THE FLICKER RESPONSE FUNCTION FOR THE TURTLE PSEUDEMYS

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I

The flicker response curve has been determined for the turtle *Pseudemys*, using the technic and procedure employed in our earlier studies with insects, fishes, newts, and man.[1] The structure of the curve, and the relations of its parameters to temperature, show several interesting features. Mean critical intensities (I_m) have been measured at constant temperature as a function of flash frequency F, with equality of light time and dark time in a flash cycle; reciprocally, F_m was obtained as a function of I. The determinations of I_m were made at various temperatures. The retina of *Pseudemys* contains no "rods," or so small a number that they have not been detected. There is thus an opportunity to test the adequacy of equations employed for the description of the flicker contour; the performance of the peripheral sensory field of the retina is not complicated by the duplexity of receptor constitution found in vertebrates hitherto studied,[2] hence a single function is available for examination over the whole explorable range.

The variability of performance of these turtles proves to be so slight that persisting individual differences in excitability are easily recognized among the ten specimens used. The group tested is thus not really a homogeneous group, as this has been defined with certain other organisms.[3] An experimental test of several features of the

[1] Wolf and Zerrahn-Wolf, 1935–36; Crozier, Wolf, and Zerrahn-Wolf, 1936–37 *a*, *b*, *c*, *d*; 1937–38 *a*, *b*, *c*, *d*, *e*; 1938 *a*, *b*, *c*, *d*, *e*.

[2] 1938 *a*, etc.

[3] 1936–37 *a*, *b*; 1937–38 *a*.

general treatment of the variability[4] of performance in response to visual flicker is therefore possible.

Young individuals of *Pseudemys scripta ssp.*, of carapace length 3.5 cm., were kept in a large terrarium near a south window, with access to water. They were fed regularly with prepared turtle food. Their activity depends upon the temperature and the light conditions; on cloudy days they are almost motionless, which has some influence upon their behavior during the tests at such times. A numbered series of 10 was selected from a group of 24 on the basis of general reactiveness.

For observation each turtle was put in a glass jar 10 cm. in diameter, with just enough water to permit an easy foothold and yet to permit extension of the head above water for breathing. If too much water is used the animals move about and the recognition of the threshold responses may become almost impossible. The jars are placed in a water thermostat (Stier and Crozier, 1932–33) for at least 1 hour, in darkness, before the measurements are made. At temperatures between 21.5° and 29° the reactions are increasingly sharp and clear as the temperature is made higher. Above 30° general activity increases, and it is necessary to wait for intervals of quiescence in which the occurrence of the index responses can be recognized with the best certainty. Below 21.5° the responses are markedly slower, but quite definite. Care is required that the rate of increase of intensity up to the critical point (or the reduction of flash frequency in the converse experiment) be not so rapid that mechanical overshooting of the end-point becomes a factor, due to the latency of the response. The determinations are made by placing the jar containing a turtle inside the striped cylinder of the apparatus,[1] which is set in motion at a fixed and controlled speed providing the desired flash frequency. The diaphragm governing the intensity of illumination is then slowly opened until an intensity is reached at which response occurs. The turtle being up to this point quiet, the threshold response to flicker consists in a sudden deflection of the head, in a direction against that of the motion of the stripes. This reaction may be of slight amplitude and of brief duration; subsequently, the head turns in the direction of motion of the opaque stripes and then jerks back to its original position. Some more active individuals show swimming movements following the turning of the head; while others give only the latter response. In some cases, especially at low illuminations, there may be shown only a rotation of the head about the body axis; this occurs most easily when the head is in contact with the wall of the container, and a gentle touch is used to move the turtle away from the wall. The occurrence of the first motion of the head is in any case taken to signalize that the critical flash intensity I_c has been found.

When F_c is measured as a function of fixed flash illumination I the animal is put in the apparatus with the selected illumination already turned on and with the cylinder rotating to give a flash frequency much above that critical for flicker, and

[4] *Cf.* Crozier, 1935; Crozier, Wolf, and Zerrahn-Wolf, 1936–37 *a*; 1937–38 *a*, etc.

thus a "uniformly illuminated" field. There is usually some initial activity, which quickly subsides. When the speed of rotation of the cylinder is slowly decreased the critical speed is recognized by the same response as in the case of increasing intensities with F fixed. The reactions are slow, however, and the decrease of the flash frequency must be produced smoothly and not too fast to avoid overshooting of the end-point. If the critical point is much overrun, the animals may be put into a state of tonic immobility from which they can be aroused only by mechanical stimulation after the light has been turned off.

Regularity and reproducibility of results, as in all such experiments, depend to a large extent upon the regularity with which the animals are fed, handled, and manipulated during use.

II

At various fixed flash frequencies F the mean critical flash intensities I_m were measured at two temperatures, 21.5° and 29.5°; the flash cycle used has equal light and dark time ($t_L = t_D$). These data are given in Table I, each value of log I_m being accompanied by the log P.E. of the dispersion of the ten individual mean values from which the I_m is computed. The general order of magnitude of the scatter of the critical intensities is not very different from that encountered in corresponding experiments with other forms;[1] certain differences in the nature and properties of this P.E._{II} as a function of I_m are considered subsequently.

The measurements in Table I are plotted in Fig. 1. It is apparent that, in contrast with the flicker response contours obtained from sundry other vertebrates thus far studied,[2] the observations fall upon a single smooth curve. They exhibit no separation into two segments, such as there has been reason to suppose with other forms represent the results of the activation of two distinct classes of irritable elements, reasonably identified with those for which respectively the excitability of rods and cones are to be held responsible.[2]

The histological evidence shows that the retinas of at least some diurnal reptiles contain no recognizable rods, or at least extremely few. The visual cells are of the "cone" type, but a certain proportion are "double cones." The tortoises (*Chelopus*, *Chrysemys*, etc.) and various lizards are said to have only retinal cones (Detwiler, 1916, 1923; Detwiler and Laurens, 1920; Menner, 1928; Verrier, 1935, 1937). The presence of a small number of rods in the turtle retina has been stated by Walls (1934). Examination of sections of light

adapted and dark adapted retinas of our *Pseudemys* shows only one general type of visual cell, cones; these are of the usual two kinds, single and "double."

These turtles were chosen for our observations because of the "rod-free" character of the retina. Unless the functional activity of the

TABLE I

Mean Critical Intensities of Illumination (I_m), Millilamberts, and the Probable Errors of the Dispersions of I_1, for Response to Flicker by the Turtle Pseudemys scripta, at Two Temperatures, As a Function of Flash Frequency (F)

F/sec.	21.5°C.		29.5°C.	
	log I_m	log PE_{I_I}	log I_m	log PE_{I_I}
1	$\bar{6}$.8221	$\bar{7}$.2472		
2	$\bar{5}$.3412	$\bar{7}$.9768	$\bar{6}$.8184	$\bar{7}$.0704
3	$\bar{5}$.7348	$\bar{6}$.1658		
5	$\bar{4}$.1635	$\bar{6}$.7687	$\bar{5}$.5760	$\bar{7}$.8397
7.5	$\bar{4}$.6103	$\bar{5}$.1402	$\bar{4}$.0803	$\bar{6}$.4183
10	$\bar{3}$.0183	$\bar{5}$.4196	$\bar{4}$.4966	$\bar{5}$.0090
	$\bar{3}$.0174	$\bar{5}$.3191		
15	$\bar{3}$.6645	$\bar{5}$.9768	$\bar{3}$.1396	$\bar{5}$.3529
20	$\bar{2}$.2036	$\bar{4}$.9146	$\bar{3}$.6764	$\bar{5}$.9764
25	$\bar{2}$.7097	$\bar{3}$.3579	$\bar{2}$.1784	$\bar{4}$.6392
	$\bar{2}$.7016	$\bar{3}$.0671		
30	$\bar{1}$.2660	$\bar{3}$.9348		
	$\bar{1}$.2368	$\bar{3}$.5710	$\bar{2}$.7036	$\bar{3}$.1623
	$\bar{1}$.2310	$\bar{3}$.3512		
35	$\bar{1}$.7569	$\bar{2}$.1804	$\bar{1}$.2240	$\bar{3}$.5637
40	0.2803	$\bar{2}$.8352	$\bar{1}$.7541	$\bar{2}$.0484
45	0.8859	$\bar{1}$.0990	0.3907	$\bar{2}$.8762
48	1.4155	$\bar{1}$.4043	0.8968	$\bar{1}$.2190
50	2.1477	0.3478	1.5973	$\bar{1}$.9853
51			2.1732	0.2762

double cones should be different from that of the typical cones, it might then be possible to find a curve of visual performance not exhibiting the composite nature encountered with other vertebrates studied,[2] which has been attributed to the duplexity of the retinal constitution.[2] It is to be recognized, of course, that the histological criteria for the distinction between rods and cones are rather un-

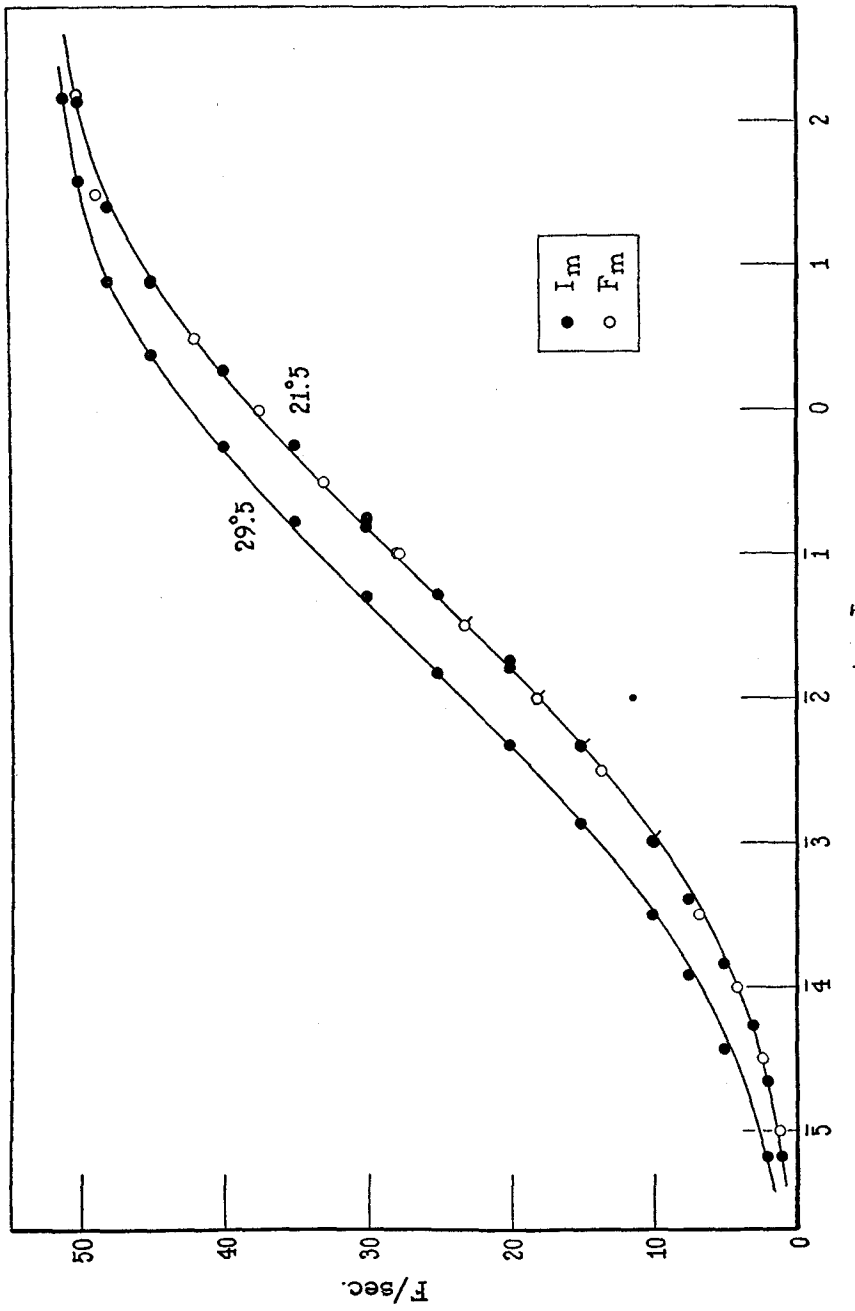

FIG. 1. Log I_m as a function of flash frequency F (solid circlets), at two temperatures (21.5°, 29.5°), for *Pseudemys scripta*, data in Table I; and F_m (open circlets) as a function of log I at 21.5° (Table II). Each plotted point is the mean of 30 measurements, 3 on each of the same 10 individuals at all points (overlapping points carry a tag). The curve drawn is the same at each temperature, a probability integral with $F_{max.} = 52.63$, $\sigma_{\log I} = 2.062$; $\tau' =$ abscissa of inflection $= \bar{2}.27$ (29.5°) and $\bar{2}.82$ (21.5°). The departures are slight (0.5 per cent at most), but are systematic; their origin is discussed in the text.

satisfactory and difficult to apply in a uniform way. The variations of human visual properties with location of the test spot on the retina[5] are indicative of a *qualitative* correlation with the associated proportions of rods and cones, and support the dissection of the visual performance curves into two portions.[5] Data on visual responses initiated by a rod-free retina should provide a conclusive test of the propriety of this procedure (particularly if supplemented by observations with cone-free animals). The precise determination of the form of the performance function in such a case is an important check upon the method of separation of presumptive rod and cone effects in the composite curves, especially if it appears that the measurements with the rod-free animal indicate a single population of sensory effects.

The curves drawn in Fig. 1 are computed probability integrals,

$$F = k F_{max.} \int_{-\infty}^{\log I} e^{-(\log I/I_0)^2/2\sigma^2} d \log I \qquad (1)$$

with the same value of $F_{max.}$ (=52.63) for the two temperatures, and the same value of $\sigma_{\log I}$ (=2.062). The abscissa at the inflection point decreases with rise of temperature, as found previously[2] for insects and fishes. We have used in some earlier papers a logistic of the form

$$F = F_{max.} /(1 + e^{-\rho \log I}) \qquad (2)$$

to describe such data, because of its formal identity with the general expression for the photostationary state,[5] although pointing out that the parameters in the latter expression do not have properties permitting rational application to the flicker contour.[6] It was also pointed out[7] that the probability integral gives a possibly better description of the data. For theoretical reasons[7] equation (1) is preferred. The simple character of the *Pseudemys* flicker curve permits a formal decision between (1) and (2), since a single function appears to cover the entire range of the data. Equation (1) gives a decidedly superior fit. It is also to be noted that the data in Table I cannot be described by a photostationary state equation; moreover,

[5] *Cf.* Hecht, 1937.

[6] 1936–37 *c*, *d*; 1938 *c*; 1937–38 *d*, *e*.

[7] Crozier, 1937; 1937–38 *d*.

the shift with temperature change (Fig. 1, and Section IV) is in the reverse direction to that called for by the nature of this formulation.[6]

III

The departures of the I_m values from the curves drawn are slight, but are consistent and significant. Moreover, at 21.5°, the departures

TABLE II

Mean Critical Flash Frequencies (F_m), 21.5° with P.E.$_1$, at Fixed Flash Illuminations (log I)

Because of the persisting individual differences, the mean values of P.E.$_{1_{F_c}}$ have been computed (*cf.* Fig. 10).

log I	F_m/sec.	P.E.$_{1_{F_1}}$	P.E.$_{1_{F_c}}$
mL.			
$\bar{5}.0$	1.19	0.0134	0.0162
$\bar{5}.5$	2.30	0.0134	0.0198
$\bar{4}.0$	4.19	0.0606	0.0554
$\bar{4}.5$	6.75	0.0321	0.0835
$\bar{3}.0$	9.82	0.0855	0.156
$\bar{3}.5$	13.54	0.0784	0.135
$\bar{2}.0$	18.04	0.0989	0.0833
	18.14	0.103	0.183
$\bar{2}.5$	23.13	0.111	0.205
	23.10	0.176	0.282
$\bar{1}.0$	27.84	0.155	0.221
	27.61	0.137	0.212
	27.82	0.171	0.304
$\bar{1}.5$	32.94	0.226	0.393
0.0	37.43	0.2147	0.353
0.5	42.01	0.201	0.324
1.0	46.02	0.291	0.304
1.5	48.85	0.153	0.280
	48.95	0.282	0.344
2.2	50.01	0.201	0.594

of the F_m data (Table II) at fixed intensities are of the same kind. This could arise from one or several different causes: the probability integral may not really describe the data; or the averages I_m may be systematically influenced by persistent individual differences among the curves for the ten turtles; or the readings of critical intensities may suffer from systematic instrumental errors.

The existence of definite differences among the individuals used is easily demonstrated. In each series of tests (I_m at fixed F, 21.5° and 29.5°; F_m at fixed I, 21.5°) rank order numbers are assigned in the sequence of decreasing sensitivities in each set of readings.[8] This means that the rank numbers 1 to 10 are given in the order of increasing critical intensities for response, at fixed flash frequencies; and in the order of decreasing critical F, at fixed intensities. In a homogene-

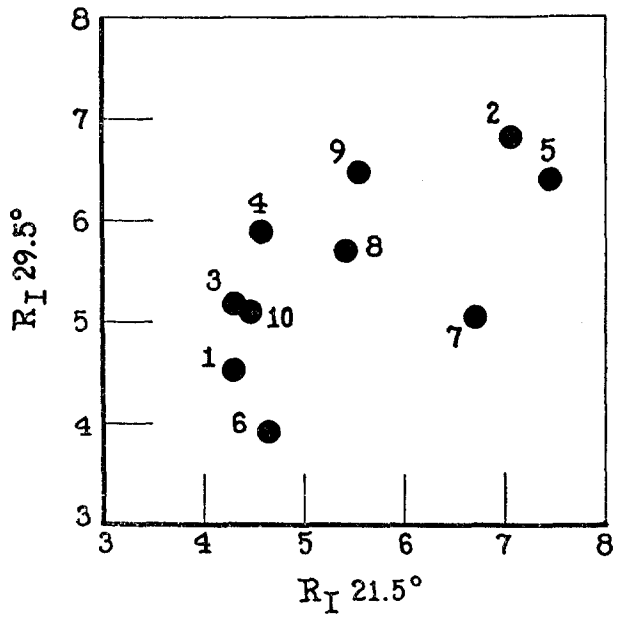

FIG. 2. Mean rank order numbers for *Pseudemys* 1 to 10 at all flash frequencies, showing correlation between relative excitabilities at two temperatures.

ous lot of individuals the rank order numbers for the lot are randomly distributed and there are no statistically significant differences between the mean rank numbers of the individuals.[8] In the *Pseudemys* experiments, however, the successive rank positions of an individual are correlated. The mean rank numbers for two individuals may differ by seven or more times the P.E. of the difference. Figs. 2, 3, and 4 show the correlation between the mean rank numbers for the

[8] 1936–37 *a*, *b*; 1937–38 *a*.

separate individuals in different tests. Fig. 2 is based on the sets of observations at 21.5° and 29.5°, Fig. 3 on the I_m and F_m experiments at 21.5°, and Fig. 4 on the determinations of I_c at two flash frequencies ($F = 20, 30$) at eleven temperatures between 12.2° and 35.8° (Section IV). It is apparent that there is a notable correlation between the mean rank order numbers of an individual in the various tests. The relative excitabilities of the individuals are retained over a period of about 4 months.

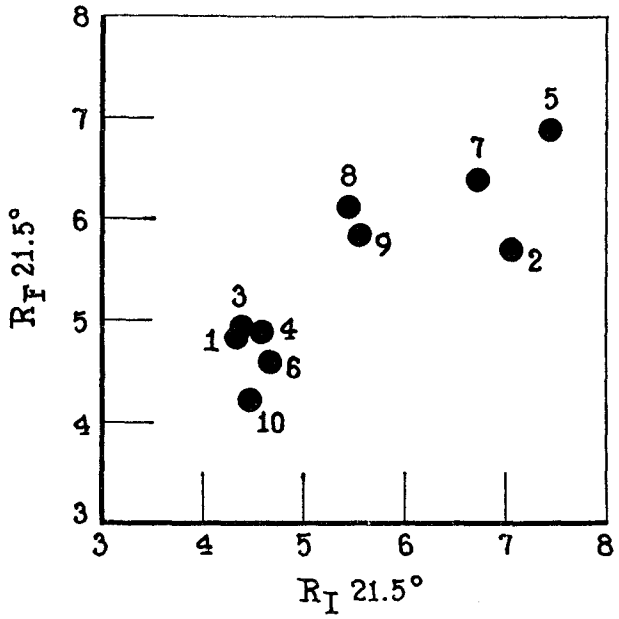

FIG. 3. Showing correlation between mean rank order numbers of individuals for determinations of I_m and of F_m at one temperature.

This individuality is further seen in the slight but definite and repeated differences between individuals in the values of $F_{max.}$ and of $\sigma_{log\ I}$ which must be taken to obtain reasonable rectilinearity upon a probability grid. Fig. 5 gives data for two individuals of nearly identical mean rank order numbers, at two temperatures, which illustrate this.

While differences of this sort could easily produce the kind of slight deviation from theory which Fig. 1 exhibits in the averaged measure-

ments, it is also true that such deviations appear in the data of practically all the individuals. They seem to be correlated with the use of particular lamps and filters to produce the different levels of intensity of illumination. Fig. 6 gives illustrations. Similar deviations occur in the F_m data (Fig. 7). The maximum single departure

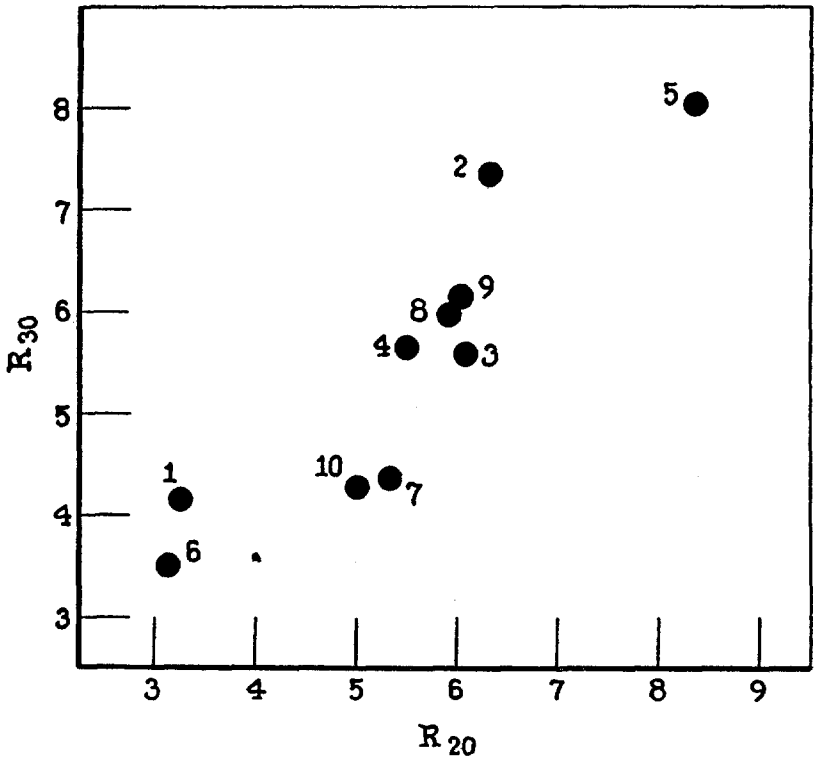

FIG. 4. Showing correlation between mean rank order numbers of the individuals in determinations of I_m at various temperatures (Table III) and at two flash frequencies ($F = 20, F = 30$).

is not over 0.06 log unit, except at the extreme upper and lower ends of the graphs. At the low intensity end it becomes difficult to make accurate observations while at the upper end it is hard to control intensity with adequate precision; in either case "overshooting" of the end-point is likely to be a factor.

The scatter of I_1 bears the same kind of relationship to I_m as we have already found with other forms.[1] The proportionality is direct (Fig. 8). There is no real evidence of a "break" in the plot, such as is found for vertebrates having both rods and cones.[1] There is no change of the level of P.E.$_{.1I}$ with long continued practice. The order of

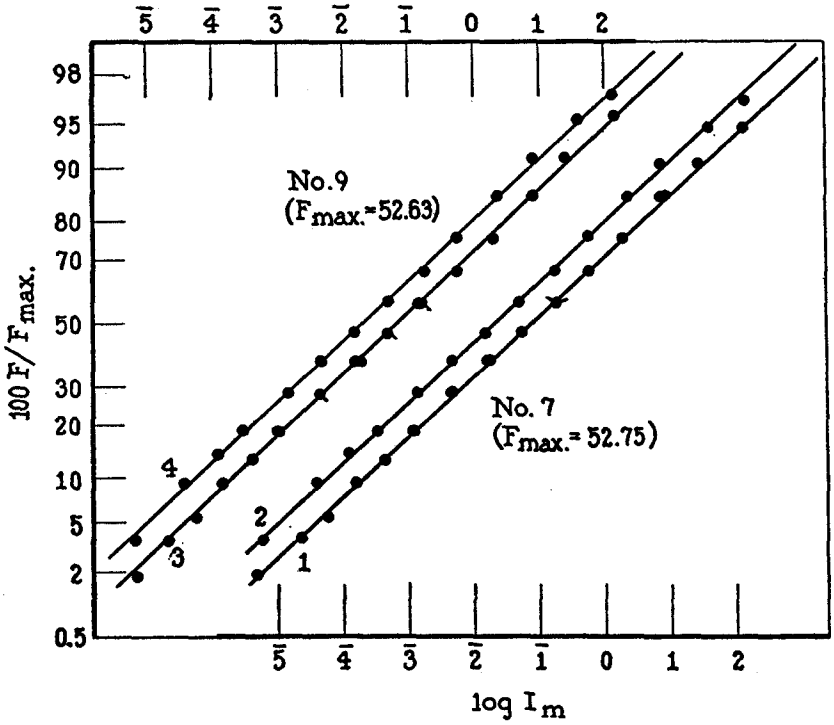

FIG. 5. Showing slight but characteristic differences between the maxima to which the F – log I_m curves for separate individuals rise, and similar differences in the slopes upon a probability grid: No. 7, at 21.5° (*1*) and at 29.5° (*2*), $F_{max.}$ = 52.75; No. 9, at 21.5° (*3*) and at 29.5° (*4*), $F_{max.}$ = 52.63.

magnitude of P.E.$_{.1I}$ is practically identical with that obtained with other animals.[1]

In cases where a homogeneous lot of reacting animals is available the nature of the variation[8] of I_1 and of F_1 in the reciprocal experiments can be shown to require that the I_m and F_m curves cannot be identical. For the data on *Pseudemys* this must be tested by measure-

ments with the separate individuals, and these should ideally be made in pairs at the same time. The graphs in Fig. 9 show that the vertical

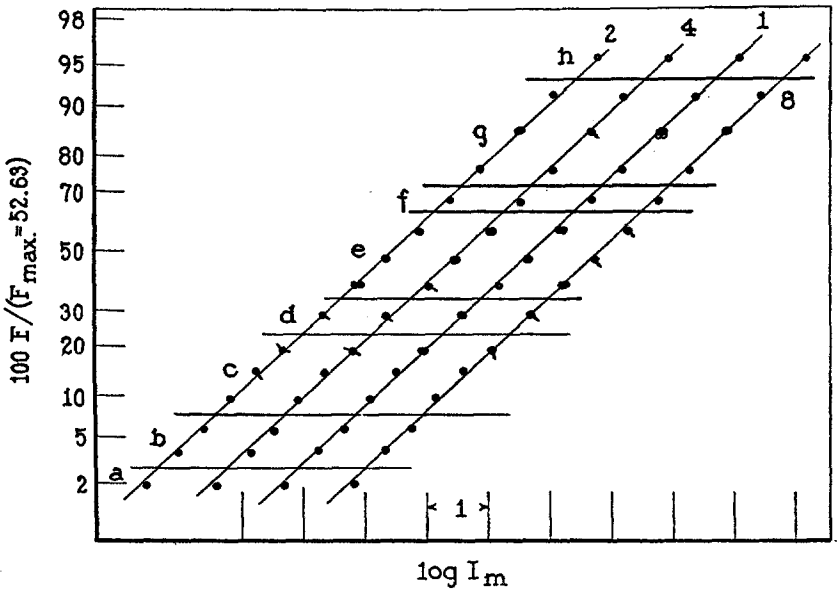

FIG. 6. Data for four typical individuals (Nos. 1, 2, 4, 8), giving log I_1 on a probability grid as a function of F, showing the consistency of certain minor departures from the fitted curve. Horizontal lines separate regions in which particular light-sources (lamps) and filters were employed. It is notable that there is a distinct correlation, probably traceable to the inescapable deficiencies in precision of calibration of lamps and filters.

	Lamp	*Filter*
a	100 w.	"1:10,000"
b	"	"1:1,000"
c	"	"1:100"
d	"	"1:10"
e	"	None
f	500 w.	"
g	1000 w.	"
h	2 × 1000 w.	"

separation of the F_m and I_m curves is definite enough, although small. This is expected since the variation of F_c (Table II) is quite small

(in correlation with the relatively low general slope of the F – log I_m curve, Fig. 1); the F_m and I_m observations were not made at the same time, and no determinations were made of F_m at measured values of I_m, consequently it is only in the central region of the graph that any consistent difference could be looked for (Fig. 9). More numerous determinations would probably show that the mean *within individual* variation in F_c (Fig. 10) would rise to a more distinct max-

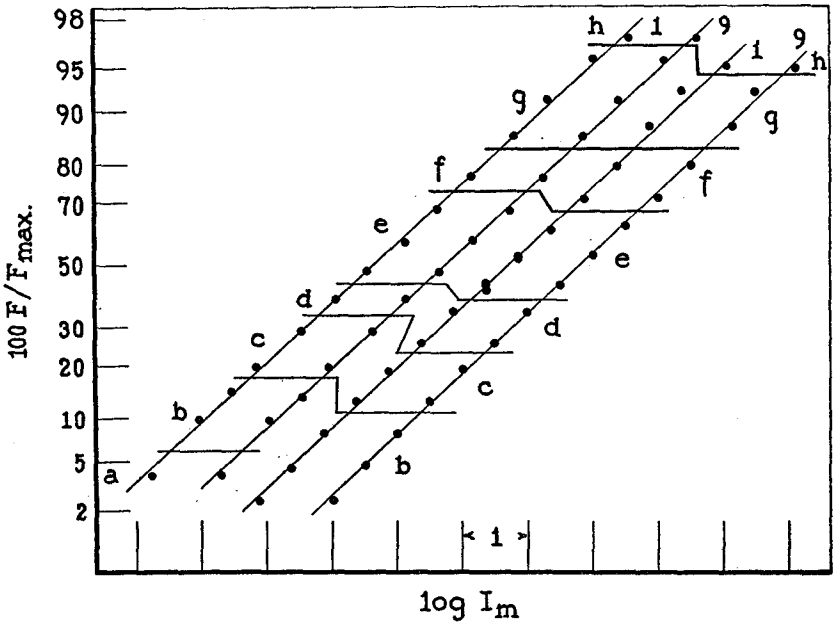

FIG. 7. Data on two individuals (Nos. 1 and 9) at two temperatures (21.5° and 29.5°), determinations of F_1 as a function of log I. The small deviations, as in Fig. 6, are correlated with the use of particular lamps and filters (*cf.* Fig. 6).

imum[1] just beyond the inflection point of the F – log I_m graph; this is obscured by the relatively larger proportion of instrumental variation which[1] becomes more important at higher levels of F and which bulks larger in this case because P.E. $_{1F_c}$ is so low.

The variation of I_1 is clearly *not* predominantly of instrumental or manipulative origin, but is due to differences between the individuals at the times of measurement, as the rank order analysis proves. The differences are of exactly the same order as those found with lots of

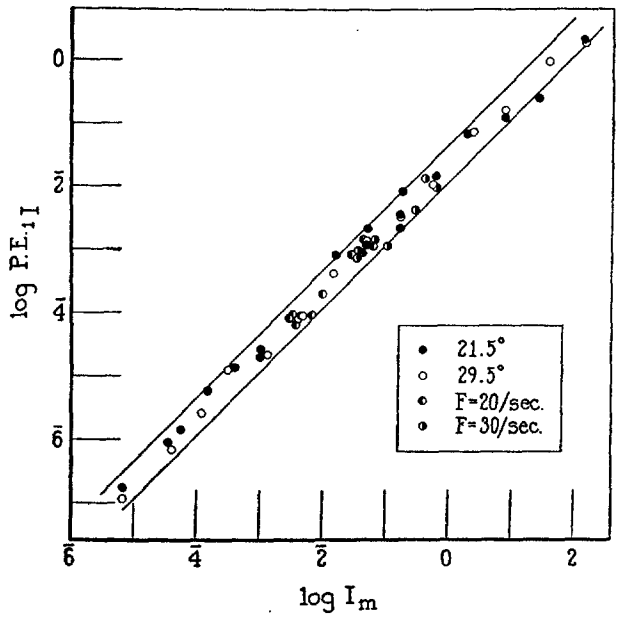

FIG. 8. The dispersions of I_1 at different flash frequencies for measurements at 21.5°, 29.5°, and at various temperatures for $F = 20$ and $F = 30$. Log P.E.$_{1I_1}$ is a rectilinear function of log I_m, with a slope of 1, over the whole range.

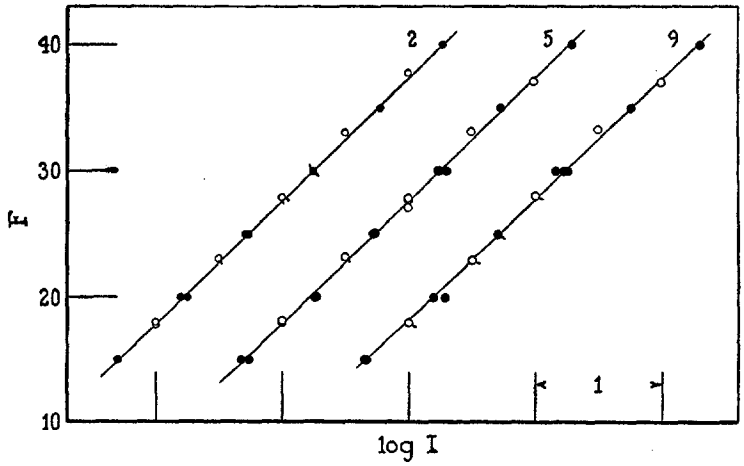

FIG. 9. The relation between F and log I, shown for individuals Nos. 2, 5, and 9, over the central part of the graph where the curve (Fig. 1) is practically rectilinear. Mean values of I_c (solid circlets) definitely tend to lie *below* values of F_1 (open circlets). See text.

other kinds of animals. With *Anax* these differences tend to persist[9] for a short time, but over longer intervals they fluctuate at random. This persistence appears in tests with different proportions of light time to dark time in the flash cycle,[10] but it is not found for animals with equivalent portions of the surface of the eye opaqued[11]—a fact for which there is a simple physical explanation,[11] and which justifies the view that the differences are of individual, organic nature.

With various species and types of fishes we have used the fluctuation in relative sensitivity is more rapid. In *Pseudemys* the individual differences tend to persist through long series of tests. Thus a kind of experimental verification is obtained for the opinion[1] that the

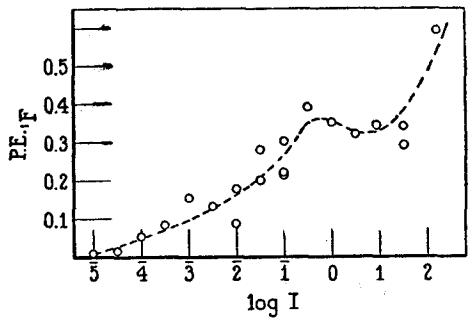

FIG. 10. $P.E._{1F_1}$ as a function of log I rises to a minor maximum in the region of the F – log I inflection (*cf.* Fig. 1).

temporary differences of similar order observed with other animals (and, in man and in *Anax* for a single individual[9]) are likewise to be regarded as essentially organic rather than merely due to "experimental error."

IV

Observations on the dragon fly larva *Anax*[12] and on the teleost *Enneacanthus*[13] showed that with elevation of temperature the curve

[9] 1936–37 *b*.
[10] 1937–38 *e*.
[11] 1937–38 *c*.
[12] 1936–37 *c*
[13] 1936–37 *d*.

of F *vs.* log I_m was moved to lower intensities without change of shape[14] and with retention of the same maximum. Figs. 1 and 2 demonstrate that this is true for the flicker curve of *Pseudemys* also. With *Anax* and *Enneacanthus* three temperatures were employed (12.4°, 21.5°, 27.3°), covering the workable range. While this was done mainly to detect any change of form which the curve might exhibit, it was also supposed that the complexity of the flicker recognition process might well make any simple analysis of the underlying kinetics in terms of the relation to temperature impossible. This was thought to be supported by the fact that the temperature coefficient of F_m is a function of I, while in terms of the activation energy (*temperature characteristic*[15]) equation the abscissa of inflection of the curve gave a non-rectilinear relationship to $1/T°_{abs.}$. The latter argument turns out to be erroneous, and, in view of our experience with many experiments bearing upon this matter,[16] was not very intelligent. The point, of course, is that in biological systems there are often encountered critical temperatures[15] with different temperature characteristics above and below this critical level;[16] data at three temperatures cannot, in general, be expected to be of much use in such a case if a critical temperature lies between the extremes.[15] The development of the theory of the flicker contour, in the meanwhile, now permits certain rational expectations to be entertained concerning the properties of more extensive measurements at a series of closely spaced temperatures.

The data are summarized in Table III. They show that the movement of the curve toward lower intensities as the temperature is increased is a regular phenomenon. The proportionate change is the same at $F = 20$ and $F = 30$, and (in conjunction with the full curves determined at 21.5° and at 29.5°) allows the contention that the form of the curve, and its maximum, are independent of temperature. The data also show, in conformity with those on *Anax*[11] and *Enneacanthus*,[13] that the $P.E._{1I}$ *vs.* I_m curve is very little influenced by temperature (Fig. 8), despite the marked influence upon the speed and amplitude of the index response (Section I); which is clearly consis-

[14] 1936–37 *d*; 1937–38 *c*.

[15] Crozier, 1924–25 *a*, p. 124; 1925–26 *b*, p. 531.

[16] *Cf.* Crozier, 1924–25 *b*; 1934–35.

tent with the view that the variation in I_1 cannot be due to experimental error.

The results of using I, temperature, and proportion of light time in a flash cycle[17] as variables require that $1/I$ be taken as a measure of excitability at a given flash frequency. It has been pointed out[18]

TABLE III

The dispersion of the measurements of critical flash frequency ($P.E._{.1F_1}$) at the inflection point of the F — log I graph is positively correlated with the slope of the curve at this level [$\Delta F/(\Delta \log I = 1)$]. This is a necessary consequence of the theory that the breadth of the band defining the relationship between F and I is due to the variability of the discriminatory performance of the organism, since $P.E._{\log I}$ is constant in this region of the curve and is practically the same for all the organisms tested (*cf.* Fig. 11). The figures in parentheses refer to "rod" portions of flicker response contours.

Animal	Slope $\Delta F/(\Delta \log I = 1)$	$P.E._{F_1}$
*Anax**	44	1.10
Enneacanthus†	18	0.76
(Sunfish)	(4	0.24)
Man‡		
C.	12	0.40
	(6.5	0.16)
W.	10.4	0.30
	(7.0	0.10)
Triturus§	9	0.37
(Newt)	(4.6	0.10)
Pseudemys	6	0.17
(Turtle)		

* Crozier, Wolf, and Zerrahn-Wolf, 1936–37 *b*
† 1936–37 *a*
‡ 1937–38 *b*
§ 1938 *d*

that I has for the flicker response the significance of ΔI in a test of intensity discrimination, and we may refer here to the fact that the corresponding use of $1/\Delta I$ as a measure of excitability can be shown to

[17] 1937–38 *d*, *e*.
[18] Crozier, 1935–36; 1936.

have decided usefulness for the analysis of differential sensitivity.[19] The effectiveness of a given I, at fixed F, will depend upon the frequency with which the excitable elements are found in a potentially excitable state. There are several ways in which this frequency could be supposed to be controlled. The relevant properties of the whole population of excitable elements could be governed by a uniform system of chemical events, so that with rising temperature the whole frequency distribution of excitability thresholds (in terms of $1/I$) should be shifted to a higher level but not changed in form. The fact that the duration of the latent period (*L.P.*) for onset of response to light, in various forms,[20] is governed by the temperature in a manner described by the temperature characteristic equation ln (*L.P.*) $= -\mu/RT + const.$ could be used as an argument for the control of the properties of an assemblage of excitable elements by the velocities of chemical events in the common medium bathing them. Or it could be supposed that the internal metabolic processes common to each excitable element are influenced in the same manner by the temperature. The latter hypothesis would, however, seem to require that each element would then be more frequently excitable by the flashes which succeed one another during an exposure; hence $F_{max.}$ would be expected to appear as a function of temperature, which is not observed. When the proportion of light time to dark time (t_L/t_D) in the flash cycle is decreased, it can be supposed[13] that the chance of finding any element in an excitable condition has been improved by the lengthening of the dark time, and in this way the observed changes in $F_{max.}$ and in the position of the flicker contour,[13,12] without change in its form, can be accounted for quantitatively. Thus the parameters of the flicker contour are affected in a significantly different way by change of t_L/t_D and by change of temperature: elevation of temperature and decrease of t_L/t_D each move the curve toward a lower intensity level, and the shift of the inflection point is directly proportional to the change in percentage light time; the slope of the curve, on a percentage ordinate ($\sigma'_{\log I}$) is not changed in either case; change of temperature does not affect $F_{max.}$, but $F_{max.}$ is a rectilinear function of $t_L/(t_L + t_D)$. Alteration of the percentage light time

[19] Crozier, 1936; Crozier and Holway, 1937, 1938; Holway and Crozier, 1937.
[20] *Cf.* Hecht, 1934.

therefore influences the total number of effective available excitable elements (by making each unit more or less frequently available, but affecting all the units in the same proportion), whereas this number is not a function of the temperature. Consequently elevation of temperature does not multiply the frequency with which each element contributes to the determination of the index response; the increase of F at fixed I with rise of temperature, in the curve between the asymptotes, is merely a mechanical consequence of the fact that the position of the curve on the log I axis has been shifted. Hence we must provisionally turn to some form of the first hypothesis, namely

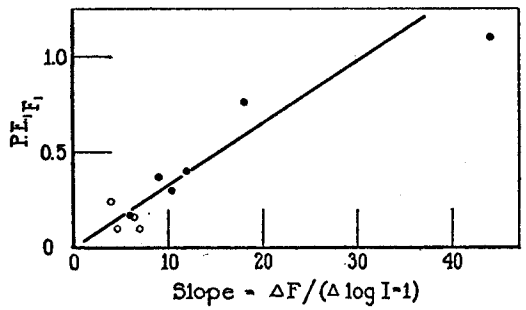

FIG. 11. Data in Table III, showing the dependence of P.E.$_{1F_1}$ at homologous points (inflections) on the flicker response curve of various animals upon the slopes of the curves at these points. The open circlets refer to "rod"-segments of flicker contours. The departures are naturally greater at the upper end, since P.E.$_{P.E._F}$ is proportional to P.E.$_F$.

that the dependence of the position of the curve on the temperature is due to the effect of conditions uniformly influencing the excitability of all the elements concerned. It is to be recognized that the elements in question are defined solely in terms of the effect produced, namely the response to flicker at critical values of F and I.

If the control of excitability by temperature is similar to that apparent in many other biological processes, we should then look for the behavior of $1/I$ to be such that the measure of excitability is proportional to the velocity of underlying chemical changes. The magnitude of the crude temperature coefficient is consistent with this view. The data are plotted in Fig. 11. To a very good approxima-

tion the mean values of $1/I$ adhere to the Arrhenius equation, with $\mu = 26{,}500$ between $t^\circ = 12.2$ and $t^\circ = 30.0$, $\mu = 12{,}400$ between 30° and 35.8°. The deviations on the intensity axis are at most = 0.05 log unit; this is about the extreme difference to be expected in duplicate determinations of I_m. The chief reason for the departures is to be found, however, in the control of the temperature. In water the turtle's temperature is not noticeably higher than that of the water,[21] but on removal from the thermostat the temperature of the aquarium changed during the 3 to 5 minutes required for an observation, and changed more the greater the difference between the experimental temperature and that of the room (21.5°). This was corrected for by taking the temperature of aquaria during the period of observation, in control tests. The change at the highest and lowest points was about 0.6°. The mean temperatures recorded in Table III are the average temperatures during the time of observation. Probably the temperature of the turtle does not change so rapidly. In Fig. 11 the same slopes have been assigned to the lines drawn for $F = 20$ and $F = 30$. These flash frequencies are situated one on either side of the inflection point of the $F - \log I$ curve. The behavior of $1/I_{infl.}$, the critical excitability at the inflection point, must therefore be understood to follow the same rule.

The measurements in Table III are brought together for comparison by dividing each $1/I_m$ at $F = 20$ by a constant (= 10.21). The resulting figures are plotted in Fig. 12, with the curves expressing $\ln (1/I_m) = -\mu/RT + const.$ It is clearly impracticable to pass a ***single*** curve through the whole course of the data. An adequate description is given by recognition of the critical temperature at 29.5°. In this region, and also at the higher temperatures as contrasted with the lower, the scatter of the determinations should be greater, as is uniformly found in such curves.[22] This particular critical temperature, 30° ± 0.5°, is one rather frequently found.[23] The behavior of $P.E._{1I}$ (Table III) is such that it is directly proportional to I_m, F constant, when I_m is changed by altering the temperature. This means that the variation of $(1/I)$ is a constant percentage of the

[21] *Cf.* also Isserlin, 1902.
[22] Crozier, 1929; 1935.
[23] Crozier, 1925–26 *a*.

mean $(1/I_m)$ at all temperatures,—the characteristic result obtained with other biological events for which a chemical control may be assumed.[22]

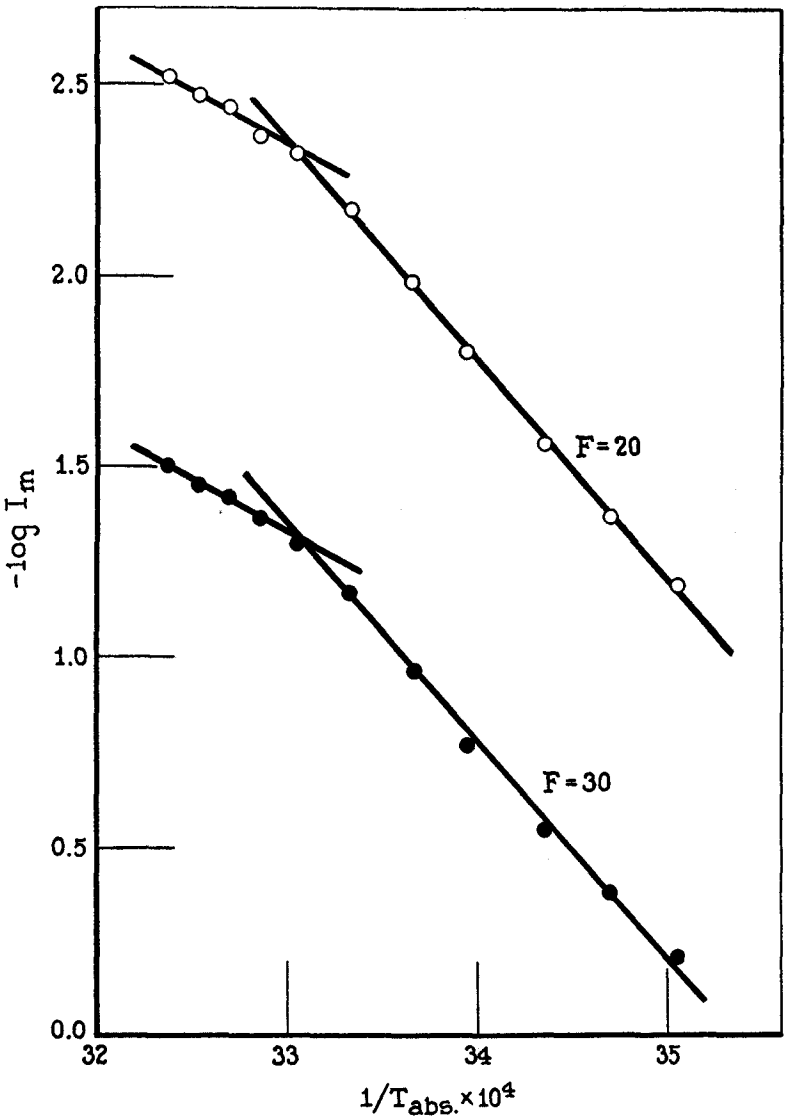

FIG. 12. Log $1/I_m$ *vs.* $1/T_{abs.}$, at $F = 20$ and at $F = 30$; Table IV. See text.

In our earlier accounts of the shift of the flicker response curve with change of temperature we have utilized[14] the conception of the building up of an effect due to a flash and its decay in the dark interval. The effect responsible for reaction to flicker is then supposed to be due to the recognition of a mean difference between E_2 (in the flash) and E_1 (in the dark interval). Elevation of the temperature leads to a more rapid decay of effect in the dark, hence at given flash

TABLE IV

Mean Critical Intensities for Response to Flicker by the Turtle Pseudemys at Flash Frequencies 20/Second and 30/Second, at Different Temperatures

The reciprocals of the mean critical intensities (millilamberts) are given for F = 30/second; for F = 20/second, shown divided by 10.21.

t°C.	F			
	20		30	
Corr.	log I_m	$1/I_m/10.21$	log I_m	$1/I_m$
12.2	$\bar{2}.811$	1.51_4	$\bar{1}.791$	1.618
15.07	$\bar{2}.634$	2.28	$\bar{1}.624$	2.377
18.03	$\bar{2}.442$	3.54	$\bar{1}.455$	3.508
21.5	$\bar{2}.204$	6.12_4	$\bar{1}.237$; $\bar{1}.231$ ($\bar{1}.266$)	5.795
23.95	$\bar{2}.017$	9.42	$\bar{1}.039$	9.141
26.90	$\bar{3}.826$	14.62	$\bar{2}.835$	14.62
29.50		20.94	$\bar{2}.704$	19.77
31.32	$\bar{3}.635$	22.70	$\bar{2}.639$	22.96
32.80	$\bar{3}.561$	26.92	$\bar{2}.582$	26.18
34.30	$\bar{3}.530$	29.51	$\bar{2}.65$	28.18
35.83	$\bar{3}.480$	32.43	$\bar{2}.498$	31.77

frequency a lower intensity is required to produce a critical balance. The excitability, as we have defined it by $1/I_m$, increases as if under the control of the velocity of some chemical transformation in a system comprising at least two discrete processes with distinct temperature characteristics.

It is instructive to inquire how this finding can be reconciled with the intensity discrimination theory of recognition of visual flicker. Qualitatively, in terms of this hypothesis, the summated action of

the nervous units involved is pictured as producing the rising segment of the "effect" curve and the decay curve for the action of a flash;[14] the velocity constant of the decay curve, other things constant, determines the level at which I must arrive to be critical. At given I, more flash-sensitive elements will be excited at a higher temperature; the flash effect curve will rise more steeply toward a higher maximum, presumably without change in its velocity constant; and to achieve marginal response to flicker each flash must be allowed to act for a shorter time; hence the critical F rises, until at a sufficiently high intensity all the elements available are activated. This works out graphically in the manner indicated in a previous paper.[14]

In the discussion of an earlier group of experiments we have pointed out that a basis can be suggested for interpreting a change in $P.E._{II}$ at constant I_m when the temperature is altered.[14] The measurements with *Pseudemys* give little evidence for such a change in this case (Fig. 11), but sufficiently complete sets of data are available for two temperatures only.

Taking the picture in Fig. 12 as due to the fact that the excitability $(1/I)$ of the neural elements concerned in marginal response to flicker is governed by the velocities of chemical events common to all of the elements concerned, we have to inquire as to the kind of mechanism which could determine control by a different process on either side of a critical temperature. The general theory of such situations has been that the two temperature characteristics each refer to a specific process (reaction), both of them involved in the control of the velocity which governs the property measured (frequency of a rhythmic activity; excitability) in its relation to temperature; below the critical temperature, one of these processes is in the slow or "master" rôle, above it the other. It was pointed out long ago[23] that the existence of particular critical temperatures makes it impossible to assume that in general the relationship between the two processes is one of mass action succession. It is necessary to appeal to something in the nature of a phase change, or to the structure of chain reactions, in some critical location[23] which affects the over-all speed of the process whose temperature characteristic is evidenced over the higher temperature segment.

The modification must then be usually such that the process giving rise to the μ observed for the upper segment has had its velocity curve abruptly lowered, without change of its μ. The occurrence of observable shifts in velocity *without* change of μ,[24] and with such changes,[25] is proof of the physical reasonableness of this conception, which also permits a rational interpretation of the experimental modifications of the shapes of temperature curves.[16] However, there is also evidence[26] of experimentally induced slow changes of critical temperatures in some instances; these are easily understood as due to a direct effect upon the mechanism responsible for the "phase change."

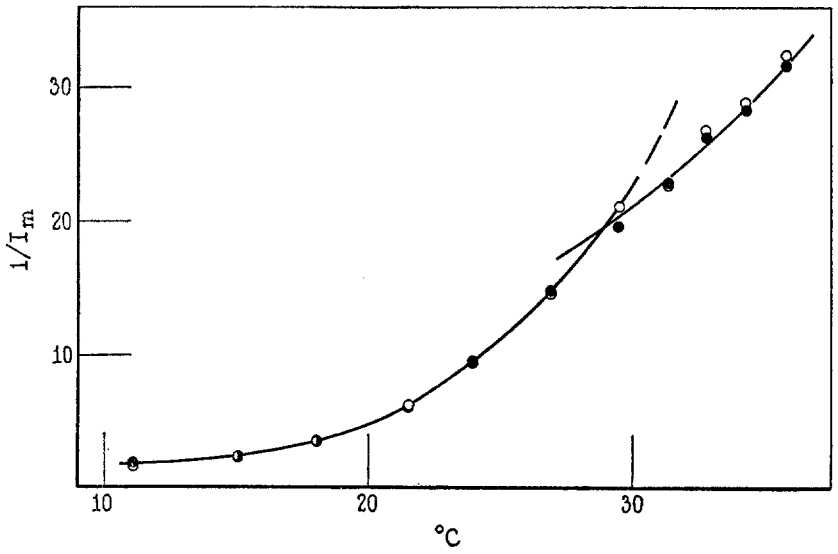

FIG. 13. $1/I_m$ *vs.* t°C.; the lines are transferred from Fig. 12. The data for $F = 20$ have been divided by 10.21 to bring the two sets of measurements together.

Certain of the relationships contemplated may be made clearer by a diagram (Fig. 13). It is necessary, in general, to regard each of the processes revealed in a composite μ-graph as an individualized entity, since it behaves as such in its response to experimental treatments.[24,27] The fact that the variation in the measured property is, over a straight section of the graph, a constant fraction

[24] Crozier, 1924–25 *b*; 1925–26 *b*; Crozier and Stier, 1924–25 *b*; Pincus, 1930–31, etc.

[25] Crozier and Stier, 1925–26; 1926–27 *a*, *b*.

[26] Experiments in course of publication.

[27] Crozier and Stier, 1924–25 *a*, *c*; Navez, 1936.

of the mean (independent of temperature) is extremely difficult to account for if the controlling process, identified by its μ, is really a composite.[22] Hence the two processes (Fig. 12) must be conceived as linked in a chain of some kind. Both are involved in the control of the appearance of the end result which is the basis of the measurements; whichever is slowest impresses its temperature char-

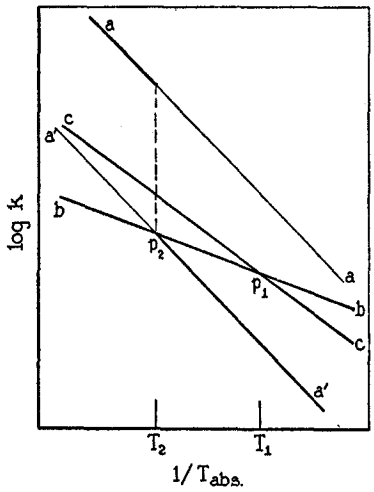

FIG. 14. To indicate diagrammatically the basis for control of the magnitude of a phenomenon governed by a system of three concurrent processes catalytically linked in the manner discussed in the text. The over-all frequency of the phenomenon, as a function of temperature, is determined by the speed (frequency) of the process (a, b, or c) which has the smallest k. Thus, if process a were to be described by $a - a$ in the figure, a critical temperature (T_1) would appear at P_1, and the Arrhenius plot for the observed frequency would be $b - P_1 - c$. Since critical temperatures markedly tend to occur at particular levels of temperature rather than in random distribution appeal must be made to shifts such as from $a' - a'$ to $a - a$ at a particular temperature (T_2); if such a shift, due to a phase change or analogous modification in some suitable location, produces a sufficient elevation of k_a, without changing the slope of the Arrhenius plot, the over-all observed frequency will follow the broken line $b - P_2 - a'$; shifts of the type $a - a$ to $a' - a'$ are known.

acteristic upon the over-all velocity of the whole. Physical models of this sort are easily contrived,[28] but are likely to be misleading. It is sufficient to suppose that we have to do with two processes which provide materials making excitation

[28] *Cf.*, *e.g.*, Hoagland, 1937.

possible; the excitability will rise with the rising rates at which each of these provide their particular substances.

The simplest assumption seems to be that we may consider a catenary set of catalytic processes, $A, B, C, \ldots$, in which the rate of the terminal process, say C, determines the velocity underlying the control of the observed phenomenon. The observed phenomenon is taken to behave as if controlled by the velocity of a transformation $X \xrightarrow{k} Y$. The velocity constant k of this process governs the behavior of the observed phenomenon (here, $1/I_m$) as a function of the temperature. The rates of the processes A, B, and C are defined in terms of the *frequencies* with which fixed quantities of their products are produced. For consistency in discussion this type of definition is necessary, since the nature of the formulation of the end result—the relation of the observed critical driving potential to temperature—depends upon the implicit definition of critical intensity as connected with the magnitude of the sensory effect which must be brought about (at a given flash frequency): this definition is obtained by means of the equation defining the dependence of I upon F, and is stated in terms of a summation of *frequencies* of elemental neural effects. The idea is specifically that, as in periodic contact catalyses, the step $B \rightarrow B'$ cannot proceed until a quantity of material $\Delta A'$ has formed from A, and cannot recommence until a new $\Delta A'$ has been formed. The *frequency* with which the reaction $B \rightarrow B'$ can begin must consequently depend upon the magnitude of k_1 in the scheme

$$A \xrightarrow{k'_1} \Delta A'$$

$$B \xrightarrow{k'_2} \Delta B'$$

$$C \xrightarrow{k'_3} \Delta C'$$

$$X \xrightarrow{k} Y.$$

It is obvious that the frequency with which $\Delta C'$ is produced, in a series of such steps, will depend upon the smallest value of k'. Thus the velocity constant k will be governed by (proportional to) the value of this particular k'. Nothing need be specified as to the mechanism whereby $\Delta A'$ releases the process $B \rightarrow B'$; it could be by covering, or rupturing, a film on a catalytically active surface, or by other means; the transformations of $A \rightarrow A'$, etc., could be, and might well be, cyclic. In general, the critical increments for k'_1, k'_2, k'_3, etc., will not be the same. Hence there is opportunity for change of temperature to alter the step for which k' is the slowest. It necessarily follows from such a scheme that if a change of this kind is brought about, each k' increasing with temperature, that the particular new k' brought into control over a higher range of temperature will have a *smaller* μ than that apparent over a lower range. Thus in Fig. 13, only the k' having a smaller μ (*i.e.* k'_c) can possibly have its curve cross that for k'_a, assumed to be smallest at the lowest temperatures.

It will be observed that this mechanism provides sharp breaks in the curve of k *vs.* $t°$, provided k'_1, k'_2, k'_3 are not too far apart and have the proper disposition of μ's. It does not, however, explain why the breaks predominate at particular temperatures, nor why there do also occur occasionally (at these temperatures) shifts of control to a μ or a k which is *higher*.[29] Catalysis, however, occurs at film-covered surfaces; the constitution of surface films is such that critical temperatures may be a prominent property;[30] the doubling of a covering barrier film could easily produce a shift of a k', with or without altering its position as smallest in a set; and thus produce a change in the over-all k with or without altering its μ; or it could, equally well, by changing one value of k' bring it (with its μ) into the governing, slowest position at higher temperatures.

There are other forms which this type of hypothesis can take, without affecting its fundamental nature. It allows for the introduction of effects which may (under some conditions) modify the position of a critical temperature, and it gives a basis for the interpretation of the known experimental modification of k and μ. Moreover, it suffices to account for the fact that the relative variation of the quantity taken as proportional to k (*i.e.* $1/I_c$) is independent of the temperature; in the present case, for example, $\sigma(1/I)\ /1/I = \sigma_I/I$, for F constant, is independent of temperature. This is easily accounted for quantitatively on the basis that the magnitude of the controlling frequency constant—*e.g.* k_B or k_C, fluctuates at random.

The basic fact about the necessary guiding assumptions is that the assumed processes (reactions) are supposed to have speeds governed by the mean rates of formation of catalysts,[31] not by mass action of the concentrations of substrates. This elementary point has been curiously overlooked by at least one critic of the "master reaction" conception.[32] The kinetic equations for successive irreversible mass action processes long ago[31, 33] made it clear that sharp breaks in the over-all rate curve could not be characteristically produced by altering the temperature, with such systems. For that reason, of course,

[29] *Cf.* Pincus, 1930–31. (Cases in which the plot of log k *vs.* 1/T is concave upward (μ increasing *continuously* with $t°$) obviously signify the concurrent influence of at least two independent processes (*cf.* Crozier, 1924–25 *b*). An instance in which the data for a case of this sort give rather striking confirmation of the theory of specific temperature characteristics, when suitably analyzed, although differently regarded by their author, is found in a recent paper by Korr (1937).)

[30] Langmuir, 1933.

[31] Crozier, 1924–25 *b*.

[32] Burton, 1936; *cf.* Hoagland, 1937.

[33] Rakowski, 1907.

and for the equally good reason that the breaks occur at definite temperatures, no schemes on this basis were ever presented; and it was early pointed out[23] that appeal must be made to a different basis for shifts from one μ to another.

SUMMARY

1. At constant temperature, with a fixed proportion of light time in a flash cycle (namely, $t_L/t_D = 1$), the mean critical intensity for motor response to visual flicker by the turtle *Pseudemys scripta* follows a probability integral (log I) as a function of flash frequency F. The fit is close and satisfactory; certain quite minor but consistent deviations are adequately explained by features of the experiments.

2. The *variation* (σ_I) of critical I is directly proportional to the mean critical intensity (I_m), over the entire explorable range.

3. These facts are consistent with the fact that the retina of this turtle is devoid of rods. It contains only cones, histologically, which, with their central representations, provide a single population of sensory effects. The properties of this population are compared with those of homologous populations deduced from corresponding measurements with other forms (various fishes; amphibian; man) which exhibit *two* such groups of sensory effects associated with the possession of retinal rods and cones.

4. Certain other formulations which have previously been applied to homologous data obtained with other organisms do not properly describe the *Pseudemys* measurements.

5. The use of a probability integral to describe the data of response to visual flicker for the dissection of the compound curves provided by animals possessing both rods and cones, is accordingly justified.

6. Persisting differences among individuals of *Pseudemys* as regards the values of the critical flash intensity under various conditions of experimentation are of the same order of magnitude as are the transitory differences found in lots of other kinds of animals.

7. Determinations of mean critical flash frequency (F_m) at fixed levels of I lie slightly above determinations of I_m at fixed values of I, as with other forms. The variation of critical flash frequency goes through a maximum as log I is increased; its height is lower than

with certain other forms, in correlation with the low general slope of the $F - \log I$ curve (more properly, band).

8. These facts are consistent with the view that the dispersions of the individual critical intensities (and flash frequencies) are determined by organic variation rather than by "experimental error."

9. When the temperature is altered the $F - \log I_m$ curve is shifted, with no change of $F_{max.}$ or of shape; the curve moves to lower intensities as the temperature is raised.

10. The reciprocal of the mean critical intensity, at fixed flash frequency, is a measure of excitability. With increase of temperature (12.5° to 36°) $1/I_m$ for given F follows the Arrhenius equation, exhibiting a "break" at 29.5° (μ = 26,700, 12.5° to 29.5°; 12,400, 29.5° to 36°). This is explained by the necessary theory that, the number of elements of sensory effect required for the index response at fixed F being constant, the ease of their excitation is governed by temperature through its control of the velocity of an interrelated system of catalyzed processes common to all of the sensory elements concerned.[34]

CITATIONS

Burton, A. C., 1936, *J. Cell. and Comp. Physiol.*, **9**, 1.

Crozier, W. J., 1924–25 *a*, *J. Gen. Physiol.*, **7**, 123; 1924–25 *b*, **7**, 189; 1925–26 *a*, **9**, 525; 1925–26 *b*, **9**, 531. 1929, The study of living organisms, in Murchison, C., Foundations of experimental psychology, Worcester, Clark University Press, pp. 45–127. 1934–35, *J. Gen. Physiol.*, **18**, 801. 1935, Déterminisme et variabilité, Paris, Hermann et Cie, 56 pp. 1935–36, *J. Gen. Physiol.*, **19**, 503. 1936, *Proc. Nat. Acad. Sc.*, **22**, 412; 1937, **23**, 71.

Crozier, W. J., and Holway, A. H., 1937, *Proc. Nat. Acad. Sc.*, **23**, 23; 1938, **24**, 130.

Crozier, W. J., and Stier, T. J. B., 1924–25 *a*, *J. Gen. Physiol.*, **7**, 429; 1924–25 *b*, **7**, 699; 1924–25 *c*, **7**, 705; 1925–26, **9**, 547; 1926–27 *a*, **10**, 479; 1926–27 *b*, **10**, 501.

Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., 1936–37 *a*, *J. Gen. Physiol.*, **20**, 211; 1936–37 *b*, **20**, 363; 1936–37 *c*, **20**, 393; 1936–37 *d*, **20**, 411. 1937, *Proc. Nat. Acad. Sc.*, **23**, 516. 1937–38 *a*, *J. Gen. Physiol.*, **21**, 17; 1937–38 *b*, **21**, 203; 1937–38 *c*, **21**, 223; 1937–38 *d*, **21**, 313; 1937–38 *e*, **21**, 463. 1938 *a*, *Proc. Nat. Acad. Sc.*, **24**, 125; 1938 *b*, **24**, 216; 1938 *c*, **24**, 221. 1938 *d*, *J. Exp. Zoöl.*, in press; 1938 *e*, in press.

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Detwiler, S. R., 1916, *J. Exp. Zool.*, **20,** 165; 1923, **37,** 89.
Detwiler, S. R., and Laurens, H., 1920, *J. Comp. Neurol.*, **32,** 347.
Hecht, S., 1934, The nature of the photoreceptor process, in Murchison, C., Handbook of general experimental psychology, Worcester, Clark University Press, pp. 704–828. 1937, *Physiol. Rev.*, **17,** 239.
Hoagland, H., 1937, *J. Cell. and Comp. Physiol.*, **10,** 29
Holway, A. H., and Crozier, W. J., 1937, *Proc. Nat. Acad. Sc.*, **23,** 509.
Isserlin, M., 1902, *Arch. ges. Physiol.*, **90,** 472.
Korr, I. M., 1937, *J. Cell. and Comp. Physiol.*, **10,** 461.
Langmuir, I., 1933, *J. Chem. Physics*, **1,** 756.
Menner, E., 1928, *Z. vergleich. Physiol.*, **8,** 761.
Navez, A. E., 1936, *Mem. Musée Roy. Hist. Natur. Belg.*, 1936, (Mélange Paul Pelseneer), 701.
Pincus, G., 1930–31, *J. Gen. Physiol.*, **14,** 421.
Rakowski, A., 1907, *Z. physikal. Chem.*, **57,** 321.
Stier, T. J. B., and Crozier, W. J., 1932–33, *J. Gen. Physiol.*, **16,** 757.
Thiersch, F., 1924, *Z. physikal. Chem.*, **111,** 175.
Verrier, M.-L., 1935, *Bull. biol. France et Belgique*, suppl. **20,** 140 pp. 1937, **71,** 238.
Walls, G. L., 1934, *Am. J. Ophth.*, St. Louis, **17,** 892.
Wolf, E., and Zerrahn-Wolf, G., 1935–36, *J. Gen. Physiol.*, **19,** 495.