adaptation factors' show the amount of this change in multiples of the normal value before selective adaptation. Recovery was followed in the dark to ensure that the thresholds returned

Table 1

No	Test colour	Blue adaptation		Green adaptation		Red adaptation	
		Adaptation factors		Adaptation factors		Adaptation factors	
		on	off	on	off	on	off
1	Green	323	15.5	17.3	1.0	10.0	4.0
	Red	23.8	15.2	23.8	2.1	6.7	15.1
	Blue	96.6	39.2	13.1	1.3	4.0	9.9
2	Green	23.6	10.0	550	91.1	142	26.6
	Red	6.6	1.5	23.8	23.3	23.8	6.6
	Blue	389	5.0	308	100	24.7	15.6
3	Green	2.7	2.7	100	244	6.4	273
	Red	4.3	3.6	1.5	2.8	36.2	159
	Blue	4.4	10.3	50.0	123	4.0	15.5
4	Green	9.7	170	9.0	1.5	3.8	2.4
	Red	3.6	12.8	1.5	1.2	2.9	4.5
	Blue	9.8	8.2	3.9	1.0	7.9	2.0

to their original value. If they did not do so the result was discarded (pressure by the micro-electrode may then have damaged the sensitive retinal structures isolated). Great precautions were thus taken to ensure optimal conditions.

A glance at table 1, illustrating the adaptation factors of four elements out of 72, will immediately show the extreme degree of variation in the design of the elements as wave-length detectors. Indeed, the retinal mosaic may almost be likened to a many-coloured Turkish carpet! The four elements have been chosen to illustrate variations in colour sensitivity as well as variations in adaptability. No wonder that simplification only is obtained as a result of statistical analysis. Clearly some process of modulation is at work but at what stage and how? Is the modulation photochemical and due merely to the plasticity of the chemical combination of a labile chromophore containing several double bonds and free electrons capable of turning the balance one way or another in addition to whatever further possibilities are provided by the linkage with receptor proteins, or is there a very limited number of, say, three or four substances modulated by interaction in the retinal switch-board of interneurons?

'Colour mixture' within an element. The appearance of modulators by the polarization technique suggested interaction (see above, p. 51) but it was nevertheless deemed important to prove by illumination experiments that

interaction between two wave-lengths of light shining on the same element could produce new patterns. This problem was attacked by means of our WRIGHT colorimeter [see e.g. WRIGHT (140)] in which two wave-lengths can be selected and made to illuminate the eye singly or together. The fully dark adapted cat was used (55) and the spectral energy for every wave-length adjusted to equal visual purple absorption in terms of the measurements by

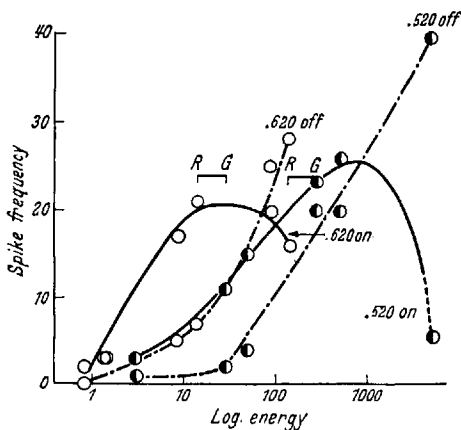


Fig. 27. Spike frequency — log intensity curves for on- and off-components of a red 6200 Å and a green 5200 Å stimulus. The logarithmic abscissae are in terms of multiples of energy necessary for visual purple threshold response. The two places marked R and G refer to experiment not to be reviewed below. [GRANIT: *Acta physiol scand.* (Stockh.) 18, 281 (1949)].

at 'on' and 'off'. A specimen is shown in fig. 27. For the two wave-lengths chosen, 5200 (green) and 6200 Å (red), impulse frequency is plotted against log energy in multiples of the visual purple threshold as abscissae. For no value on the abscissa can the impulse frequencies be matched in the red and the green.

What happens when, with such types of elements, the red and the green are thrown in simultaneously after reduction of the energy by one half so as to maintain the total energy for the double stimulus constant in terms of visual purple absorption. Do the impulse frequencies of the two add, do they strike a mean between the singles or does either of the two "win" in the sense that it succeeds in impressing its value upon the sum? The results were perfectly clear cut: provided that the two wave-lengths chosen were sufficiently far apart so as to differ in impulse frequency, when adjusted in energy according to visual purple absorption, either of the two mostly succeeded in imposing its frequency pattern upon the sum. The other one behaved as if non-existent from the point of view of specificity of impulse pattern or off/on-ratio.

DONNER and myself (28), published above in fig. 14, and obtained by the same apparatus.

In several elements it was immaterial whether the spectrum was used at full intensity or if neutral filters were interposed. An equation of two colours in terms of equal visual purple energy values gave the same spike pattern for all wave-lengths, whatever the intensity level. Clearly then these elements can only have contained visual purple and their frequency-log intensity curves must have been identical for all wave-lengths, as, indeed, proved to be the case whenever they were determined. But in a considerable number of elements equal visual purple energy values did not give equal responses in terms of impulse frequency

Table 2 compares the full and half values for a green at 5200 Å with the corresponding values for a red at 6400 Å and with the sum of the red and the green stimulus, both halved so as to maintain the total energy constant in terms of visual purple. It also presents the off/on-ratios. The values have been determined with our automatic frequency counter recording the impulses separately at 'on' and 'off'. By means of this device a large number of readings

Table 2.

	G, full	G, half	R, full	R, half	Half (R + G)
On-off	27—16	21—9	25—30	21—16	23—18
Off/on-ratio	0.6	0.4	1.2	0.8	0.8

can be quickly assembled. Clearly the sum of red and green at half intensity has reproduced the off/on-ratio as well as the absolute frequencies of the red half value.

In such experiments it is not difficult to find elements of the type for which the off-components are more marked in the red. When this is the case there is nearly always a smaller rise of the off-response in the violet too. It is also relatively easy to find elements in which the red dominates in the combinations of the two, less commonly one encounters elements with green dominance in the sum but even in such cases the green stimulus may give a lower off/on-ratio. The variations in off/on-ratio with wave-length may as such be an aid in discrimination. They, of course, signify that the frequency-log intensity curves are running a different course for different wave-lengths. When an animal turns its eye towards the coloured light these differences will emerge in the pulsations of frequency set up by the stimulus.

These experiments on 'colour mixture' within a single element are of interest from the point of view of the psychophysics of colour mixing but in this connexion they are chiefly taken up because of the principle involved and its significance for the understanding of the nature of the modulators. [The reader is referred to the original paper for details (55).] They establish the fact that at least at *suprathreshold intensities* a zone of overlap between the spectral absorption curves of two substances in different receptors converging towards the same ganglion cell may lead to interference altering the shape of the absorption curves responsible for the primary effect in the receptors.

Wave-length and spike frequency-time curves. DONNER (27) has recently made the important observation that the spike frequency as a function of time can be used to detect specific colour sensitivities in the retinal elements of the fully dark adapted cat. He developed an electronic shutter for our automatic frequency counter and by means of this apparatus selected a

counting period of 0.1 sec. and shifted it along the course of the on- or the off-discharge. By means of this device the temporal course of the spike frequency in about one hundred elements could be studied without the irksome analysis of several thousand metres of film. In the on-elements the impulse frequency rose along similar curves for all wave-lengths (adjusted for equal visual purple stimulation). But in a considerable number of on/off-elements

specific changes took place with a change in wave-length.

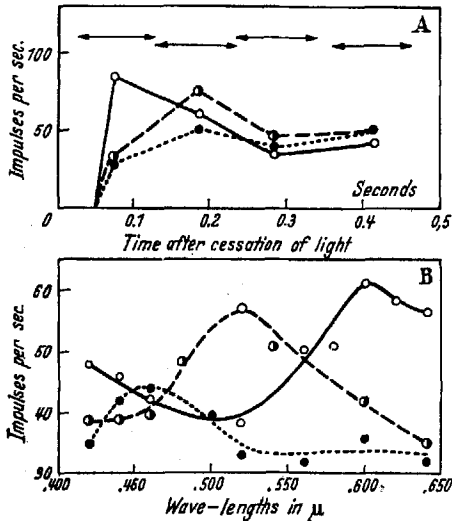


Fig. 28. A. Spike frequency-time curves for the off-effect of single retinal element at three wave-lengths: 6000 Å: line drawn in full, 5200 Å: broken line, 4600 Å: dotted line. Duration and location of counting periods marked above record. B. Analysis of 9 elements. Averaged spike frequency for counting period of 0.1 sec. located on top of the three maxima for red, green, and blue as explained in text. [DONNER: *Experientia* 5, (1949).]

A case in point is the element shown in fig. 28 A. The impulse frequency for the red stimulus (6000 Å) rises along the curve drawn in full. The frequency for the green stimulus (5200 Å) is shown by the broken line and that for the blue stimulus (4600 Å) by the dotted line. Since the energy values for red, green and blue were adjusted for equal visual purple absorption the curves ought to be identical (as, indeed, they were for the pure on-elements and several on/off-elements). But these curves are not. Sometimes, too, there was an early rise, probably due to visual purple stimulation, detectable for all colours which then separated themselves according to the principle established by fig. 28A: the red maximum is always the first one, next follows the green maximum, and, finally, a great deal later, the blue has the highest

frequency of the three. The blue maximum is not definite in the example chosen but in some of DONNER's curves it is quite prominent. The temporal location of the three maxima was fairly stable from element to element. It was not necessary to maintain the adjustment for equal stimulation of visual purple to see the three humps separate themselves. DONNER found them also in a number of experiments in which the spectrum was adjusted for equal energy. As soon as an intensity level had been reached at which the humps became visible they often maintained themselves in their relative location within a considerable intensity range.

DONNER's result made it possible for him to select the average loci for the three maxima in the frequency-time curve and study each independently in the whole spectrum by delaying the counting period in an appropriate fashion. The question attacked by this method is clearly as follows: within

what spectral range can the specific 'red', 'green' and 'blue' maxima be demonstrated? The answer is provided by fig. 28B, an average for 9 elements. The red maximum is seen to be best marked for wave-length 6000 Å, then to diminish rapidly towards the green and to rise again in the violet. The rise in the violet is particularly interesting in view of the fact, mentioned above, that elements with a high off/on-ratio in the red also tend to have a rise in the violet. The green maximum is at its best at 5200 Å, the blue one at 4600 Å. Again the maxima coincide with those obtained by myself with the aid of the indirect methods of selective adaptation and polarization. Besides, by the direct method, the maxima at 6000 and 5200 Å were found in the pure cone eye of the snake. [*Added in proof.* DONNERS curves should be compared with those of MOTOKAWA [*J. of Neurophysiol.* 12, 291 (1949)] for man and frog. They also have the rise in the violet.]

It is clear that these curves cannot represent the primary photochemical substances although, taken in conjunction with all the other evidence presented above, they emphasize the necessity for a minimum of three primary photochemical compounds to account for the ever-recurring three preferential regions in the spectrum. From another point of view DONNERS results are still more interesting. This is the question as to how discrimination is possible in view of the fact that different colour receptors very definitely are connected to the same cable destined for the higher centres. I have already pointed out that in such a system discrimination can be facilitated by variations in the off/on-ratio signifying different rates of rise of the frequency-log intensity curves. DONNERS results show that additional clues are provided by the frequency differential with respect to time.

Sensation-time curves have been a favourite topic of psychophysical research [see e.g. a monograph by Bills (12)] and it is well known that colour sensations develop at different rates, the red being the fastest one, then follow green and blue, all thus in the same order as the rates of rise of spike frequencies in the cat.

Theoretical considerations. For *the scotopic dominator* it is easy to point to the existence of an equivalent photochemical compound, be it the visual purple or visual violet. In this case there is merely the question as to what extent can we be certain that visual purple occurs in one form only in the dark adapted retina, i.e. the most stable form that corresponds to the extractable substance. We have seen in figs. 15—17 that, at the absolute threshold of vision where interaction might be supposed to be at its minimum and cones are supposed to be less sensitive, there are humps on the electrically determined curves. Are these humps due to a natural chemical process of variation within the visual purple compound, a kind of photochemical modulation, or do they signify that cone substances are more sensitive than one might have thought? I cannot be convinced that the traditional distinction

between rods and cones, inherited from the duplicity theory, can aid us so much in interpreting our experiments.

MORTON and his collaborators (7, 91) have shown that other points of view are likely to be more profitable. Before reviewing them I want to draw attention to the curious fact that the centres of gravity of the red and green spectral modulator regions of the visual purple eye correspond with the maxima of visual violet itself and its photopic dominator at 6000 Å suggesting that visual purple easily is transformed into something that absorbs at these particular maxima. I have pointed out this fact before (52) and still think it highly significant. Now one of the breakdown products after illumination of visual purple in solution is the carotenoid called retinene₁, if formed from visual purple, and retinene₂ when formed from visual violet [WALD (125, 127 to 129)]. MORTON and GOODWIN (92) set out by demonstrating that these retinenes are the aldehydes of respectively vitamin A₁ and vitamin A₂. By the oxidation of vitamin A, BALL, MORTON and GOODWIN (8) produced retinene and could now consider further steps of analysis based on the pure substance. Thus, by combining retinene₁, dissolved in aqueous alcohol, with suitable proteins or amino-acids, they obtained absorption maxima around 4400—4600 Å after acidification; with p-aminobenzoic acid the maximum was found around 5350 Å and this substance was easily decomposed by light. Modulator analogues were produced by dissolving vitamin A or retinene in concentrated sulphuric acid or syrupy phosphoric acid at temperatures near 0° C. In fact, the 'modulator analogues' exhibited narrow maxima in fairly good agreement with those obtained from various eyes by the physiological method (see above fig. 23) and the substances were light sensitive, unfortunately also thermolabile [BALL, COLLINS, MORTON and STUBB (7)].

MORTON and his colleagues do not claim to have discovered the photochemical substrates of the modulators. They fully realize that their methods have been unphysiological. The great value of their work lies in the freshness of the approach and the new experimental ideas. Their work has introduced the many interesting concepts and possibilities of modern structural chemistry into this field. By discovering the chemical relationship between vitamin A and WALD's retinene they transferred general experimentation with colour reactions into work with known chemical structures. Our chemical knowledge about the significance of the number of double bonds and free electrons within a chromophore for the colour of the latter now stands available for the further analysis of these problems.

This is not to be a review of the photochemistry of visual purple. Enough has been said, however, to make it intelligible that molecular changes within the chromophore and in its combination with proteins can produce spectral shifts and variations of colour sensitivity. Even visual purple itself may be

modulated in such a manner and this effect, inasmuch as it is due to free electrons, would not show up in the substance finally extracted after treatment of a retina with digitonin or saponin. It seems likely — and this hypothesis is to-day in the centre of most photochemical work on the retina — that all rod and cone substances are structural variations on the same theme and it may well be that the variations at the absolute threshold of reception of the scotopic modulator, shown in fig. 15—17, may be a kind of Nature's attempt to try out a mechanism of photochemical modulation that has led to a stable result in the pure cone eyes. The humps on the scotopic dominator may be further emphasized by the internuncial mechanisms of interaction between receptors of different spectral sensitivity joined to the same element.

The photopic dominator presents a particularly interesting problem. Just as the scotopic dominator, it can hardly in itself be responsible for any colour discrimination. Its spectral absorption curve is too broad to mediate more than an over-all increase in impulse frequency, probably responsible for brightness. The interesting problem here is that the work with the retinæ of rats and guinea pigs (I) so clearly showed that a considerable number of cones is necessary for a photopic dominator to exist in an eye, (II) that e.g. a red modulator at 6000 Å may be present without a dominator at 5600 Å, and further, that (III) the work with the cat retina, reviewed above, suggested that the dominator is a compound curve because modulators confined to three preferential areas could be obtained by indirect methods such as selective adaptation, polarization and DONNERS method based on the frequency-times curve; and yet, in spite of all this, we have evidence in the work of WALD (126) and BLISS (14) to the effect that a substance, their iodopsin, with the general absorption curve of the photopic dominator can be extracted from the eyes of chicken. One might say that such a substance is unnecessary! It provides us with an *embarras de richesse* of possibilities because we can quite satisfactorily account for the photopic dominator by a process of neural combination, as pointed out above. Is the iodopsin too a compound substance (we gather that its spectral photosensitivity is not as well known as that of visual purple) or does the internuncial system act in two ways: (I) to increase the discrimination provided by a limited number of substances as well as (II) also to allow these substances to deliver their primary photochemical distribution of sensitivity in the shape of dominators?

Whatever the solution of these problems the photopic dominator is a physiological reality in the response that is forwarded to the higher centres by ganglion cells forming optic nerve fibres and so cannot be left out of account in our attempts to understand the processes of vision.

The modulators provide a somewhat similar problem. We can account for a certain amount of modulation by neural interaction although it is uncertain whether at the threshold of photoreception, this process of interaction is

strong enough to create the narrow curves recorded. But the basic evidence for the existence of a neural process of interaction capable of sharpening the colour bands is now definite enough in some types of the experiments described above. On the other hand, it seems probable that within the next few years retinal photochemistry will make further progress and provide new possibilities. In addition to the substances produced by MORTON and his collaborators (7), there is LYTHGOES transient orange [LYTHGOE (88), LYTHGOE and QUILLIAM (89)], WALDS new substance (130) with maximum at 5300 Å, some results in interesting reports by BLISS (13, 15, 16). A minimum of three primary photochemical compounds in addition to visual purple would seem necessary to account for the three preferential regions but there may well be a greater number of such compounds accounting for the variation that expresses itself in the modulators and in the extreme variability of specific colour sensitivity in GERNANDTS experiments on adaptation (35, 36). The chemical methods of extraction can as yet neither deal with single elements nor with the problem of free radicals.

A few words might be added about the so-called cone substances of v. STUDNITZ [see e.g. his summary (121)]. I am taking these words from the paper by BLISS (14): "In recent years several investigators have reported the extraction of cone pigments from tortoises, chickens and frogs. The first claim was made by von STUDNITZ (1937) who reported that ether extracts of tortoise and frog retinas contained a photosensitive substance with a maximum absorption at 570 m μ . However, this claim cannot be accepted because the height of this absorption maximum is little greater than the sensitivity of his galvanometer (1 mm. deflection for 0.1 lux). In his figure of the absorption spectrum of cone substance from frogs the absorption peak at 570 m μ absorbs 0.3 per cent more light than the minimum on the long wave side. Since the total illumination is 4.5 lux, a difference in absorption of 0.3 per cent would cause the scarcely detectable change in deflection of only 0.14 mm. The insignificance of this peak is further emphasized by the fact that von STUDNITZ ignores the existence of an absorption at 630 m μ actually greater than that at 570 m μ .

So much has been made of these findings by von STUDNITZ (1940) that the experiments seemed worthy of repetition even though HOSOYA, OKITA, and AKUNE (1938) and WALD (1943) have already failed to confirm them. An ether extract of frog retinas was prepared following the procedure in his report and was measured on SHLAERS spectrophotometer (1937). The solution yielded only a smooth absorption curve into the violet and showed no photosensitivity."